UNIVERSITÀ DEGLI STUDI DI PALERMO
Tecnologie e Scienze per la Salute dell'Uomo
Dipartimento Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza "G. D'Alessandro" PROMISE
Farmacia

NOVEL DRUG DELIVERY SYSTEMS FOR TREATMENT-RESISTANT SCHIZOPHRENIA

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CICLO XXXIII
ANNO CONSEGUIMENTO TITOLO 2020

To my own ambitions

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Part 1. Introduction

Chapter 1. Route of Administration and Drug Delivery System

Pharmaceutics (the science of dosage form design) is one of the four pharmacy's disciplines of preparing new or old drugs into modern medicaments that can be safely used by patients. This field usually investigates chemical, physical, physiological, and formulation factors that can affect drug absorption, distribution, and elimination processes determining *in-vivo* performance (Rowland Tozer; 2005). Drug absorption indicated the journey of a drug from the site of administration to the auction site. The rate of drug absorption affects the onset of drug effect, therefore studying it, is essential to ensure that the amount of drug responsible for therapeutic effect reaches systemic circulation (Van de Waterbeemd et al.;2001). Regarding physical properties, drug solubility and permeability are of importance as the drug needs to be in solution to cross biological membranes. It has to possess balanced hydrophilicity and lipophilicity to diffuse and be absorbed (Dahan et al.; 2009).

Drug administration aims to exert either a local or a systemic effect. Local drug delivery implies that the drug's impact is at the site of application, and no further distribution in another part of the body is needed. On the other hand, systemic administration requires that the drug is absorbed to reach systemic circulation and be transported to the effect site or sites (Brunton et al.;2013).

Drugs are usually administered by enteral, parenteral, and topical application, and, with an exception for intravenous and arterial administration, once the drug is administered is had to be absorbed and then distributed in the systemic circle. Choice of the parenteral route depends on the emergency of initiating treatment or on physicochemical properties of the drug that might affect its absorption via gastrointestinal tract (Gulati and Gupta; 2011).

The significant challenges in drug delivery include overcoming drug absorption barriers, such as biological membranes, and of drug transportation from the administration site to site of action. Overcoming drug absorption barriers is essential in the understanding of structure and function of membranes, epithelia, and tissues, which is necessary to improve the absorption process. The endothelial cell has a lipid bilayer that consists of amphipathic lipids, phospholipids, glucolipids, steroid and proteins, that creates cell membrane. This complex organisation allows permeation of lipophilic molecules; however, protein channels or complex could allow incorporation of hydrophilic molecules or generate a cascade of electrochemical steps that create a therapeutic response (Madani et al.;2011). The body surface is cover with epithelia that are layers of proteins which connect cell membranes of adjacent cells, leaving a small space (i.e. gap junction) between them filled with

extracellular fluid (Sperelakis and Kaneshiro; 2012). Passage of molecules through the membrane is selective for nutrients, vitamins, mineral and other functional compounds, and those can be absorbed with a different mechanism that is used by drugs as well. Main tools are passive diffusion, carrier-mediated and para-cellular, and those were schematised in Figure 1 (Hedaya; 2012).

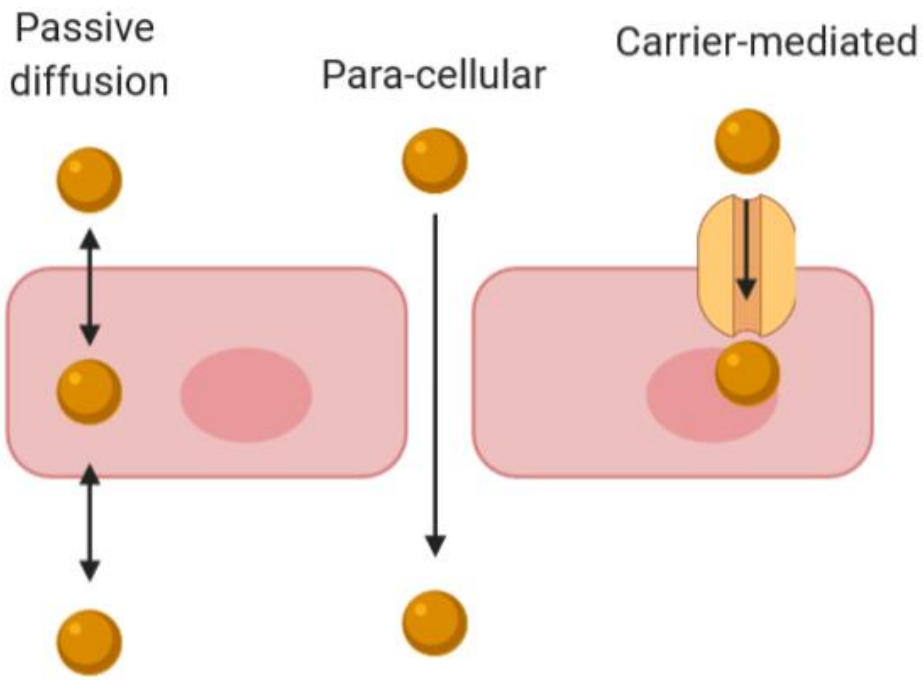


Figure 1. Schematic representation of common mechanism for drug across membranes. Image is drawn via BioRender and adapted from Mohsen and co-workers (Hedaya; 2012).

Passive diffusion is a process that allows a molecule to move from a higher to lower concentration according to the concentration gradient. Passive diffusion does not require energy and the distribution through the membrane is described by Fick's law; equation 1 (Le; 2019).

$$Rate\ of\ Diffusion = \frac{dA}{dt} = DKS/h\ (Cabs - Cp) \qquad \text{Equation 1}$$

Where dA/dt is rate or diffusion across membrane, D is diffusion coefficient that is drug-specific, K is drug partition coefficient, S is membrane surface area, h thickness and ($C_{abs} - C_p$) is difference between the concentration of drug in absorption site and plasma.

It is clear that the rate of absorption is proportional to drug lipophilicity and surface area available but decreases with the increase of membrane thickness. Therefore, when administrating a drug in the gastrointestinal tract, the leading absorption site is represented by the small intestine that possesses a large surface area (Thompson et al.;2014). From the other hand side, carrier-mediated

transport usually includes the transport system as a protein in which drug passage implies energy consumes, while the para-cellular route is the movement of small molecules through gap junction.

As mentioned above, most common administration routes are enteral, parenteral, and topical. Oral drug delivery is most common due to its high patient compliance and handles formulation strategies. Once the medication is taken, it undergoes different stage before being absorbed, and those are described in the next chapter of present work. Parenteral delivery is considered an emergency administration producing a fast onset of action and requires intervention by clinicians as it is highly invasive. However, it is the only route available for drugs, like insulin, lidocaine and fentanyl that degrades in the gastrointestinal tract (Gavhane and Yadav; 2012). The most significant advantage is that drug directly reaches system circulation, by-passing physical or biological barriers. Such administration route has risks of injury, infection at the site of injection, phlebitis, and thrombosis (Homayun et al.;2019).

Moreover, formulation administered by this route should not contain particles larger than five µm, must be water-miscible, sterile, isotonic, and pH. Sterility, isotonicity, and pH must be respected as a variation of one of these parameters could cause blood clots, infection, thrombosis and stroke (Smith and Mendel; 1920). Topical dosage forms present advantage of being applied on site of action, avoiding both systemic circulation and hepatic metabolism. The drug is absorbed through a thicker layer of skin; therefore, the absorption rate is lower and strongly influenced by drug lipophilicity. This route is used for drugs with low molecular weight (Mw), adequate lipophilicity and can be applied to a broader category of drugs if formulated with appropriate excipients named “enhancer” (Benson et al.;2019). Recent research was directed towards the alternative of both oral and parenteral route whit aim of by-passing hepatic metabolism and increases absorption rate to achieve an early therapeutic onset of action, named transmucosal delivery.

The concept of drug absorption and its delivery to the site of action to cause a therapeutic effect is defined as bioavailability and is calculated as “Area Under Curve” (AUC). Bioavailability of drug represents the quantity of a drug which reaches the systemic circulation, and when a drug is a delivery by the parenteral route, this reaches a theoretical value of 100%. At the same time, oral administration is influenced by absorption rate through membranes, as schematised in Figure 2.

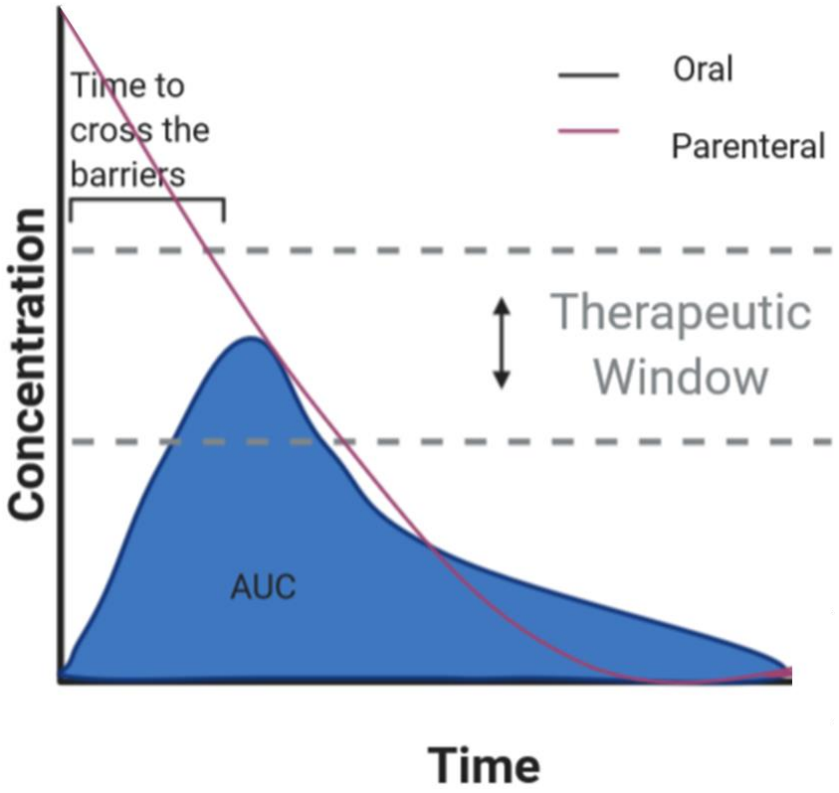


Figure 2. Comparison of absorption rate of oral (in blue) and parenteral (in purple) route.

1.The gastrointestinal tract

The tradition oral route (i.e. drug delivery via the gastrointestinal tract) is the most commonly used in drug delivery due to high patient compliance, acceptability and ease of administration and formulation. Its efficacy depends on different factors such as drug solubility and intestinal permeability, that affects the quantity of the drug that permeate membranes to reach the systemic circulation. However, oral absorption is usually slow and does not ensure enough drug bioavailability as the drug is diluted into physiologic fluids through the gastrointestinal tract (Goodman et al.; Michael; 2007). The digestive tract includes four main anatomical areas: oral cavity, stomach, small and large intestine (Figure 3). Presence of villi increases the absorption surface of the small intestine to 200 $m^{\wedge 2}$, representing drug and nutrients absorption site.

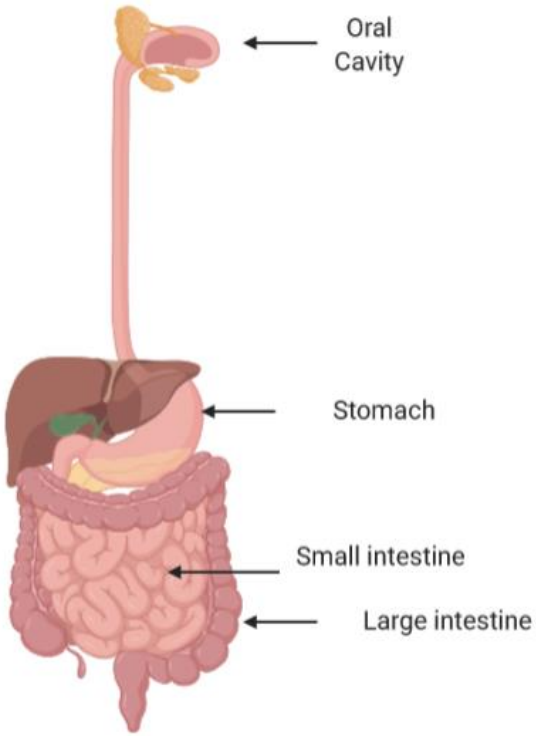


Figure 3. Gastrointestinal tract is composed of four regions: oral cavity, stomach, small and large intestine and designed via BioRender.

The epithelial covering the intestine are four: mucosa, submucosa, muscularis propria and propria. Mucosa represents absorption site, and both physiological factors and drug physicochemical properties affect oral drug absorption, thus bioavailability. The physiological factors are gastrointestinal motility, metabolism, presence of food, pH, and individual variation (such as gender, age, race, and medical condition). Along with gastrointestinal tract pH varies from acid (stomach) and basic in the intestine; moreover, presence of food modified pH in the stomach where from typical values between 0.8 and 2, rises to 4-5. While intestine pH is between 6 and 7. Gastrointestinal pH influences drug dissolution, solubilisation, and absorption. Drug absorption is therefore influenced by drugs physicochemical properties such as partition coefficient, pKa, Mw, chemical stability, and solubility (Artursson et al.;2007). As described above, drugs can be administered via different administration routes which selection depends on physical drug properties such as permeability and solubility. In the following sections, those are described and discussed, as well as conventional solid dosage forms used for drug delivery through the oral route and oral cavity.

1.1 Drug solubility

Solubility is a physical property of molecules in a solvent that correlates with drug solid-state that could be either crystalline or amorphous, and its chemical structures. Chemicals can be acids or bases, and their solubility increase as their degree of ionisation increases. Therefore, their solubility could be predicted in a specific solvent applying the following equations.

$$pK - pKa = \log\left(\frac{S-So}{So}\right) For\ acids \qquad \text{Equation 2}$$

$$pH = pKa - \log\ (\frac{(So)}{(S-So)})\ for\ basis \qquad \text{Equation 3}$$

Where at a pH above pKa solubility of acids increases and pH values below pKa solubility of bases increases.

As described above, drug absorption through the membrane depends by different factors such as diffusion coefficient, partition coefficient, membrane surface and thickness. However, drugs have to be in solution to cross epithelial, and its absorption rate relates to how fast it dissolves in human fluids at the absorption site. Dissolution rate is, therefore, the controlling steps of all process and is strictly correlated with drug solubility. It is estimated that more than 40% of the drug is poorly soluble in water. Thus pharmaceutical research focuses on improving the solubility of poorly soluble drugs to ultimately increase dissolution rate, absorption and bioavailability (Kalepu and Nekkanti; 2015). Foundation of this line of research relates with Noyes-Whitney equation (equation 4) which explains that a drug dissolves more rapidly when it possesses a high surface area, achieved by reducing its particle size.

$$\frac{dm}{dt} = kS\ (Cs - Ct) \qquad \text{Equation 4}$$

Where dm/dt is dissolution rate, i.e. amount of drug dissolved per unit time, K is dissolution rate constant, S is the surface area of drug particles; Cs is saturation solubility of the drug; Ct is the concentration of dissolved solute (Martinez and Amidon; 2002).

This equation indicates that reducing particle size results in an increase of dissolution rate, thus absorption. Once a drug is dissolved, it can cross the membrane, and the flux of absorption can be predicted and calculated through Fick's law (equation 1).

Drug solubility depends as well on its solid-state, which can be amorphous or crystalline. Amorphous materials possess short-range molecular order and high kinetic energy, and their weaker attractive intermolecular forces result in bonds which are easily broken. This allows molecules to be freed on the solid-state surface into a liquid medium resulting highly soluble and having a faster dissolution rate (Shah et al.;2006). From another hand side, in crystalline state molecules possess long-range molecular order which defines a low energy state resulting in higher stability and allowing an adequate physical form control during formulation and storage processes (Sinko; 2011). Amorphous compounds are, therefore, characterised by a lack of long-range molecular order and it is an undercooled liquid, thermodynamically unstable. Disordered amorphous structure implies molecular spatial fluctuations. Such fluctuations cause bonds breaking and spatial reorganisation toward an equilibrium named stable equilibrium, which is typical of the crystalline compound. In fact, as per the second thermodynamic law, matter tends to minim energetic conformation; therefore, amorphous compound potentially mutates to crystal. However, this modification is time-dependant and could be improved or prevent by modifying condition such as temperature and pressure (Franks et al.;2007).

APIs are categorised based on their solubility and permeability through membranes in Biopharmaceutical Classification Systems (BCS). BCS is an experimental model design for oral drug delivery as 90% of the marketed dosage form are designed for oral delivery. According to BCS, drugs are organised in four classes, from I to IV, with almost 60% of them listed in II and IV classes, due to their high hydrophobicity, as schematised in Figure 4. A drug is considered highly soluble if the highest clinical dose is soluble in 250mL at a pH between 1 and 7.5 at 37°C (Ku; 2008).

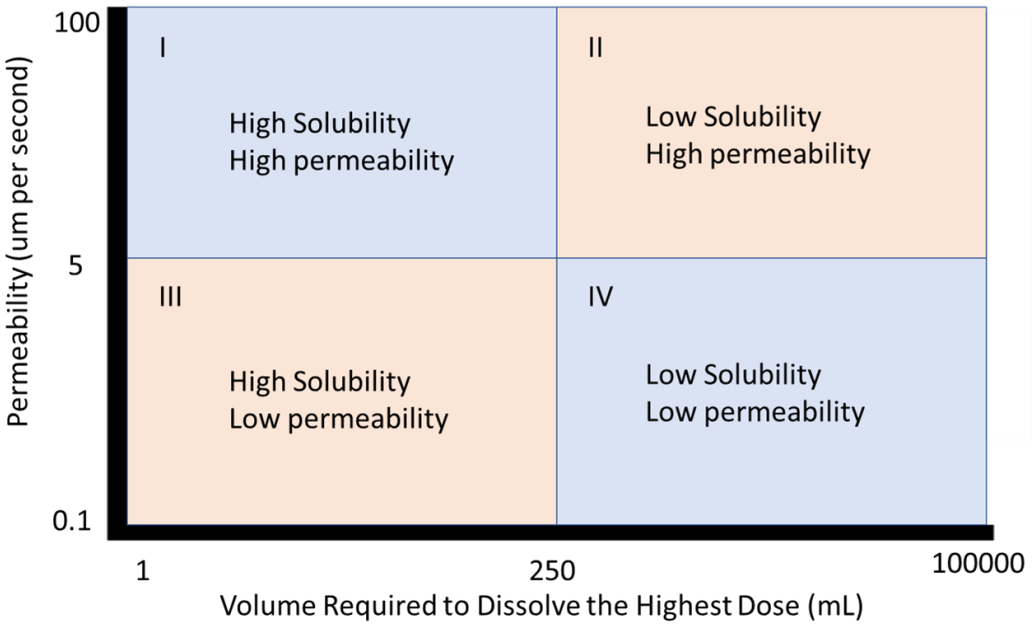


Figure 4. BCS classification of drugs according to their solubility and permeability. In 2011, it was calculated that BCS class II represent 60% of marketed dosage forms, while class I just 20%.

Solving solubility related issue is considered a significant challenge in drug-delivery, considering that the majority of drugs have low solubility, but about 90% of marketed dosage forms are formulated for an oral dose. Therefore, several formulation strategies are employed, and they are discussed in the following section (Samie et al.;2017; Campardelli et al.;2017).

1.2 Oral solid dosage forms

Approximately 90% of marketed drugs are administered by the oral route as a liquid or solid dosage forms. Liquid dosage forms are useful when required for rapid and efficient absorption, as molecules are already dispersed with a small surface area. Those formulations present the main problem that most of the drug is poorly soluble; therefore, their suspension or solution in a solvent requires used of several solvents and excipients to maintain stability. Moreover, liquid dosage forms are fewer patients acceptable as challenging to carry, and it is easy to mistake dose. From a pharmaceutical point of view, liquid formulation usually present sugars and water, representing a potential cause of bacterial growth once opened (Haywood and Glass; 2013). Therefore, liquid dosage forms are not discussed in this work.

Most commonly, medicines for the oral route are in the form of tablets or capsules, which once reached the stomach, or intestine disintegrates in granules or small particle before being dissolved in human fluid to be absorbed. In solid dosage form, absorption is affected by particle size as well as by the presence of excipients or additives such as disintegrating, granulating agents, lubricants, and others (Michael; 2007). Tablets are usually obtained by compression of granules or powder containing drug and excipients which facilitate the manufacturing process. After oral tablet administration, drug dissolution is slow due to small surface area and once tablet starts to disintegrate in small granules or particle dissolution rate increases. Then absorption and diffusion mechanisms depend on physical drug properties, as described above. For example, many drugs are not stable in acidic pH of the stomach; therefore, coated tablets were developed to avoid disintegration of the tablet in the stomach allowing the release of drug in the intestine (Sinha and Kumria; 2003). Tablets formulation are the most used medicine both for immediate and prolonged release, as well as targeted and controlled release, to reduce systemic side effects.

Moreover, it is possible to modify the tablet surface to improve taste and swallowability. Even if tablets are convenient to use for patients, their manufacturing requires several formulation units and product loss during the process (Allen et al.; 2013), and those are not suitable for children and elderly demographic that usually have swallowing difficulties. Moreover, tablet efficiency is affected

by poor patient adherence, as they can be easily forgotten and confused (Jimmy and Jose; 2011). From a physical point of view, during tabletting process drugs undergo physicochemical alteration by water absorption on tablet surface that might lead to drug solubilisation and recrystallization.

Globally, capsules are the second most common oral solid dosage forms and contain uncompressed drug and additives in a gelatine shell. Capsules dissolve faster in gastrointestinal, providing a faster absorption and onset of therapeutic response (Hedaya; 2012). They could contain solid, powder, semisolids, and liquid. Capsules offer the advantage of avoiding the compression process of tablet manufacturing, and they might contain liquid improving oral bioavailability. However, they could have problems associated with liquid fill as well as its weight, drug content and homogeneity (Jones; 2008).

Drug bioavailability is affected by both physical-chemical and physiological factors, related to the site of administration, which reduces it due to loss of drug during absorption and permeation process. It was also discussed how these properties are studied by pharmaceutical scientists to be overcome. For example, medicines which degrade into stomach could formulate such as enteric-coated tablet and water solubility could be improved by reducing particle size increasing surface area. However, once a drug is administered by the oral route, it encounters two other causes of bioavailability reduction that could not be overcome with formulation strategies in pharmaceutical dosage forms, such as hepatic metabolism and gastrointestinal drug transporters which are efflux or influx system present on membrane surfaces which help to absorb nutrient and eliminate toxins. Mainly, those systems such as glycoprotein-P could reduce the absorption of drugs through the membrane (Hunter and Hirst 1997).

Once the drug is absorbed in gastrointestinal, it is delivered through the portal vein to the liver before it can reach systemic circulation and being directed to the site of action. Part of drug it is also lost due to degradation and metabolism by microbiota and enzymes and in part excretion in faeces (Gavhane and Yadav 2012). This effect is known as “first-pass metabolism”, which is summarised in Figure 5, and that can decrease the amount of drug needed for therapeutic effects. Once the drug reaches the liver, it passes through a metabolic reaction catalysed by cytochrome P450 that hydrolyses, oxidases, reduces and conjugates drug creating metabolites. This effect could be small or large depending on drug chemical structure, affecting bioavailability (Pond and Tozer 1984).

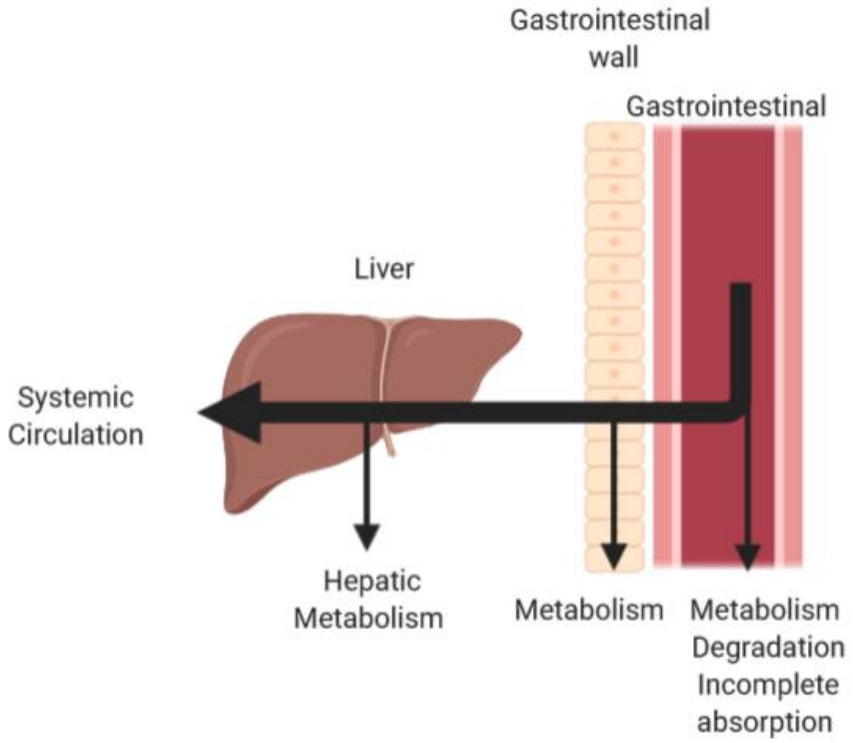


Figure 5. Schematic illustration of causes of loss of drug after oral administration. Image is redrawn from "Basic Pharmacokinetics" via Bio Render (Hedaya 2012).

Conventional solid dosage forms represent the most common drug delivery system despite several issues. However, oral cavity represents a new administration site proposing to achieve a systemic delivery and improve absorption, thus bioavailability. Potential of this route is discussed in the following section.

2. The oral cavity: An alternative route of administration for systemic drug delivery

The oral route is the most common administration route due to patient compliance and the several formulation strategies available. However, not all drugs are suitable for oral delivery due to discussed hepatic metabolism and loss in the gastrointestinal tract. Drug bioavailability depends on the absorption rate, which is influenced directly by surface area and indirectly by the thickness of the absorption site. The oral cavity includes lips, lining inside the cheeks, the front of the tongue, upper and lower gums, and floor of the mouth under the tongue. It presents advantages that suggest this route as a targeted site for drug delivery.

Local drug delivery in the oral cavity is recognised as oral transmucosal delivery which aims to drug systemic delivery through the mucosal surface covering the oral cavity. There are different application sites such as sublingual floor and buccal mucosa that are the epithelia of cheeks, gums,

and upper/lower lips. Sublingual delivery is characterised by a permeable membrane which allows absorption of small and lipophilic molecules such as nimesulide, atenolol, and metoprolol (Goswami et al.;2017). This route is favourable for immediate onset of action, as the administration of fast dissolving tablet of betamethasone as first AID to tackle an allergic reaction. From the other hand side, the buccal route offers a controlled release profile, and it is usually dispensed as an adhesive patch (Martin 2003).

The oral mucosa presents three layers such as epithelium, lamina propria and submucosa, as represented in Figure 6, and the epithelium could be keratinised or not. Keratinisation protects from mechanical and chemical stress, while non-keratinised epithelium is more flexible and permeable. Therefore, recognised as pertinent for drug delivery purposes, such as sublingual and cheeks. Lamina propria is constituted by connective tissue and communicates with submucosa made prevalently by blood vessels. Blood is then collected by three veins that flow into jugular vein allowing the drug to pass into the systemic circulation (Gibson et al.;2001).

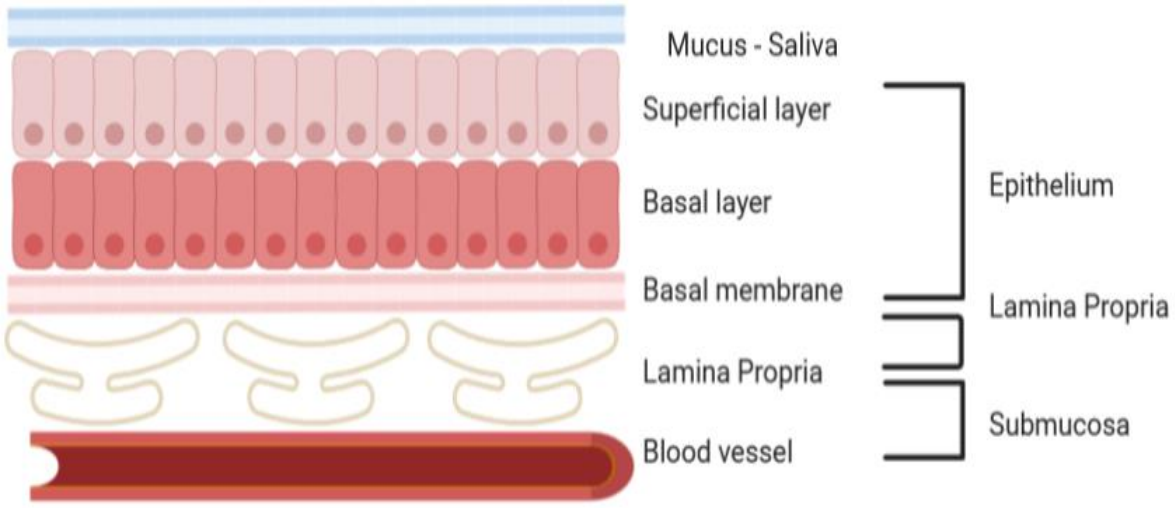


Figure 6. The physiological structure of the oral cavity which presents three layers. From the oral cavity, there are epithelium, lamina propria and submucosa rich of blood vessels. The image was drawn via Bio Render.

Drug absorption through this route is affected by various physiological factors. Mucous film covering mucosa could represent the first barrier. However, this involves only drugs which bind mucin (the major component of mucous) and with a large Mw (>1 kDa). Main influencing factors are permeability and thickness of the epithelium, which permeability relates with both keratinisation and presence of intercellular lipids. Absence of keratinisation and existence of such fats increase permeability allowing the sublingual region to be more permeable than buccal. The buccal mucosa is

also thicker (500-800 µm) than sublingual (100-200 µm), confirming a faster absorption, bioavailability, thus onset of action for the sublingual route (Kraan et al.; 2014).

The most significant advantage of the oral cavity as administration route compared with conventional oral way is represented by avoidance of hepatic metabolism and high surface area available. Mainly, buccal mucosa presents a smooth surface which enables retention of dosage form, providing a site of administration for controlled release. Such retention could be allowed by the formulation of adhesive medicaments.

2.1 Transmucosal drug delivery

Pharmaceutical research and development of transmucosal drug delivery is growing due to potential offered by this route to improve drug bioavailability by avoiding first-pass metabolism and allow systemic delivery, thus the faster onset of action respect conventional oral route. Drug entering systemic circulation could be affected by several parameters of dosage forms itself which influence drug bioavailability. The buccal route is suitable for this scope at is permeable, does allow a fast absorption offering retention for controlled release dosage forms. Oral transmucosal drug delivery presents several advantages: high surface area of 100cm^2, it is accessible for drug delivery for application and removal and usually offers a simple design due to the space available. They are more accepted compared with other transmucosal drug delivery systems such as suppositories and intravaginal.

Moreover, the buccal route presents an abundant blood supply which ensures rapid absorption and maintains sink condition. Sink condition is the ability to dissolve at least three times of drug respect amount in dosage forms. In the buccal cavity, this is allowed because once absorbed. The drug is immediately directed in circulation, creating a favourable gradient of concentration to be release from dosage forms (Hao and Heng 2003).

Compared with the conventional oral route, buccal absorption is affected by less variability, such as the presence of food, intestinal motility, extreme pH variation. It is also more resistant to mechanical and chemical stress, therefore, less irritable. From a clinical point of view, this route is suitable for patient with a compromised gastrointestinal tract, patients with swallowing difficulties, nausea and vomiting (Zhang et al.;2002). In contrast, from a pharmaceutical point of view, it is useful when formulating drug which undergoes massive metabolism or that are unstable at gastrointestinal pH (Patel et al.;2011). Oral cavity pH is between 6.8 and 7.4. Finally, this route of administration presents a few disadvantages such as clearance from saliva, which could be overcome by using the adhesive system, and mucus layer could bind specific drugs (i.e. antimicrobial (Butnarasu et al.;2019)).

Moreover, there are still a few products (i.e. lofexidine (Al-ghananeem; 2009), fentanyl (Jobdevairakkam; 2005), detomidine (Huhtinenet; 1993)) on the market, delaying development and marketing of similar dosage forms.

2.1.1 Oral Transmucosal patch as a buccal drug delivery system

As described above, oral transmucosal delivery systems have to provide a prolonged adhesion to buccal mucosa to allow a controlled release profile through the oral membrane. The oral transmucosal route will enable drugs to be released into the oral cavity into the buccal mucosa, composed of an epithelium layer (150–250 µm) and connective tissues (i.e., lamina propria and submucosa), as schematised in Figure 6. Just two formulation were discussed and produced so far, such as films and patches.

Patches can allow a multi-directional release (Michael; 2007) and unidirectional (Hillery; 2013), displayed in Figure 7. The multi-directional patch allows release both through the mucosa and, in saliva, which represents a disadvantage as the drug could be diluted and swallowed following conventional oral route bringing to a substantial drug loss. Unidirectional patches reduce release in saliva by application of an impermeable backing layer restricting absorption into the area of use. However, the implementation of the backing layer could reduce the flexibility of the patch leading to patient discomfort.

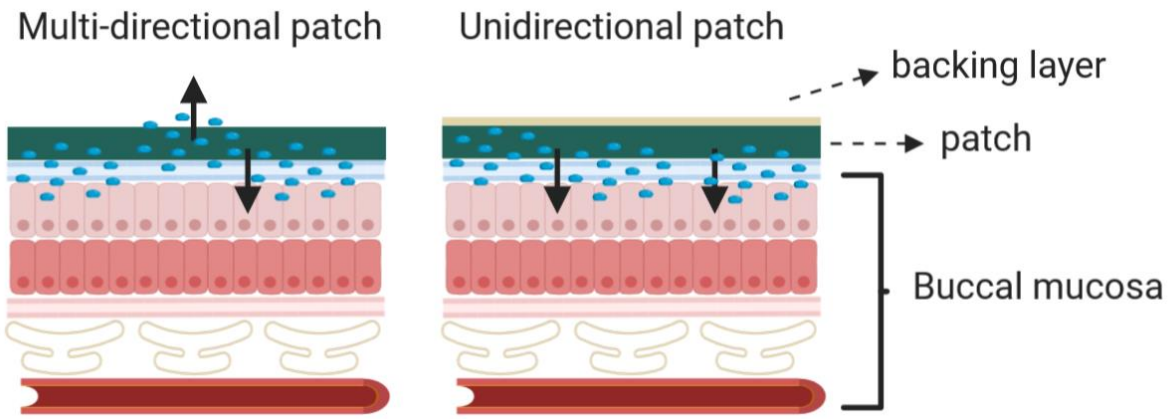


Figure 7. Two types of patches available which differ for release that could be multi-directional (both mucosa and saliva) or unidirectional (through buccal mucosa).

Those patches could regulate the release of drug through the creation of a drug-polymer matrix that is easy to formulate or by using a reservoir system. In devices which release is matrix-controlled, drug molecules are disposed on the device surface and available for release as particles

have to migrate along with the thickness of patch resulting in controlled-release over time (Hong et al.;2011). Dissolution rate is controlled by the dissolution of the drug within matrix according to first Fick's Law, discussed previously.

The drug is absorbed in the relation of surface area available, and usually, patches present a surface area between 1 and 5 $cm^{\wedge 2}$ (Hong et al.;2011). The patch needs to be adhesive to ensure drug absorption, to the buccal mucosa and to allow drug permeation. Mechanism of adhesion depends on physical entanglement of adhesive polymer with glycoprotein chains of mucous and with the creation of chemical bonds (either electrostatic, hydrogen, hydrophobic) (Kumar et al.;2017). In the formulation of buccal transmucosal patches most used adhesive polymers are cellulose derivates such as poly (hydroxyethylcellulose), poly (sodium carboxymethylcellulose) (Cui et al.;2009), acrylic acid derivates such as poly (acrylic acid) and poly (methacrylic acid) (Laulicht et al.;2012), polyvinylpyrrolidone (PVP) (J. Lee 2005), polyvinylalcohol (PVA) (Peppas and Mongia; 1997), α,β-poly (N-2-hydroxyethyl)-D, L-aspartammide (PHEA) (Lo Monte Aet al.; 2012), Soluplus (SLP) (Salawi and Nazzal 2018). Polymer viscosity in media (saliva) affects adhesion time; moreover, presence of an impermeable backing layer provides higher adhesion time due to protection offered by this layer to solubilisation of matrix, usually composed of hydrophilic polymers such as PVA.

Different theories were explored to explain the mechanism of adhesion of polymers to membranes, and all are considered valuable. The process is based on the creation of electronic forces between the adhesive polymer and negative electric charge of mucus (Dodou et al.;2005), but also the formation of a physical bond. Lee and co-workers considered adhesion a diffusion-based process when polymer chains interact with a substrate which degree depends on cross-link, Mw, and temperature. Such a mechanism required that polymers present both hydrophilic and lipophilic groups to be able to interact at the molecular level with phospholipids of cell membranes (named interpenetration theory) (Lee et al.;2000).

2.1.1.1 Assessment of patch mucosal adhesiveness

Mucosal adhesiveness is mainly influenced by polymers properties such as concentration, hydration, presence of the functional group, electrical charge, pH. For example, PVA possesses a concentration-dependent adhesion when formulates as a solid dispersion to create micro-particles reaching 18 nano Newton (nN) at a concentration of 20% w/w (Du et al.; 2011; Peppas and Mongia 1997). According to interpenetration theory, polymer hydration promotes interpenetration and relaxation of polymeric chains, improving adhesiveness (Sigurdsson et al.;2006). Also, the presence of hydrophilic groups such as NH2, COOH, and OH increase creation of electrical bond, thus adhesion,

such as PHEA, PVA and SLP. These groups, also in an aqueous environment, as saliva and mucous of the oral cavity, are dissociated creating a charge that could improve the interaction between mucosa and polymer (Capra et al.; 2007). As acids and bases dissociation is influenced by pH, as demonstrated, and explained with equations 2 and 3, it is considered that polymers which pKa is higher than pH (7.4) are adhesive in the oral cavity (Sudhakar et al.;2006).

When formulating patch for buccal drug delivery, it is fundamental to evaluate adhesiveness. Adhesion could be assessed both *in-vitro* and *in-vivo,* which is now in disuse. Main *in-vitro* techniques are the measurement of detachment force and adhesion strength. First one calculates the energy required to separate two slides covered with polymers and mucous, and it is, therefore, named pull-out quantification (Beach et al.;2002). The second one analyses polymer and mucin interaction using polarised light. The newest technology that uses adhesion strength measurement is called Atomic Force Microscope (AFM) in quantitative mode (AFM-Qi) to evaluate forces in micro and nanometer size with a pN resolution. AFM uses spectroscopy to analyse surface topography by using a cantilever-mounted tip and recreates an image which shows areas with significant interactions (Patel et al.;2000).

2.1.1.2 Assessment of drug permeability through buccal mucosa

The drug can permeate membranes through paracellular route, favourite by hydrophilic drugs, and transcellular route, preferred by lipophilic drugs. Rate of drug permeability is often evaluated via *in-vitro* study in which drug passes from a donor compartment to a receiving chamber separated by a membrane layer that can be an animal specimen or a suitable membrane such as cellulose acetate (Borbás et al.;2015) and novel biomimetic PermeaPad (di Cagno et al.;2015). At every time point, a particular volume is withdrawn and characterised to quantify the amount of drug permeated. This experiment is often carried out once formulating transmucosal dosage forms to ensure formulation advantage of improving permeation, thus absorption. The research is conducted via vertical Franz Cell and permeability is calculated as apparent permeability (P_{app}) according to equation 5 and 6: the fractional amount of permeated drug (dQ) was calculated over a time interval (dt, expressed in seconds). Drug flux (J) through the barrier was normalised by the membrane surface area (A) (Di Cagno et al.;2015).

$$J = \frac{dQ}{A*dt} \qquad \textbf{Equation 5}$$

P_{app} was calculated by normalising flux over-concentration of the drug in the donor compartment (C_0) as described by

$P_{app}=J/C_0$ **Equation 6**

3. Use of solid dispersion technology to improve solubility and permeability of BCS II and IV drugs

Fick's law describes that drug rate of diffusion through the membrane is proportional to its lipophilicity and the surface area available; however, it decreases by increasing membrane thickness. Drug absorption through the layer it is possible just if the drug is dissolved or molecularly dispersed from the dosage form. Dissolution performance is described by Noyes and Whitney equation, which explains that the dissolution rate is directly proportional to the drug surface area, which increased by decreasing drug particle size (Goldberg et al.;1966). Once the drug is dissolved in human fluids, it can diffuse through the membrane, be absorbed, and reach it action site generating therapeutic effect. As described in chapter 1, the amount of drug that goes systemic circulation is named bioavailability, and it is affected both by physiological and drug physical parameter. Once physiological parameters could be studied to analyse, which is the best administration route; the significant role of the pharmaceutical scientists is to modify drug and dosage form physical properties to improve bioavailability. Noyes and Whitney's equation suggests that a substantial part is played by drug solubility, as described in section 1.1 (Dahan et al.;2015). Drugs with low solubility, BCS class II and IV, represent a significant challenge in the drug-delivery formulation, and several pre-formulation strategies being employed to overcome this issue, such as salt formation (Samie et al.;2017), micronisation (Campardelli et al.;2017), and most commonly solid dispersion (Modica de Mohac et al.;2017).

Solid dispersions are described as a solid product containing a hydrophilic matrix and a hydrophobic drug (Modica de Mohac et al.;2018). Their first definition in literature is dated back to 1961 when Sekiguchi and Obi, described them as presenting the medicine in a microcrystalline state (Sekiguchi and Obi; 1961). Later, Goldberg and co-workers (Goldberg et al.;1966), described it as a system where the drug is dispersed in a polymer possessing high aqueous solubility. Advantage of a solid dispersion relates with is drug release mechanism as once exposed to an aqueous media, hydrophilic carrier dissolves, releasing drug as fine particles. Most recently, a stable dispersion was described as a delivery system whereby the drug is dispersed in a biologically inert matrix, adding to the definition that polymers must be biocompatible and not be toxic (Craig; 2002; Serajuddln; 1999).

Solid dispersion is widely used pharmaceutics due to a high number of advantages. In particular, if they are produced in the form of particles with minimal dimensions, this increases the specific surface of solid and consequently, causing a higher dissolution rate (Kamalakkannan et al.;2010). The suitability of solid dispersion manufacturing is proved through years by approbation by

the Food and Drug Administration agency (FDA). Many products available on the market, such as Kaletra® (containing lopinavir/ritonavir and produced by Abbott), Intelence® (containing etravirine and produced Tibotec), Certican® (containing everolimus and produced Novartis), and Rezulin® (containing troglitazone and produced Sankyo) (Zhong et al.;2018).

Solid dispersions are classified into four different generations according to drug and carrier morphology, and the fifth generation of multicomponent solid dispersion (multi component solid dispersion) was proposed by Modica de Mohac and co-workers, in 2020 (Modica de Mohac et al.;2020). Identification of this generation is based on the physical state of drug, thus if it is in the amorphous or crystalline state. Such information is fundamental in pharmaceutics as solid-state drug influence it solubility, hence dissolution, thus bioavailability, as discussed in section 1.1. Composition of each generation is described in Table 1.

The term multi component solid dispersion was first used in 1987 by Shadrina and co-workers but is definition was proposed just in 2006 by Yoo and co-workers and Pongpeerapat. They defined multi component solid dispersion as a "ternary solid dispersion", prepared with more than two polymers in carrier matrix (Pongpeerapat et al.;2006; Yoo et al.;2009) Singh and Van den Mooter, in 2013, described multi component solid dispersion as a system with a third or even fourth excipient, which would improve solid dispersion performance (Singh and Van den Mooter; 2016). In 2016, Haneef and Chadha described that multi component solid dispersion formulation implicates a therapeutic advantage as a drug-drug cocrystal compacted in the supramolecular complex where two or more compounds possessing a therapeutic effect. The complex represents a solid method to improve the physicochemical properties of drug delivery systems to achieve therapeutic advantages[55, 56]. However, this definition included just formulation where both drugs and polymers are in the crystalline state. This condition is not represented in most common drug delivery systems, in which amorphous polymers and drugs are used. Furthermore, this definition implied that physical complex between drug and polymer achieves a therapeutic advantage. Still, no scientific evidence demonstrated the effect of polymers in enhancing system potential.

Table 1. Classification of solid dispersions according to the latest report from Modica de Mohac and co-workers 2020(L. M. Modica de Mohac; 2020)

Generation	**Composition**
First	Eutectic, in which both drug and carrier are in the crystalline state (Haneef and Chadha; 2017).
Second	Both drugs and carriers in the amorphous state with prescription supersaturated in the molten matrix (van Drooge et al.;2006).

Third	System of multiple carriers that present self-emulsifying properties which let to improve drug solubility and system stability (Pouton; 2006).
Fourth	Solid dispersion prepared using the amphiphilic chemical structure of SLP (Shamma and Basha; 2013).
Fifth	A solid system in which one or more drugs are dispersed in a carrier matrix composed by at least two compounds whose possess properties capable of modifying or enhancing drug delivery system. This system improves drug release and permeability through membrane or increase system adhesivity to the mucosa. The latest definition of the fifth generation of solid dispersion as an multi component solid dispersion was provided early in 2020 by Modica de Mohac and co-workers.

They defined an multi component solid dispersion as "solid system in which one or more drugs are dispersed in a carrier matrix composed by at least two compounds whose possess properties capable of modifying or enhancing drug delivery system in term of improving drug release, improving drug permeability through membrane or increase system adhesivity to mucosa". Those represent an evolution of solid dispersion that has as main aim increasing of drug release profile suggesting a therapeutic effect of polymer or biologically active compounds forming solid dispersion matrix.

Solid dispersion advantage of improving drug solubility and dissolution profile depends on polymer selection and the release mechanism.

3.1 Polymers commonly used in solid dispersion technology

In preparation of novel dosage forms, polymers are selected during pre-formulation studies, in which potential effects are evaluated such as the ability to increase the dissolution rate of drug, stabilizer or dispersing agent for formulation itself (Poozesh et al.;2017). Different polymers are used in solid dispersion manufacturing, and example on their use is reported below in alphabetic order.

3.1.1 PHEA

PHEA is a polymer with a protein-like structure, high water solubility, non-toxicity, and biocompatible, chemical structure reported in Figure 8. It is suitable to formulate drug systems with a prolonged, controlled, and targeted release. It was successfully used to prepared solid dispersion of hydrophobic drugs such as beclomethasone dipropionate and flutamide and coat gold nanoparticles

(Licciardi et al.;2012; Cavallaro et al.;2015). PHEA unique advantage is a combination of a lipophilic structure with a hydrophilic group; this amphiphilic structure allows micelles formation within a diameter of 10-100nm which achieve high solubility enhancement effect. PHEA can be derivatised and complexed with many polymers such as PLA and PVA. Such derivates were used to produce both nanoparticle and micro-particles, increasing solubility of α-tocopherol and beclomethasone (Buscemi et al.;2017; Lo Monte A. et al.;2012).

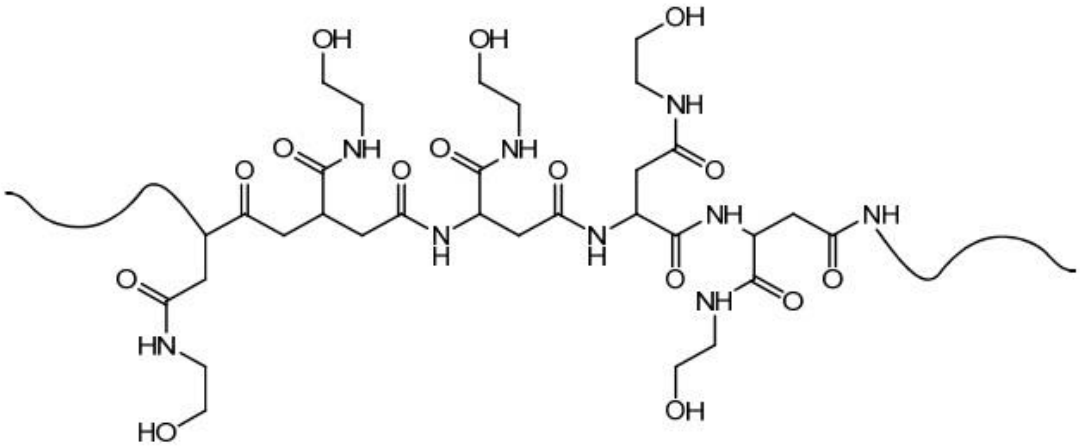


Figure 8. Chemical structure of polymer PHEA.

3.1.2 Cellulose acetate phthalate (CAP)

CAP is used as a tablet-coating material employed to produce enteric films. It presents a chemical structure which resists to gastric fluid pH but dissolves in intestinal environment pH five or pH 5.5. Derivatives of cellulose are most common and widely used polymers for controlled drug release, which are introduced into a dosage form as a coating agent, viscosity modifier, and mucoadhesive agent (Paudel et al.;2013). Moreover, CAP is used to formulate controlled-release dosage form (Modica De Mohac et al.;2019). I was employed at 75% w/w to prepare solid dispersion of itraconazole increasing its solubility in the intestine by avoiding drug release in the stomach (DiNunzio et al.;2008).

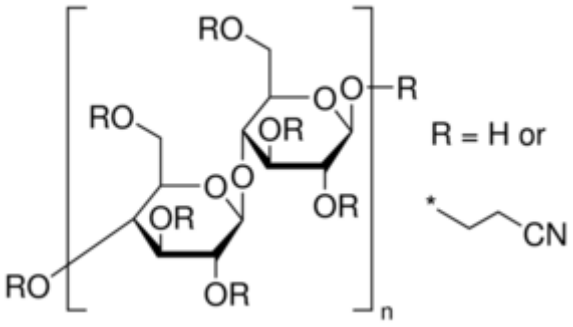


Figure 9. Chemical structure of polymer CAP.

3.1.3 Inulin

Inulin is a polysaccharide which targets intestinal tract where shows a protective effect on the intestinal mucosa. Inulin possesses several applications in food and pharmaceutics and its chemical structure, with β 1,2 links, is not hydrolysable by human digestive enzymes but just from intestinal microbiota (Petrovsky; 2010; Mensink et al.;2015). Due to its hydrolysis in the intestine, inulin is used as prebiotic and colon targeting material in controlled drug delivery system (van der Beek et al.;2018; Oliveira et al.;2011). It also presents protective properties against diarrhoea, the most common side effect of several drugs. Inulin was firstly used for solid dispersion preparation by Visser and co-workers, which improved permeability and absorption of HIV protease inhibitor TMC20. Such increment is due by high solubility that inulin shows when in the formulation at more than 2.5% w/w (Visser et al.;2010).

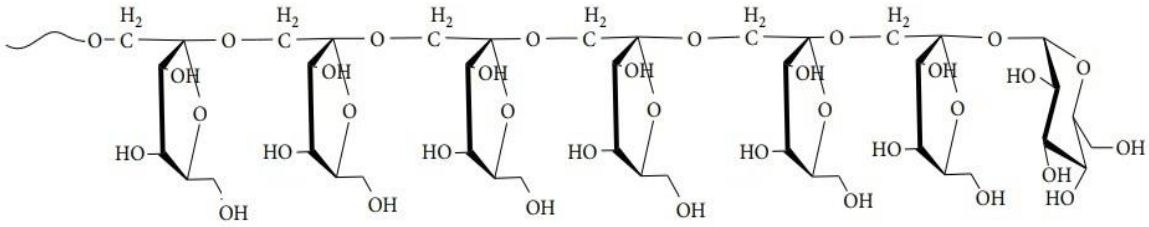


Figure 10. Chemical structure of polysaccharide Inulin.

3.1.4 Maltodextrin

Maltodextrin is a polysaccharide produced from starch by enzymatic hydrolysis of starch, constituted by amylose and amylopectin. It presents glucose bonds $\alpha_{(1\rightarrow4)}$ and $\alpha_{(1\rightarrow6)}$ every 24 to 30 glucose units. Maltodextrins are a coating agent and highly soluble in water up to 35.5% w/w.

Maltodextrins are used in direct compression of tablet and have hygroscopic properties. They were employed in further research to improve the solubility and dissolution rate of the drug such as glimepiride meloxicam, and artemisinin. In each example, particle obtained presented high-water content due to hygroscopicity of this polysaccharide. It is also suitable for different formulation strategies, such as spray-drying and freeze-drying (Surin et al.;2018; Ainurofiq et al.;2016; Meliana et al.;2020).

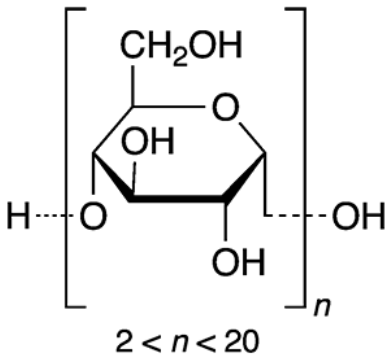


Figure 11. Chemical structure of maltodextrin.

3.1.5 Poly (methyl methacrylate-co-methacrylic) acid (PMMA)

PMMA is methacrylic acid, used as a coating agent to protect the drug from the acidic environment of the stomach. It is often used to modify release as it dissolves at a pH higher than 5. PMMA was used to increase the dissolution of griseofulvin and spironolactone at a pH value of 6.8 (Kawakami et al.; 2020; Balogh et al.; 2017). PMMA is suitable for different formulation strategies such as hot-melt extrusion, freeze-drying and electrospinning for fibres formation.

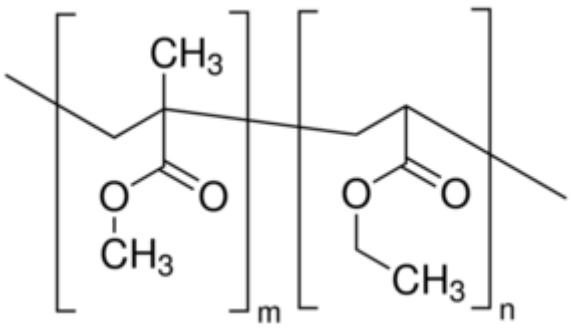


Figure 12. Chemical structure of PMMA.

3.1.7 Poly-lactic acid filament (PLA)

PLA is a biodegradable polymer, considered as a smart polymer used as a controlled delivery system and as a polymer for implant generation as it is non-toxic and biocompatible. Smart polymers use to respond to external stimuli such as the human environment to modify their properties and improved drug release profile. PLA is more commonly used as a filament for three-dimensional (3D) printing of solid dispersion of diclofenac or as nanoparticle of docetaxel. Nanoparticle had a particle size between 100 and 200 nm, with a controlled release over ten days (Surini et al.;2018; Arias et al.;1994). It presents a melting point of 150-160°C.

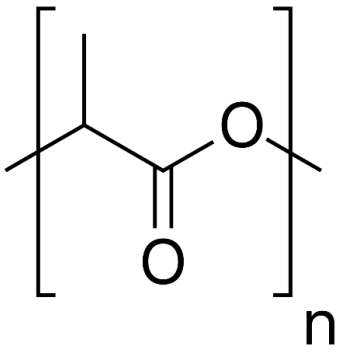


Figure 13. Chemical structure of PLA.

3.1.8 PVA

PVA is a water-soluble polymer which is widely used in film preparation by solvent casting method and electrospinning. It is high biocompatible as it was used for different in-vivo studies for soft contact lens material, artificial heart linings, artificial cartilages, and pancreas membrane. Because of its biocompatibility, drug compatibility, water-solubility, film-forming, excellent mechanical and swelling properties, PVA was deeply studied in the literature (Kumar et al.; 2017). Moreover, it possesses mucoadhesive properties (Peppas and Mongia; 1997; Shalini et al.;2012). It was used to prepare the buccal transmucosal patch to modify the release of simvastatin. It was also used as solubility enhancer in the formulation of itraconazole, ibuprofen and indomethacin (Mishra and Kumar 2012; Jaipakdee et al.;2018). Chew and co-workers, and Goyanes and co-workers, recently used PVA as a filament for 3D printing, which effects in controlling drug release was proved (Goyanes et al.;2015; Chew et al.;2019).

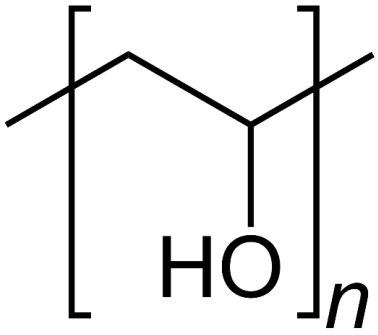


Figure 14. Chemical structure of PVA.

3.1.9 SLP

SLP, was introduced to the market due to its influence on the enhancement of drug bioavailability and dissolution rate of drug with low aqueous solubility (Basf; 2010; Kolter;2012; Reintjes; 2011). It is a polyvinyl caprolactam-polyvinyl acetate-polyethene glycol graft copolymer with amphiphilic structure, and it is suitable to solid dispersion production through spray-drying. SLP is a water-soluble polymer; its addition in stable dispersion formulation always led to a solubility enhancement of drug's solid dispersion such as carvedilol, increasing its dissolution rate (Shamma and Basha; 2013). Most recently, in 2017, many authors used this polymer to reach the goal of increased drug bioavailability and solubilities, such as nifedipine and sulfamethoxazole (Altamimi and Neau; 2017), indomethacin (Ogawa et al.;2018), and itraconazole (Davis et al.;2017).

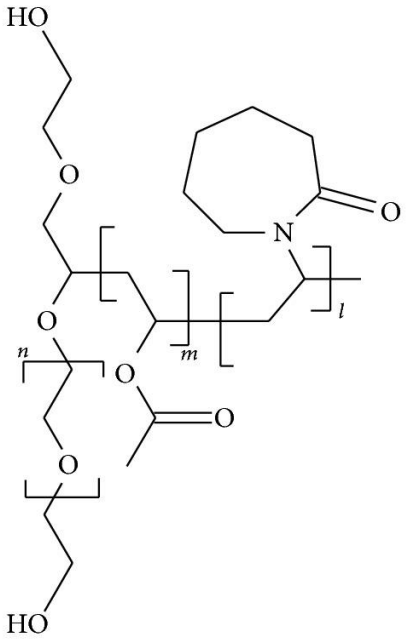


Figure 15. Chemical structure of SLP.

3.2 Solid dispersion drug release mechanism

The main advantage of solid dispersions is an improvement in dissolution rate, with concomitant implications for improving the bioavailability of poorly water-soluble drugs. Such improvements in dissolution rate are often considerable, with increases of up to four hundred-fold were reported (Craig; 2002).

There are several hypotheses regarding the mechanism of drug release from solid dispersion. Most relates with different characteristics of the final product such as particle size and carrier. Particle size reduction causes an increase of particle surface area, causing an accumulation of dissolution rate, according to Noyse and Whitney equation. Carrier, as described above, could influence product water content, hygroscopicity and release profile. However, the most argued question relates to the hypothesis of two different mechanisms of drug release, carrier-controlled or drug-controlled (Figure 16).

This hypothesis is based on critical findings by Corrigan and co-workers. They found that the dissolution rate of the drug in polymer and polymer alone were identical, leading to the suggestion of carrier-controlled dissolution in phenobarbitone- (poly)ethylene glycol (PEG) solid dispersion (Corrigan et al.;1985). The same carried-controlled mechanism was identified by a different author who used PEG as a carrier (Dokoumetzidis and Macheras; 2006). This hypothesis suggested that interfacial layer between dissolution surface and the aqueous environment is depleted in more rapidly dissolving component (the carrier), leading to the creation of a surface layer rich in one element through which other must diffuse before releasing into bulk phase, Figure 16a. Moreover, Craig suggested that, if a system underwent carrier-controlled dissolution, then physical properties such as the initial particle size of drug or crystallinity should be mostly irrelevant (Craig; 2002).

The second scenario is that release is drug-controlled, where drug diffusion into the polymer is comparatively slow, and the drug is released as solid particles. Therefore, dissolution does not relate with polymer but with drug characteristic such as size, and physical form. Therefore, other features are fundamental such as wettability and zeta potential. Understating this process would lead to a better possibility of solid dispersion to enhance drug dissolution rate through a preferential selection of appropriate carrier (in this case polymer) or a combination of carrier and carrier types (Yan et al.;2016).

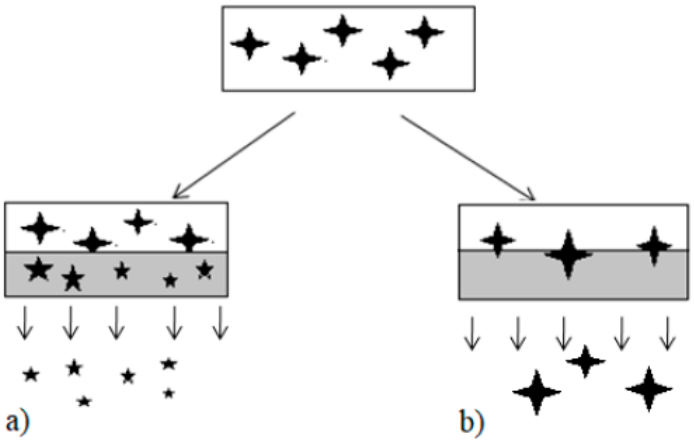


Figure 16. Schematic representation (a) Carrier-controlled dissolution and (b) drug-controlled dissolution (Adapted with permission from (Craig; 2002)).

3.2.1 Dissolution studies

The determination of the release profile is described in pharmacopoeias which describe test apparatuses for different dosage forms. A typical dissolution test includes paddle apparatus and time limits are established according to part of the body aimed to be mimicked. Dissolution studies are considered as a pharmaceutical quality test, ensuring that dosage form provides a reproducible and predictable behaviour in-vitro (Hillery; 2013). The study has to be relevant. Therefore, appropriate dissolution media and conditions have to be created. Analysis, mimicking gastrointestinal environment presents a pH of 1-2 (obtained by a chloride acid HCl solution 0.1N), while intestine requires a phosphate base solution with pH 6.8. From the other hand side, oral cavity environment requires a neutral pH of 7.4 (Talukder et al.;2011; Dressman et al.;1998; Marroum; 2012).

3.3 Solid dispersion manufacturing

The most significant advantage of solid dispersion manufacturing relates to a variety of formulation strategies that can be employed for their products such as by melting (fusion), solvent-based, or melting-solvent method. This work focused on the solvent-based process such as spray-drying (Lavra et al.;2017), freeze-drying (Nireesha et al.;2013), solvent-casting method et al.;2016), electrospinning (Brako et al.; 2018), and fusion method of 3D printing (Chew et al.;2019). Those techniques were widely used to prepare solid dispersion as reported in the literature. In the following section, each method is critically discussed.

3.3.1 Spray-drying

Spray-drying is a formulation technique where a mixture of excipients, commonly a polymers dispersion, containing a drug, is atomised, sprayed and dried in a chamber by a heated gas stream (generally air) (Encina et al.; 2018). Schematic of mechanism in Figure 17. This technique offers several advantages as it is suitable for thermolabile compounds because heat inlet gas gets in touch with inlet sample, which possesses high humidity, allowing rapid solvent evaporation and, consequently, cooling down of chamber temperature. Spray-drying influences final product characteristic calibrating different parameters such as inlet and outlet temperatures, pump velocity and aspiration percentage and solvent selection, as recently Reviewed by Paudel and co-workers (in 2013) (Paudel et al.;2013) and Singh and Van den Mooter (in 2016) (Paudel et al.; 2013; Singh and Van den Mooter; 2016). Parameters' effects on spray-dried particles are summarised in Table 2.

Table 2. Spray-drying parameters effects on particles

Temperature effects	Higher inlet temperatures and a low difference between inlet and outlet temperatures allow obtaining dried particles. Increase of this difference might affect particle water content with high humidity resulting (Singh and Van den Mooter; 2016).
Pump and Aspiration	Increase in pump velocity decreases outlet temperature; this enhances the difference between inlet and outlet temperature so increase the humidity of the product. Low aspiration reduced particle water content (Baysan et al.;2019).
Solvents	Solvents with a low boiling point are easy to evaporate and allow to obtain dried particles (Encina et al.;2018).

Spray-dried solid dispersions were widely studied in the last decades as an option to improve dissolution rate and in turn bioavailability of poorly water-soluble drugs such as one classified as BCS class II and IV. This technique is useful to obtain spherical particles whit small size and narrow distribution. Those characteristics are not only due to the spray-drying procedure itself but are also affected by polymers ratios and properties (Wlodarski et al.;2015; Takeuchi et al.;2018; Ogawa et al.;2018). Spray-drying was used by several researchers, such as Beak and co-workers (2012) who produced amorphous solid dispersions containing dutasteride and various excipients including CAP, cyclodextrin, hydroxypropyl-methylcellulose (HPMC) and PVA (Beak and Kim; 2012). Mahmah and co-

workers demonstrated enhancement of felodipine's dissolution rate by using spray-drying and both PVP and HPMC as polymeric carriers (Beak and Kim 2012; Mahmah et al.; 2014).

In last two decades, researchers studied the use of spray-drying to obtain pharmaceutical products and formulated a drug delivery system as solid dispersion for inhalation (Porzio et al.;2017), stabilize insulin (Porzio et al.;2017) and thermolabile vaccine (Peabody et al.;2017), incorporate additives for drug control release (Palmieri et al.;2001).

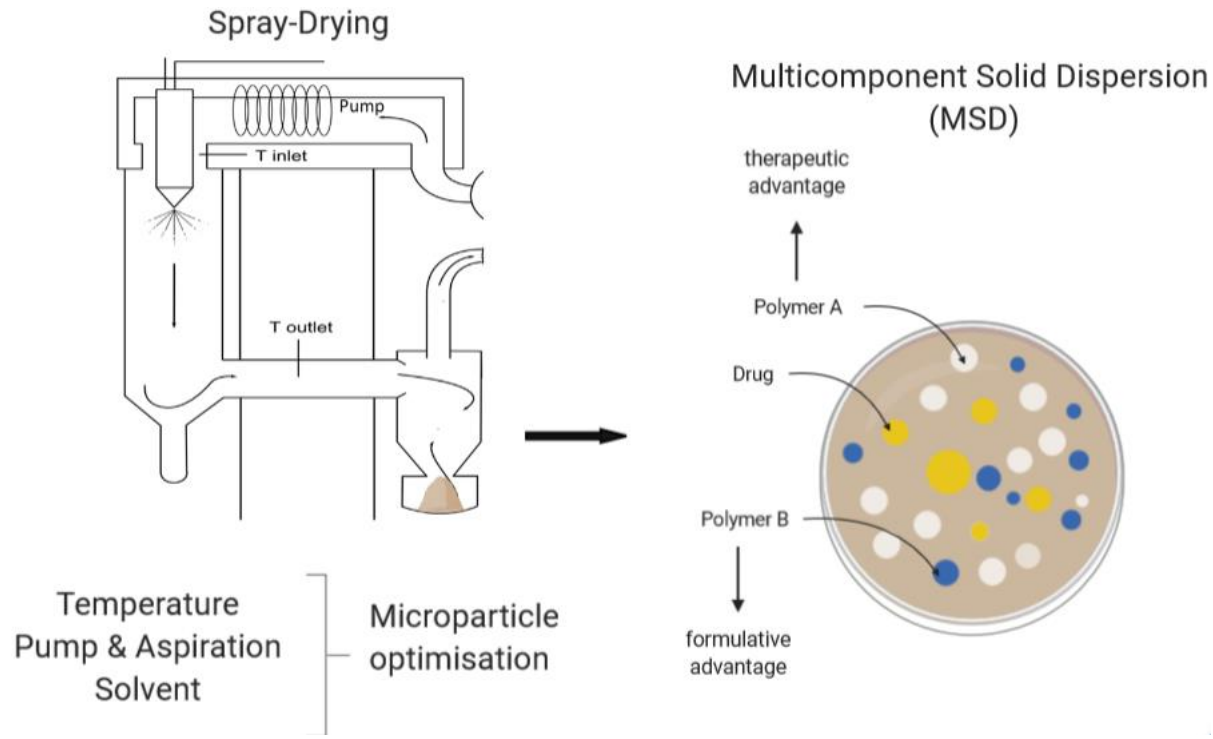


Figure 17. Schematic representation of solid dispersion manufacturing via spray-drying. Parameter effect on particle physical-chemical characteristic was described in Table 2.

The use of spray-drying to produce solid dispersion presents several advantages. As it is just a single step manufacturing, it possesses the capacity to incorporate, at a molecular level, multiple components (i.e. drugs, polymers, excipients) into a particle. Particle characteristics could be easily modified in terms of dimension, wettability, and density by adjusting different parameters of the production process, as described in Table 2 (Zhu et al.;2019; Mondal et al.;2019; de Melo Ramos et al.;2019; Arrighi et al.;2019). Moreover, the spray-drying method is, as well, suitable for thermolabile products, either drugs or excipients, by optimizing inlet and outlet temperatures. In particular, by modifying inlet temperatures, although outlet was found to affect several physical properties such as solubility and to decrease in bulk density, moisture content, and water activity (Maas et al.; 2011). Results are manufacturing of a solid product with suitable particle size (to targeted administration route), excellent dispersibility and solid-state properties. Spray-drying also gives a great advantage to

obtain particle wholly dried. Preparation of stable dosage forms as a solid dispersion requires to be carried with suitable solvents (organic or not) that evaporate allowing the formation of dried powders. The preferred solution used for spray-drying is water (Paudel and Van Den Mooter; 2012; Wu et al.;2011).

3.3.2 Freeze-drying

In this process, the solvent (commonly water) is frozen and then removed by sublimation and desorption, resulting in the creation of porous materials (Nireesha et al.; 2013). Pharmaceutical companies often use freeze-drying to increase the shelf life of products, increasing sample stability (Shivanand Mukhopadhayaya; 2017; Emami et al.; 2018).

More than 150 FDA approved biopharmaceutical products available on the market are made by freeze-drying (Zhong et al.; 2018). It presents different advantages as the process is usually performed at low temperatures which protect drugs, can be completed in sterile conditions, and the final product can be rapidly rehydrated due to its porous characteristics. Freeze-drying is often used to produce solid dispersions, to increase both drug solubility and stability, using drugs such as lovastatin, Δ9-tetrahydrocannabinol, and glyburide (Naik and Mokale 2014; van Drooge et al.; 2005; Allen et al.; 2013). However, this technique is expensive due to the amount of energy needed to freeze, sublimate, and maintain shelf temperature at the same value along the process. The freeze-drying process is driven by ice nucleation and crystal growth rates in a supersaturated drug solution, and the method may result in both crystalline or amorphous solids (Felix; 2007).

In previous work, Eddleston and co-workers highlighted the advantage of freeze-drying to avoid problems caused by differences in solubilities. They utilised it to prepare cocrystal containing two or more pharmaceutical compounds, with the final aim to create a novel mixture of caffeine and theophylline (Eddleston et al.; 2013). Such formulation, if brought to market, could bring to combination therapy of two molecules in the treatment of bronchoconstriction.

3.3.3 Solvent-casting method.

It is the oldest solvent-based technology used in solid dispersion formulation and was developed over a century ago, driven by needs in the emerging photography industry (Collins; 1990). First reported use of the solvent method for pharmaceutical applications was in 1951, by Wolff and co-workers who produced pharmaceutical films of amylose (Wolff et al.; 1951). Later, Saldanha and Kyu, studied use of this technique using polycarbonate and PMMA (Saldanha and Kyu; 1987). Lee and

co-workers used solvent-casting to formulate heparin derivatives for medical devices (i.e. cardiopulmonary bypass circuits, heart-lung oxygenators, and kidney dialysers) (Lee et al.; 1998). This is a manageable and easy technique where the drug can be dissolved or dispersed in a polymer solution, and the solvent evaporated overnight leading to film formation. Another advantage of this technique is that experiment could be conducted at room temperature under a fume hood. Therefore, it is convenient while using thermo-liable drugs. It offers a big surface area, thus high dissolution performance; however, drug particle size could not be directly affected during process as sedimentation and aggregation could occur during evaporation (Taylor et al. 2017; Saldanha and Kyu 1987).

The solvent casting method is often used to prepare pharmaceutical films which offer advantages when administered in the oral cavity. When in contact with saliva, they dissolve, releasing the drug. Drug-loaded pharmaceutical films are known to be accepted by patients who cannot swallow or have absorption diseases of the gastrointestinal tract (Kianfar et al.; 2011). This method was used to prepare different poorly water-soluble drugs such as gliclazide and tromethamine, promoting dissolution behaviour by improving drug wettability and hydrophilicity (Bruni et al.; 2018). Its advantage in increase solubility and bioavailability was also proved by Chadha and co-workers, who prepared felodipine solid dispersion as a pharmaceutical film (Haneef and Chadha 2017).

3.3.4 Electrospinning

Electrospinning is a versatile but straightforward process that utilises electrostatic forces to generate polymeric fibers and was used for over 100 years to create synthetic fibers (Teo et al.; 2011; Zelkó et al.; 2020). Electrospinning involves pushing a viscous polymer or polymer/drug solution through a spinneret (narrow gauge syringe needle) at a constant flow rate. A voltage is applied to the polymer solution, creating repulsive forces between like charges in solution and attractive forces between charged solution and grounded collector. When electrostatic forces are equal to the surface tension of the liquid, a Taylor cone is formed (Li and Xia 2004). As fiber jet accelerates towards the collector, it undergoes chaotic whipping instability which increases transit time and path length to the collector, allowing the solvent to evaporate leaving solid, thin fibers. Electrospinning mechanism is schematised in Figure 18. Cheng and co-workers presented electrospinning as a novel processing method to generate functional nanomaterials with many applications ranging from wound healing and medical textiles, to production of oral-dispersible film solid dispersion and controlled delivery systems (Cheng et al.; 2018).

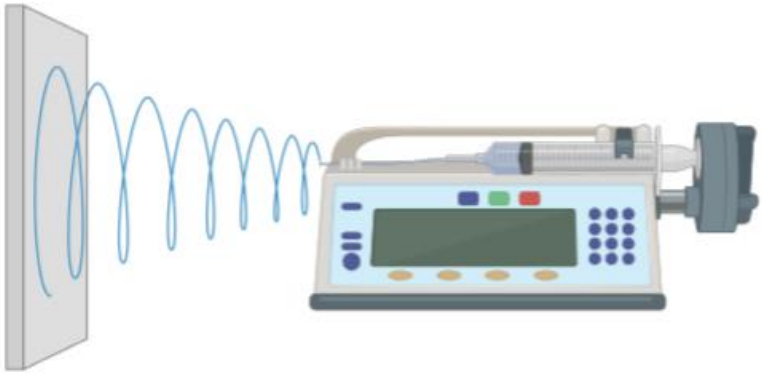


Figure 18. Schematic representation of an electrospinning device which consists of a pump to set up flow rate; a syringe which needle is charged by high voltage and a collector.

There are different types of electrospinning. In mono-axial setup, polymer and drug in solution or suspension are dispensed through a single needle resulting in a monolithic product, with medication and polymer typically evenly and homogeneously blended throughout fibers (Williams;2018). In Verreck and co-workers' study, nanofibrous solid dispersion of Itraconazole-HPMC was prepared, and the release mechanism was assessed as drug-controlled (Verreck et al.; 2003). PVP-acetaminophen solid dispersion developed by electrospinning were compared to traditional solid dispersion prepared by freeze-drying. It was showed an advantage of electrospinning process over the conventional method being unique microstructural characteristics of electrospun nanofibres generated.

Coaxial and Multi-Axial Electrospinning involves the use of a two-needle spinneret, with one needle concentrically nested inside another. Fires generated tend to have greater versatility in terms of the range of materials used, therapeutic agents incorporated (ranging from small molecules to biological molecules), size (e.g., from 100nm to 300µm), and can offer complete drug encapsulation and higher drug stability compared to mono-axial fibres (Shen et al.; 2011; Qi and Craig; 2016). Coaxial and multi-axial electrospinning approaches were applied in drug delivery with great success to generate targeted release (Yu et al.; 2013) and multifunctional materials (Lin et al.; 2018). Electrospinning was used to create nanofibrous ferulic acid-cellulose acetate as a depot system (Ji et al.; 2010). Tri-axial electrospinning has also been successfully used to fabricate nanofibres of lecithin (known to be un-spinnable), diclofenac sodium with CAP as carrier matrix for oral colon targeting (Williams et al.; 2013).

3.3.5 3D printing - Fused Deposition Modelling

3D printing is a manufacturing method where the 3D product is produced by deposition of material in successive layer. Nowadays, the use of 3D in pharmaceutics has become of interest after the FDA approved the use of 3D printed fast dissolving tablet of levetiracetam (Spritam®). This technique presents several advantages as freedom in design geometries, shapes, the amount of product printable (by selecting the respective infill). The 3D printer works by printing a design previously developed as a Computer-aided Design (CAD) and extracting it as a Stereolithography (STL) file. Two leading different 3D printing technology is used in pharmaceutics: inkjet printing, was a liquid is divided into droplets; and fused deposition modelling (FDM), which deposits solidifying material in layers of the desired form. The most significant disadvantage of inkjet printing relates to the need to use solvents to create the printable liquid. Therefore, solvent boiling point, explosion temperature and toxicity have to be taken into account as well as solubility of both drug and polymers in the mixture (Prasad and Smyth 2016).

In FDM, the material is molten inside a heating extruder and then deposited layer-by-layer on a platform where solidifies, schematic representation in Figure 19. The main parameters to take into consideration once using 3D printing, are the physical characteristics such as melting point (T_m) and glass transition (T_g) and the infill density. The term "infill", indicates the amount of volume that is printed in the designed product and could chance from 0% (where just the external wall is built) to 100 % (where the printing material fills all the volume) (Mohanty et al.; 2015).

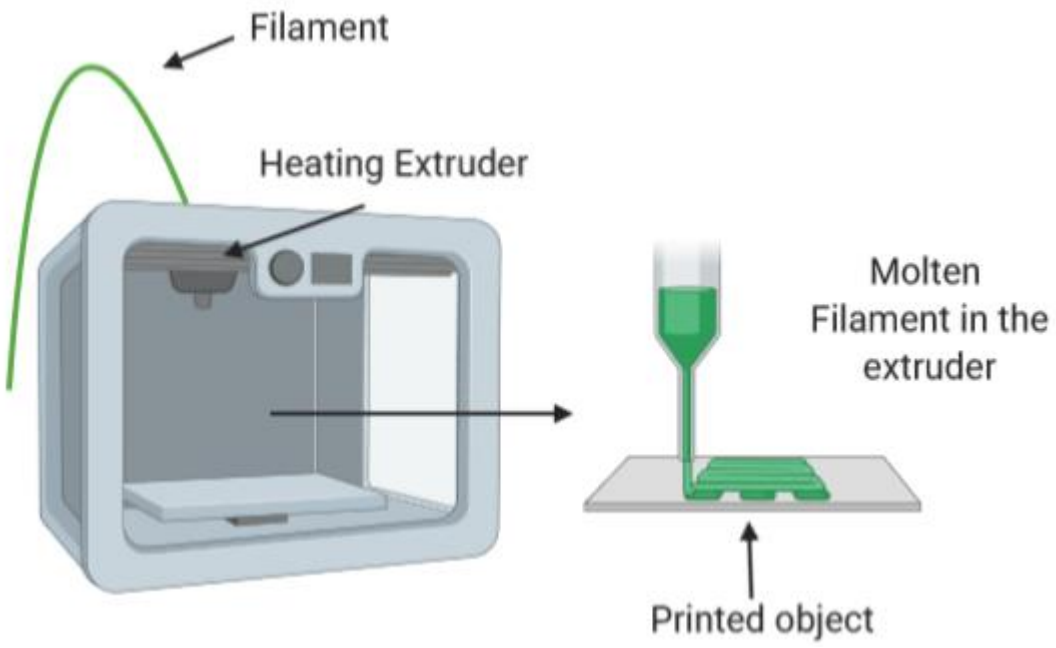


Figure 19. Schematic representation of an FDM 3D printer.

Two main polymers are used in 3D printing of pharmaceuticals PVA and PLA, which are both available for FDM printing process as filaments. The filament usually is impregnated with a drug saturated solution overnight, and the influence of different solvent polarity on the drug loading was recent, 2019, investigated by Chew and co-workers showing that methanol produced higher drug loading compared with ethanol (Chew et al.; 2019). Once the filaments are soaked with the drug, they can be printed through the 3D printer device and design accordingly with the CAD characteristic. The impregnation method was successfully used by Goyanes and co-workers, who produced tablet impregnated by fluorescein and aminosalicylic acid. The study also evaluated the effect of the infill on the dissolution rate, proving that increasing the infill could be of advantage for prolonged-release (Goyanes et al.; 2015). Moreover, it was found a relation between shapes and dissolution profile, which suggested that shapes as sphere and pyramids provide the slowest and most controlled release (Goyanes et al.; 2015). This technique allows the customisation of products representing a new trend for patient-centric dosage form manufacturing.

3.3.5.1 Developing Patient-centric Formulations Using 3D printing

Patient-centric medications are personalised dosage forms where the product provides the benefits for the individual patient's need. Customisation of dosage form comes to a hand as the patient is the final user of the medication and as it proved that parameters of the most conventional dosage forms, such as shape and size, affect medication acceptability. The problem of medication acceptability and related dosage forms issues are discussed in chapter 4.1.

A recent example of the patient-centric 3D printed tablet was reported in early, 2020 when tablet with braille signs was successfully printed for visually impaired patients. While Khaled and co-workers in 2015, produced a fixed-dose combination of five drugs (ramipril, pravastatin, atenolol, aspirin, and hydrochlorothiazide) a polypill with both immediate and controlled release profile. This novelty pill suggested an improvement in the quality of life of patients affected by hypertension, who usually use multi-therapy to manage symptoms (Khaled et al.; 2015). From the other hand side, Awad and co-workers printed miniprintlets, to both provide multi-drug controlled release and to manage size-related non-compliance toward medication (Awad et al.; 2019). In 2017, capsules were printed in the nano-size range to produce solid dosage forms of deflazacort using 50% of infill to maximise drug loading (Awad et al.; 2019). This novel methodology also allows the manufacturing of transdermal patch for wound dressing purposes, as proved by Muwaffak and co-workers (Muwaffak et al.; 2017).

3D printing flexibility in modification and customisation of the dosage form is now understudy in a Central London hospital to design patient-centric shape and size (FabRx; 2019).

The following chapters discuss the pharmaceutical issues towards schizophrenia medication, highlighting the area of further improvement.

Chapter 2. Pharmaceutical Challenges in Schizophrenia

1. Schizophrenia: demographic and physiopathology

Schizophrenia definitions are discussed since years varying in specify its characteristics. In 1998 National Institute of Mental Health Schizophrenia (United States of America, USA) defined it as a chronic and severe mental disorder that affects how a person behaves, feels, and thinks. People with schizophrenia may seem like they have lost touch with reality (National Health Report; 1998). In 2009 it was described as a dimensional spectrum disorder within a broader psychosis umbrella that includes bipolar affective disorders, with positive, negative and cognitive symptoms (Howes and Kapur; 2009). It is usually defined also as a significant psychiatric disorder (or cluster of diseases) that affects an individual's perception, thoughts, affect and behaviour (Gaebel; 2014). Recently, in 2016, schizophrenia was described, by National Health Society (United Kingdom, UK), as a severe long-term mental health condition that causes a range of different psychological symptoms, and this definition is one that I refer to in this manuscript (NHS; 2016).

Schizophrenia is undoubtedly the most common form of psychotic disorder and its mean incidence, reported in the latest epidemiological study published in Lancet in 2017, is 0.11 per 1000 (Gaebel; 2014; James et al.; 2018). It affects 1% of the population, and 10% of them are children and adolescents (McGrath et al.; 2008). This study was based on geographical demography that includes 195 countries and territories grouped into 21 regions and seven super-regions (James et al.; 2018). Evidence suggested that incidence peak both genders is between 15–24 years old; while a second peak is observed in male between 55 and 64 (John McGrath et al.; 2004). It was also found a trend showing that males have 30-40% higher risk to develop schizophrenia (Aleman et al.; 2003). Schizophrenia is considered a global burden as in 2016 were registered 20.9 million cases and between 30 and 50% of patients affected attempt to commit suicide, and more than 10% of them die of suicide (Charlson et al.; 2018). World Health Organization (WHO) estimated that 2% of world death relates to schizophrenia-related suicide (Lu et al.; 2020; Ventriglio et al.; 2016). The principal cause for such high suicidal risk depends on low medication adherence and compliance, and those will be discussed along with text (Hawton et al.; 2005).

Schizophrenia is characterised by positive, negative and cognitive symptoms that are thought to occur relative to varied dopamine levels in mesolimbic and mesocortical circuits (Goodman and Gilman; 2013). Positive symptoms could include: hallucinations in any of five senses (vision, hearing, smell, taste, or touch); delusions which persist even if there is evidence that beliefs are not valid or logical; disorganised thinking as an incapacity to connect thoughts logically; movements disorder with

repetition of motions over and over till patients become catatonic. From the other hand side, negative symptoms could be confused with depression as they are associated with disruptions to normal emotions and behaviour as reduced feelings of pleasure and speaking, difficulty in the beginning and controlling activities. For some patients, cognitive symptoms might occur as well, such as low ability to understand information and use it to make decisions, trouble on focus or keep the attention and short memory (Andreasen; 1982; Liddle; 1987). Those symptoms are related to neurotransmitters (NT) abnormal function.

There is a significant hypothesis on schizophrenia pathophysiology based on different NT: dopamine, serotonin, amino-gamma-butyric acid (GABA), glutamate and acetylcholine (ACh). Dopamine hypothesis is more accredited and relates to the increase of dopaminergic activity and a high number of dopamine receptors, as demonstrated in basal ganglia of deceased schizophrenic patients (Owen et al.; 1980). Figure 20 illustrates a schematic representation of the dopamine hypothesis.

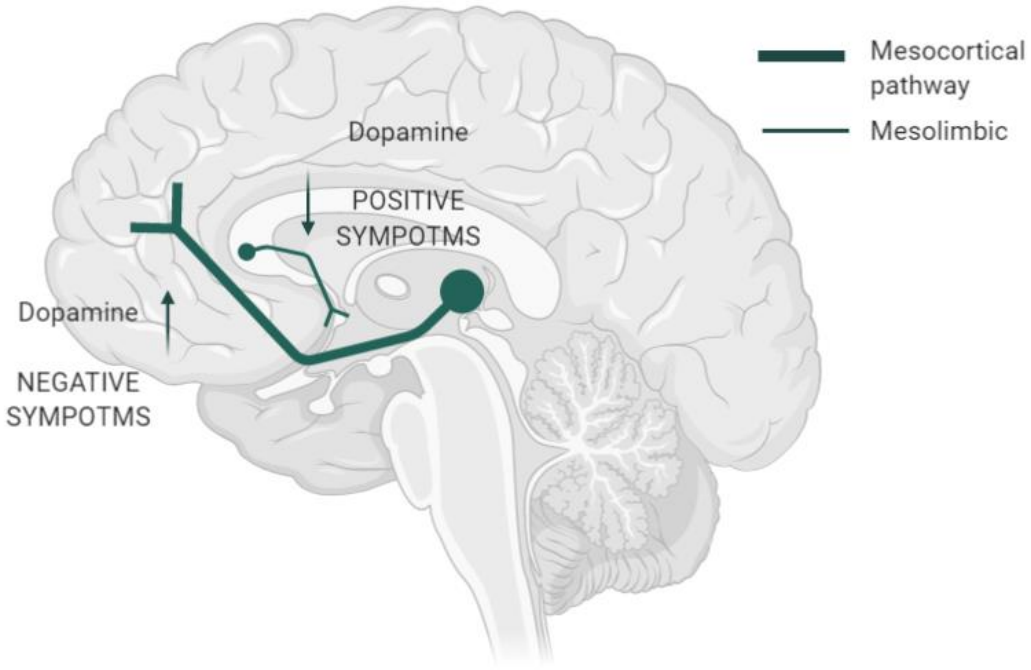


Figure 20. Schematic representation of dopamine mechanism of schizophrenia. The image was drawn via Bio Render.

The increase of dopamine causes hyperactivation of subcortical projection on mesolimbic (resulting in hyperstimulation of D2 receptors and positive symptoms) and hypoactivation of mesocortical projections to the prefrontal cortex (resulting in hypo stimulation of D1 receptors, negative symptoms, and cognitive impairment). From another hand side, an increase in serotonin

receptors, in particular, was also found in the majority of post-mortem prefrontal cortex of patients affected by schizophrenia (5HT1a, 5HT2a, and c) (Carlsson et al.; 2001; Reynolds and Beasley; 2001). It is, then, possible to understand that physiopathology of schizophrenia could not be just related to the dopamine hypothesis as dopamine itself is regulated or regulate other NT pathways. For example, it was found that a reduction in functionality of N-methyl-D-aspartate (NMDA) receptor results in a decrease in inhibition of GABA interneurons that means an over-activation of dopamine neurons projecting to the striatum (Cohen et al.; 2015). As a result of the increase of dopamine activity, a state of aberrant salience occurred, and patients develop delusions.

Meanwhile, a lack of top-down control pathway from prefrontal cortex might contribute to the maintenance of psychotic symptoms (Friston; 1995). Recently, schizophrenia has also been linked to changes in muscarinic Acetyl-choline system. At that systems-level, schizophrenia can be viewed as disconnection syndrome, with first striatal interactions specifically postulated to be crucial in symptoms formation (Lowe et al.; 2018). However, schizophrenia mechanism is not well understood, and both prefrontal and temporal-limbic cortexes seemed to be involved in the neurobiology of disease as well as abnormal brain development (Beerpoot et al.; 1996). Causes and risk factors of this disease are still under investigations such as birth complication (i.e. hypoxia) (Zornberg; 2000), parental age (Zammit et al.; 2003), infection and immune system alteration (Yolken and Torrey; 1995), autoimmune disease, and use of drugs (Messias et al.; 2007). While 70% of patients who experienced schizophrenia responded to therapy, approximately 20-30% of patients do not respond to an initial antipsychotic trial. (Dold and Leucht; 2014) This condition is known as treatment-resistant schizophrenia and will be discussed in following sections.

2. Pharmacological interventions in Schizophrenia: First- and second-generation antipsychotics

Since initial evidence that dopamine brain level alteration was the leading cause of schizophrenia, first-generation of antipsychotics were used to the antagonist D2 receptor. Such an effect was presented from drugs like haloperidol, chlorpromazine, promazine, perphenazine, and flupentixol (Chakos et al.; 2004). While the impact on D2 receptor does calm down positive symptoms, a misregulation of dopamine in other pathways (tuberoinfundibular, mesocortical and nigrostriatal) causes a lot of side effects such as extrapyramidal syndromes, hypotension, a decrease of secretions and weight gain (Landry et al.; 2010). Nevertheless, first-generation antipsychotics represent first-line treatment in all different stages of schizophrenia, even when the new generation of antipsychotics (amisulpride, aripiprazole, clozapine, olanzapine, quetiapine, risperidone, and ziprasidone) is available, with a most complex pharmacological profile. They are, in fact, not just able to block D2

receptor but also to block different NT pathways such as serotonin receptors, involved, as discussed, in schizophrenia physiopathology. Thanks to their profile, second-generation antipsychotics can control negative symptoms reducing as well many side effects (Tandon et al.; 2008; Chakos et al.; 2004). Despite this evidence, World Psychiatric Association Pharmacopsychiatry in 2008 declared to be skeptical and stated: "an inconsistent superiority for treatment of negative, cognitive, and depressive symptoms was revealed for second-generation in comparison to first-generation" (Tandon et al.; 2008).

Consequently, the choice of an antipsychotic for administration has to take into a consideration side effect of each drug, and specific and personal vulnerability. Different pharmacological approaches are now used to overcome such interpersonal differences in drug administration and drug effectiveness. According to Conley and co-workers (R. R. Conley and Buchanan 1997), first-generation antipsychotic resulted in fewer than 5% of patients achieving a satisfying therapeutic response. Clinical strategies in treat schizophrenia centered on either modifying doses of conventional antipsychotics or using adjunctive agents (combination or augmentation), such as lithium, beta-blocking drugs, anticonvulsants, and benzodiazepines (Barnes et al.; 1991). However, when symptoms do not usually regress a high-dose of treatment is used, this increases antipsychotic dose above officially approved dose range (off-label prescription, dose-escalation) and cannot be recommended as an available treatment option for management of patients that are resistant to therapy. To treat such patients, clinicians also used polypharmacy strategies such as a combination of drugs (two drugs of the same group such as two antipsychotics) or augmentation (two drugs of different classes). In a meta-analysis comprising 14 double-blind, randomised, placebo-controlled clozapine combination studies with second-generation antipsychotics, Taylor, and co-workers found a statistically significant superiority of combination treatment compared to placebo (Taylor et al.; 2012). However, in both cases of combination or augmentation, it is registered an increase in side effects caused by antipsychotics such as comorbid depressive or obsessive-compulsive syndromes. A study conducted by Waddington and co-workers in 1998 also showed an increase of death associated with polypharmacy (Waddington et al.; 1998). Therefore, such strategies are not covered by pharmacological guidelines which strongly suggest antipsychotic monotherapy (Zink and Englisch; 2012). However, some patients appear to benefit from a switch of medication (Hatta et al.; 2018; Agid et al.; 2013).

The standard treatment for patients, who seem to resist to medication, is, as described, to increase dosage or number of drugs administrated per day, a one-drug therapy should be first consideration after the failure of conventional drug therapy. When clozapine was introduced in the market, in 1972, it showed its superiority in reducing psychotic symptoms respect other drugs as well

reported from Kane and co-workers (1988) and Leucht and co-workers (2013) (Leucht et al.; 2009; Kane; 1988). Clozapine advantage, to manage both positive and negative symptoms, has elevated it to be described as gold-standard to treat schizophrenia. Due to clozapine side effects such as neutropenia and agranulocytosis, its use in clinical practice it always delayed and reserved to patients who are classified from suffering on treatment-resistant schizophrenia . Definition of this condition was controversial and would be discussed later in the manuscript that has as main issues use of clozapine in clinical practice.

3. Use of Clozapine in Clinical Practice

Lieberman and co-workers (2003) carried out a flexible-dose study in which compared the use of clozapine and chlorpromazine in treatment-naive first-episode patients. This study showed that patients treated with clozapine remitted faster than patients receiving chlorpromazine (Lieberman et al.; 2003). Girgis and co-workers conducted a similar survey in 2011, and their findings support comparability in effectiveness between clozapine and chlorpromazine. Still, clozapine had more excellent tolerability in the treatment of first-episode psychosis (Girgis et al.; 2011). But from both studies, it is not possible to understand the effectiveness of clozapine as first-line treatment against schizophrenia because, at the start of treatment, it is challenging to identify refractory patients. While clozapine is suggested as a 1st-line treatment in treatment-resistant schizophrenia , in clinical practice and from World Federation Society it was reported that "clozapine should not be used as a 1st-line treatment in first-episode schizophrenia patients". It is usually used just as a secondary option after haloperidol, risperidone, and olanzapine (Siskind et al.; 2016; Šagud; 2015; Okhuijsen-Pfeifer et al.; 2018; Hasan et al.; 2012).

Usually, as 2nd-line treatment for schizophrenia two possibilities are chosen from clinical practitioners: a first-generation antipsychotic such as haloperidol is administered or a long-acting injectable medication (risperidone/paliperidone/aripiprazole) is offered to patients (Jones et al.; 2018). There is limited reported evidence on the use of clozapine as 2nd-line(Buckley et al.; 2007). A most recent study, in 2018, showed that remission of symptoms could be achieved using sequential administration of amisulpride and clozapine and concluded after patients should be introduced to clozapine when a single-antipsychotic trial failed and not more (Kahn et al.; 2018). As 3rd-line usually a first- or second-generation (as lurasidone or aripiprazole) antipsychotic is suggested and if those would not be effective, clozapine is prescribed (NHS; 2008). It was reported that clozapine is used as a 3rd-line treatment in two studies, one from 1990 and one from Canadian compendium, such choice was explained by fair of agranulocytosis effects of the drug (Tiihonen et al.; 2009). Underutilisation of

clozapine was also reported in UK literature since its use was recommended as 3rd-line treatment in schizophrenia in national and local guidelines (S. Gee et al.; 2014).

A study on 200 patients by Grover and co-workers showed that usually three antipsychotic drugs are given to patients before considering clozapine, and 27.5% of patients receive polypharmacy before clozapine. In particular, it was found that the majority (93.5%) of patients fulfilled criteria for treatment-resistant schizophrenia, but clozapine was started with 1.5 years delay (Grover et al.; 2015). A study from Texas University showed that only 5–25% of patients who should be initiated on clozapine were on treatment (Buckley et al.; 2007). Even more, delays were highlighted from Taylor and co-workers in 2003, where 112 patients retrospective analysis showed that use of clozapine was delayed for up to 5 years and those patients received more than five antipsychotics or polypharmacy, on average, before being prescribed clozapine, that then achieved remission in 6 months (Taylor et al.; 2003). Similar findings were reported from different authors (Paton et al.; 2008; Howes et al.; 2012; Elkis; 2007). Another retrospective-chart from Alessi-Severini (Alessi-Severini et al.; 2013), in 2003, said that 68% of patients received at least three antipsychotics before starting with clozapine; while Wheeler and co-workers reported that 37% of patients received at least five antipsychotics before being prescribed with clozapine (Wheeler et al.; 2014). A most recent study (2015) from 243 consultant psychiatrists registered with Royal College of Psychiatrists in the UK showed that 40.5% preferred to use several other antipsychotics before considering clozapine, rest felt it was not safe to start clozapine. Such misuse was assessed to concerns about side effects, patients not wanting to have blood tests, lack of experience or knowledge were reported, and 20% were not aware of its benefit in reducing suicidal risk (Tungaraza and Farooq 2015; Gee et al.; 2014). Siobhan and co-workers investigated patients-related excuse for underused of clozapine in 2017. They found that patients would be more likely to accept clozapine if they might have the opportunity to start treatment at home and blood tests do not appear as a barrier against clozapine initiation (Gee et al.; 2017). Some patients also found it helpful to speak with other patients who used clozapine (Tiihonen et al.; 2009).

4. Treatment-Resistant Schizophrenia

Patients affected by schizophrenia who do not respond to treatment are considered refractory, and their pathology has defined as treatment-resistant schizophrenia . Definition of treatment-resistant schizophrenia was challenging till consideration made by Kane and co-workers in 1988 who described it as failure to respond to at least three periods of antipsychotic treatment in preceding five years with a dose of 1g per day of chlorpromazine. This definition, known as "Kane criteria" was then modified by Suzuki and co-workers in 2011 who accepted such criteria but for six

weeks non-responding to at least to antipsychotic trials (Suzuki et al.; 2011). NICE guidelines now define treatment-resistant schizophrenia as a disease in which patients with schizophrenia who, “despite at least two adequate trials of classical neuroleptic drugs, have persistent moderate to severe positive, or disorganisation, or negative symptoms together with poor social and work function over a prolonged period” (Raedler et al.; 2007; Berman et al.; 2007). In the majority of clinical trials, this condition is prolonged for at least six weeks (Kane; 1988).

Moreover, it was reported from Lownamn and co-workers that patients who have not responded to initial neuroleptic therapy have a 9% chance of returning to another classic neuroleptic drug (Lowman and Montgomery; 1998). treatment-resistant schizophrenia represents one of the most important clinical challenges in the pharmacological management of schizophrenia (Taylor and Duncan-McConnell; 2000). Chronicity frequently is taken as a synonym of refractoriness, and some authors use parameters such as the number of patient hospitalisations or chronic hospitalisations or an increase cortical atrophy compared with responder's patients to define treatment-resistant schizophrenia (Conley and Kelly; 2001; Steen et al.; 2006). Even if its pathology of schizophrenia, and so of treatment-resistant schizophrenia , is not well understood, it was suggested that its neurobiology involved dopaminergic pathways. Over the years scientists have noticed some distinct tracts from patients affected by treatment-resistant schizophrenia such as birth complications, motor, and language retardation, use of recreative drugs or alcohol, high intellective quotient and brain abnormalities (Weinberger; 1987; Elkis; 2007). In particular, Mitelman and Buchsbaum notice ventricular enlargement and decrease in grey matter, corpus callosum, internal capsule, and putamen (Mitelman et al.; 2007). Another discussed parameter is a plasma or cerebrospinal fluid level of one dopamine metabolite, homovanillic acid that was found to be at a lower level in non-responsive patients respect to responsive (Ottong and Garver; 1997). Recently, it has also been possible to conduct a meta-analysis on D2 receptor occupancy after two weeks of treatments using brain single-photon emission tomography. This analysis showed, not only that more than 65% receptor occupancy reflected patients that responded to therapy but also that a higher occupancy is just related to an increase of side effects (Yilmaz et al.; 2012). So, much effort should be involved in a better understanding of such tracts, thus achieving faster identification of refractory patients, and developing the right regiment.

4.1 Use of clozapine in patients with treatment-resistant schizophrenia

The introduction of clozapine was a significant step forward in the treatment of refractory schizophrenia, making it "gold standard" for this group of patients. In a trial by Rosenheck and co-

workers (1997), clozapine-treated patients showed to be significantly more compliance than were haloperidol-treated patients to continue their assigned treatment for a full year (Rosenheck et al.; 1997). More than 20 years ago, a randomised trial by Kane and co-workers investigated the effects of clozapine compared with chlorpromazine in patients with treatment-resistant schizophrenia (Chakos et al.; 2004). Nevertheless, antipsychotic clozapine is effective even in patients who failed to respond to conventional antipsychotics (Taylor et al.; 2000). Clozapine is still the only drug with proven efficacy in treatment-resistant schizophrenia , and it also reduces violent behaviour (Frogley et al.; 2012), tardive dyskinesia and risk of suicide (Meltzer; 2003). Effect of clozapine in lowering suicide rate made it only medication approved from United States FDA as capable of preventing suicide (Buchanan et al.; 2010). Spivak and co-workers, in 2003, also demonstrated that the reduction of suicide index is related to the removal of aggression and impulsiveness (Buchanan et al.; 2010). Even if exact mechanism with which clozapine acts against suicidality, this could be linked with its unique pharmacological profile that blocks 5HT2a causing an increase of dopamine and serotonin in limbic regions and prefrontal cortex counterbalancing rise of dopamine in sub-cortex and nucleus-accumbens observed in schizophrenic patients (Shiloh et al.; 1999; Brisch et al.; 2014). It is recommended as first-line treatment for treatment-resistant schizophrenia in many guidelines such as Maudsley Prescribing Guidelines in Psychiatry. According to this guideline, initial clozapine dose should be started at 12.5 mg once per day and then the dose is increased to 112.5 twice a day, and then 25-30mg per day until reach 300mg dosage, further increase of 50-100 mg could be achieved if necessary. Plasma level should be aimed to be 350 µg/L (Taylor et al.; 2018). Such plasma levels found agreement in the literature (Bell et al.; 1998; Spina et al.; 2000). Despite clozapine numerous advantages, it has lower tolerability compared to other drugs. Patients could commonly experience sedation, constipation, sialorrhea, weight gain, metabolic syndrome, sinus-tachycardia, as well as uncommonly, but seriously agranulocytosis, seizures, myocarditis, ketoacidosis adverse events (Trevor; 2015). Kane and co-workers reported that those side effects caused patients to discontinue clozapine treatment even before it has reached target plasma concentration. But it is now known that almost all patients receiving clozapine demonstrate less rate of side effects than reported by Kane(Kane; 1988). Despite recommendations in clinical guidelines, and probably due to safety concerns, clozapine remains underutilised in patients with schizophrenia (Warnez and Alessi-Severini; 2014) and its use is often substantially delayed (Howes et al.; 2012). So, the overall question "is clozapine gold standard?" remains debated. Still, it shouldn't be due to its unique capacity to reverse years of illness, as shown by Wilson and co-workers (1995) (Wilson; 1995; Volavka 2012). Even today, based on current and still growing evidence, clozapine remains gold standard (Dold and Leucht 2014; Taylor et al.; 2017) and

first-line treatment in patients with treatment-resistant schizophrenia . (Hasan et al.; 2012) But it should be introduced sooner in practice.

In schizophrenia treatment, clozapine represented a step forward in reducing patients' risk of suicide. Due to its complex pharmacology, its mechanism is not understood, but clozapine effects on the clinical issue of treatment-resistant schizophrenia have lifted it as the gold standard. Although it is underused in clinical practice as its first use is delayed, such delay was related to clinicians' fears about compliance or medical complications. While patients seem to be more likely to accept this treatment due to therapeutic advantages and low need to introduce other medications during the treatment reduction of suicidality and absence of extrapyramidal syndrome should be taken in high considerations. Evidence that clozapine is started with such delay in clinical practice for refractory patients seems more and more related to a lack of interpretation of main tracts that usually characterised treatment-resistance.

4.2 Available Clozapine Dosage Forms: Licensed and Unlicensed used

Clozapine is available in the market as a tablet, oral disintegrating tablets and oral suspension under most common targeted names of Clozaril®, FazaClo®, and Versacloz®. Patients affected by treatment-resistant schizophrenia and that refuse to start an oral therapy can receive an off label intramuscular injection that is now just offered by Sussex Partnership under a protocol established by South London and Maudsley NHS Trust (Ray 2016). Clozapine bioavailability via the oral route is 50%, for this reason, administration of clozapine as systemic immediate (i.e. injection) release is considered dangerous and to be prescribed just on individual circumstances (Professional Practice; 2017). More commonly, when present patient difficulties or aversion in swallowing a tablet, an off label used of clozapine is authorised. Mainly, tablets are crushed and dispersed in water for administration. This scenario is usually monitored at hospital level as reported from different guidelines. However, high vigilance must be sought as clozapine is not soluble in water, therefore desired concentration of drug could not be achieved (Horne 2018; The National Institute for Health and Care Excellence; 2019). Crushing tablets is not just believed dangerous for patients, but health care professional deputed to breaking. In 2012, it was reported that a pharmacy team member (location nondisclosed) was diagnosed with a chronic pneumonitis due to both active ingredient and excipients of powdered medicine (Lewis et al.; 2012). It is well-established that clozapine is the only medication available in treatment-resistant schizophrenia , and it is well-know that medication discontinuation is the leading cause of therapy inefficacy. Therefore, in 2019, Till and co-workers, proposed “assertive approach to clozapine” suggesting administration of drug via the use of nasogastric tubes. This method is, however,

enforced, and patients described it as a cruel and unnecessary alternative (Till et al.; 2019). The introduction of Denzapine® oral suspension reduced the disadvantage connected with crushing the tablet and improved taste (Oloyede et al.; 2019).

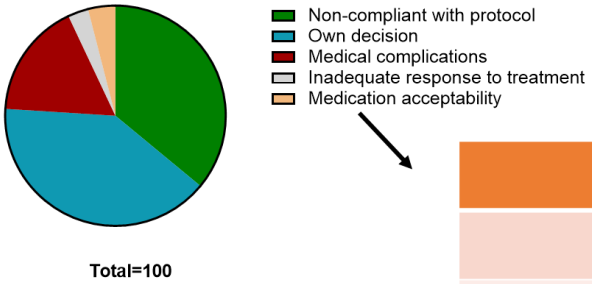
5. Pharmaceutical challenges in treatment-resistant schizophrenia

70% of patients affected by treatment-resistant schizophrenia are usually classified, at first, as non-adherent to medication, if they do not take their medications correctly, or stop taking them (Haddad et al.; 2014). This behaviour is controversially described both as non-adherence or poor compliance (Chakrabarti; 2014). However, both terms, adherence, and compliance are often misused and confused. WHO, in 2003, offered a definitive definition of adherence as "the extent to which a person's behaviour, taking medication, following a diet, and executing lifestyle changes, corresponds with agreed recommendations from a health care provider" (Hsu et al.; 2013). Therefore, patients may be nonadherent during different stages of their treatment and may decide not to fill their prescriptions in pharmacy and not start their treatment. While compliance definition is dated back in 1997 as "the extent to which patient's behaviour matches prescriber's recommendations" (Fenton et al.; 1997). Though the term "compliance" is frequently employed to describe medication-taking behaviour, considering patient playing a passive role. However, there are many barriers to compliance, such as lack of comprehension of treatment benefits and low health literacy (Miller; 2016). More generally, adherence reflects the decision of the patient to do not take medication even if understood the reason for prescription while compliance indicates that patient does not accept medication for lack of comprehension of treatment or not acceptance of dosage from (Tilson; 2004). Both terms could be considered equivalent in term of outcome as both attitudes indicate a medicine-taking behaviour that leads to medication discontinuation as prescribed and agreed with clinicians (Horne et al.; 2005). Therefore, in this thesis I refer to term "medication discontinuation" as result of non-adherence and low compliance.

Medication discontinuation with antipsychotic is a frequent problem in the management of schizophrenia and was reported to be intentional or unintentional (Campbell; 2005). Intention or no in adhering to medication was said to be usual in response to day-by-day challenges of ordinary living, such as the need to reduce side effects or to forgot medicines (S. Gibson et al.; 2013). It leads to poorer patient outcomes, including an increased risk of relapse and reduced quality of life. This usually occurred because patients are not motivated, and this was found to be related mainly to side effects of drugs, multidrug administration and fear of relapse or that drugs would not have effects (Wroe; 2002). Medication discontinuation contributes to significant cost-effectiveness in treatment of

schizophrenia due to fact 53% of non-adherent patients experience a relapse of an acute psychotic episode and this cause 153,000 deaths per year (Gilmer et al.; 2004; Taylor et al.; 2009). It also has an impact on the health economy with a cost for medication non-adherence ranged from $2512 to $25 920 as reported in different studies (Robinson et al.; 2006; Jiang and Ni; 2015). As non-adherence depends on patients behaviour (even if unintentional), it is usually confused with treatment-resistant schizophrenia condition, that possesses, instead, biological, and physiological background.

In treatment-resistant schizophrenia cause of medication discontinuation has been widely investigated and described as a result of two main barriers: clinical-related and patients-drug related (Farooq et al.; 2019). Pai and co-workers, in 2012, told clinical-related barriers as a combination of patient decision and medical complication, inadequate response. A review of currently available literature revealed that patient-drug related barriers are side effect for 28% of case and about 19% with dose and duration of treatment (Pai and Vella; 2012). A schematic representation of medication discontinuation in treatment-resistant schizophrenia is reported in Figure 21.



Medication Acceptability	
Side Effect	28%
Dose – Duration - Polypharmacy	19%
Other Illness	13%
Death	13%

Figure 21. Schematic representation of medication discontinuation causes. The chart was redrawn from Pai and co-workers (Pai and Vella 2012), while the table is comprehensive of the leading cause of medication acceptability from the literature evidence.

The most common side effects are sedation, neutropenia, tachycardia, dizziness, sedation, nausea, hypersalivation and weight gain (Legge et al.; 2016; Davis et al.; 2014). A significant impact on medication acceptability relates to dose and duration of therapy (Taylor et al.; 2009). Usually, in psychiatry, treatments are chronic and life-long. In the case of clozapine, maintaining therapeutic drug level is not tolerated as many tablets per day must be taken. Clozapine is a low soluble drug with 50% of bioavailability. Therefore, it is administered with several doses along day to ensure that therapy efficacy, however, it was noticed that effectiveness relates to side effects. An example of medicine intake is reported below Table 3 from Maudsley Hospital guideline to initiate patient to clozapine.

Table 3. Clozapine initiation protocol provided by Maudsley Hospital in South West London (David M. Taylor; Barnes; 2018)**.**

Day	Morning (mg)	Evening (mg)
1	-	12.5
2	12.5	12.5
3	25	25
4	25	25
5	25	50
6	25	50
7	50	50
8	50	75
9	75	75
10	75	100
11	100	100
12	100	125
13	125	125
14	125	150
15	125	150
16	125	150
17	125	200
18	150	200
19	150	200
20	150	200
21	200	200
22	200	200
23	200	200
24	200	200
25	200	200
26	200	200
27	200	200
28	200	250

The issue related to patient non-adherence is well-recognised and different solutions have been proposed so far as electronic monitoring, control pharmacy records, and controlling blood plasma level (Sajatovic et al.; 2010). While from a clinical point of view adherence level could be monitored during a consultation with patients and their carer, from a pharmaceutical point of view a lot could be done to modify administration route and let it be more suitable for the patient requirement. Lately, British Journal of Psychiatry suggested formulation of the controlled release dosage form to both improve patient acceptability and enforce achievement of therapeutic window (Mortimer; 2011). Other studies demonstrated that maintaining treatment with a long-acting antipsychotic is useful, and only 1 of 10 reviews reported case of discontinuation. However, no dosage forms for prolonged and controlled release of clozapine is available, and no study has been undertaken to propose such a novel system.

5.1. General Background on Medication Acceptability

Even if the cause of medication discontinuation has been studied, a clear understanding of patient-drug barrier in treatment-resistant schizophrenia has not been achieved. WHO suggests that medication acceptability of formulation itself could influence patient compliance and treatment efficacy and safety (Lallemant; 2018). Mainly, European Medicines Agency (EMA) defines medication acceptability as 'the overall ability and willingness of the patient to use and its caregiver to administer medicine as intended', and it is considered related to characteristics of a medicinal product (Klingmann et al.; 2013). As well, FDA reported, in 2015, that a percentage of drug discontinuation relates with the pharmaceutical composition of a dosage form such as taste and shape, size, and a dose of medicament (U.S. Department of Health and Human Services Food and Drug Administration (CDER); 2013).

5.1.1 Dose, size, and shape

In patients that consume large amounts of medicines, their acceptability is crucial to avoid adverse side effects and life-threatening incidents caused by overdoses or low doses. However, the quantity of solid dosage forms administered in patients under chronic treatment is reported as the main problem (Mistry and Batchelor; 2017). WHO said that patient could experience sight aversiveness based on the external characteristic of dosage form, causing non-compliance and low medication acceptability. Mainly, a study from Adams and co-workers, conducted in Tanzania, showed that patients would prefer to assume one tablet per day than multiple (Adams et al.; 2013).

Size and shape can both affect patient acceptability of medication as it is estimated that about 3% of the population worldwide is affected by swallowing difficulties and 4% of those (~ seven million people) have discontinued therapy because tablets and capsules were challenging to take (Cho et al.; 2015). Both size and shape. Size and shape influence transit of medication through pharynx and oesophagus and directly affecting the patient's ability to swallow the drug. A study conducted at the University of Copenhagen demonstrated that patients prefer the smallest size of tablet or capsules, indicating an overall preference for oblong/oval, and coated tablet (Overgaard et al.; 2001). Moreover, the large tablet could transit slowly in the first gastrointestinal tract (i.e. oesophagus) improving the chance of starting in the advance disintegration process of a tablet with release of a drug that may be toxic or create injuries. Smaller and oval tablets were proved to transit faster in oesophagus compared their larger counterparts (Channer and Virjee; 1986).

In recent years, technology as 3D printing is investigated for its advantage to customise products shape and size. Many studies are, in fact researching acceptability of different shapes on patient acceptability. FDA, moreover, reports that different shapes present different release profile and therefore useful to tailor dosage form with the patient requirement. For examples, Goyanes and co-workers, in 2015, proved that 3D object printed as a cube, cylinder, torus and sphere present different release profile (Goyanes et al.; 2015). Dosage forms shape and size are easily manageable towards most common formulation strategies.

5.1.2 Taste

Taste is one of five senses stimulated in the mouth by the compound's chemical characteristic and identified in brain-based on information provided by taste receptors (Bradbury; 2004). This sense reception is constituted by a group of 50-100 epithelial cell with express a specific kind of receptors, namely G-protein-coupled receptors (T2R). Once the receptor is stimulated, it triggers a cascade of signals that leads to neurotransmitter release, which transmits a message to the brain (Mennella et al.; 2013). Individually, when a compound binds a T2R expressed in the buccal membrane of receptor cells, that is activated. Figure 22 presents the taste receptor representation and taste signal. Once the receptor is activated an electrochemical signal is transduced by intermediaries such as α-gustducin creating a response that phosphorylates adenosine diphosphate in adenosine triphosphate (ATP) that drives serotonin release transmitting the message to gustatory nerves, thus to the brain (Oka; 2018).

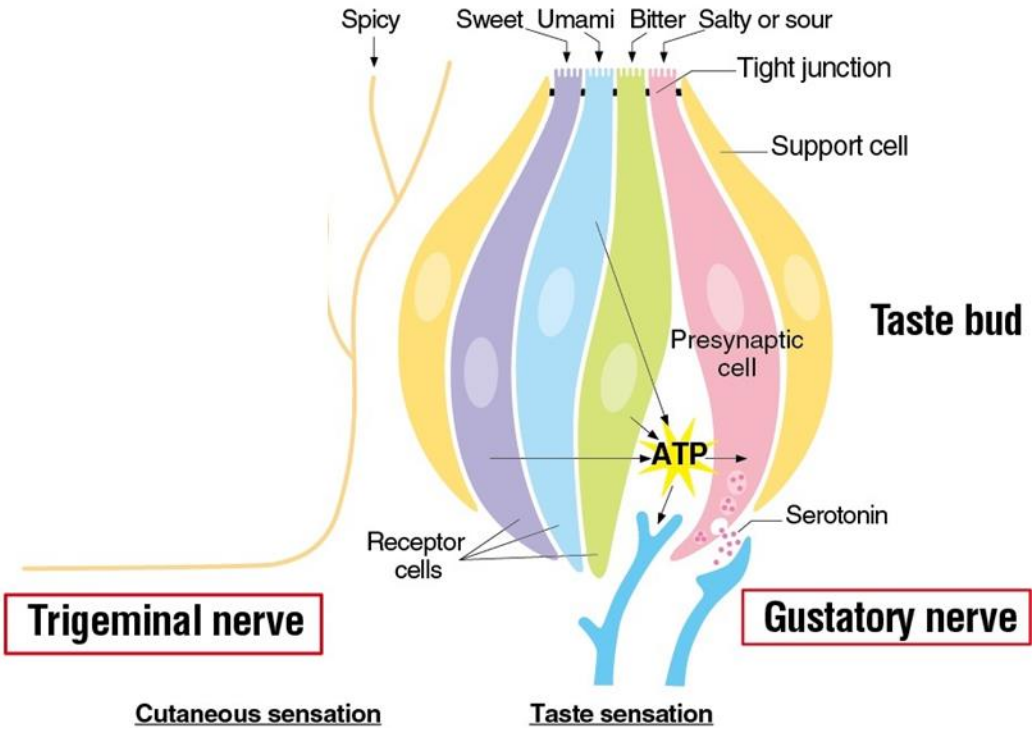


Figure 22. Taste receptor cells on tongue and taste signal. Image modified from Oka and co-workers, 2018 (Oka 2018).

So far were recognised five different taste where bitter is one of the underlying feelings, along with sweet, sour, salty and umami (Mullan; 2000). Signal transduction of flavours bitter, sweet, and umami depends on T2R, while salt and acid are transduced via ion channels (Oka 2018; Nolden and Feeney; 2020). Receptor family (TAS2Rs) is a family of 25 receptors in which it was demonstrated that difference in gene expression for one or all receptor creates an individual perception of taste and affects perception of different compounds (Shaw et al.; 2018; Soares et al.; 2013). Moreover, it has to be acknowledged that even patient personality and experience play a role in taste perception. It was recognised that when people talk about "taste", they are referring to "flavour" that is the perception of a taste and the consecutive behavioural and physiological reaction in the oral cavity (Rozin; 1982). Therefore, the formulation of the drug for a therapeutic purpose considered taste perception as a critical point to understand medication acceptability.

4.1.2.1 Challenges in drug development: Formulation Taste

In pharmaceutics, over 350 drugs from different categories were identified to be bitter (Wiener et al.; 2012; Schiffman; 2018), such as antihypertensive, antipsychotics, endocrine and diabetes drugs as well as vitamins, minerals, nutrients and related compounds (Schiffman; 2015). Taste plays a critical role in medication palatability, and aversive taste often results in patient

avoidance of medication itself. For example, in the pediatric population was reported that more than 90% of non-compliance depends on drug's taste (Milne and Bruss; 2008).

The WHO considers the lousy taste of medication as one of the leading causes of non-compliance and, therefore, low therapeutic efficacy (Hsu et al.; 2013). The infield of low compliance towards clozapine medication, it was suggested that drug aversive taste is one on significant issues (Reddy et al.; 2011). Information about clozapine taste is provided by Netherlands Clozapine Collaboration Group in "the guideline for the use of clozapine" in 2013 (Abeelen; 2013) and from Ramuth and co-workers in 1996 (Ramuth and Flanagan 1996). Clozapine marketed dosage forms described above has listed “bitter taste" as a side effect of the dosage form. However, data about a quantitative and solid assessment of clozapine taste or its formulation counterpart are missing. Nonetheless, literature presents quite a few pieces of work that refer to it as an aversive drug or to cause taste impairment.

Even though many solid oral dosage forms could encapsulate or mask the taste of drugs using coating agents, this cause increase in the size of a tablet that could not be swallow easily. Therefore, different methods are used to assess both drugs and dosage forms taste, as discussed in the following chapter.

4.1.2.2 Dosage forms taste assessment

Drug taste assessment it is a trending topic in the last decades, and nowadays, there are a few methods available to establish drug bitterness both in-vitro (Podrażka et al.; 2017) and in-vivo (Keeley et al.; 2019). Those strategies could be used to determine the taste threshold, which is the minimum drug amount causing an aversive taste perception (Dale et al.; 2001). Identifying the taste threshold leads to an understanding of drug aversiveness or bitterness in comparison with two well-known bitter compounds quinine and strychnine. Both present a threshold of, respectively 0.008 m*M*, and 0.0001 m*M* (Martin; 2013).

In-vitro analytical methods for taste assessment

In-vitro strategies include a biochemical assay of α-gustducin carry on artificial saliva (Anand et al.; 2007). Assay studies activation of α-gustducin,t that is one of the primary transmitter involved in taste signal. Its activation relates to most bitter compounds but not all such as glycine, monosodium glutamate, citric acid or potassium chloride (Ruiz-Avila; 2000). Therefore, it is not considered a precise method.

The newest invention in sensory pharmaceutics is an electronic tongue that consists of a sensor array and a detector known as a sensor chip (Anand et al.; 2007). The latest version of this machine is Astree electronic tongue system equipped with seven sensors to quantitative and qualitative assess compounds taste thresholds. Taste is identified based on sensor physical properties which generate a voltage potential when meeting an Ag/AgCl reference electrode (Latha; 2012). The system can also be combined with a dissolution bath to evaluate the taste of substance once dissolved. Even if this innovation would address problem relates with the *in-vivo* characterisation (which is discussed below), its results are still questionable as it does not take int consideration individuals' variation on taste perception (Mennella et al.; 2013). Moreover, sensor array systems present limitations concerning compatibility to sample solutions and sensor damage can occur (Woertz et al.; 2011a; Latha; 2012).

Lately, sensory pharmaceutics shifted in predicting taste of compounds and drugs by using a database BitterBD. This was introduced in 2011 by Wiener and co-workers (Wiener et al.; 2011) and included about 500 compounds for which provides information regarding molecular properties that use to predict bitterness. BitterDB also possesses data on 25 aversive taste receptors, and it relates to the genome browser to improve its efficacy. Overall, the system considers molecules as aversive if they have a molecular weight (MW) $< = 700$ and hydrophobicity (AlogP) range $-3 < = \text{AlogP} < = 7$ (Wiener et al.; 2012; Dagan-Wiener et al.; 2017; Wiener et al.; 2011), indicating compound with a taste higher than 0 as bitter and more elevated than three as very bitter. It then, compared results obtained with available literature.

In-vivo taste assessment methods

In-vivo taste assessment could be animal or human-based. Animal preference tests are used to determine a preference in taste dose-related by animals such as rats and mice (Tordoff; 2003). Some studies have studied the capability of those animals to detect weak bittern compound dissolved with a low concentration in sucrose solution, demonstrating rats possess a high sensitivity (Contreras et al.; 1995). A newest and more precise *in-vivo* model was recently developed by Boughter and co-workers named brief-access taste aversion (BATA) model, which reduce intake of the sample by reducing volumes and measuring behaviour response as licking rate. By presenting small amounts of taste samples and measuring immediate behavioural reactions. Quantification is allowed by evaluating licking rate of aversive compound solution through a lickometer (Woertz et al.; 2011b; Soto et al.; 2016). This method is usually correlated with a modified dissolution apparatus which mimic the oral cavity. Even if animal models are becoming more accurate showing a good correlation with human

taste data, human studies are considered the more precise assessment technique (Mohamed-Ahmed et al.; 2016; Ali et al.; 2020)

Human taste panel study investigates the taste of chemicals and drugs by analysing the response of healthy human volunteers in standardised ethically approved operation. This kind of research is sensitive in measurement and are designed to reduce bias by recruiting volunteers of difference gender, age, and race. Volunteers assess taste intensity from different solutions or dosage by using an adjective scale, rating taste perception. The study is not meant to influence candidate psychologically; therefore, patient usually can type a comment to improve their rating. Rate is generally from zero that indicates no flavour at all, to 100 that represents the maximum intensity of taste perception that they are asked to identify (Schiffman 2018; 2015). Human taste panel studies are considered extremely accurate because they take into account interpersonal variability connected with both physiological and psychological preferences.

The WHO considers that any medication causing undesirable inpatient behaviour or unpleasant experience should be suppressed. The organisation recognises taste, and sight as the leading cause of medication discontinuation and non-compliance. Due to high effect that drug taste has on patient behaviour towards medication and due to availability of different methods of taste assessment, those should be used more widely as a tool to improve patient acceptability towards the drug.

Part 2. Aim and Objectives of PhD

In the Introduction of this thesis, two significant pharmaceutical challenges were identified in treatment-resistant schizophrenia namely the daily dose requirement and palatability of clozapine (the gold-standardin the UK, US and Italy). Clozapine in treatment-resistant schizophrenia offers the advantage of reducing the risk of suicide as well as improving symptoms. Clozapine is categorised as BCS class II (i.e. with low solubility and high permeability) and is marketed as an oral dosage form.

The aim of this PhD is to generate and characterise patient-centric formulations to reduce daily dose requirements and overall improve palatability and patient acceptance. Therefore, it seeks to apply different formulation strategies to produce a novel patient-centric formulation as solid dispersions which reduces dosing per day by providing a controlled-release formulation of clozapine. The aim is two-fold: the reduction of clozapine dose per day and the investigation of clozapine palatability, as concerns about drug taste are considered affecting medication acceptability.

Obtaining an optimised dosage form requires the evaluation of different formulation methods. In the present Thesis, different formulation strategies and polymers combinations were investigated to optimise a patient-centric clozapine dosage form.

To reach the PhD aim, each year was organised in objective as follow.

1. Investigation of the use of spray-drying due to good advantage in term high solubility and permeability. In this study, irinotecan is used as model drugs due to its low solubility and permeability (BCS class II). The focus of this investigation is to evaluate the effect of spray-drying parameters on drug dissolution and permeation. Obtaining this information is crucial to develop an optimised patient-centric formulation.

2. Dosage form optimisation requires the comparison of the most common formulation methods for solid dispersion manufacturing in term of both obtained particle size, homogeneity, and dissolution performances. Therefore, an objective of this work is to compare solid dispersions formulation strategies such as spray-drying, using parameter optimised in term of pump efficacy and temperature, freeze-drying and solvent cast method. The study produces reliable data by using three model drugs, all classified as BCS class II such as dexamethasone, triamcinolone, and olanzapine. This step establishes which method is the most suitable to produce a patient-centric dosage form for clozapine prolonged release.

3. Oral transmucosal delivery system, as discusses, is of benefit in buccal absorption. This system is prepared by using biocompatible polymers in the formulation. Therefore, this crucial step has the objective to investigate two polymers, PVA and PHEA as biocompatible polymers for patches

generation. These polymers present both solubilising and adhesive properties, and this work seeks to examine the effect of the different polymeric combination in producing and optimising the creation of an oral transmucosal delivery system for controlled clozapine release. The oral transmucosal delivery system is usually prepared as a solvent cast film and fibres, manufactured through the electrospinning. The advantage of this novel dosage form is currently under study, as described in the Introduction, as an administration site for controlled dosage forms. Fibres offer higher surface area compared with solvent cast film, therefore high dissolution performances. As one of the main features of the transmucosal patch relates to its adhesiveness and the generation of adhesive forces, this section hypothesises that different polymers combinations create various adhesive forces. AFM-Qi possesses potential in obtaining high-quality data and topographic analysis of surfaces to assess adhesiveness. Then, clozapine dissolution performances are analysed to identify which polymer combinations and formulation strategies provide a controlled release dosage form.

4. A transmucosal patch can provide a multi-directional release of drug in both buccal mucosa and oral cavity or unidirectional, preferring drug permeation through buccal mucosa and avoiding drug clearance in the oral cavity. Manufacturing of unidirectional patch with electrospinning is considered challenging as well as customisation in term of patch size and thickness. The last part of the present work has as objective the use of 3D printing technology to produce unidirectional transmucosal patches provided with a hydrophobic backing layer of PLA. 3D printing presents an advantage to create intricate design allowing customisation and size management. PVA, which solubilising and adhesive properties are going to be assessed at point 3, aims to be used as a primary polymer of the 3D printed product. As described in the Introduction, different printing infills can modify the drug release profile. Therefore, it is hypothesised the effect of varying infill percentages on clozapine dissolution profile as dissolution mechanism could be a carrier- or drug-mediated as discussed in the Introduction, this part of the thesis objective to establish which mechanism drives clozapine release by loading in the patch different drug strengths. The optimised patches, loaded with a dose of 10 mg, aim to produce a controlled release profile of clozapine from the unidirectional transmucosal patch. *In-vivo* absorption is mediated by two mechanisms, dissolution in the media, which represents the first limiting step and permeation through membranes. Therefore, as the final objective of the present work, permeability studies provide information on *in-vivo-in-vitro* correlation, and such data can be provided by using both a combination of cellulose acetate membrane and the biomimetic membrane Permeapad™.

5. The final objective of the PhD present thesis has the objective to investigate clozapine taste perception due to the discussed impact of aversive drug tastes on medication acceptability. This analysis seeks to conduct a structured literature review estimating the extension of the role of

clozapine taste in non-compliance. Confirmation of the structured literature review is intended to be validated based on the described advantage of human taste studies in assessing drug aversive taste threshold. This method allows volunteers to rate taste according to an adjective scale and to comment supporting their rating. This research required ethical approval by the University College of London Ethical Committee.

Part 3. PhD Laboratory Experimental Chapter

Chapter 1. Physical-chemical characterisation of raw material

1. Background

In the Introduction Chapter, different types of solid dispersions were highlighted where the difference between them was solid-state of the polymer matrix, and drug, i.e. both drug and polymer could be in the crystalline or amorphous state. It is well-known that amorphous materials possess short-range molecular order and high kinetic energy. Amorphous materials are generally more soluble and have a faster dissolution rate (Shah et al.; 2014) compared to their crystalline counterparts which possess a low energy state resulting in low solubility and longer stability (Michael; 2007; Sinko; 2011). Despite amorphous benefit, it possesses limited stability, which relates to factors such as thermodynamic and kinetic energy. Thermodynamic studies solids since 1930 when the thermal analysis was suggested as a method to predict solid-state solubility. Due to high kinetic energy possessed by amorphous solids, once heated they liquefy in a long thermal range named T_g; while lower energy state causes sudden melting process recognised as T_m (Bhugra and Pikal; 2008).

The present thesis aims to produce a controlled-release formulation of drug clozapine. Optimisation of final dosage forms included different studies which were conducted using poorly-water soluble drugs as a model drugs such as irinotecan, dexamethasone, triamcinolone, and olanzapine. In present work, dexamethasone and triamcinolone were used as a model drug due to their poor solubility and solid crystalline state. Triamcinolone differs in its chemical structure from dexamethasone just for an OH group. This chemical similarity was used as an advantage in present work to produce consistent data. Irinotecan is a poorly water-soluble drug classified as BCS class II and in this study is used as a model drug to verify spray-drying potential in improving drug dissolution profile and permeability rate. Therefore, olanzapine was used due to its chemical similarity with clozapine. It was used as a model drug while collecting preliminary data on suitable solid dosage forms manufacturing to obtain a controlled release formulation (Modica de Mohac et al.; 2016).

This first experimental Chapter aims to physical-chemically characterise drugs and polymers, used as unformulated compounds in this thesis, through differential scanning calorimetry (DSC) and Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR). Such characterisation is fundamental to evaluate the solids state of raw materials used in present work. Compounds solid-

state affects solubility and dissolution behaviour. Thus, final dosage forms response once in the human body, as explained in Introduction. Therefore, thermal analysis via DSC was performed to identify solid drug state to assess that all drugs used in present work were crystalline, as target drug clozapine.

Most of the analytical techniques used to characterise materials in solid-state provides information about the state of material (i.e. amorphous, crystalline or a mixture of both) and rely upon thermal analysis via DSC, dynamic mechanical analysis and thermogravimetric analysis (TGA). DSC is used to characterise pharmaceutical solids and builds upon change solid-state of a material as it is heated; while TGA calculates water content of each material to evaluate component hygroscopicity (Groenewoud; 2001). Solids are also characterised investigating hydrogen bonding formation via ATR-FTIR (Storey; 2011). This technique allows a molecular-level analysis which provides information by probing molecular-level interactions within each component of the system, dosage form (De Lima and Filho; 2016). ATR- FTIR is extensively used for characterisation of pharmaceuticals in solid-state to identify hydrogen bond formations between components of a formulation such as a drug and a carrier and to identify any specific functional groups that might cause physical or chemical interaction (Minnes et al.; 2017).

In following section physical-chemical characteristics of unformulated drug and polymers are discussed to establish both materials solid-state, which could affect drug dissolution performances and presence of functional group which could determine hydrogen bond formation between drug and polymer during formulation strategies applied as explained in Chapters 2 to 6.

2. Materials and Methods

In present work, different drugs to both carry pre-formulation studies and to improve dosage forms for treatment-resistant schizophrenia , using clozapine. Model studies were conducted on BCS class II drug such as Irinotecan (Irn), Olanzapine (Olz), Dexamethasone (Dsm), Triamcinolone (Trm), were purchased from Sigma-Aldrich.

Different polymers were used and investigated to obtain different properties in each formulation. CAP, Mw 2534.12g/mol, Mannitol Mw 182.172g/mol, MDX, Mw 34,000 g/mol, inulin from chicory Mw 522.453g/mol, PMMA Mw 34000g/mol, PVA, Mw 44.053 g/mol were purchased, from Merck KGaA (Darmstadt, Germany). PLA Mw 60.000 g/mol was purchased as a filament by RS. PHEA (Mw 85000 g/mol) was synthesised by the Department of Biological, Chemical, and Pharmaceutical Sciences and Technologies (STEBICEF). SLP, Mw 344.536 g/mol was kindly provided from BASF USA.

2.1 Infrared- analysis

Conventional FTIR studies carried out to identify any molecular chemical group of drugs Irn, Dsm, Trm and Olz and polymers CAP, inulin, MDX, mannitol, and PMMA. Samples were prepared by compression of a thin circular tablet using as 1:99 drug: KBr ratio. Samples were placed on the holder, and nitrogen gas was used to reduce carbon dioxide peak and spectra were collected using a JASCO FTIR-6000 spectrometer, from 4000 to 300cm^{-1} with 264 scans (same as background) at the resolution of 2cm−1 for each sample. Experiments were carried out in triplicate.

ATR-FTIR studies were conducted to identify the molecular structure of drug Clozapine and polymers PHEA, PVA and SLP. Samples were placed on ATR crystal, sufficient pressure was applied to sample to enable proper contact with ATR crystal, and spectra were collected using a Bruker Vertex 90 spectrometer, from 4000 to 550 cm $^{-1}$ with 264 scans (same as background) at a resolution of 2 cm^{-1} for each sample. Experiments were conducted under ambient conditions, and each sample was examined in triplicate.

2.2 DSC

Thermal analysis was conducted using a DSC 2920 Modulated DSC machine (TA instruments). Modulated DSC was predilected as it uses two simultaneous heating rates, a linear heating rate and a modulated heating rate allowing to plot a time-based derivative of temperature per minute, which is more accurate. DSC analysis was conducted at a rate of 5°C/min, and samples were heated from 20 to 300°C. nitrogen purge gas used had a flow rate of 130mL/min. Samples were also held isothermally for 5 minutes. Aluminium hermetically sealed pans used were from TA Instruments. Each sample was analysed in triplicate. Data analysis was conducted using a TA Universal Analysis software (version 5.1.0).

3. Results and Discussion

3.1 Characterisation of drugs as unformulated compounds

At first, chemical groups were investigated, and FTIR spectra were recorded to analyse functional groups which could lead to chemical bonds during formulation. Then, DSC analysis was performed to examine both drug and polymers solid-state, which allows identification of solid dispersion generation generated. Creation of a chemical bond between drug and polymer would cause

the creation of new chemical entities that was not intended in this project. Drugs are discussed in order of subsequent experimental Chapters' explanation. All drugs spectra were reported in Figure 23, while detailed peak assignation was provided in Table 4.

At first, Irn spectra showed most significant peaks at 3511cm^{-1}, assigned to OH vibration, 2940cm−1 assigned to CH2 symmetric stretching vibration, 2876cm^{-1}, due to CH stretching, 1747, 1686, 1661, 1611 and 1417cm^{-1} well-known as Irn C=O and C-C vibration. Then peaks at 1452 and 1435 were assigned to methyl groups; peaks at 1216 and 1160cm^{-1} were assigned to CH in-plane bending vibration. As it is known, peaks from 1200 to 1000cm^{-1} refer to aromatic's groups 1106, 1065 and 1007cm^{-1} were assigned to stretching ring vibrations of group C-C and C-N. Aromatic CH in-plane bending modes of benzene and derivatives are observed in region 1300–1000cm^{-1} Irn shows such peaks at 1334, 1313, 1260 and 1192cm $^{-1}$. Peak 2940cm $^{-1}$ is reported to be an acceptor group of Irn and therefore considered capable of interacting with the donor group when in the formulation (Thomas et al.; 2004). Each peak was correspondent to existing literature (Chinna-Babu et al.; 2012).

Dsm and Trm are corticosteroid molecules with the chemical difference that Trm presents two hydroxides on two nearer carbons in the aromatic ring. Both Dsm and Trm offer the vibration of alcohol vibration at 3400 cm $^{-1}$, where Trm gives a double-peaks referring to two OH groups. They both perform a CO stretching of carbonyl between 1700 and 1674cm $^{-1}$. Dsm shows a peak at 1268 cm $^{-1}$ related CF stretching while it does appear at 1057-1080cm $^{-1}$ CF stretching for Trm. Those findings were confirmed in the literature (Rodrigues et al.; 2009; Shin et al.; 2000). FTIR spectra of Olz, showed characteristics peaks (Silverstein et al.; 2005) at 3239 cm $^{-1}$ referring to NH and OH stretching vibration and, 2929 cm-^{1} to CH stretching. Different peaks identified olz aromatic structure in the region of 1550-1580 cm $^{-1}$ as well as at 1450cm $^{-1}$.

Clozapine analysis was performed by using ATR-FTIR for further precision required in the formulation of dosage forms intended for systemic delivery. In spectra were observed peaks at 774 cm $^{-1}$ (C-Cl bond), 1025cm $^{-1}$ and 1357cm $^{-1}$ (C-N stretching vibration) and 1545 cm $^{-1}$ (C=N stretching vibration) were identified (Ali et al.; 2015). In Figure 24, a zoomed-in section of relevant peaks.

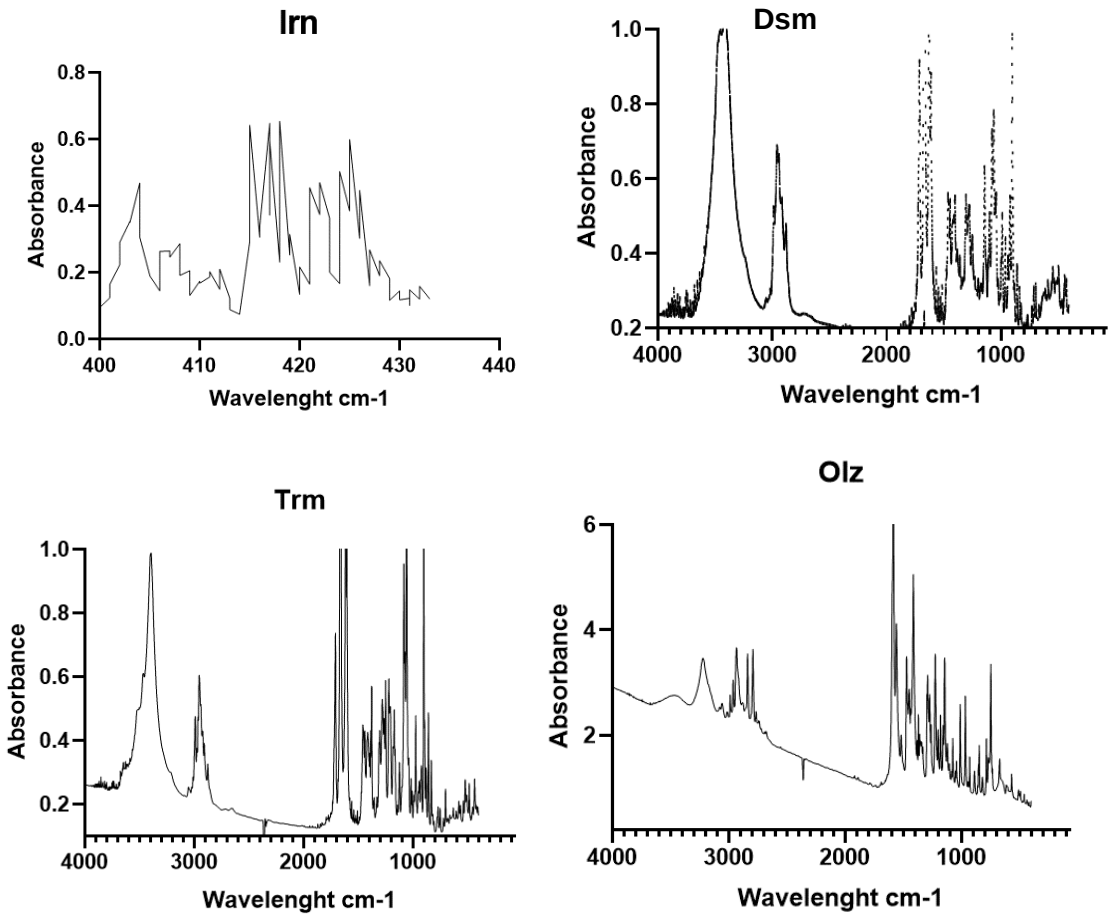


Figure 23. FTIR spectra for Irn, Dsm, Trm and Olz.

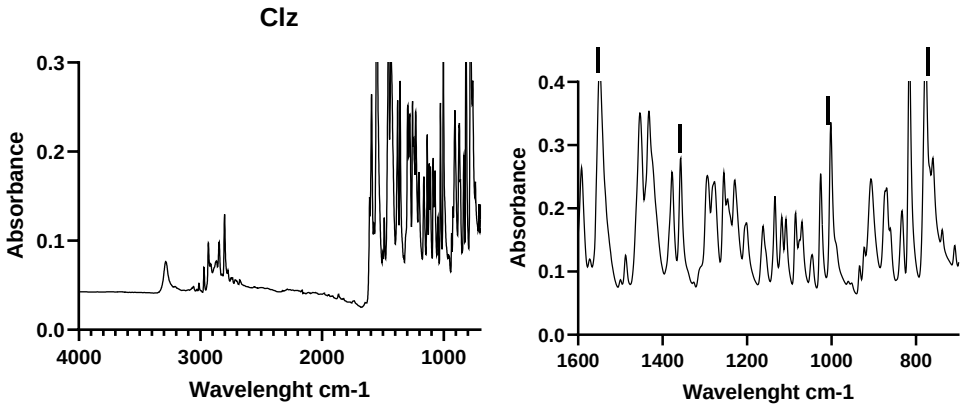


Figure 24. ATR-FTIR spectra of clozapine, on the, left full spectra presenting; on the right, most relevant peaks were zoomed in and highlighted.

Table 4. each drug peaks assignation and definition. From the top, Irn, Dsm, Trm and Olz which peaks were identified by using conventional FTIR method; at end Clozapine which peaks were studied through ATR-FTIR.

Infrared peaks assignation	
Irn cm $^{-1}$	
3511	OH alcohol vibration H bond
2940	CH2 symmetric stretching vibration
2876	CH Stretching
1747 and 1686	CO stretching carbonyl
1661, 1611 and 1417	C=C stretching aromatic ring
1452 and 1435	methyl groups
1216 and 1166	CH in-plane bending vibration
1334, 1313, 1260 and 1192	aromatic C–H in-plane
Trm cm $^{-1}$	
3464-3397	OH alcohol vibration H bond
1707-1662	CO stretching carbonyl
1456	CH methyl
1375-1302-1278	COC acid alcohol
1057-1080	CF stretching
Dsm cm -1	
3407	OH alcohol vibration H bond
2981-2953-2932-2866	CH methyl
1704-1659	CO stretching carbonyl
1617-1603-1436	C=C stretching aromatic ring
1268	CF stretching
Olz cm $^{-1}$	
3221	NH stretching
3078-3060	CH aromatic ring stretching
2931	CH3 methyl stretching
1584-1558	C=C asymmetric aromatic stretching
1470-1446-1412	C=C aromatic ring stretching
1289-1223	CN bonding
Clozapine cm $^{-1}$	
1545	C=N stretching vibration
1357	C-N stretching vibration
1025	C-N stretching vibration
774	C-Cl bond

Thermal property of each drug was then analysed to investigate solid-state of the compound. As crystalline and amorphous compounds have different internal energy levels, information regarding their physical state is needed to assess the stability of drug to interact with polymers during formulation process as well as with aqueous environment once final formulation would be tested.

Drugs thermograph is reported in Figure 25. All measures were obtained in triplicate in the thermal interval from 20 to 300°C. Irn showed a T_m at 270.56 ± 5.22 °C (Ishtikhar et al.; 2016); graph showed an endothermic event at 100°C for each replicate, this related to water loss. Melting point required a heat capacity or enthalpy of fusion (Δ_{cp}) of 37.65 ± 7.89 J/g. In the next Chapter, Irn was used in formulation at 1% w/w, this amount of drug in the mixture is usually below the detection limit in DSC analysis. Therefore, it was used Δ_{cp} as a method of quantification (Gaisford; 2007). Using DSC, the traditional approach for quantification of content is based on the determination of Δ_{cp} (Dilworth et al.; 2004). drugs Dsm, Trm and Olz were also analysed for thermal properties investigation. Each drug was proved to be in crystalline solid-state, presenting respectively T_m value of 267.63 ± 0.87°C (DrugBank; 2020), 292.24 ± 2.30°C (Li and Pustaka; 2009) and 193.98 ± 1.36°C (Modica de Mohac; 2016). Dsm, Trm and Olz were used to perform pre-formulation studies intended to evaluate which techniques between spray-drying, solvent-casting and freeze-drying improve most dissolution rate of water poorly soluble drugs. In the study, each drug was used in the mixture at more than 10% w/w. DSC analysis of clozapine showed a characteristic endothermic peak associated with its melting point (Dias et al.; 2015) at 185.44 ± 0.89°C. resulting peaks once drugs were formulated, were visible, and heath capacity was not used as a quantification method; detail of each thermal event is summarised in Table 5.

The results confirmed that all drugs selected as a model drug to investigate the potential of the use of different techniques to produce a controlled release dosage form of clozapine were crystalline. As a crystalline compound, they possess low solubility and dissolution rate. Therefore, their use as a model of drugs could ensure data reliability during pre-formulation studies.

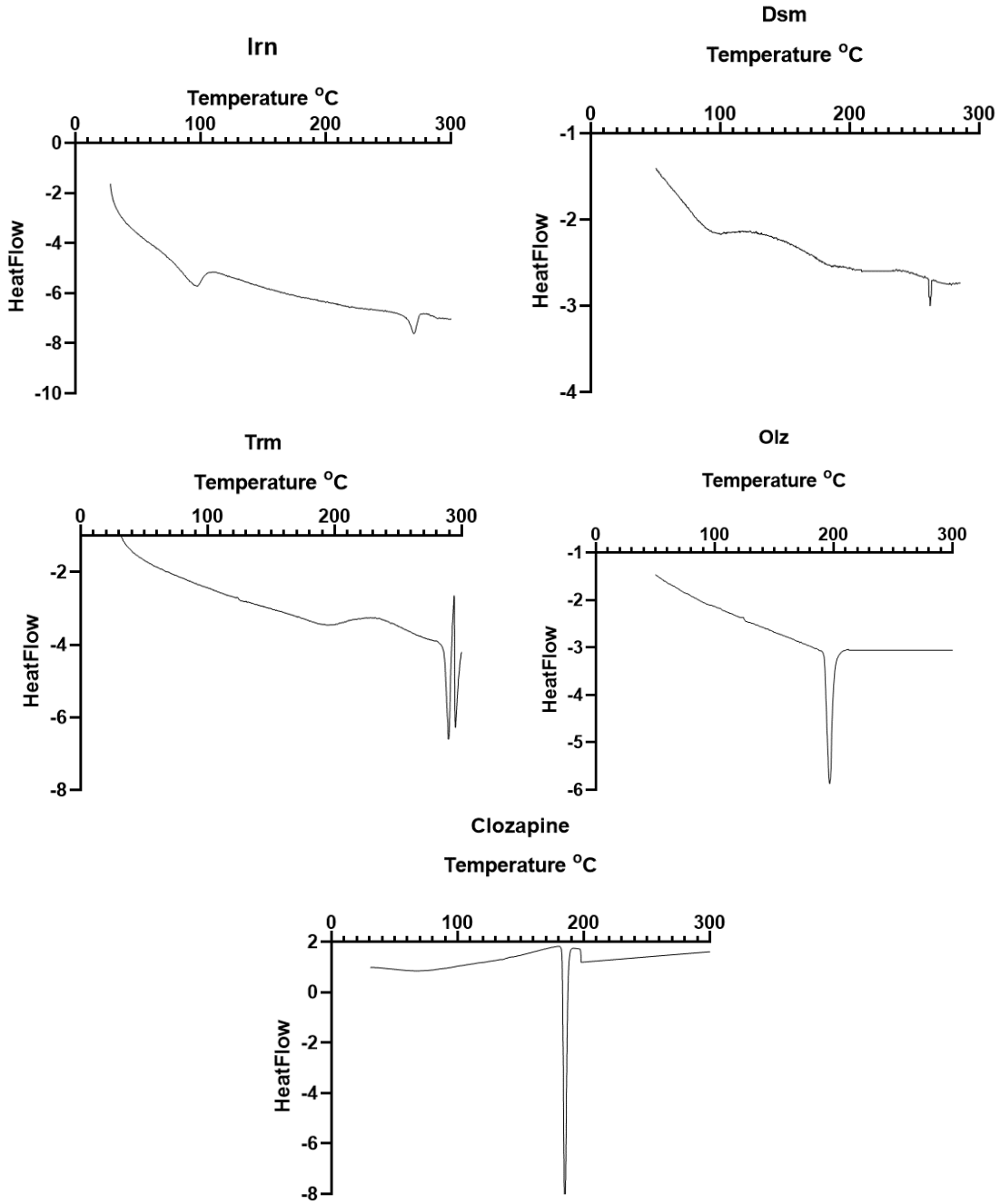


Figure 25. DSC thermographs of Irn, Dsm, Trm, Olz and Clozapine are showing respectively that each drug was in the crystalline state due to presence of peculiar Tm.

Table 5. Thermal properties of drug as raw material and heat capacity necessary for the event to occur.

	T_m (°C)	Δ_{cp} (J/g)
Irn	270.56 ± 5.22	37.65 ± 7.89
Dsm	267.63 ± 0.87	18.54 ± 10.65
Trm	292.24 ± 2.30	10.01 ± 3.45
Olz	193.98 ± 1.36	15.85 ± 8.34
Clozapine	185.44 ± 0.89	122.39 ± 4.52

3.2 Physical-chemical characterisation of the unformulated carrier used in solid dispersion manufacturing

As discussed in Introduction, solid dispersion can be formulated, generating different generation which presents different solid-state compound as well as diverse polymer/s mixture. For example, the use of SLP leads to the formulation of fourth-generation solid dispersion with enhanced solubility carrier-related; while the use of excipient with a therapeutic effect leads to the formulation of multi component solid dispersion named the fifth generation. Therefore, in this Chapter, polymers solid-state was investigated to identify crystalline or amorphous nature and later recognise relevant solid dispersion generation. However, during formulation steps, drug and carrier can provide hydrogen bond interactions that could lead to the creation of new chemical entities. Therefore, carrier functional group were analysed to identify main chemical groups that could lead to bond formation via FTIR and ATR-FTIR as described in Methods. All polymers spectra were reported in Figure 26 while detailed peak assignation was provided in Table 6 CAP is a mixed ester of cellulose obtained through phthaloylation of cellulose acetate. It is utilised in different domains, as a pharmaceutical excipient, due to its pH-dependent solubility in aqueous media. FTIR study showed a strong H-bond stretch in the alcoholic group at 3445 cm^{-1}. CAP presents different OH acid groups in its chemical structure and those were highlighted at 2640 and 2528cm^{-1}; all CAP free OH groups may be involved in hydrogen bonding with CO group on other molecules (Dobos et al.; 2012).

In present work, were used three different polysaccharides molecules: inulin, mannitol and maltodextrin (MDX). Inulin is usually used to improve intestinal microbiota, trough FTIR typical peaks were observed around 800–1200 cm^{-1} which are characteristic for polysaccharides at OH acceptors group, identified as hydrogen bond site, at 1132 and 1031 cm^{-1} and 989 and 936 cm^{-1} vibration C-C-C group. Inulin also showed peaks at 3343, and 1642 cm^{-1} referred to OH groups, recognised to be typical acceptor groups of molecules (Fischbach and Sonnenburg; 2011; Oscarson and Sehgelmeble; 2002; Pourfarzad et al.; 2015). Another saccharine unit used in the present work is mannitol. It presents typical CH deformation vibration between 1280 and 1300 cm^{-1} as well as donor groups alcoholic OH groups highlighted at 3405, 3391 and 3293 cm^{-1} (Shchodryi et al.; 2019). Finally, MDX was analysed by FTIR and peaks in the "fingerprint" region of sugar were identified at 930 and 843 cm^{-1}, referring to C-H bending 1,4-disubstituted (Sritham and Gunasekaran; 2017). Moreover, a band at 1642cm^{-1} was identified as indicative of extended β-sheet structures (Oldenhof et al.; 2008). In saccharine molecules, the presence of free OH allows the formation of glycosidic bonds. Therefore, such groups

are considered able to donate an electron, so more likely to create hydrogen bond once formulated (Weiss et al.; 2015).

PHEA is an adhesive polymer, which adhesiveness was investigated by ATR-FTIR by Saiano and co-workers, in 2005. PHEA presents characteristics “fork peaks” of C-N stretching vibration 2130, 1953 and 1279 cm^{-1}. The molecule has amidic groups repeated along the chain. FTIR spectra showed a signal of the carbonyl group (1063 and 1013 cm^{-1}), that is recognised as an electron-donor group, therefore free for bond (Saiano et al.; 2002).

Other polymers as PLA, PVA, PMMA and SLP were used in this work for their solubilising effect as well as suitability to different formulation strategies. PLA is a biocompatible polymer with high employability in implant production and tissue regeneration (Buscemi et al.; 2017; Gavini et al.; 2004). spectra showed typical peaks at 1750 and 1080cm^{-1} regarding stretching of C=0 group and fingerprint peak at 756cm^{-1} (Dukali et al.; 2014). PVA FTIR spectra showed a strong alcohol vibration H bond at 3290cm^{-1}; this hydroxide is considered as a proton donor group (Zelkó et al.; 2020). Another significant peak refers to the stretching of the C-O group at 1086cm^{-1} (Pal et al.; 2007). PMMA presents a proton acceptor hydrogen at 3389cm^{-1} (Dukali; 2014) from a free hydroxide and a strong signal at 1272, 1245 and 1196 forester group (Rao et al.; 1999). Finally, SLP was characterised, and main peaks referring to aromatic and CO stretching were identified (Palmieri et al.; 2001).

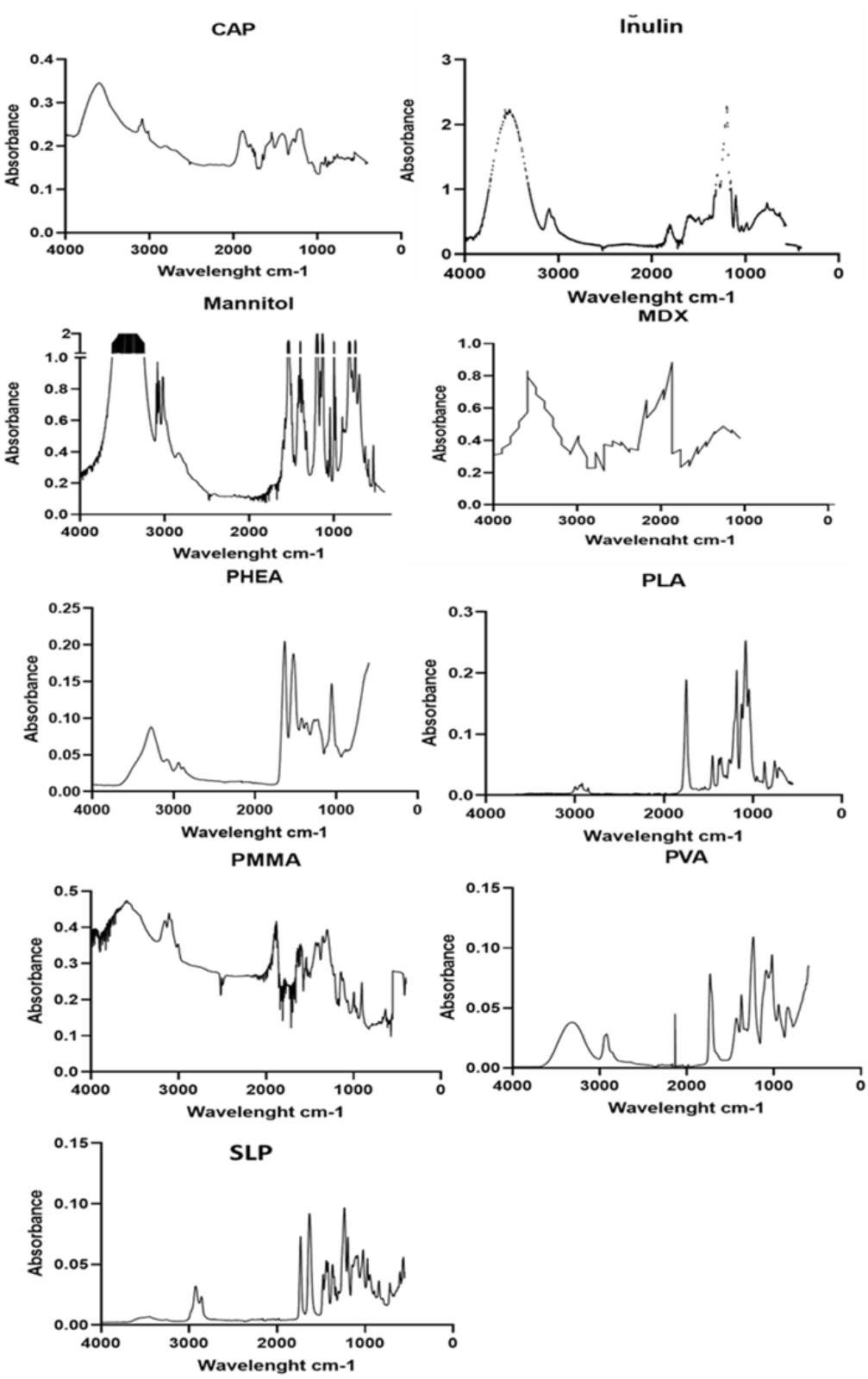


Figure 26. FTIR spectra of each polymer acquired between 4000 and 700 cm $^{-1}$ with 264 repetitions for each sample. Main peak assignation was reported in detail in Table 6.

Table 6. Table summarised peaks assignation for each polymer. Results were confirmed with existing literature.

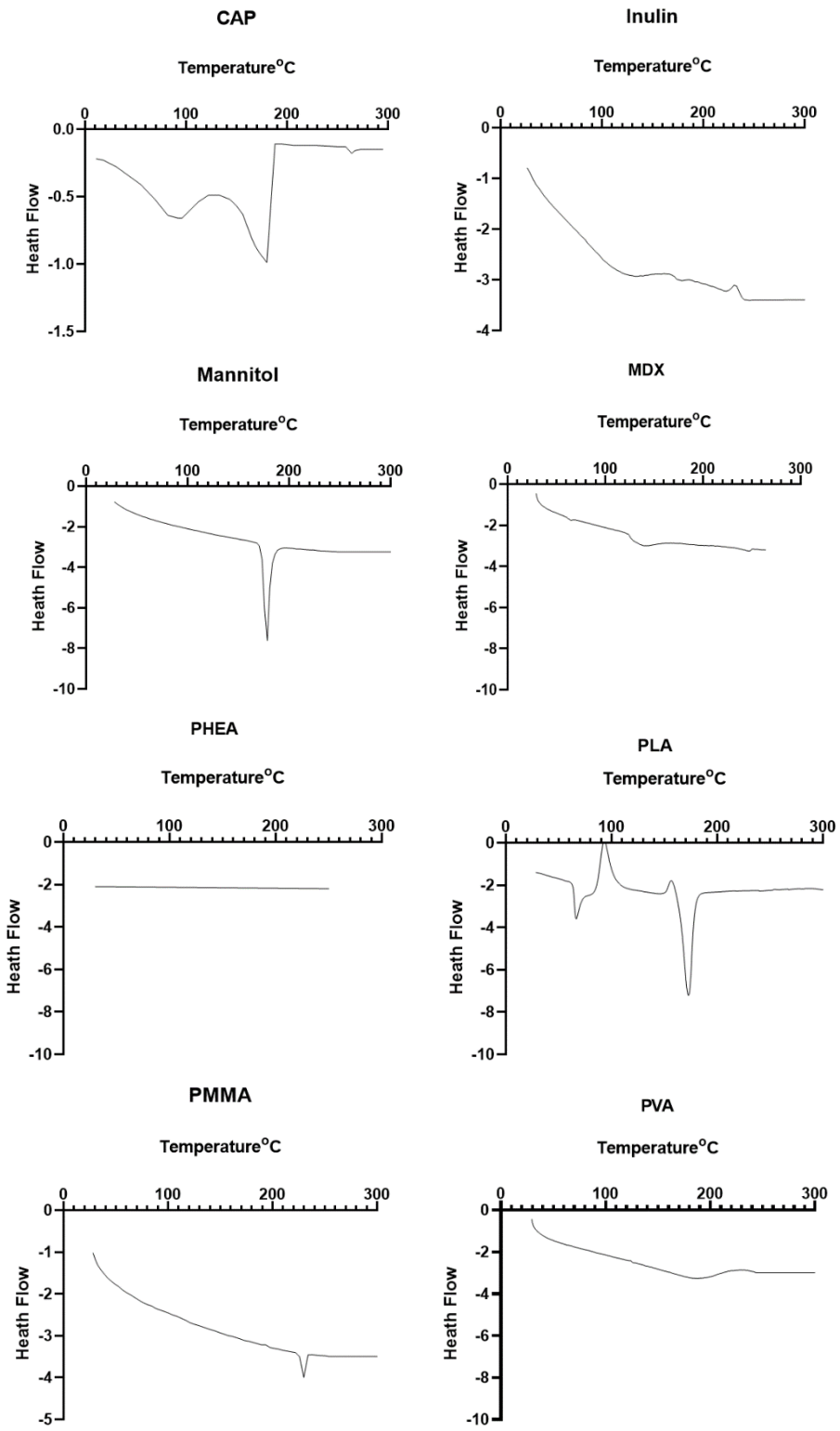
FTIR peaks assignation	
CAP	
3445	Strong H-bond stretch in alcoholic OH
2640 and 2528	OH acids
1720	Stretch ketone acyclic
1383	CH bending
1256	C-O stretching ether
1038	CO Ester two bands peak
Inulin	
3343	OH alcohol vibration H bond
1642	OH-bending (in-plane)
1327	C-O stretching ether
1132 and 1031	C-O stretching
989 and 936	Vibration C-C-C group
Mannitol	
3405, 3391 and 3293	OH alcohol vibration H bond
2970, 2946 and 1419	CH stretching
1281, 1257 and 1301	CH deformation vibration
1079 and 1019	Strong C-O Stretch
MDX	
3396	OH alcohol vibration H bond
1642	OH-bending (in-plane)
1456 and 1418	CH blending
1157 and 1030	C-O stretching ester
930 and 843	C-H bending 1,4-disubstituted
PHEA	
3371	OH alcohol vibration H bond
3085	OH alcohol vibration H bond
2130, 1953 and 1279	C-N stretching vibration
1655	C=C asymmetric aromatic stretching
1407	C=C aromatic ring stretching
1063 and 1013	CO stretching carbonyl
PLA	
1750 and 1080	C=0 stretch
1363 and 1452	CH3 symmetric bending
756	CH bending
PVA	
3290	OH alcohol vibration H bond
2939	asymmetric stretching of CH group
1421	symmetric bending of the CH2 group
1086	stretching of C-O group
PMMA	

3389	OH stretching of lattice water
2998 and 2950	CH stretching
1724	C-O carbonyl
1449	CH3 Stretching
1272, 1245 and 1196	C-O stretching ether
SLP	
2926	aromatic CH stretching
1732 and 1633	C-O stretching
1477	C-O-C stretching

Then, the thermal properties of each polymer were analysed to identify at first their solid-state and then the type of solid dispersion created once the formulation occurred. Thermal analysis showed that polymers CAP, Inulin, MDX, PVA and SLP were in the amorphous state. Therefore, they were considered of further advantage in increasing solubility of drugs used for this work. Mainly, each drug resulted in being in the crystalline state, thus provided with low solubility. For each compound, T_g was identified and reported in Table 7. T_g values found correspondence in current literature, respectively for Inulin, MDX, PVA and SLP (Cooper et al.; 2013; da Silva dos Passos et al.; 2015; Reguieg et al.; 2020; Thakral et al.; 2012).

Interestingly, CAP showed (Figure 27), an endothermic event a 190°C that is related to its degradation (Karlsson and Singh; 1998). Such a phenomenon was not registered for any of other polymers, which resulted in being more stable to thermal changes. Interestingly no T_g was identified for polymer PHEA; such data resulted in being lacking in literature as well. DSC present detection limit for a compound with a low specific weight (Kasap et al.; 2017).

Mannitol, PMMA and PLA resulted in being in crystalline form. Mannitol is used in pharmaceutical formulations as a diluent (10–90% w/w) in tablet formulations and used in tablet direct compression. It is highly water-soluble used to prepare dispersions by solvent casting method (Arias et al.; 1994; Das et al.; 2011). Mannitol and PMMA showed respectively, narrow peaks related to the endothermic event of T_m at 168.75°C (Ye and Byron; 2008) and at 229.31°C (Poomalai et al.; 2007). Finally, PLA showed a more complicated graph with both a T_g and a T_m. Thermograph presents a T_g at 66.58 ± 1.24 °C followed by polymer crystallisation and then by a melting point. This result is consistent with literature finding and demonstrated that polymer is present as a physical mixture of both form amorphous and crystalline (Poomalai; 2007; Xie et al.; 2016).


CAP
Temperature°C
0
100
200
300
0.0
-0.5
-1.0
-1.5
Heath Flow
Inulin
Temperature°C
0
100
200
300
0
-1
-2
-3
-4
Heath Flow
Mannitol
Temperature°C
0
100
200
300
0
-2
-4
-6
-8
-10
Heath Flow
MDX
Temperature°C
0
100
200
300
0
-2
-4
-6
-8
-10
Heath Flow
PHEA
Temperature°C
0
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-2
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-8
-10
Heath Flow
PLA
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-2
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-6
-8
-10
Heath Flow
PMMA
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Heath Flow

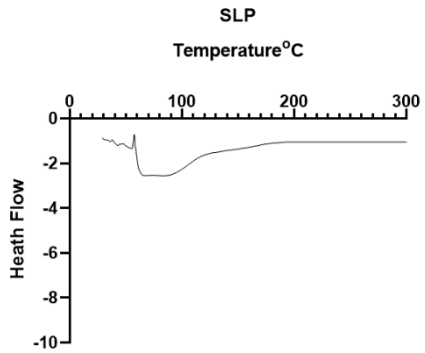


Figure 27. Thermographs were investigated with DSC instrument, and each sample was analysed in the range between 20 and 300°C. Data showed that polymers CAP, Inulin, MDX, PVA and SLP were in amorphous solid-state. At the same time, Mannitol and PMMA presented a crystalline structure identified by T_m detection, respectively at 168 and 229°C. PLA demonstrated to be a mixture of amorphous and crystalline, showing both a T_g and a T_m. Data are consistent with literature findings.

Table 7. thermodynamic properties of each compound expressed in term of T_g and T_m and related to Δ_{cp} needed for event occurrence.

Carrier	T_g (°C)	Δ_{cp} (J/g)
CAP	89.12 ± 0.91	31.52 ± 3.41
Inulin	120.90 ± 5.67	2.76 ± 4.98
MDX	50.01 ± 12.53	4.98 ± 3.72
	142.13 ± 6.23	18.22 ± 0.58
PVA	192.46 ± 7.73	30.61 ± 14
SLP	57.85 ± 1.25	0.87 ± 0.06
	T_m (°C)	Δ_{cp} (J/g)
Mannitol	168.75 ± 6.34	7.98 ± 3.21
PMMA	229.31 ± 7.39	8.89 ± 7.23
PLA	172.89 ± 2.57	40.76 ± 9.25

4. Conclusion

Physical-chemical characterisation of raw materials is a fundamental step in formulation sciences as it allows the understanding of their properties. In present work, at first, each material was characterised in term of chemical evaluation through FTIR and ATR-FTIR analysis aimed to identify a chemical group capable of bond formation. Data obtained showed that both drugs and polymers used present spectra compatible with literature findings and that drug Irn presents typical group able to donate electron for bond formation, as well as all sugary compounds used and polymer PHEA. Thermal analysis was conducted to evaluate the solid-state of each compound. Thermograph showed that each drug was used in the crystalline state. Such solid-state implies low solubility and high stability of the product. This finding is coherent with literature and with reported low solubility of each drug used in the present study. Form other hand side, polymers CAP, Inulin, MDX, PVA and SLP used were found to

be amorphous, while mannitol and PMMA were in the crystalline state. PLA was then proved to be a mixture of amorphous and crystalline.

The data acquired in this section allow solid dispersion identification in the next Chapters.

Chapter 2. Model study on spray-drying to improve solubility and permeability of poorly water-soluble drug

1. Background

In last decades pharmaceutical technologists have proved the suitability of solid dispersion manufacturing as formulation strategy for drug dosage forms (Marano et al.; 2016; 2017; Modica de Mohac et al.; 2016; Pina et al.; 2014). Spray-dried solid dispersions were widely studied in the last decades as an option to improve dissolution rate and in turn bioavailability of poorly water-soluble drugs such as one classified as BCS class II and IV. Spray-drying is considered as a suitable method to produce multi component solid dispersion, as described in the Introduction of this thesis. This is due to flexibility in the selection of solvents, range of temperatures, kind of liquid dispersions injectable and pumps speeds, as recently reviewed by Paudel and co-workers (in 2013) and Singh and Van den Mooter (in 2016) (Paudel; 2013; Singh and Van den Mooter; 2016). This technique is useful to obtain spherical particles whit small size and narrow distribution. Those characteristics are not only due to the spray-drying procedure itself but are also affected by polymers ratios and properties. Spray-drying influences final product characteristic by using different parameters such as inlet and outlet temperatures, pump velocity and aspiration percentage and solvent selection. Changing of these parameters was proved to modify final product properties improving particle size homogeneity, wettability, moisture content, and solubility, through reduction of particle size.

Due to crescent needs in clinical practice to use oral dosage forms to increase patient's compliance towards their medication this part of the thesis focused on the formulation of drug-loaded multi component solid dispersion with the aim of both increase drug solubility and permeability through the intestinal barrier. multi component solid dispersion was formulated by use of spray-drying where the effect of two parameters in obtained multi component solid dispersions was investigated: pumping efficacy, in term of amount of feed dispersion, loaded in the heating chamber and feed dispersion concentration. Irn was used as a model drug as it is classified as BCS class II (Slatter et al.; 2000). multi component solid dispersion was characterised in term of particle size, solid-state and in-vitro dissolution profile and ex-vivo permeation studies were conducted.

2. Materials and Methods

Irn, inulin from chicory, PMMA, CAP was purchased from Merck KGaA (Darmstadt, Germany). Ethanol, Dulbecco buffer phosphate (DPBS), chloride acid (HCl), potassium bromide (KBr), dimethyl

sulfoxide (DMSO), sodium chloride and sodium hydroxide were obtained from Merck KGaA (Darmstadt, Germany). Water used was Milli-Q ® (Millipore).

2.1 Sample preparation

Empty microparticle formulations were prepared by spray-drying with mannitol and inulin as based-polymer, with the addition of different ratio of PMMA and CAP. Polymers were dispersed in various solvents (water, basified water at pH 9.0 and 80:20 v/v water/ethanol mixture) and stirred magnetically overnight. Then, dispersions were homogenised using Microfluidics M-110P (Newton, Massachusetts, USA). Polymers dispersions (and consequently obtained microparticles) were named Polymers Dispersion (PD) and presented following composition: Mannitol/Inulin 90/10 (PD1); Mannitol/Inulin/CAP 85/10/5 (PD2); Mannitol/Inulin/PMMA 85/10/5 (PD3). Excipients concentrations were selected according to 'Handbook of Pharmaceutical Excipients' (Raymond and Paul; 2009). PDs were formulated at 1% and 10% w/v, to evaluate the effect of feed dispersion concentration of final microparticles. After homogenisation, liquid PDs were spray-dried through a BUCHI Mini Spray-Dryer B-290, with the co-current regime and a two-fluid nozzle atomiser, connected with an absorption air-dryer (Ultrapac; 2000). Inlet Temperature (T_{inl}) of 110°C and outlet Temperature (T_{out}) of 70°C and two different values of the pump, 10%, and 20%, were used.

For drug-loaded microparticles preparation, 1% w/v Irn was added to liquid PDs. Dispersions were stirred overnight by magnetic stirring and then homogenised using Microfluidics M-110P. Drug-polymer distributions were then spray-dried at inlet Temperature (T_i) of 110°C and T_{out} of 70°C with 20% of pump efficacy. Collected samples have then been stored under reduce humidity condition (30% RH).

2.2 Scanning electron microscopy (SEM)

SEM images were recorded on freshly prepared microparticles by using a Phenom ProXSEM**.** Particles were analysed using the same parameters (i.e. contrast and luminosity) and same stub for at least four samples. Average diameter (*d*) of microparticles was determined from the mean value of 100 measurements using ImageJ (the USA, version 1.46 v). From diameter measurements have then been possible to evaluate polydispersity index (PDI) by formula (Gaumet et al.; 2008):

$$(PDI) = \langle d^2 \rangle / \langle d \rangle^2 \qquad \text{Equation 7}$$

2.3 FTIR analysis

FTIR studies carried out to identify any molecular interaction between Irn and polymers. Samples were prepared by compression of a thin circular tablet using as 1:99 drug: KBr ratio. Samples were placed on the holder, and nitrogen gas was used to reduce carbon dioxide peak and spectra were collected using a JASCO FTIR-6000 spectrometer, from 4000 to 300 cm^{-1} with 128 scans (same as background) at the resolution of 2 cm^{-1} for each sample. Experiments were carried out in triplicate.

2.4 DSC and TGA

DSC analysis was performed using a LABSYS Evo STA (simultaneous thermal analysis) TGA-DSC, at heating rates of 7°C/min between 30°C and 500°C and alumina crucibles were used in all experiments. Nitrogen purge gas was used with a flow rate of 5 mL/min. TGA studies were carried in triplicates out with calculation of water content of prepared microparticles.

2.5 Drug loading evaluation

Proper amount (2 mg) of microparticle samples were dissolved in 2 mL of DMSO, filtered through a 0.45 mm syringe filters with cellulose acetate membrane (VWR International, USA) and drug content of each sample was determined via UV-VIS spectra. Spectra were recorded by 2401 PC Shimadzu Recording UV Spectrophotometer, in 600-200 nm spectral range. A calibration curve was used for quantification of drug content in the concentration range of 0.1-0.0001 mg/ml of Irn standard solutions in DMSO (R=0.999) and spectra recorded at 254 nm.

2.6 Permeation studies

Colon sections were obtained from healthy male Winstar rats (300−350 g body weight). All animal tissues were collected by Istituto Zooprofilattico Della Sicilia 'A. Mirri' (Palermo, Italy) according to protocols approved by the National Bioethical Committee. Rats were sacrificed previous anaesthesia by intraperitoneal administration of ketamine (100 mg/kg). Then colon segments were collected immediately after animal sacrifice, washed twice with normal saline, and directly used for permeation experiments. Appropriate sections of the colon were mounted in vertical jacketed Franz type diffusion cells (5mL acceptor volume) with 5mm diameter orifice (0.20 cm^2 area). Tissue specimen was equilibrated initially at 37±0.1°C between donor and acceptor chambers both filled with DPBS

solution at pH 6.80. Afterwards, DPBS solution was carefully removed from the donor compartment and replaced with drug-loaded microparticles dispersion in DPBS pH 6.80 (1 ml; drugs concentration 0.20 mg/ml). At scheduled time intervals, aliquots (200 µl) were withdrawn from the acceptor chamber and immediately replaced with fresh buffer solution to maintain sink conditions. Subsequently, the filtrate was analysed spectrophotometrically at 254 nm, and Irn quantised using a calibration curve above described. Each experiment was carried out in triplicate at 37±0.1°C for eighth under continuous agitation in an orbital shaker. An identical procedure was used to evaluate drug colon permeation by using free drug dispersions (Irn 0.20 mg/mL). Results are expressed in term of percentage of drug permeated as a function of time.

At the end of each *ex vivo* permeation experiment, the amount of drug entrapped into colon tissue was quantified by organic solvent extraction. Colon sections were removed from Franz cells and washed with DPBS at pH 6.80 to remove eventual drug remained on its surface. Subsequently, colon specimens were stirred in 2 mL of organic solvent DMSO overnight at 37°C. Extraction liquor was transferred into a flask and analysed spectrophotometrically at 254 nm.

2.7 In-vitro Irn dissolution and release studies

In-vitro dissolution studies were carried out by using the basket method, in gastrointestinal mimicking condition at pH values according to European Pharmacopeia (European Pharmacopeia 7.6; 2012). the gastric environment was mimed by 70mL of a pH of 1.2 acid solution for 2 hours, then 30 mL of DPBS was added to reach a pH of 6.8. During the experiment, a rotation speed of 100 rpm and a temperature of 37.0±1 °C was maintained. At predetermined intervals, 1 mL of solution (0.20 mg/mL) was withdrawn and filtered through a 0.45 µm cellulose acetate syringe filter and replaced with the same amount of fresh buffer. Subsequently, the filtrate was analysed spectrophotometrically at 254 nm. All in-vitro dissolution studies were performed under sink conditions. The calibration curve was conducted in the concentration range of 0.1-0.0001 mg/ml of Irn standard solutions in both acid solution and DPBS pH 6.80 (R=0.999) and spectra recorded at 254 nm.

2.8 Statistical Analysis

A one-way analysis of variance (ANOVA) was applied to compare different samples. Data were considered statistically significant with a value of p below 0.05, and differences between groups were compared using Bonferroni t-test. Each test was developed in triplicate.

3. Results and Discussion

3.1 Preparation and characterisation of multi component solid dispersions: external solid-state and water content

For the preparation of multi component solid dispersions, attention was focused on optimising spray-drying methodology to obtain spherical particles with an average particle size between 0 to 6 µm. It was proved by Barltorp and co-workers (1979) that particles within this diameter values might be better absorbed and retained by tissue (Barltrop and Meek; 2013). In particular, it was proved that microparticle with 3 µm diameter is detained in the mucosa (Jenkins et al.; 1994; Reineke et al.; 2013).

At first, feed dispersions concentration and pump efficacy were analysed in the spray-drying process. Two different feed concentrations (1 and 10%) and for each, two different pumping rates (10 and 20%), were used for producing microparticle samples. As a result, twelve PDs as multi component solid dispersion were formulated via spray-drying. PD used for consequent microparticles production by spray-drying technique, were numbered from 1 to 3 as summarised in Table 8. particles were then named according to parameters used during the spraying process, as in Table 9.

Table 8. Summary of the composition of polymer dispersions and solvents ratios used.

	Name Polymers	Polymers ratios w/w	Solvents ratios v/v
PD1	Mannitol/Inulin	90:10	Water
PD2	Mannitol/Inulin/CAP	85:10:5	Basic Water
PD3	Mannitol/Inulin/PMMA	85:10:5	90:10 Water: Ethanol

Table 9. Microparticle names after the spray-drying process. Particles -A were obtained using 1% of feed solution concentration and 10% pump efficacy; B were obtained using 1% of feed solution concentration and 20% pump efficacy; -C were obtained using 10% of feed solution concentration and 10% pump efficacy; - D were obtained using 10% of feed solution concentration and 20% pump efficacy.

Name	Feed Concentration	Pump Efficacy
PD1-A	1%	10%
PD1-B	1%	20%
PD1-C	10%	10%
PD1-D	10%	20%
PD2-A	1%	10%
PD2-B	1%	20%

PD2-C	10%	10%
PD2-D	10%	20%
PD3-A	1%	10%
PD3-B	1%	20%
PD3-C	10%	10%
PD3-D	10%	20%

The obtained microparticle samples were collected to be characterised in terms of particle size and PDI, which were evaluated by SEM and analysed by ImageJ software allowing calculation of PDI values. Figure 28 summarises mean average particle size of PDs and related values of PDI. As was previously stated that the particle size of below 6 µm achieves the best absorption in the intestine, the authors decided to study the effect of feed dispersion concentrations on particles diameters. Ability to control particle size and density, even after formulation, is the main advantage of spray-drying as it was proven form authors in the field of pulmonary delivery (Items et al.; 2018). In particular, particles obtained from polymers dispersions with a 1% w/v concentration PD_{1-3}-A present a particle size lower than 3 µm and PD_{1-3}-B lower than 4 µm resulted in being smaller than one obtained with 10% w/v concentration. Overall particles received when preparation a solution at 10% w/v have a diameter 1.80 fold (p-value <0.0001), although in current literature is reported that higher feed dispersion concentration leads to smaller particle size (Hashib et al.; 2015). However, when the solvent represents the majority in dispersion, it has a low density that is reported to cause the formation of a particle with a small diameter (Ekdahl et al.; 2019). Particle -C and -D presented a size around 6 µm, therefore considered of use to mucosa permeation.

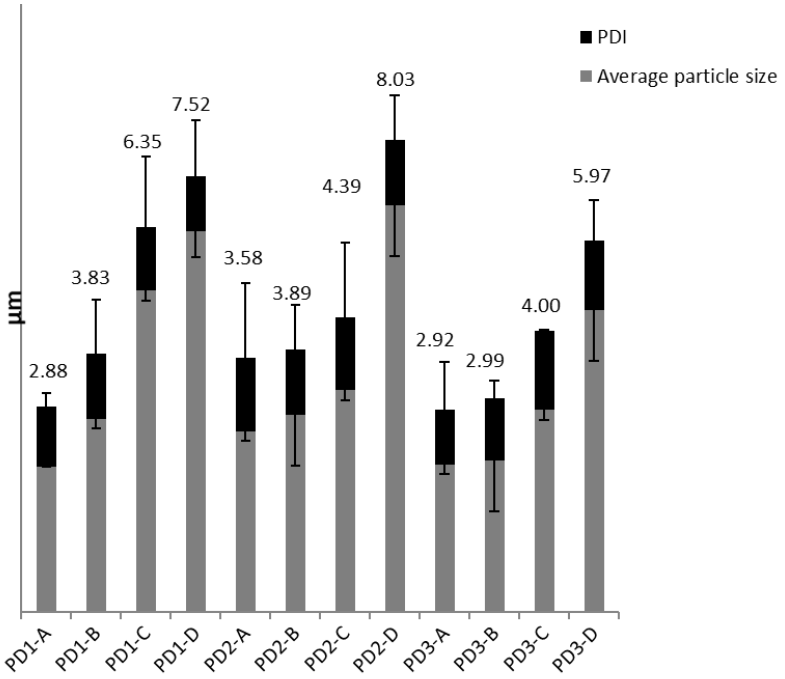


Figure 28. Graphical summary of particle size and PDI of each multi component solid dispersion. Data showed that particles obtained with 10% w/v feed dispersion concentration have higher (around 6 µm) respect than one produced with 1% w/v (about 3 µm).

However, it is well-known that an increase of feed dispersion viscosity, as happened by increasing feed dispersion concentration, affect the solvent evaporation process, leading to obtaining particle with high density and low diameter. Moreover, the effect on pump efficacy on particles humidity was evaluated. As previously stated, the peristaltic pump percentage affects the difference between T_{inl} and $T_{out.}$ So, the water content of the obtained microparticles was investigated using TGA; results showed in Table 10. Data showed that particle -D has a lower water content respect to other empty multi component solid dispersions, due to the use of ethanol in the solvent mixture. EtOH presents fast evaporation in spray-drying due to its low boiling point, and it is reported that adding ethanol a solvent in the spray-drying process reduces the water content of the produced product (Gilani et al.; 2005).

Table 10. Water content obtained by TGA analysis.

The water content of each PDs obtained by TGA					
PD1-A	PD1-B	PD2-A	PD2-B	PD3-A	PD3-B
2.50 % ± 0.30	3.22% ± 1.22	2.42% ± 0.60	1.98% ± 3.50	2.20% ± 0.10	3.56% ± 2.36
PD1-C	PD1-D	PD2-C	PD2-D	PD3-C	PD3-D
1.95% ± 0.65	1.80% ± 1.56	2.16% ± 3.75	0.74% ± 0.30	1.50% ± 3.65	0.86% ± 2.56

The production of multi component solid dispersion was obtained using spray-drying to formulate particles with a mean diameter below 6 µm. The particle was characterised to detect their particle size, homogeneity in distribution (through PDI values calculation) and water content. Such an investigation could suggest that the use of more concentrated feed dispersion and higher pump efficacy, might allow obtaining particle with bigger particles size and less water content. Therefore, multi component solid dispersions PD1-D, PD2-D, and PD3-D were used for the successive step of drug loading, as was noticed that higher pumping efficacy is the lowest water content is detained. A relation between particle size and pump efficacy percentage was also seen, regarding the fact that in most produced multi component solid dispersions a pump efficacy of about 20% increased particle size. This founding is coherent with literature insights that explained that water content depends on how much time the sample has spent in atomiser (Maa et al.; 1997; Sweeney; 1998).

3.2 Preparation and characterisation of Irn-loaded multi component solid dispersions: external solid-state and water content

For the preparation of drug-loaded microparticles, three PDs formulation (PD1-D, PD2-D, and PD3-D) obtained with same spray-drying conditions (D), was selected to prepare final drug-loaded formulations because of those with a size between 4 and 6 µm, with lowest residual water content. Drug loaded formulations (F) were named: F1 from PD1-D+Irn; F2 from PD3-D+Irn and F3 from PD2-D+Irn.

SEM images of F1, F2, and F3, were acquired to measure the mean particle size of multi component solid dispersion, and results are provided and summarised in Figure 29. It is interesting to notice that the particle size of multi component solid dispersions containing Irn resulted smaller than empty correspondent formulation. F1 was 1.51-fold (p-value <0.001) lower than PD1-D, as well as F3, has a particle size of 1.60-fold (p-value <0.001) inferior of its correspondent not loaded MDS. While F2 and PD2-D had similar particle size and no significance was found. Reduction in particle size could be due to alteration in density and viscosity of feed dispersion once Irn is added to the formulation (Gilani; 2005). However, such properties were not characterised.

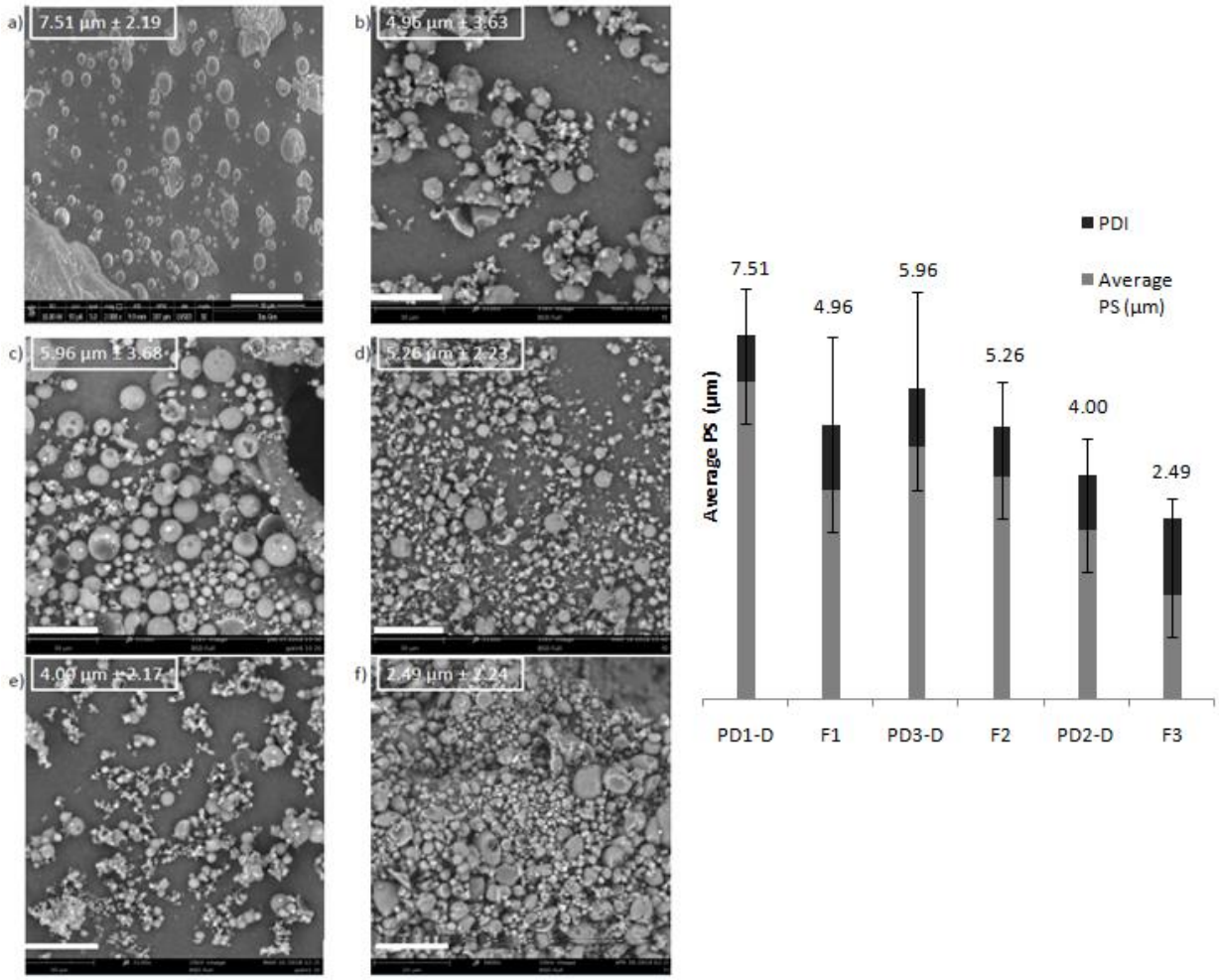


Figure 29. Left panel: SEM images of empty (PD1-D, a; PD2-D, c; PD3-D, e) and drug loading (F1, b; F2, d; F3, f) multi component solid dispersions. Right panel: Graphic comparing obtained mean average particle size with relevant standard deviation values expressed by errors.

Then, the physical characteristics of loaded multi component solid dispersion were evaluated in term of drug-excipients interaction, drug solid-state, and water content.

Overall DSC analysis of drug loading multi component solid dispersions identified one main thermal event related to mannitol T_m. Such an event is coherent with raw material characterisation even if in F3 T_m moved to a slightly lower temperature value. In Figure 30, DSC thermograms and zoom of area related to Irn T_m are shown.

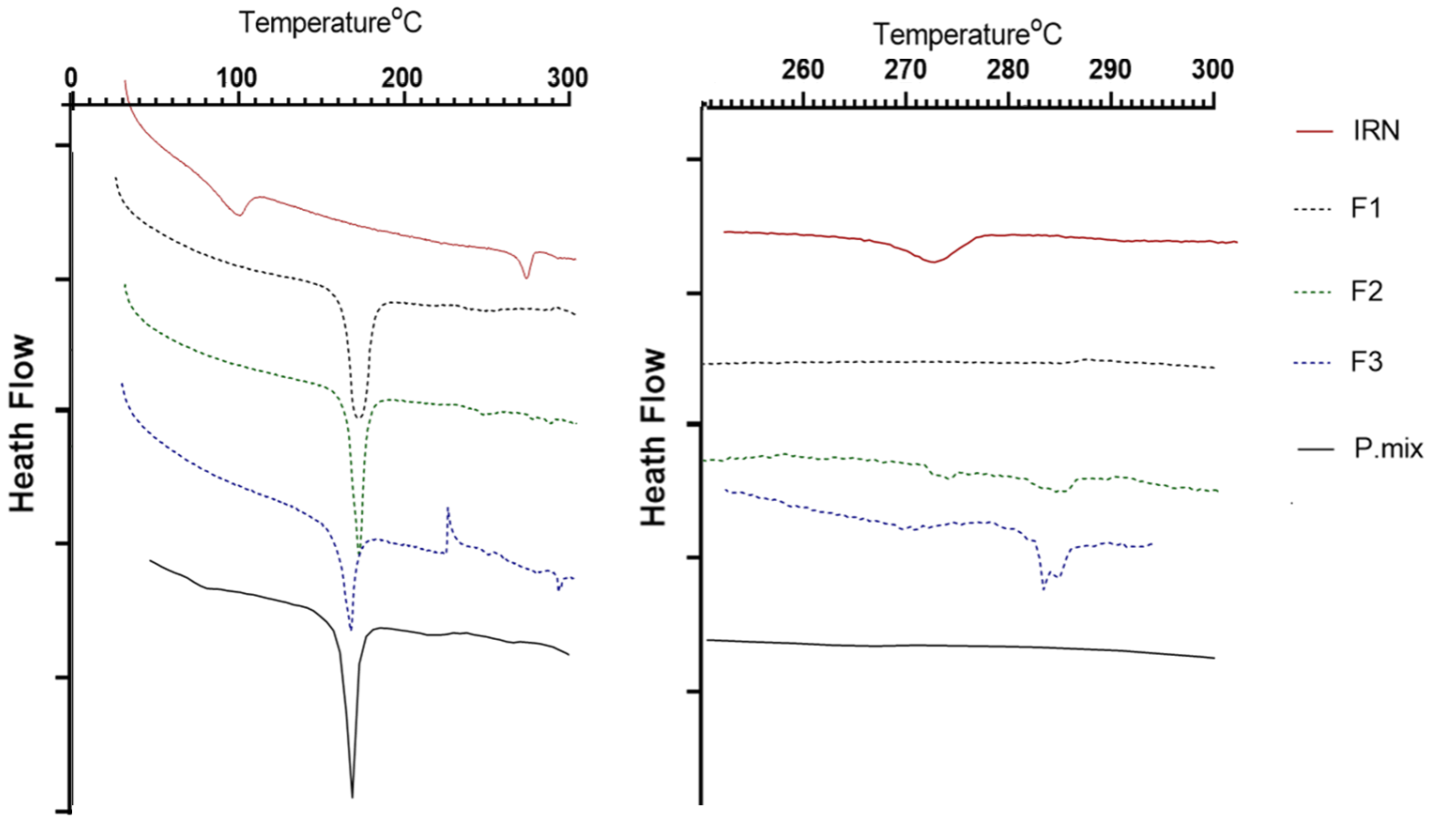


Figure 30. a) Comparison of multi component solid dispersion formulations and pure drug thermograms. From above: F1, physical mixture (PM) of F1, F2, F3, and Irn. b) Zoom of the region from 250°C and 300°C.

From the other hand, identification of drug T_m resulted almost complicated due to low drug loading in all formulations (~ 1%). DSC curve of Irn was reported as well to discuss obtained results easy. As shown in the zoomed region (Figure 30b), for formulations F2 and F3, it was possible to detect endothermic events associated with Irn T_m. A confirmation of this was obtained evaluating Δ_{cp} of pure drug T_m and comparing it with values obtained from F2 and F3. In particular, Irn T_m and Δ_{cp} were respectively about 270°C and 49 J/g while for F2, and F3 values were respectively about 273°C and 1.2 J/g and 276°C and 1.3 J/g. That evidence appeared to happen closer to pure drug T_m and Δ_{cp} values and resulted in being coherent with the amount of drug-loaded in F2 and F3. However, for F1, it was challenging to find an event well associated with Irn endothermic peaks. To be sure that fact highlighted for F1 was related to Irn T_m, PM with polymers presented in F1 and with 10% of drug loading was analysed as a control. PM thermogram showed an endothermic peak at 265°C, value at which small event in F1 occurred, with a Δ_{cp} of 4.7 J/g. value is coherent with drug percentage in PM and helps to ensure drug identification in F1 thermogram. TGA studies were involved in evaluating water content changing after drug loading. Results, shown in Figure 31, demonstrated that drug addition to polymers dispersion caused an increase in humidity of 1.74-fold for F2 (p-value < 0.0001) and of 2.75-fold F3 (p-value < 0.0001), but unchanged for F1. Increasing water content could be explained since Irn could detain water, as suggested by Hermann and Robert in 1972 (Hermann; 1972).

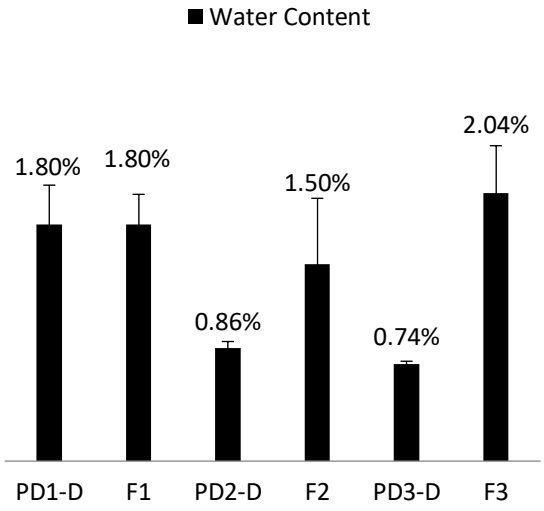


Figure 31. water content of multi component solid dispersion before and after drug loading

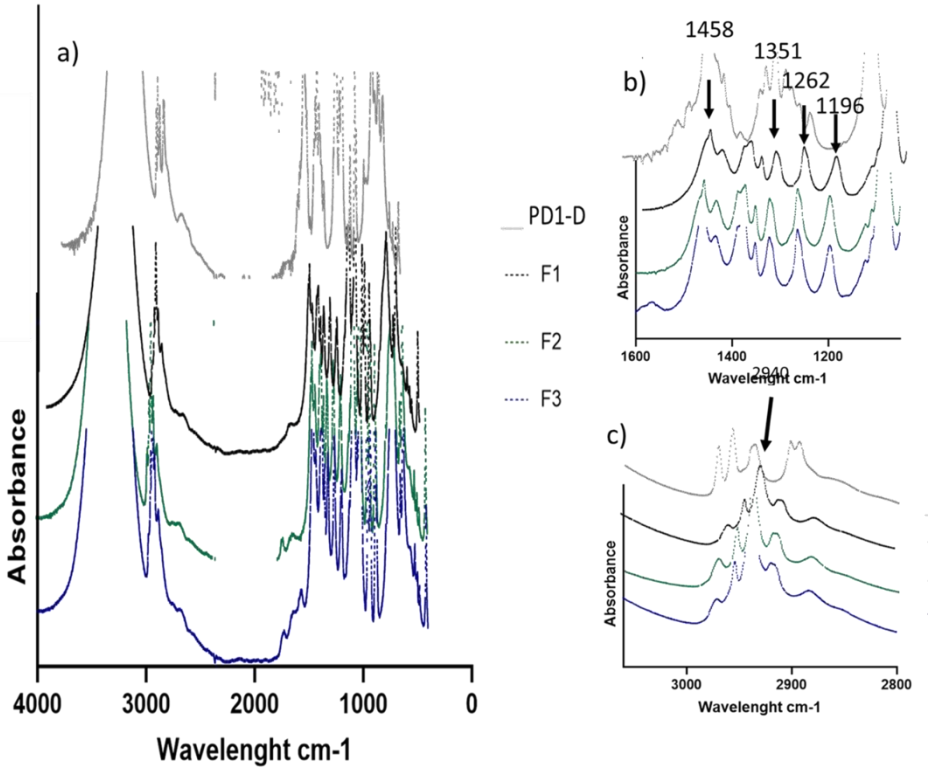


Figure 32. a) FTIR spectra from above of PD1-D, F3, F2, F1. b) FTIR spectra zoom from 1595 cm-1 to 1047 cm-1 of F3, F2, F1. c) FTIR spectra zoom from 3061 cm-1 to 2773 cm-1 of F3, F2, F1.

FTIR spectra were analysed to evaluate the existence of any interactions occurring between drug and polymers used in each formulation. To a better understanding of obtained data, FTIR spectra of drug-loaded multi component solid dispersions (F1, F3, and F3) were compared with empty one (PD1-D), as it mannitol and inulin. In Figure 32, from left, a comparison of FTIR spectra of Irn, F1, F2, F3, and PD1-D is illustrated. It is possible to notice a difference between all formulations and frequency of PD1-D (see regions highlighted in circles and zoomed-in Figure 32b and 32c). In Figure 32b, it is possible to notice three peaks that are not present in PD1-D, and that comes out in all formulations containing Irn. Those peaks at 1321, 1262 and 1196 cm^{-1} were assigned to Irn aromatic groups and come out from signals overlap of inulin peak at 1327 cm^{-1} and mannitol CH deformation at 1257 cm^{-1}.

Moreover, it was possible to notice a peak enlargement at 1458 cm^{-1} of F2; this could be due to Irn peak at 1452 cm^{-1}. Another central peak in all formulation that is not present in PD1-D is one at 2940 cm^{-1} (Figure 32c) assigned to Irn CH2 symmetric stretching vibration. However, this peak appears to be of different intensity and shape. So it was related to a possible bond created between donor groups of mannitol and acceptor group of Irn (Thomas et al.; 2004).

3.3 multi component solid dispersions drug loading and dissolution profiles

The drug loading studies were performed dissolving all formulations in DMSO and evaluating drug absorbance at 254 nm. Table 11 summarised all drug loading contents that resulted in being all-around 1% which corresponds to a 100% encapsulation efficiency.

Table 11. Drug loading of each multi component solid dispersion formulations.

Formulations	*Drug Loading*
F1	0.93% ± 0.56
F2	1.11% ± 2.31
F3	1.10% ± 1.65

The evaluation of the dissolution profile of loaded-multi component solid dispersions allowed assessment of the potential of use spray-drying to enhance poorly water-soluble drug dissolution rate, compared with the unformulated drug. Figure 33 shows dissolution profiles of F1, F2, and F3 compared with pure Irn within 10h (Figure 33a), and an overview of first 120 min of the experiment conducted in the gastric-mimicking environment (Figure 33b). F1 increase Irn dissolution profile with a potential increase on its gastric permeation, and it was not considered capable of reaching purposed aim, as 60% of the drug was released in an acid environment. A different profile can be observed for F2 and F3. F2's profile had an initial fast drug release at acid pH (within 2h) with a maximum of drug release percentage reached of about 80%. Therefore, it was not considered as suitable with the purpose of protecting Irn release from the gastric environment. However, F3 was able to reduce drug release in the gastric environment and allowed to release 100% of Irn when pH was jumped, accordingly to pharmacopoeia suggestion, to intestinal pH. As the main aim was to increase Irn dissolution rate in the intestinal environment and then its permeation in the colon, dissolution studies were conducted reproducing gastrointestinal environment according to European pharmacopoeia to evaluate gastro-protective efficacy of PMMA and CAP. From F1 and F2 were possible to notice an initial fast drug release at acid pH (within 2h) with a maximum of drug release percentage reached of about 80% and therefore it was not considered as suitable with the purpose of protecting Irn release from the gastric environment. Such behaviour was not noticed, from another hand side, from F3, which exhibited a shallow dissolution behaviour in the gastric environment. At the same time, at pH 6.8, it was able to release a maximum of drug percentage of about 100%. This behaviour could be useful to increase selective drug delivery to the colon, where colon cancer is located. At this regard, inulin should confer a synergistic action as colon-targeting excipient when associated with CAP as enteric disregard of microparticles.

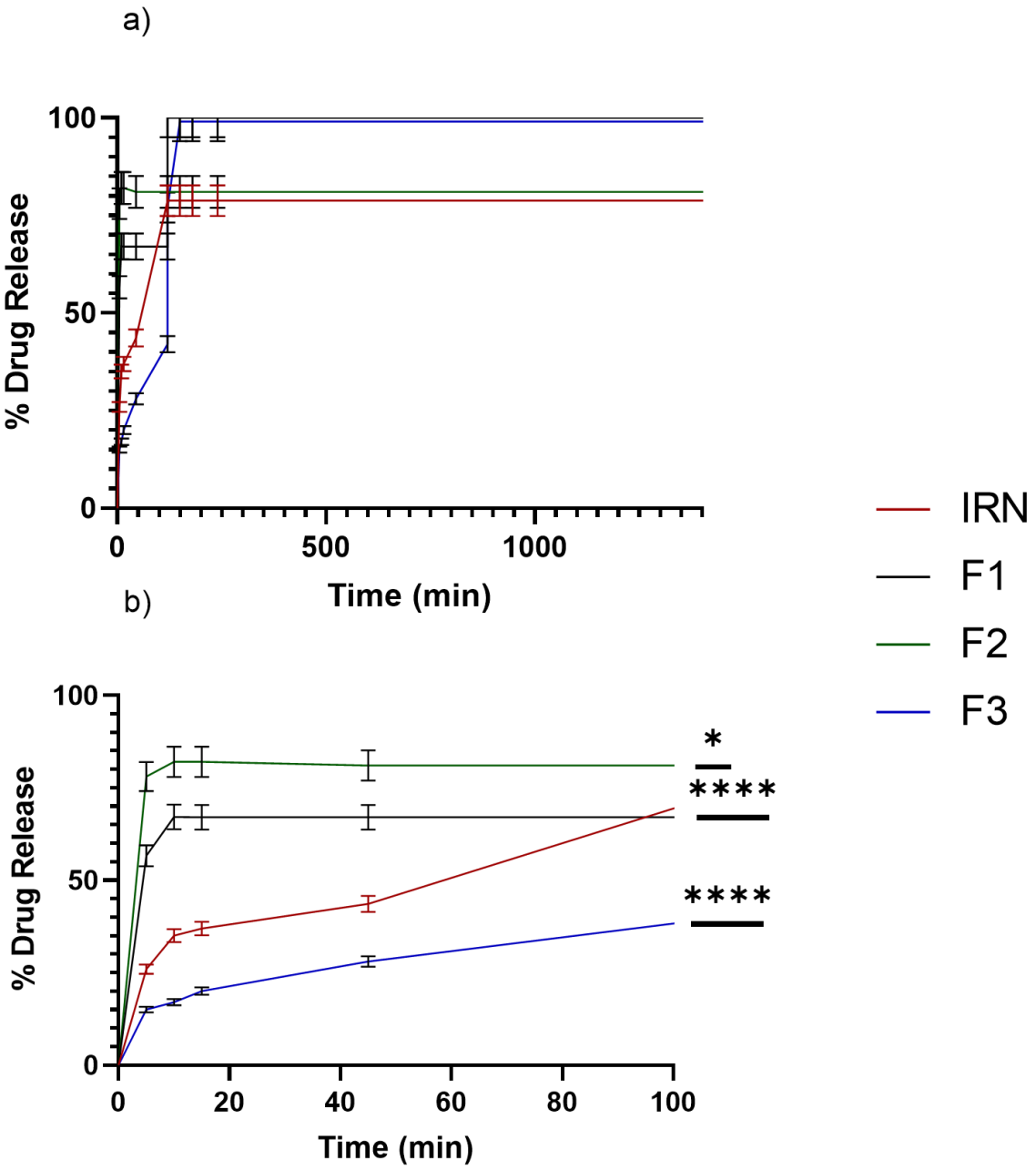


Figure 33. Drug release percentage: a) of F1, F2 and F3 compared with pure Irn within 10h. b) Zoom on the first 120 min of the experiment in the gastric-mimicking environment

Ex-vivo studies (summarised in Figure 34), demonstrated the real capability of prepared multi component solid dispersions to increase drug permeation and absorption through colon specimens.

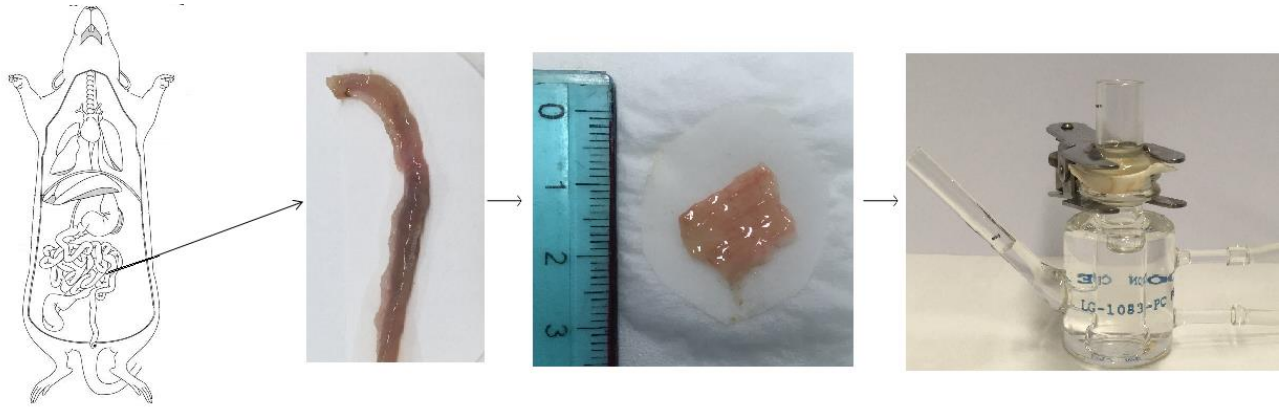


Figure 34. Experimental scheme of ex-vivo permeation studies.

Interestingly, each obtained formulation showed a significant increase in drug permeation across physiological tissue. As shown in Figure 35, the percentage of permeated drug always increases when formulated as multi component solid dispersion, up to experiment time (10hs). Furthermore, the fact that Irn was not found in colon specimens suggests that permeation could have continued until completion. It cannot be excluded that improvement of drug absorption generated by three formulations might be associated with the development of Irn solubility because of the presence of mannitol that acts as solubilising agent and diluent. As mannitol possesses high solubility, it increased Irn solubility in the donor compartment allowing a higher amount of drug molecules readily available for permeating across colon specimens. Interestingly F2 achieved the best result allowing 50% of drug permeation after just 300 min. presence of pH-dependent PMMA could show an enhancer effect attributed to mucoadhesive properties of this copolymer that could interact with mucus glycoproteins by forming physical interactions resulting in the formation of a strengthened mucus gel network, able to promote drug diffusion (Cui et al.; 2006).

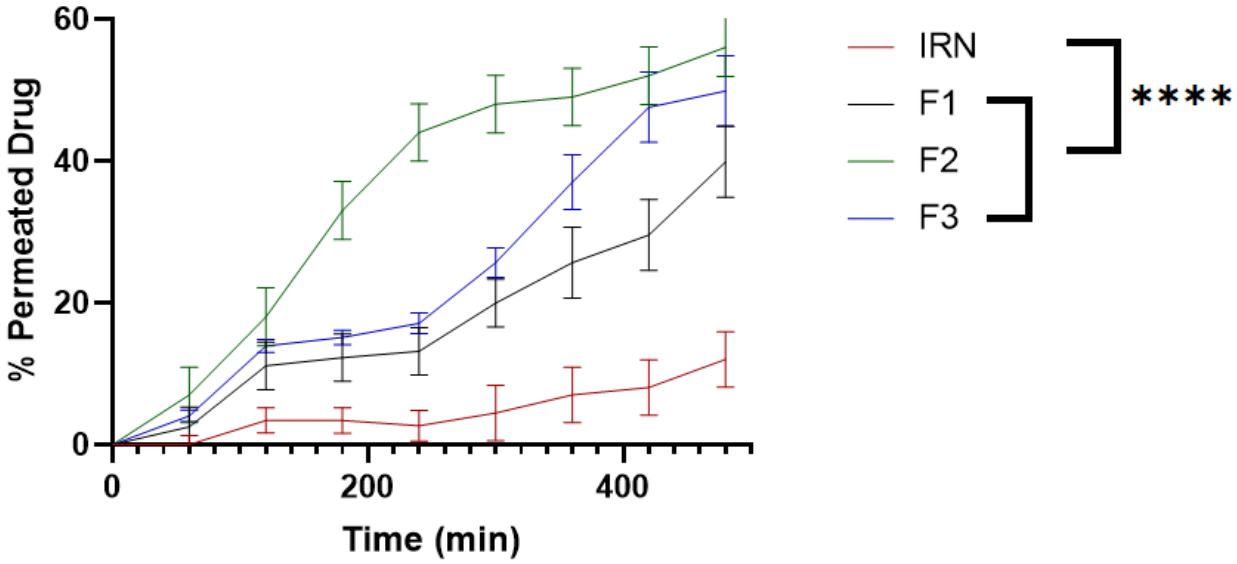


Figure 35.Percentage of permeated drug trough colon specimens.

4. Conclusion

This part of the project detected the use of spray-drying as a formulation strategy to produce multi component solid dispersions able to increase solubility and permeability of poorly-soluble drug. Irn was used as a model drug due to its low solubility and permeability. Due to advantages described in Introduction, multi component solid dispersions were prepared empty and characterised to ensure selection of best final products. Twelve empty particles were formed via spray-drying, and mannitol and inulin were used as based-polymers in each preparation. Particle size and water content analysis showed that particles obtained with 10% w/v feed dispersion concentration and 20% spray-drying pump efficacy generated multi component solid dispersions in range of 4 and 6 µm and low water content. Therefore, those particles were loaded with the model drug. By varying two formulation parameters, improvement of Irn dissolution and release profile in gastrointestinal mimicking conditions was demonstrated. Obtained results confirmed that an increase of feed dispersion concentration produces an increase of particle size and that pump efficacy has, in most cases, an equal effect.

The dissolution profile of each formulation has shown an increase of drug release percentage in comparison to pure drug and *ex-vivo* permeation experiments demonstrated a significant enhancement in drug permeation through colon specimens respect unformulated drug. Whole obtained results suggested a potential use of spray-drying to increase in oral absorption of the poorly water-soluble drug. However, spray-drying is not only formulation strategies to improve the solubility

of poorly soluble drugs. Therefore, further investigation of its advantages compared with other leading methods is highly recommended to identify the most suitable for solid dispersion manufacturing.

Chapter 3. Comparison of Traditional Processes in Enhancement of Drug Dissolution Rate.

1. Background

All drug delivery systems are composed of two components, drug that produces physiological effects, and excipients, that allow drug administration and distribution within the physiological environment. Drugs with low solubility represent a significant challenge in drug-delivery, and several pre-formulation strategies are usually employed to overcome this issue (Campardelli et al.; 2017; Samie et al.; 2017). In particular, a most common strategy is based on the formulation of solid dispersions (Marano; 2016; 2017; Pina; 2014) and its preparation can be categorised into a general grouping based on equipment or strategies used (Lu et al.; 2017). Advantages and disadvantages of each formulation strategies were discussed in the Introduction section of the present thesis. All techniques are used to produce solid dosage forms in a big scale from pharma companies. In Chapter 2, it was proved spray-drying efficacy in both increasing dissolution and permeability of a poorly water soluble drug. However, just a few studies compared this technique with the other available and none more than two simultaneously. One of the purposes of this thesis was to analyse and compare the most common formulation methods to understands their weakness and strength when formulation solid dosage forms.

Therefore, this thesis section aims to compare spray-drying, freeze-drying and solvent-casting method, as they represent the most common formulation strategies used to prepare drug-loaded solid dispersions. Pharmaceutical advantages proposed by these techniques were investigated, with focus placed on faster drug release profiles, improve surface homogeneity, increase drug incorporation capability of three different model drugs: Olz, Dsm and Trm. These drugs, classified as BCS class II, were selected due to their low solubility and their widespread use in medical practice (Clinical; 2019; Fuentes et al.; 2018).

2. Materials and Methods

Olz, Dsm, Trm, MDX, PVA, Ethanol, phosphate buffer saline (PBS) tablets, hydrochloric acid, KBr, DMSO, sodium chloride and sodium hydroxide and were purchased from Merck KGaA (Darmstadt, Germany). Water used was produced using Milli-Q ® system (Millipore).

2. 1 Sample preparation

A 10% w/v solution of PVA:MDX (50:50 weight ratio) mixture was prepared. polymers were added to 20 mL of water and stirred overnight and then heated for 40 minutes in an ultrasonic water bath. Water was used as solvent-based due to its suitability for selected preparation techniques. Polymer solutions were processed as follows: 5 mL was placed into a Petri dish (5 cm diameter) and placed under a laminar flow of 0.45 mL/min for 24 hours, to allow for water evaporation. Another 5 mL of solution was freeze-dried using 30 °C as a shelf temperature. A further 7 mL of solution was spray-dried (at T_{inl} of 120 °C, 20% pump and 100% aspiration).

Drug loaded solid dispersions were obtained by adding each pure drug to the polymer solution, followed by evaporation, freeze-drying or spray-drying procedures. A small amount of each polymer solution (containing drug) was withdrawn and tested to evaluate the polymer's influence on drug solubility.

2.2 SEM

SEM images were obtained for each freshly prepared sample to investigate their surface homogeneity, solid-state, and size, using a Phenom ProXSEM. Each sample was deposited onto a carbon-coated steel stub and dried under vacuum (0.1 Torr) before analysis.

2.3 FTIR

Samples were prepared by compression of thin circular tablet using as 1:99 drug: KBr ratio. After samples were placed on the holder, nitrogen gas was used to reduce carbon dioxide peak. Spectra were collected using a JASCO FTIR-6000 spectrometer, from 2θ 4000 to 300 cm^{-1} with 128 scans (same as background) at a resolution of 2 cm^{-1}. Spectra were recorded in triplicate.

2.4 DSC and TGA

DSC studies were performed, and measurements taken with n=3 repetitions, using a LABSYS TGA-DSC and heating rates of 7 °C/min. Experiments were performed between 30 °C and 500 °C, with alumina crucibles used in all tests conducted in triplicate. Nitrogen purge gas was used with a flow rate of 5 mL/min. TGA studies were carried out to measure the water content of prepared samples using the same instruments.

2.5 Determination of drug solubility equilibrium in polymer solutions

The aqueous solubility of each drug was investigated in the presence of PVA and MDX. Excess amounts of pure drug powders were dispersed in aqueous polymer solutions for 72 h and stirred using a shaking incubator (150 rpm, 37 °C). polymer solutions (10% w/v of the drug in a polymer mixture of PVA: MDX 50:50 weight ratio) were then filtered through a 0.22 mm Millex-GP filter (Merck Millipore, UK) and their concentrations determined spectrophotometrically using calibration curves for each drug (concentration range of 0.1–0.0001 mg/mL in drug standard water solutions (R = 0.999). Ultraviolet (UV) spectra were recorded on a 2401 PC Shimadzu Recording Spectrophotometer in 600–200 nm spectral range. Spectra were recorded at 253 nm for Olz, 242 nm for Dsm and 241 nm for Trm.

2.6 Drug-loading evaluation

Drug-loading of prepared formulations were determined after weighing 2 mg of each sample was dissolved in DMSO for optimum solubilisation of selected drugs. Dispersions were agitated on an orbital shaker for three days at room temperature and covered with aluminium foil to avoid light interaction. Following this, 1 mL of solution was withdrawn and filtered using a 0.45 mm syringe filter with a cellulose acetate membrane (VWR International, USA). Drug loading of each sample was determined spectrophotometrically. Drug loading of each sample was determined spectrophotometrically, as aforementioned, with n=6.

2.7 In-vitro release studies

In-vitro drug release studies were carried out in triplicate using basket apparatus method. European Pharmacopeia (European Pharmacopeia 7.6; 2012) was used to determine experimental conditions, such that they would mimic those found within intestine *in-vivo*. DPBS at pH 6.8 was used to achieve the desired intestinal pH. Each formulation was added to 100 mL of dissolution medium (20 mg to 100 mL) and stirred (100 rpm, 37 ± 1 °C). This drug concentration represents a conventional dose of each drug, given at the beginning of a therapeutic regimen. At predetermined time intervals, 1 mL of solution was withdrawn and filtered through a 0.45 µm cellulose acetate syringe filter and replaced with the same amount of fresh buffer. Subsequently, the filtrate was analysed spectrophotometrically at a specific wavelength for each drug and the amount of drug present

calculated using a calibration curve. Drug release of each sample was determined spectrophotometrically, as aforementioned.

2.8. Statistical Analysis

The *f2 E*quation was used to compare dissolution performance between drugs, as well as corresponding formulations (Raimi-Abraham et al.; 2015). This similarity factor is calculated using the following Equation (Shah et al.; 1997):

$$f2 = 50*\log\{[1+ (1/n)\sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} 100\} \quad \text{Equation 8}$$

Where n is the number of time points, R is dissolution value of reference t batch at time t, and T is dissolution value of test batch t at time t.

When *f2* is below 50, profiles are considered different, and when *f2* is above 50, compared release profiles are considered equivalent. Two-way analysis of variance (ANOVA) was applied to compare different samples. Data were considered statistically significant with a value of p below 0.05. Each test was conducted in triplicate.

3. Results and Discussion

3.1 Effect of polymeric excipients on drug solubility

Three different methods to produce solid dispersions were investigated by using three different model drugs as showed in Table 12. For clarity throughout the text, A= OLZ, B=DSM, C=TRM, 1=spray-drying, 2=solvent-casting and 3=freeze-drying. To evaluate which of preparation methods used (spray-drying, solvent-casting, and freeze-drying) improved drug dissolution rate in an aqueous environment, some parameters such as drug particle size, drug-loading capacity and surface homogeneity were evaluated.

Firstly, the effect of polymers (PVA and MDX with a weight ratio of 50:50) on drug solubility was evaluated to predict drug behaviour in the physiological environment. Improvements of more than 100% (p-value <0.0001) in water solubility were noticed for each drug, as shown in Table 13. PVA and MDX are well-known solubility enhancers and used widely for this scope formulation of dosage forms of poorly-water soluble drugs (Ganesan et al.; 2015; Sahoo et al.; 2009). Particularly, MDX has a ratio dependant behaviour when in polymer combination at more than 20% w/w (Oktavia et al.; 2020). Polymers used in this work used to decrease the surface tension of aqueous solutions or for

adsorption onto colloids with steric interactions (Dong and Johnson; 2003). Such interactions could be repulsive, with a stabilising effect on the system, or otherwise attractive, increasing solubility of adsorbed molecules (Agama-Acevedo and Bello-Perez; 2017).

Table 12. Summary of prepared solid dispersion formulations and their composition.

Sample Name	Drug	Methods	Composition
A1	Olz	Spray-drying	All formulations contain a 10% w/w drug loading and polymeric matrix formed by PVA: MDX with a weight ratio of 50:50.
A2	Olz	Solvent-casting	
A3	Olz	Freeze-drying	
B1	Dsm	Spray-drying	
B2	Dsm	Solvent-casting	
B3	Dsm	Freeze-drying	
C1	Trm	Spray-drying	
C2	Trm	Solvent-casting	
C3	Trm	Freeze-drying	

Table 13. Drug solubility expressed as g/mL in water and polymer solutions (PVA and MDX with a weight ratio of 50:50).

	Drug solubility in Water g/mL	Drug solubility in polymers solution g/mL
Olz	0.0023 ± 1.35	0.09 ± 0.02
Dsm	0.0008 ± 0.86	0.45 ± 1.72
Trm	0.0013 ± 0.35	0.19 ± 3.42

3.2 Characterisation of solid dispersions produced

SEM was used to evaluate surface homogeneity and solid-state of each sample. Shape and surface solid-state of solid dispersion samples prepared by spray-drying were reproducible for all drug molecules as the same polymer-based composition was used for drug loading. However, the size of these spray-dried microparticles was not found to be homogeneous, with diameters ranging from 7 to 30 µm (see SEM images A1, B1 and C1 of Figure 36). Freeze-drying samples (see SEM images A3, B3 and C3 of Figure 36) reproduced surface solid-state, with pores of ± 1 µm. Conversely, surface analysis of pharmaceutical films obtained using the solvent-casting method (see SEM images A2, B2 and C2 of Figure 36), showed the presence of spherical drug aggregates, differing in abundance and diameter for each loaded drug. Films containing Dsm (image B2 of Figure 36) and Trm (image C2 of Figure 36) showed a homogeneous distribution of drug aggregates in pharmaceutical films, with a diameter of 5 µm for B2 and 10 µm for C2 respectively. It was proved that the particle size of drug in a final

formulation depends on Mw, smaller Mw, smaller obtained particle size (Choi et al.; 2008). Dsm presents a lower Mw respect to Trm (Dsm, Mw 392.46 g/mol, Trm, Mw 434.50 g/mol). While Olz has an even smaller Mw (312.43 g/mol), corresponding sample, A2, showed a non-homogeneous distribution of drug aggregates, with diameters ranging from 2-15 µm compared with films loaded with Dsm and Trm, indicating that drug affected solid-state of the final product.

Freeze-drying and solvent-casting methods allowed shape and surface solid-state to be reproduced, the spray-drying process needs further optimisation, considering various experimental variables in sample preparation, as seen in a previous study (Modica De Mohac et al.; 2019).

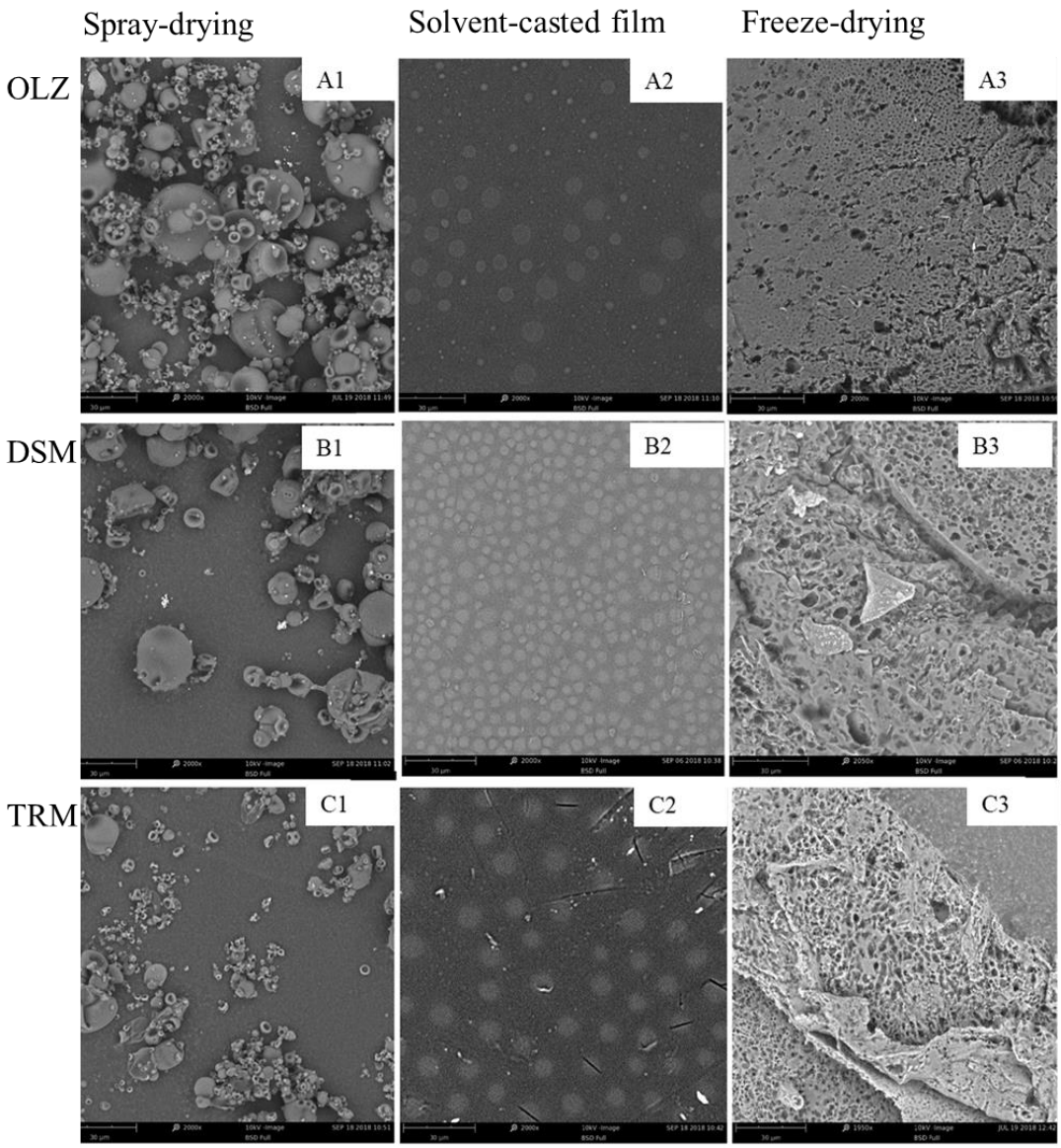


Figure 36. SEM images of solid dispersion samples loaded with Olz (A1-3), Dsm (B1-3) and Trm (C1-3) respectively, recorded at 2000x magnification. Samples prepared by spray-drying (A1, B1 and C1), solvent-casting(A2, B2 and C2) and by freeze-drying (A3, B3 and C3). Scale bar 30 µm.

The physical-chemical characterisation of each formulation investigated the presence of drug-excipient interactions, occurring between specific chemical groups within their structures. Table 14 summarises peaks assignment of each pure drug and respective peaks found in each formulation. This experiment was also conducted to prove that the drug was in the polymer's mixture. Identified peaks found relevant correspondence in literature and no abnormal chemical interactions were detected, as further confirmed by thermal analysis (Hiriyanna et al.; 2008; Rodrigues et al.; 2009; Shin et al.; 2000). Figure 37 showed central peak identified in each formulation. A1, A2 and A3 showed Olz peak at 1584 cm^{-1} related to C=C asymmetric aromatic stretching as well as a peak at 2931 cm^{-1}. No shift in signal was reported. Samples containing Dsm showed main peaks associated with CO stretching

carbonyl and C=C stretching aromatic ring, as well as CF stretching. Same typical groups confirmed the presence of Trm in samples C1, C2 and C3 where CF stretching is showed at 1080 cm^{-1}. No peak shifts were observed in any samples.

Table 14.FTIR peak assignment for each drug molecule and solid dispersion formulation.

Infrared peaks assignation				
Olz cm^{-1}	A1 cm^{-1}	A2 cm^{-1}	A3 cm^{-1}	
3221	3221		3221	NH stretching
3078-3060				CH aromatic ring stretching
2931	2939	2931	2936	CH3 methyl stretching
1584-1558	1584	1586-1561	1582	C=C asymmetric aromatic stretching
1470-1446-1412	1415		1418	C=C aromatic ring stretching
1289-1223				CN bonding
Dsm cm^{-1}	B1 cm^{-1}	B2 cm^{-1}	B3 cm^{-1}	
3407			3407	OH alcohol vibration H bond
2981-2953-2932-2866	2936-2862	2932		CH3 methyl
1704-1659	1705	1662	1655	CO stretching carbonyl
1617-1603-1436	1616-1602	1620-1603-1425	1620-1610	C=C stretching aromatic ring
1268	1268	1254	1268	CF stretching
Trm cm^{-1}	C1 cm^{-1}	C2 cm^{-1}	C3 cm^{-1}	
3464-3397	3375			OH alcohol vibration H bond
1707-1662	1715-1654	1704-1659	1718-1660	CH stretching
1456			1454	CH3 methyl
1375-1302-1278	1375		1375	COC acid alcohol
1057-1080	1078	1080	1080	CF stretching

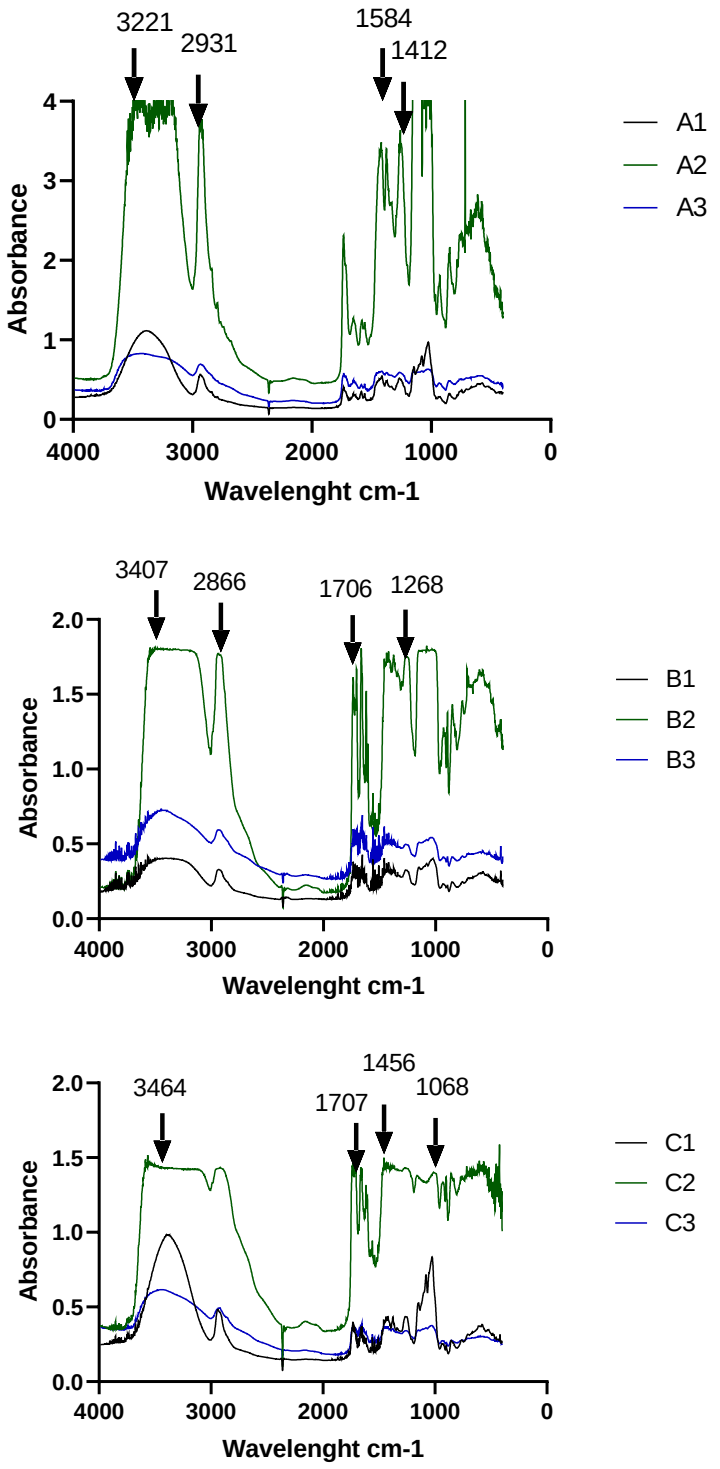


Figure 37. FTIR spectra of formulations A1, A2 and A3; B1, B2 and B3; C1, C2 and C3. Per each group of main peaks were highlighted in correspondence of pure drugs peaks.

The physical-chemical characterisation allows solid-state of pure drugs (Chapter 1) to be ascertained. Internal solid-state of each drug was then studied by DSC analysis. Data show all drugs remained in their crystalline state, as can be seen by retention of T_m values associated with this state. Therefore, formulation processes did not affect drug solid-state, while both polymers were

maintained in their amorphous conformations. Such drug-polymer solid-state are referred to in the literature as second-generation solid dispersions, in which carriers, generally polymeric, are in the amorphous state (Modica de Mohac et al.; 2020). At the same time, the drug is dispersed molecularly within the inert matrix in a crystalline form (Modica de Mohac et al.; 2018). Table 15 reports thermal analysis properties such as T_m, Δ_{cp} and water content of pure drugs and solid dispersions. No T_g was recorded, as formulation strategies did not cause drug amorphisation over time. This finding allowed each prepared formulation to be classified as a second-generation solid dispersion (both drug and carriers in an amorphous state) according to the classification proposed by Meng and co-workers and Modica de Mohac and co-workers (Hallouard et al.; 2016; Meng et al.; 2015; Modica de Mohac; 2018). For all samples, it was noticed that, while drugs as pure compounds did not present water (therefore pure and well-stored), once formulated, water content increased. This finding relates to the reduction in surface tension of aqueous solutions caused by polymers used. It is also proved that both PVA and MDX are hygroscopic molecules that cause water absorption (Asem et al.; 2018; Hofman et al.; 2016).

Interestingly, samples with Olz, the present lowest increase in water content (from 8 to 30-fold increase), with the highest growth for samples prepared via the solvent-casting method (A2). Such finding represents one of the disadvantages of the technique itself as present in the Introduction section (Boateng et al.; 2009). For samples containing Dsm and Trm, an increase in water content was from 30 to 250-fold (p-values <0.0001), with a 145-fold increase (p-values=0.0023) for both samples produced by freeze-drying. This finding could be due to freeze-drying residual moisture content considered as a disadvantage of the process (Seligmann and Farber; 1971). Overall, the highest enhancement of water content was highlighted in formulations produced by spray-drying, which cause a 250-fold increase in B1. Fold increase varies consistently between different drugs and formulation processes. It is well-know that hygroscopicity depends as well on compound Mw, and therefore results are coherent with reported Mw for each drug used in the present study (Lesvi et al.; 2009). Moreover, it is described that while Olz is a non-hygroscopic molecule (Lesvi et al.; 2009), while Trm and Dsm are most of the corticosteroid molecules (Davidson et al.; 2017).

Table 15. Thermal analysis data for each pure drug and drug-loaded formulation.

	Sample name	Water content %	Tm (°C)	Δ_{cp} (J/g)
Olz sample	A1	0.24 ± 0.03	193 ± 0.95	10.11 ± 4.71
	A2	0.60 ± 1.12	191 ± 0.87	11.43 ± 3.21
	A3	0.17 ± 0.67	192 ±1.21	14.93 ± 9.76
	Olz	0.02 ± 0.09	193 ± 1.35	15.80 ± 2.56
Dsm sample	B1	4.95 ± 0.23	271 ± 0.23	4.95 ± 0.93
	B2	1.49 ± 1.04	267 ± 0.52	1.71 ± 2.43
	B3	2.82 ± 0.89	265 ± 0.78	1.82 ± 0.09

	Dsm	0.06 ± 0.01	267 ± 0.95	18.5 ± 9.98
Trm sample	C1	1.74 ± 0.53	292 ±1.35	2.11 ± 1.67
	C2	0.64 ± 0.42	298 ±2.09	9.13 ± 4.86
	C3	2.95 ± 0.86	290 ± 0.64	1.29 ± 3.45
	Trm	0.16 ± 0.23	292 ± 0.24	10.01 ± 6.12

3.3 Drug loading and release studies

Each solid dispersion formulation was prepared such that drug made up 10% of the total. As expected, corresponding drug loading values were obtained for formulations developed by solvent-casting and freeze-drying techniques. In contrast, lower drug loading was detected in all formulations prepared by spray-drying (samples A1, B1 and C1, Table 16). results suggest that solvent-casting method allowed for highest and most consistent drug loading, while freeze-drying and spray-drying methods resulted in drug loss during the formulation process. According to drug loading, each formulation was evaluated in terms of its drug release profile. The dissolution rate of each formulation was compared with that of the respective pure drug within a buffer solution. It was found that even if all formulation strategies allowed 70% release of each drug after 60 minutes, the only solvent-casting method achieved a release of 50% after 5 minutes and 100% after just 120 minutes, as shown in Figure 38. These results were confirmed statistically by evaluation of similarity factor, *f2* (values are reported in Table 17), which was calculated to be less than 50 for each formulation when compared to pure drug alone, confirming differences in dissolution profiles. Data relevance was assessed by two-way ANOVA, with a p-value lower than 0.001 for each pure drug dissolution profile compared with the formulation. Overall, each formulation achieved a faster drug dissolution than the particular pure drug alone. However, this work aimed to detect formulation strategy that produced the most enhancement of release profile of model drugs. Dissolution rate enhancement was observed in pharmaceutical films prepared by solvent-casting, because drug molecules are more readily soluble since they are more accessible to aqueous solvent, being closer to the surface of solid dispersion than in other formulations (Siemann; 2005). Similarity factor validated findings, *f2*, which was found to be less than 50 for each of solid dispersions prepared.

Table 16.Drug loading values for all solid dispersion formulations prepared by spray-drying (samples A1, B1 and C1), solvent-casting(samples A2, B2 and C2) and freeze-drying (samples A3, B3 and C3). Respectively.

Sample name	Drug Loading
A3	9.20 ± 0.2
A2	9.89 + 2.56
A1	5.96 ± 1.37
B3	8.54 ± 1.95
B2	9.12 ± 0.98
B1	5.23 ± 1.49
C3	7.56 ± 2.62
C2	9.74 ± 0.81
C1	8.39 ± 1.79

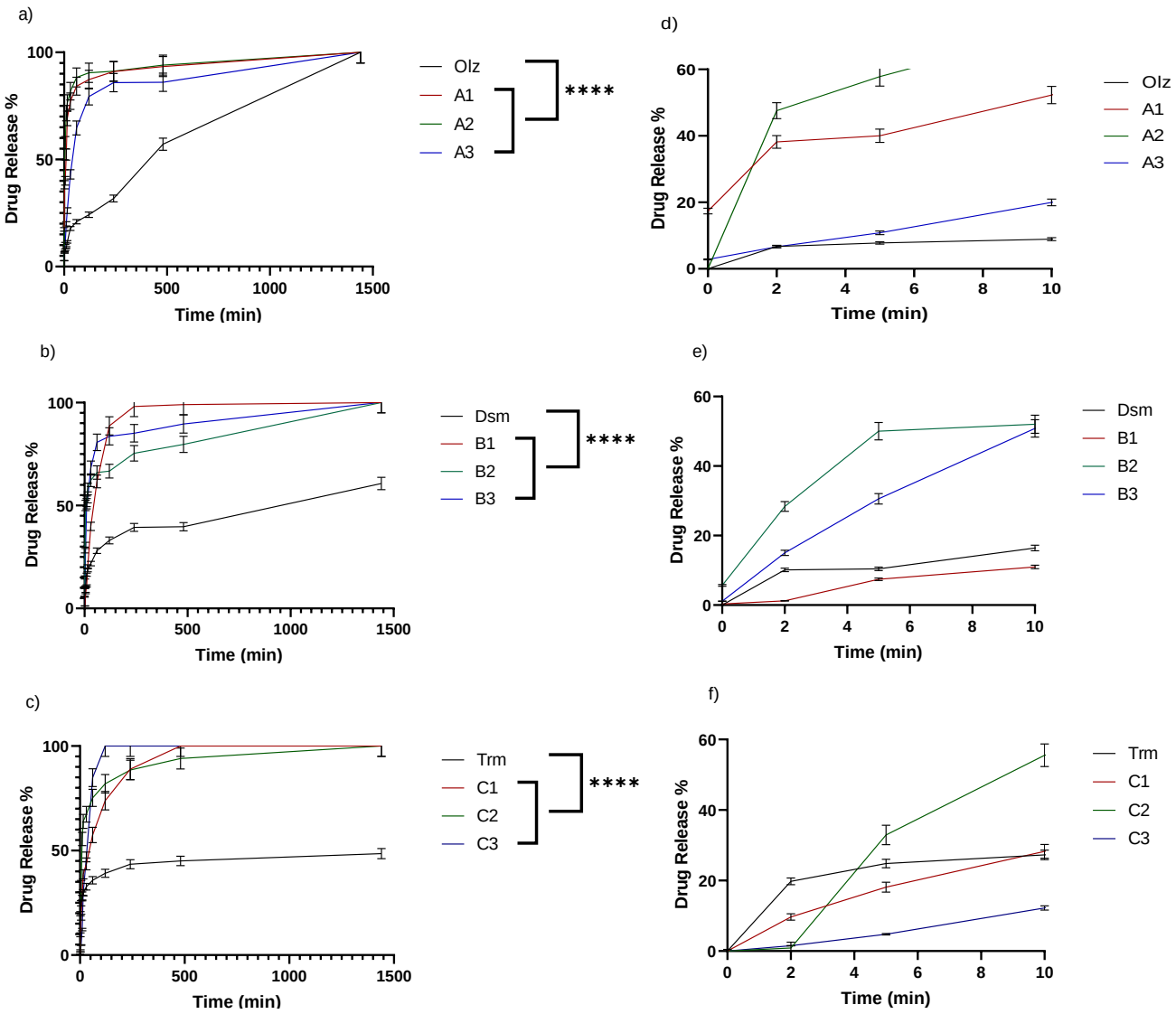


Figure 38. Drug release profiles depicting percentage drug release over time (minutes). The pattern of each pure drug alone is compared to that of individual drug formulations over 24 hours (a, b and c), as well as for first 10 minutes of drug release (d, e and f).

Table 17. Similarity factor, *f2*, for each drug formulation.

Sample Name	*f2*	Sample Name	*f2*	Sample Name	*f2*
A1	16.58026	B1	23.01202	C1	22.49841
A2	14.02035	B2	26.09391	C2	19.36046
A3	26.34483	B3	20.59854	C3	18.45019

4. Conclusions

More than 40% of commercialised drugs are poorly soluble. Solid dispersions are used to increase dissolution rates of BCS class II and IV drugs. They can be produced using several formulation strategies, with the three most common techniques (spray-drying, solvent-casting, and freeze-drying) being compared in this study. This study aimed to investigate pharmaceutical advantages of different methods described above, in terms of achieving faster drug release, improved surface homogeneity and drug incorporation capability using three different active molecules: Olz, Dsm and Trm.

Firstly, techniques were compared to evaluate differences in surface homogeneity, drug incorporation capability and drug stability during the formulation process. Freeze-drying and solvent-casting methods allowed to obtain a more homogenous and reproducible surface solid-state and pore/particle size, while spray-drying did not generate particles of the same size or shape. Moreover, spray-drying caused drug loss during the production process, while the other two methods produced the best drug loading results. All methods were able to preserve each drug in its original crystalline solid-state showing to be suitable formulation strategies for model drugs used. Concerning the dissolution rate, of all processes, solvent-casting generated the fastest drug dissolution/release rate for all model drugs used. These results suggest that solvent-casting still represents the most efficient and reproducible productive method of solid dosage form, such as solid dispersion. Further investigations and innovations are needed to make the technique industrially scalable.

Chapter 4. PVA-PHEA electrospun nanofibres – Synthesis, characterisation and evaluation of their adhesive and drug delivery potential

1. Background

In treatment-resistant schizophrenia , a significant clinical challenge is poor patient compliance with clozapine medication due to both its daily dose and taste (Oloyede et al.; 2019). Clozapine is usually administered as a tablet, but due to its low solubility in the gastrointestinal tract and its hepatic metabolism, it must be taken by multiple doses per day. Drug delivery to the oral cavity is a promising and attractive route of drug administration (Chinna Reddy et al.; 2011; Kraan et al.; 2014). Mainly, buccal transmucosal delivery is a favourable route as drug avoids gastrointestinal tract; therefore, bypasses first-pass metabolism (Morales and McConville; 2011). Oral mucosa (inner lining of cheek) has a stratified and non-keratinised epithelium that makes drug absorption via this route more natural compared to keratinised epithelium (Hooda et al.; 2012; Jiang and Turner; 2016). For this route of administration, solid dosage forms such as films and patches are favourable, especially for controlled drug release (Lam et al.; 2014). The potential of electrospinning at commercial scale is well discussed in the literature, and there is an increasing drive to discover suitable excipients for an electrospun transmucosal dosage form that possesses desirable properties such as adhesion, improved drug dissolution and in-turn oral bioavailability (Persano et al.; 2013; M. J. Taylor et al.; 2010).

The work detailed in this chapter aimed to synthesise, characterise, and evaluate PVA-PHEA based nanofibres patches for their adhesive and drug delivery potential. PHEA is a novel biocompatible, low toxicity, the solubilising and mucoadhesive polymer, which properties were discussed in the Introduction of this thesis (Carfì Pavia; 2014; Civiale; 2009; Licciardi et al.; 2012; Lo Monte A. et al.; 2012; Saiano; 2002). PHEA elecrospinnability an effect as solubiliser agent was proved during a parallel study reported in Appendix 1. Though PHEA has low electro-spinnability, this has shown to be improved when co-electrospun with polymers, such as PVA (Aytac and Uyar; 2017; Brough et al.; 2016; Buscemi, Damiano, et al.; 2017; Buscemi; 2017; Golafshan et al.; 2017; Lo Monte A. et al.; 2012; Wali et al.; 2018; Yu et al.; 2011). PVA use in electrospinning, especially for drug delivery applications, has increased over the years, most likely due to its positive effects in improving aqueous drug solubility and high electro-spinnability (Aytac; 2017; Brough; 2016; Golafshan; 2017; Wali; 2018; Yu; 2011). Mucoadhesive properties of PHEA-PVA film formulations were evaluated using AFM-Qi (Jiang; 2016). SLP was included in this study to improve drug release properties, PHEA elecrospinnability and an investigation into its role in adhesion was also evaluated using AFM-Qi.

The new oral nanofibrous transmucosal polymeric patches were prepared both via wet electrospinning and solvent casting film method with the purpose to increase clozapine solubility as well as prolong its release. Two different formulations made via electrospinning and solvent casting were compared. In Chapter 3, the solvent casting method was recognised as the most suitable technique to produce big surface area, particle size homogeneity along the film surface, and high dissolution performance. However, the drug particle dimension is difficult to optimise, and the drug can aggregate during the process (Saldanha and Kyu; 1987). Nanofibres are known to possess advantageous properties such as the high surface area to volume ratio (Gareth R. Williams; 2018), malleability, porosity (Brako et al.; 2015), mechanical properties (Zhu et al.; 2010) and release profile tailoring (Williams et al.; 2012; Yang et al.; 2017) compared to the older system such as solvent cast films. In the present section, it was therefore investigated the effect of PHEA in physical formulation properties such as adhesiveness, particle size dimension and finally in controlling clozapine release, which was evaluated via *in-vitro* dissolution studies.

2. Materials and Methods

NHS Maudsley Hospital kindly provided clozapine, STEBICEF synthesised PHEA, PVA (Mw 145000 g/mol) was purchased from Sigma-Aldrich and SLP was kindly supplied from BASF USA. PBS tablets with pH 7.4 were purchased from Sigma-Aldrich. EtOH was purchased from Sigma-Aldrich. Water used was distilled.

2.1 Sample preparation

Clozapine, PVA, PHEA, and SLP were initially combined as PM by gentle mixing using a mortar and pestle with drug-polymer ratios displayed in Table 18. SLP was added to formulations to improve PHEA spinnability (Nagy et al.; 2012). Physical mixtures were analysed to evaluate their influence on drug dissolution rate. Thus, the formulations were formulated both as pharmaceutical films and nanofibrous patches. Those were generated in ratios highlighted in Table 18 by solvent casting method and wet electrospinning. Films and nano-fibres formation was achieved by adding to physical mixtures PBS pH 7.4 and stirring magnetically overnight. Obtained dispersions were then placed in plastic Petri dishes and left overnight under fume cupboard to obtained films and electrospun with 18kV and with a flow rate of 0.1 mL/h to collect nanofibres.

Table 18. Drug-Polymers ratios w/v and formulations composition.

Composition				Physical Mixture	Film	Nanofibres
PVA	PHEA	SLP	Clozapine			
7.5	/	/	10	A	F1	F2
7.5	2.5	/	10	B	F3	F4
7.5	5	/	10	C	F5	Not Spinnable
7.5	2.5	2.5	10	D	F6	F7
7.5	5	2.5	10	E	F8	F9

2.2 DSC

The analysis was conducted using a DSC 2920 Modulated DSC machine (TA instruments). DSC analysis was held at a rate of 10°C/min, and samples were heated to 200°C. nitrogen purge gas used had a flow rate of 130mL/min. Samples were also held isothermally for 5 minutes. Aluminium pans used were from TA Instruments. Each sample was analysed in triplicate.

2.3 ATR-FTIR

The analysis was conducted using a Perkin Elmer Frontier FTIR spectrometer. This was to identify any interactions occurring between molecules, such as hydrogen bonding. A total of 64 scans were taken at a resolution of 2 cm^{-1} and using a wavelength range of 4000-600cm^{1}. Each sample was analysed in triplicate.

2.4 Particle Size analysis

Dynamic light scattering measurements analysed particle size with 12 mm cell (DTS 0012). Each sample was sonicated for 5 minutes before reading. Particle size was determined with a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., GB) and analysed by a Malvern analytical Instrument.

2.5 SEM

The images were recorded on freshly prepared microparticles by using a Phenom ProXSEM. Particles were analysed using the same parameters (i.e. contrast and luminosity) and same stub for at least four samples. The *d* of microparticles was determined from the mean value of 100 measurements using ImageJ (the USA, version 1.46 v).

2.6 AFM

AFM was performed on a Bruker Nanowizard 4 system (Bruker Nano GmbH) operated in Quantitative Imaging (QI) mode. An RTESPA-300 cantilever with a nominal spring constant of 40N/m was calibrated and checked on a sample of polystyrene of a known modulus value. Images were acquired at a resolution of 512 x 512 and processed in JPKSPM data processing software.

2.7 PVA and PHEA effect on clozapine saturation solubility

The effect of PVA, different concentrations of PHEA and SLP on the aqueous solubility of clozapine was investigated (in ratios presented in Table 18). Excess amounts of clozapine powders were dispersed in aqueous polymer/s solutions for 72 h and stirred with 150 rpm at 37 °C using a shaking incubator (SciQuip, UK). Polymer solutions were then filtered through a 0.45 mm Millex-GP filter (Merck Millipore, UK) and concentration of clozapine was determined spectrophotometrically at 257 nm.

2.8 Drug loading evaluation

Proper amount (2 mg) of samples were dissolved in 2 mL of EtOH, filtered through a 0.45 mm syringe filters with cellulose acetate membrane (VWR International, USA) and drug content of each sample was determined spectrophotometrically. UV-VIS spectra were recorded by Perkin Elmer Lambda UV/VIS Spectrophotometer, in 300-200 nm spectral range. A calibration curve was used for

quantification of drug content. The calibration curve was performed in the concentration range of 0.1-0.0001 mg/mL of clozapine standard solutions in EtOH (R=0.999) and spectra recorded at 257 nm.

2.9 In-vitro dissolution and release studies

The studies were carried out in not sink condition by using 24 well-plates placed in a shaking incubator provided by SciQuip Ltd at 37°C and with 50 rpm of shaking. Samples were weighed and placed in well-plates to obtain 3 mg of clozapine in each plate. According to European Pharmacopeia and literature evidence, each sample was placed in a PBS solution at pH 6.8 to mimic buccal environment (Abruzzo et al.; 2012; Koland et al.; 2010; Langoth and Kalbe; 2003). At predetermined intervals of 15, 30, 45, 60, 90, 120, 180, 360, 480 and 1360 minutes 500µL was withdrawn and diluted in 5mL and filtered through a 0.45 µm cellulose acetate syringe filter and replaced with the same amount of fresh buffer. Subsequently, the filtrate was analysed spectrophotometrically at UV with a wavelength of 257 nm. The calibration curve was performed in the concentration range of 0.1-0.0001 mg/mL of clozapine standard solutions in PBS pH 6.80 (R=0.999) and spectra recorded at 257 nm.

2.10 Statistical Analysis

A one-way analysis of variance (ANOVA) was applied to compare different samples. Data were considered statistically significant with a value of $P < 0.05$, and differences between groups were compared using Bonferroni t-test. Each test was developed in triplicate. *f2* was calculated using Equation 1 in Chapter 3 (Shah; 1997) and was used to compare dissolution performance between drugs, as well as corresponding formulations (Raimi-Abraham; 2015). When *f2* below 50, profiles are considered different, and when *f2* above 50 compared release profiles are considered equivalent.

3. Result and Discussion

3.1 Use of atomic force microscopy quantitative imaging to assess adhesiveness

Oral transmucosal dosage forms are available since the 1980s, and their prerogative is to be adhesive to buccal mucosa (Patel et al.; 2011; Torres-Lugo and Peppas; 2000). PHEA has both solubilisation and mucoadhesive properties. Its mucoadhesive features were confirmed uniquely using ATR-FTIR by observation of bond formation between PHEA and mucin molecules (Cespi et al.; 2010; Dias; 2015; Saiano; 2002). SLP effect on mucoadhesion was significantly demonstrated on solvent cast film by Alopaeus and co-workers, 2020 and Salawi and co-workers, 2018, by a mucin-

interaction test (Alopaeus et al.; 2020; Salawi and Nazzal; 2018). Particularly both literature findings described that SLP possesses adhesive properties when used at 0–75% w/w. In this section, it was assessed adhesiveness of PHEA-PVA films at different concentration with and without SLP using AFM-Qi. AFM-Qi allows for simultaneous topographical mapping and quantitative nanomechanical analysis of the sample's surface. It also represents the only method available to investigates intrinsic adhesive forces of compounds (Beach et al.; 2002). Adhesion forces are defined as the pull-off force when the tip of the needle (of the AFM) is moved closer to the surface of the material and then suddenly repelled (Jiang; 2016). In this study, adhesive forces assessed at the nanoscale were measured in sub nanonewton (nN) range.

Figure 39 shows AFM-Qi phase images and a corresponding plot of the adhesive force (nN). 7.5% w/v PVA films had an adhesive force of 14.96 nN. This finding relates with findings in the literature where 15 and 20% w/v PVA films presented a work of adhesion of 15 nN (Ikeuchi-Takahashi et al.; 2017; Padday and Uffindell; 1968; Peppas and Mongia; 1997). Particularly, increasing polymer concentration leads to increment density that is correlated with an increase of adhesiveness due to augmentation of average bond energy that improves adhesive bond strength (Brenman and Lerchenthal; 1976). However, the work of adhesion does not provide direct information on the magnitude of the adhesion range. Therefore the tradition AFM-based pull-off cannot be used for fine calculation that is allowed by the newest AFM-Qi that does consider surface topography (Bhat et al.; 2018). Addition of 2.5% w/v of PHEA increased adhesive force from 14.96 ± 1.57 nN to 19.02 ± 9.41 nN ($p<0.0001$), a 1.27-fold increase, respect 7.5% w/v PVA patch. When increasing PHEA concentration to 5% w/v, adhesiveness increased 3-fold ($p<0.0001$) to 45.79 ± 13.07 nN. PHEA adhesive properties were previously analysed using FTIR and a mucin solution; therefore, findings are shown here represent first available data on the intrinsic adhesive force of this polymer (Lee; 2005; Saiano; 2002). Polymer combination was then augmented with 2.5% w/v of polymer SLP to improve PHEA spinnability. However, it was observed that adding 2.5% w/v of SLP to patch prepared with 7.5% w/v PVA and 2.5% w/v of PHEA caused 2-fold increased respect to patch without SLP. SLP adhesive and mucoadhesive properties are thought to be related to its plasticising effect that leads to a decrease in intermolecular forces which improves flexibility and chain mobility (Alopaeus; 2020; Vieira et al.; 2011). High flexibility is favourable to adhesion, as proved in the case of a buccal-adhesive tablet of carvedilol (Yamsani et al.; 2007). However, when SLP is added to the formulation containing 7.5% w/v of PVA and 5% w/v PHEA, the adhesive force was reduced to 32.37 ± 9.61 nN ($p<0.0001$), a 1.4-fold decrease. This event could be due to a reduction of the plasticising effect of SLP when used in polymer combined with a w/v ratio higher than 3.5% w/v (Alopaeus et al.; 2019). Overall, it was possible to assess that increasing PHEA concentration to 5% increases patch adhesiveness and that

there is a relation between the increase of its ratio and increase of adhesive forces. Particularly, increasing PHEA concentration from 2.5% to 5% w/v leads to an increase of adhesive force from 19.02 nN to 45.79 nN, with an overall 2.40-fold increase ($p<0.0001$), representing a potential to increase the adhesiveness of 24% for each increase of concentration of 2.5% w/v. This result could be attributed to the intrinsic adhesive effect of PHEA. Such force depends on polymer surface interaction, thus zeta potential when solubilised (Ault et al.; 2019; Craparo et al.; 2010). When in aqueous solution presents a zeta potential of -13 mV, previously determined by Cavallaro and co-workers who demonstrated that dispersing a polymer in distilled water of PVA 2% w/v reduced the zeta potential of PHEA leading to a more interactive surface (Gennara Cavallaro et al.; 2015; Craparo; 2010). In the present work here it was used a higher concentrated PVA solution (7.5% w/v) and, in 2017 it was found that using PVA in the ratio between 2% and 15% w/v offers higher adhesiveness (Ikeuchi-Takahashi; 2017). This finding suggested that PVA and PHEA combination could be of use in the preparation of adhesive patch for buccal delivery. Overall, it was possible to assess that increasing PHEA concentration to 5% increases patch adhesiveness. A relationship between the increase of PHEA ratio from 2.5 to 5% w/v and the increase of adhesive forces were identified. Polymer combination containing 7.5% w/v of PVA and 5% w/v of PHEA led to highest values of 45.79 nN that could be a promising potential for successful adhesiveness to the buccal mucosa. Particularly, Sumarokova and co-workers demonstrated that a force of 2.50 nN is sufficient to create adhesion between a layer of endothelial cells and the untreated tip of the AFM (Sumarokova et al.; 2018). Such compliance would allow patch enough to contact with epithelium to prolong drug release allowing a systemic delivery of drug.

Through this analysis, it was possible to observe a relation between the increasing concentration of PHEA and increasing adhesiveness effect. It was noticed that 7.5% of PVA w/v and PHEA at 5% w/v in formulation increased mucoadhesive property up to a maximum of 45.79 ± 13.07 nN. Such a result suggested that PVA and PHEA combination could be of use in the preparation of adhesive patch for buccal delivery.

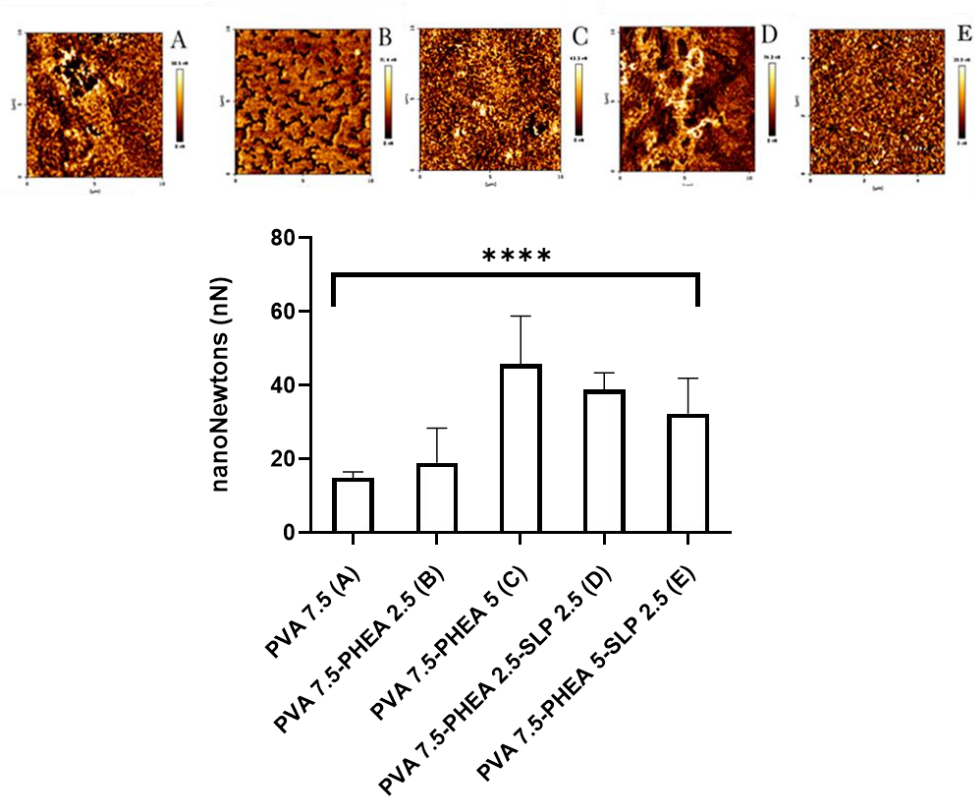


Figure 39. AFM in QI mode analysis was used to investigate mucoadhesive properties of different polymer combinations in the nanoscale range. The investigation was conducted on polymer-based films with polymer combination reported in Table 18. It was observed that film is containing 7.5% w/v of PVA and 5% w/v of PHEA present highest adhesiveness of 45.79 nN. Two-way ANOVA showed the highest significance (****) when comparing each result.

3.2 Fibre characterisation: Solid-state and fibre diameter

SEM studies were conducted to confirm fibre solid-state and diameter changes with polymers w/v ratio used. Figure 40 presents SEM images of randomly aligned fibres successfully generated with PVA (F2) and with increasing concentrations 2.5% (F4) and 5% (F7) w/v of PHEA and SLP (F9) well in the nano range. Addition of 2.5% w/v of PHEA (F4) appeared to reduced fibre diameter to 536 ± 166 nm, while at 5% PHEA fibres could not be spun due to precipitation of PHEA when adding pressure from the pump system. Though PHEA spinnability is well reported in the literature, it showed to be spinnable when dissolved in pure organic solvents (such as Dimethyl-sulfoxide)or water/organic solvent mixture with water lower than 30%v/v (Buscemi; 2017; Licciardi; 2012). However, the addition of 2.5% w/v of SLP (F9) increased PHEA spinnability, and fibre diameter was 606 ± 212 nm. SLP ability to increase polymers solution or dispersion by stabilising it and reducing precipitation, as proved by Paveer and co-workers (Paaver et al.; 2014). No clear trend was observed upon the increase of PHEA concentration and the resultant effect on nanofibre diameter.

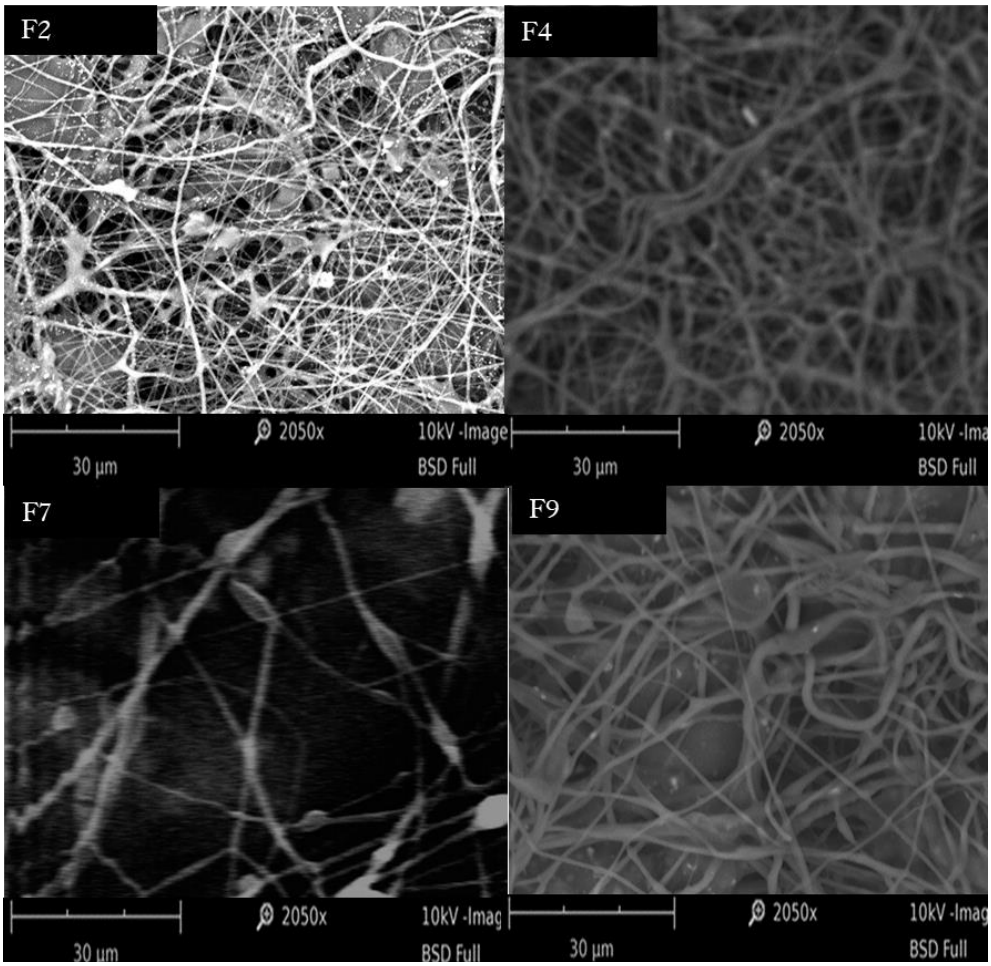


Figure 40. SEM images of electrospun nanofibres F2, F4, F7 and F9. Images were obtained with x2050 magnification

3.3 Clozapine particle size analysis and solid-state determination of clozapine in films and nanofibrous patches

Drug crystallinity is considered an advantage when formulating a product to improve shelf-life longevity. In this study, it was investigated the effect of formulation method on resultant properties of clozapine, namely, hydrogen-bonding interactions between clozapine and polymers, solid-state and particle size.

The presence hydrogen bond interaction between polymers PVA, SLP and PHEA and clozapine within formulations F1-F9 was evaluated in ATR-FTIR and DSC studies, respectively. Figure 41 shows the ATR-FTIR spectra of clozapine and formulations F1 -F9. Characteristic ATR-FTIR characteristic peaks for crystalline clozapine (Cavallaro et al.; 2004) were observed at 774 cm^{-1} (C-Cl bond), 1025cm^{-1} and 1357cm^{-1} (C-N stretching vibration) and 1545 cm^{-1} (C=N stretching vibration) in formulations F1-F9. No hydrogen bonding interactions were observed between clozapine and polymers (i.e. PVA, PHEA and SLP) in any of formulations.

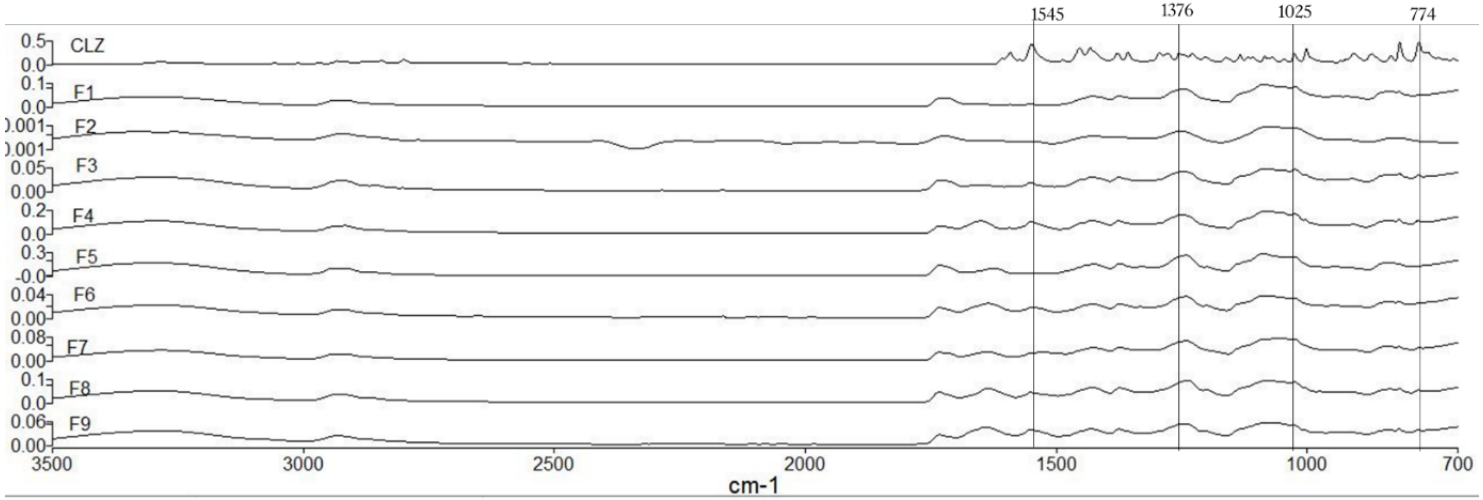


Figure 41. ATR-FTIR spectra of clozapine, film and nanofibrous formulations F1 – F9 from 4000 – 700 cm^{-1} with characteristic clozapine peaks highlighted at 774 cm^{-1} (C-Cl bond), 1025 cm^{-1} and 1357 cm^{-1} (C-N stretching vibration) and 1545 cm^{-1} (C=N stretching vibration).

DSC analysis of clozapine (raw material) exposed a characteristic endothermic peak associated with its melting point (Licciardi et al.; 2012) at 185 ± 0.75°C (Δ_{cp} 122.39 ± 4.52 J/g). This endothermic peak corresponding to the clozapine melting point was observed in formulations F1 to F7 irrespective of the method of preparation (Figure 42). However, in formulations, F8 and F9 (both containing 7.5% w/v PVA, 5%w/v PHEA, 2.5% w/v SLP and 10%w/v clozapine) a step-change in the baseline characteristic of a glass transition (T_g) at 40.36°C and 41.54°C respectively was observed. Temperature range of this thermal event occurred ~10°C lower than reported T_g clozapine, which would be expected in a system where additives (i.e. polymers) providing a plasticising effect. (Caron et al.; 2013; Triolo et al.; 2017; Wood et al.; 2016). In F8 and F9, the Presence of clozapine in the amorphous state lead to the creation of amorphous solid dispersion and could be due to the addition of 2.5% w/v of SLP. SLP presents property of preventing drugs (i.e. ibuprofen and carbamazepine) from re-crystallising over time (Cho et al.; 2015; Hussain et al.; 2018). This effect could be due to the amphiphilic and solubilising properties of SLP that could create a complex with the drug preventing clozapine recrystallisation. Even if drug crystallinity is considered advantageous as it would lead to an improved dosage form stability in terms of shelf-life, amorphous solid dispersion presents the advantage of a higher solubility that influences dissolution behaviour.

As ATR-FTIR analysis demonstrated no interactions between polymers and drug, it is possible to conclude that identified thermal events are correlated with clozapine. An evaluation of drug solid-state is essential to a better understanding of the drug release profile. Usually, an amorphous drug tends to give a burst in release while crystalline form gives a slower profile (Ignatious et al.; 2010; Shi et al.; 2019). The release profile is influenced not just from solid-state but also from its particle size (Dokoumetzidis and Macheras; 2006).

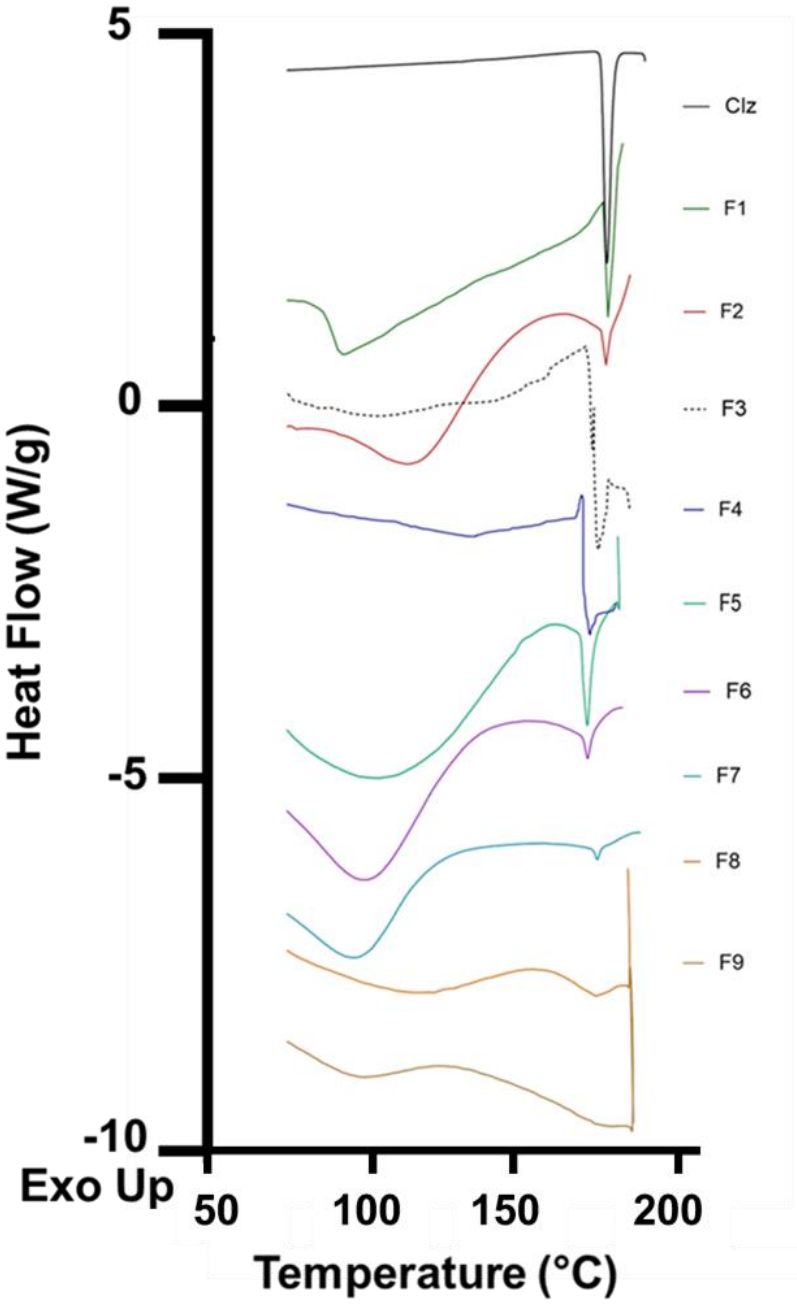


Figure 42. DSC thermograph of clozapine and all F1- F9 formulations.

An evaluation into particle size post formulation would confirm if in one or both types of formulations, micro- or nanocrystalline solid dispersions have been created. These types of solid dispersions have been shown to have increased advantages over amorphous solid dispersions due to their increased stability. It was proved that these systems combine the benefit of long term stability provided by a crystalline compound with the enhancement of dissolution profile provided by formulation (Modica de Mohac et al.; 2020; Modica de Mohac; 2016; 2018). Clozapine particles extracted from F1-F9 formulations were analysed via dynamic light scattering and compared with unformulated clozapine), data reported in Figure 43. Unformulated clozapine average particle size was 1.95 ± 1.02 µm. Initially, formulations F1, F3 and F6 achieved a particle size above

the range of 1 µm. However, no statistical significance was found running a one-way ANOVA test between clozapine alone and F1. These formulations were prepared with the solvent casting method, and both F3 and F6 showed 2.5% w/v of PHEA in the formulation.

All formulations prepared by electrospinning (F2, F4, F7 and F9) reduced drug particle size significantly (between 500 nm and 1 µm) compared to drug alone dissolved in same media ($p<0.0001$). This reduction is related to electrospinning capabilities of reducing drug size due to a combination of needle diameter as well as of high voltage applied to evaporate solvent (Ball and Woodrow; 2014; Ignatious; 2010). Interestingly, the film F5 and F8 (both containing 5% of PHEA) showed a particle size in the nano-size range between 60, a 16-fold decrease ($p=0.0023$) and 90 nm, an 11-fold reduction ($p<0.0001$). This size could be due to micellisation properties of PHEA, which can create micelles within the range from 10 to 100 nm (G. et al. Cavallaro; 2004). It was observed that formulations developed by solvent casting method (F1, F3 and F6) gave a larger particle size compared counterpart electrospun fibres. With solvent casting method the particle dimension could be influenced by dispersibility of particle within solvent and agitation force applied while stirring initial dispersion. Therefore, control of particle diameters is more challenging to achieve, resulting overall in size higher than 1µm (Prasad et al.; 2017). Using electrospinning provides the advantage of modifying parameters such as voltage and flow rate to affect the size. Notably, the application of high voltage (Deitzel et al.; 2001) and a low flow rate (Haider et al.; 2018) is proved to achieve a smaller particle size. These set of experiments, allowed us to identify formulations F2, F4, F5 and F7 as solid nanocrystalline dispersion which could aim to improve shelf-life and prolonged release profile.

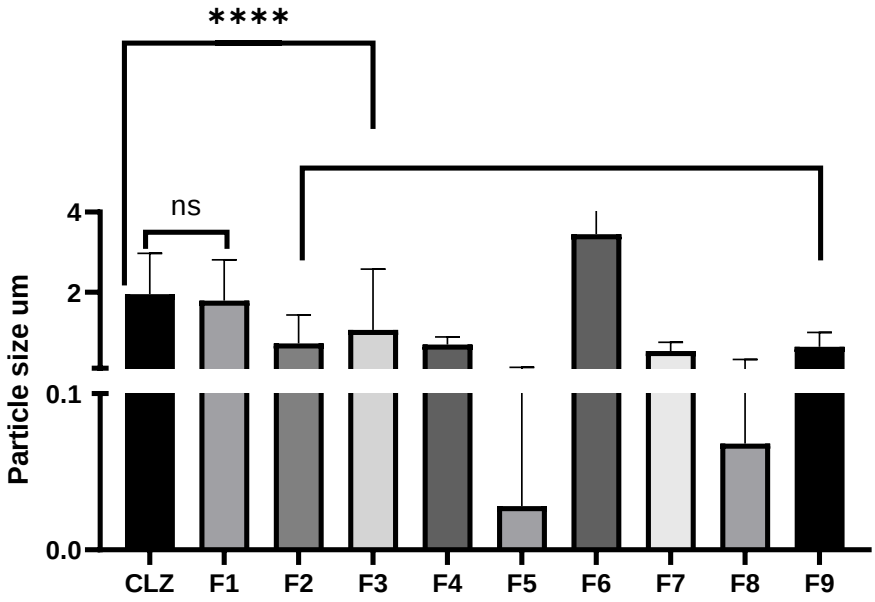


Figure 43. Particle size per each formulation compared with clozapine unformulated. The analysis was developed using Nanosizer, and value is expressed in µm. Statistical analysis showed the highest significant (****) comparing clozapine pure drug with all formulation apart from F1 where no significance occurred (ns).

3.4 PVA, PHEA and SLP effect on clozapine saturation solubility

Saturated solubility studies were conducted on clozapine dissolved in aqueous pH 7.4, PVA, PHEA, PVA-PHEA and PVA-PHEA-SLP solutions. Solution combinations were chosen to mimic drug-polymers ratios w/v and formulation compositions used in the study (as displayed in Table 18). Clozapine aqueous (pH 7.4) solubility at 25°C resulted to be 0.018 mg/mL. All subsequent clozapine saturated solubility as compared to this value. It was observed that addition of 7.5% w/v PVA increased clozapine saturated solubility 11-fold to 0.21 mg/mL ($p<0.0001$). This increment is due to pH-independent solubility of PVA and its electron density which was shown to improve the solubility of poorly water-soluble drugs (Brough; 2016; J. Lu et al.; 2008).

The analysis of solution containing 2.5% w/v PHEA increased clozapine saturated solubility 5-fold increase to 0.09 mg/mL ($p<0.0001$). Due to the amphiphilic nature of PHEA, it can successfully encapsulate and solubilise hydrophobic drugs such as clozapine through micelles formation. Particularly, PHEA has a critical aggregation concentration at about 0.037 mg/mL, and in present work, it was used at higher concentration (250 mg/mL) (Andrews and Jones; 2014; G. et al. Cavallaro; 2004; Gennara Cavallaro et al.; 2003; Civiale; 2009; Triolo; 2017). At concentration above 0.037 mg/mL PHEA forms micelles that are well-known as a pharmaceutical strategy to improve drug solubility (Duan et al.; 2011). PHEA chemical structure is fundamental in carrying hydrophobic drug as clozapine because as it presents a hydrophobic core to entrap drug and hydrophilic shell able to interact with the aqueous environment of the human body (Torchilin; 2006). Clozapine saturated solubility in a PVA-PHEA solution increased 19-fold to 0.35 mg/mL ($p<0.0001$). Interestingly, increasing PHEA concentration to 5% w/v in combination with PVA further increased clozapine saturated to 0.20 mg/mL ($p=0.0007$). Addition of SLP (2.5% w/v) to PHEA (2.5% w/v)-PVA clozapine saturated solubility increased 8-fold compared to clozapine in pH 7.4 ($p<0.0001$). Increasing PHEA and SLP concentration i.e. PHEA (5% w/v)-PVA-SLP (2.5% w/v) further increased clozapine saturated solubility 13-fold ($p<0.0001$) to 0.23 mg/mL. These findings confirm that PVA, PHEA and SLP combinations overall increase clozapine saturated solubility.

3.5 Drug loading and In-vitro dissolution studies

At first, drug loading and drug encapsulation efficacy were calculated to allow clozapine quantification during dissolution studies. Each formulation was prepared with a theoretical concentration of 10% of clozapine. Through drug loading study, it was possible to calculate that all formulation present ~12.53 ± 0.62 %; therefore, 100% of encapsulation efficacy was ensured. Table 19 summarised each drug loading value.

Table 19. Drug loading expressed in percentage (%) obtained in each formulation prepared both as a physical mixture and as the formulated film. The expected drug loading was 10%.

A	**B**	**C**	**D**	**E**
11.19 ± 0.55 %	7.83 ± 0.39 %	9.56 ± 0.47 %	9.15 ± 0.45 %	8.85 ± 0.44 %
F1	**F2**	**F3**	**F4**	**F5**
12.06 ± 0.60 %	6.11 ± 0.30 %	25.99 ± 1.29 %	21.11 ± 1.05 %	8.90 ± 0.67 %
F6	**F7**	**F8**	**F9**	
14.62 ± 0.73 %	21.52 ± 1.07 %	7.99 ± 0.39 %	10.56 ± 0.52 %	

In-vitro dissolution studies comparing unformulated clozapine to clozapine-polymers PM (Table 18) were conducted in non-sink conditions. The experiment was conducted under the non-sink condition to allow the creation of a supersaturated condition; strategy usually used when analysing poorly soluble drugs BCS class II (i.e. indomethacin and itraconazole) (Augustijns and Brewster; 2012; Liu et al.; 2013; Sun et al.; 2016). Figure 44 illustrates *in-vitro* dissolution profile of unformulated clozapine and PM. All PM except from E (containing 7.5% w/v PVA, 5%w/v PHEA, 2.5%w/v SLP and 10% w/v clozapine) had increased clozapine dissolution profile. Mainly, *f2* similarity factor was studied to investigate differences in dissolution profile of clozapine and each of PM and reported in Table 20. Comparing clozapine release profile with PM, it was noticed an *f2* below 50 for PM C (containing 7.5% w/v PVA, 5% w/v PHEA and 10% w/v clozapine) and E implying diversity in release respect to the unformulated drug. *f2* is a calculation approved by the FDA policy to detect alteration of more than 10% in the release profile of approved drugs (Shah et al.; 1999). Particularly values above 50 are considered equivalent to control pathway, while below are deemed different, and lowest value most significant difference in profile (Dubois and Ford; 1985; Shah; 1999). Analysing release mg/mL at time points 15, 30 and 45 min, considered of importance in oral formulations (Hermans et al.; 2017; Rams-Baron et al.; 2018), it was noticed that C and E achieved a constant release of drug from of 0.005 ± 0.0006 mg/mL and 0.005 ± 0.001 mg/mL, respectively. These time points are fundamental, according to the FDA guideline, as 75% of drug release within the first 45 minutes is considered as an immediate release. Both FDA and the International Pharmaceutical Federation established that the dissolution study should mimic the in-vivo condition and for dosage form applied in the oral cavity this implicates resembling the intraluminal environment that the drug passes through during administration. Such an environment is not considered achieved after 45 min in the oral cavity (Hermans; 2017; Marroum; 2012).

Overall C and E exhibited a constant at every time point, suggesting the use of PHEA at 5% w/v possesses an effect of controlling release profile. While PMA (containing 7.5% w/v PVA and 10% w/v clozapine), B (containing 7.5% w/v PVA, 2.5% w/v PHEA and 10% w/v clozapine), and D (containing 7.5% w/v PVA, 2.5% w/v PHEA, 2.5% w/v SLP and 10% w/v clozapine) displayed similar pathways with clozapine. Such effect could be due to a concentration of PHEA of 2.5% w/v, while at its highest concentration (of 5% w/v) it is due to the controlled release of clozapine. Such potential is a novelty in the field of treatment treatment-

resistant schizophrenia as only formulations available of clozapine are immediate-release oral dosage forms (Abeelen; 2013; Bachmann et al.; 2017; D. M. Taylor; 2017).

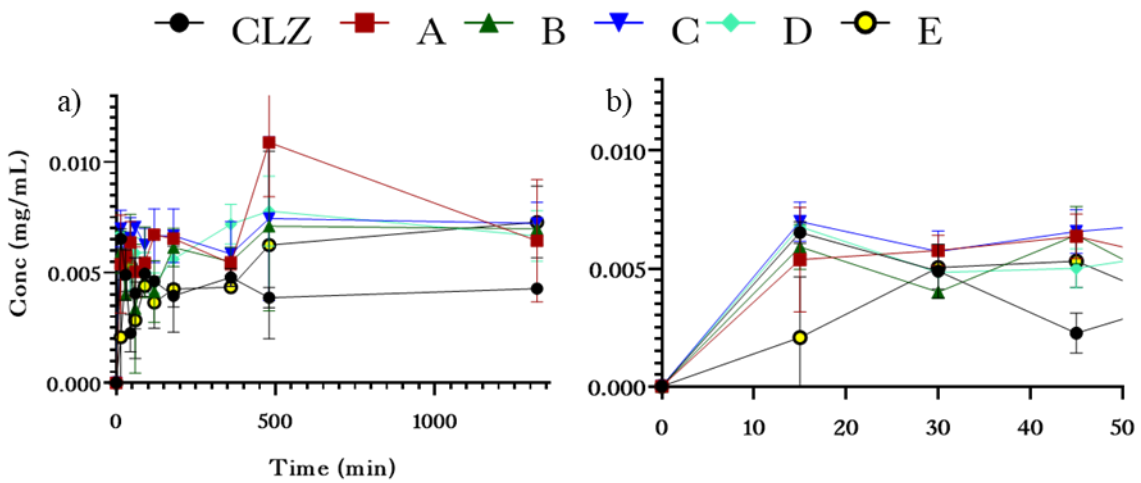


Figure 44. In-vitro dissolution study of PM (drug-polymer ratio in table 18) and clozapine pure drug express in mg/mL. The release profile was evaluated in non-sink condition and experiment conducted for 24 hours (1440 minutes). Graph b) a zoomed-in of first 50 minutes of release, allows a better understanding of release pathway from each formulation.

Table 20. Similarity factor f2 per each PM.

	f2
A	52.28
B	59.62
C	45.89
D	77.73
E	40.46

The results obtained from PM dissolution studies were used as a guide of understanding behaviour of clozapine once released from formulation prepared as nanofibres and film, graph reported in Figure 45. Therefore, *in-vitro* dissolution studies revealed different release profiles between formulations and pure drugs. Such differences could be noticed, using *f2* and time points till 45 minutes, between solvent casted film and fibres produced with same compound ratios. Similarity factor reported in Table 21. At first it was noticed that F1 and F2 both containing PVA 7.5% w/v and 10% w/v clozapine, and F6 and F7 (containing 7.5% w/v PVA, 2.5% w/v PHEA, 2.5% w/v SLP and 10% w/v clozapine) have a f2 below 50, resulting of releasing clozapine with equivalent pathway as if it was not formulated. From another hand side, F3 and F4 (containing PVA 7.5% w/v and PHEA 2.5% w/v and 10% w/v clozapine) presented an *f2* of 33.18 and 30.67, respectively, achieving an immediate release of the drug. This data is coherent with finding, discussed in the previous paragraph, that combination of PVA 7.5% w/v and PHEA 2.5% w/v caused an increase of clozapine saturation solubility of 19-fold. This result was not the purpose of present work that intended to create a controlled release delivery system.

The 45-minute time point is an important reference time point in an oral formulation, a slight increase in release profile could be seen for F4 with 0.007 ± 0.0001 mg/mL release, higher than what observed for F3 0.006 ± 0.0006 mg/mL. This overall enhancement of 1.16-fold (p=0.0034) relates to the higher surface area available in nanofibre formulation (F4) compared to solvent cast film (F3) (Deng et al.; 2018). F5 (containing 7.5% w/v PVA, 5% w/v PHEA and 10% w/v clozapine), F8 and F9 (containing 7.5% w/v PVA, 5% w/v PHEA, 2.5% SLP and 10% w/v clozapine), shows a *f2* value of 28.81, 26.00 and 31.39, respectively. Interestingly, all these formulations present PHEA at 5% w/v and most constant and prolonged release of clozapine from the patch. F5 has been identified as solid nanocrystalline dispersion. It presents a more constant release profile over time with an overall release of 0.0007 ± 0.00008 mg/mL/min along with all study. From the other hand side, film F8 contains the drug in amorphous conformation, and profile presents a burst in the release, as observed as well in formulation F9. However, F8 did not exhibit a constant trend after 45 minutes while F9 achieves this. As expected from the pre-formulation study on PM C and E, correlate formulations F5 and F9, showed a constant trend overall experiment, achieving a modified and prolonged release.

In-vitro dissolution studies presented different release profiles between formulations and pure drugs. Such differences could be noticed between solvent casted film and fibres produced with the same compound ratios. In particular, it was seen that as the drug is present in crystalline state release profile is more constant over time (F5), while when the drug is in amorphous conformation profile presents a burst in the release, as formulation F9. Nanofibre F9 offers the advantage of a higher release rate 0.008 ± 0.0009 mg/mL/min compared with F5, a 1.14-fold increase ($p<0.0001$) due to the upper surface area available from in electrospun fibres and to the addition of 2.5% w/v SLP to the formulation. These results are coherent with the solubilising effect of SLP that was proved and discussed in the polymer solubility section of the present work.

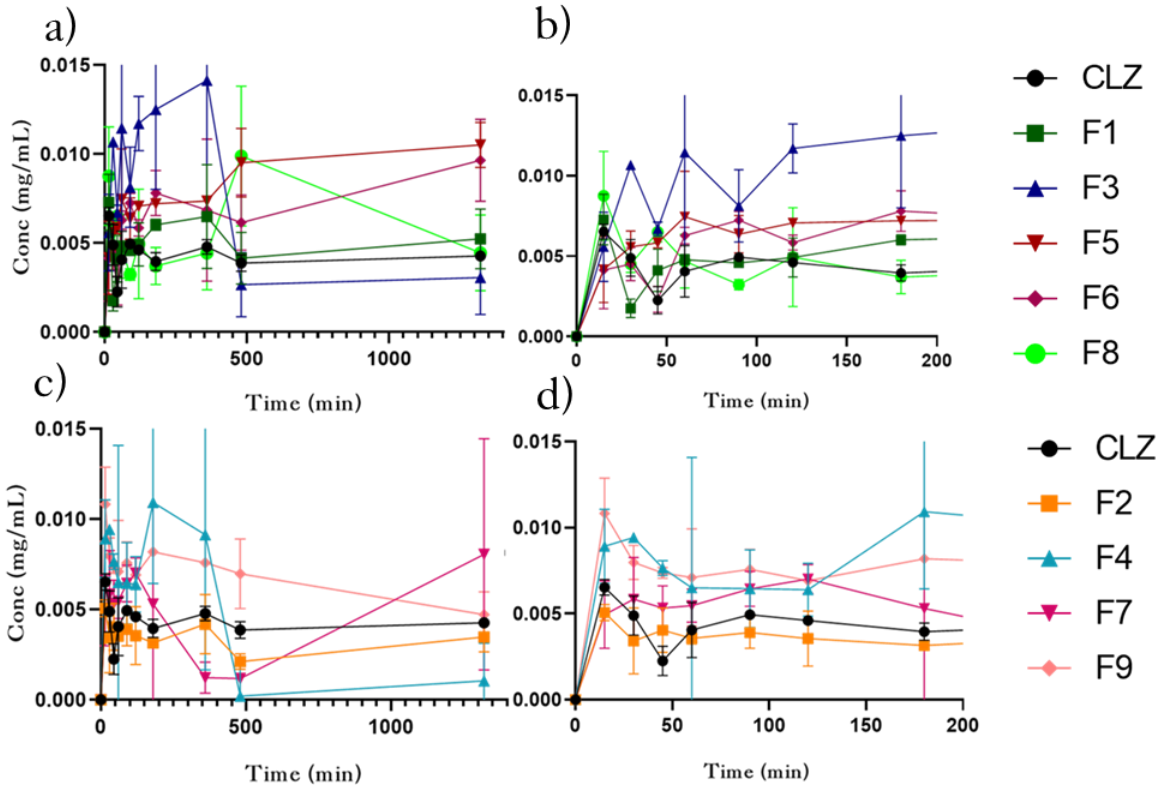


Figure 45. In-vitro dissolution study of formulations prepared by solvent casting method (F1, F3, F5, F6, F8) and electrospinning (F2, F4, F7, F9) compared with clozapine pure drug express in mg/mL. Graphs b) and d) represent a zoomed-in of first 50 minutes of release.

Table 21. *f2* similarity factor for each formulation.

	f2
F1	58.81
F2	50.62
F3	33.18
F4	30.67
F5	28.81
F6	84.78
F7	73.4
F8	26.00
F9	31.39

4. Conclusion

In conclusion, this study developed a new oral transmucosal polymeric patch containing clozapine aimed to reduce drug daily dosage to treat treatment-resistant schizophrenia. In particular, the effect of varying concentrations of polymer PHEA on drug solubility, patch adhesiveness and dissolution performance were tested. Therefore, it was possible to conclude that PHEA possesses a solubility enhancement effect and that achieved higher solubility enhancement potential when mixed with 7.5% w/v of PVA. PHEA possesses a

concentration dependant effect on adhesiveness providing most top adhesive forces when in a mixture of 7.5% w/v of PVA and 5% w/v of PHEA. Such polymer combination also provided control in clozapine release resulting in a potential advantage in terms of patient acceptability and compliance in this demographic group. However, patient compliance is well-known to be affected by drug taste. Transmucosal patch here produced has the potential to release a small amount of drug in the oral cavity and cause alteration in taste perception. Therefore, it is considered fundamental to assess the taste of clozapine to investigate if drug release in the mouth could influence negatively patient compliance when the patch is scaled up and progress in further formulation for human production. Moreover, patch production toward customisable technique should be evaluated for optimal patient compliance.

Chapter 5. 3D Printing Technology to Produce a Novel Polymeric Patch for Oral Controlled Release of Clozapine: An Investigation into the Effect of Infill Percentage on Clozapine Release Profile from Patches with a Surface Area of 4cm ^{2}

1. Background

Chapter 4 demonstrated that formulating clozapine as a transmucosal patch achieved a prolonged drug release and a potential advantage in improving patient compliance towards medication. The main advantage of the use of electrospinning in oral drug delivery is to create a nanofibrous oral dosage form with a high surface area which allows a high dissolution rate. However, the method presents difficulties in producing customisable 3D structure as fibres deposition is most commonly achieved using a cylindric structure (collector) which low level of product customisation (Nune et al.; 2017; S. Sahoo et al.; 2009). While specific collector has to be produced on purpose, 3D printed technology allows for shape and size modification by designing the appropriate CAD file.

3D printing is a manufacturing method to produce 3D structured, which is used in pharmaceutics for drug delivery is a new and developing trend(Trenfield et al.; 2018). 3D printing allows flexibility in dosage form shape, dimension and design, which represent advantageous when formulating patient-centric formulation(Rui et al.; 2016). In recent years such flexibility of using 3D printing in pharmaceutics is under investigation, and a range of 3D printed structures have been designed to study drugs release profile(Goyanes, Wang, et al.; 2015; Khaled et al.; 2015a; 2015b).

Moreover, 3D printing provides chances for scale-up by printing multiple devices/dosage forms in the same operation; and its use in the clinical environment is under evaluation due to smooth operation process of designing, developing and dispensing(FabRx; 2020; Ku and Dulin; 2012). This technique allows dosages modification by physically altering design shapes, dimensions or infill percentage (Goyanes et al.; 2014). Mainly, the infill percentage (infill %) is a printing parameter that indicates the quantity of total volume of thermoplastic polymer that filled the final product during printing as a percentage. When the total amount is printed, this is referred to as 100% infill. Depending on the 3D printer used, reduction in printed volume could be select between 100 and 0%.

This work aimed to use 3D printing manufacturing technology to produce a transmucosal patch capable of prolonged delivery of clozapine. 3D printing is advantageous when creating intricate 3D structures, and in this part of this thesis, it was used to print micro-pyramids on top of flat patches. Effect of different infill % and differential geometry to dissolution performance were evaluated. Physical properties of each patch produced were analysed to ensure reproducibility. Patches have final goals to allow systemic and prolonged administration of clozapine, a formulation that does not exist in the market yet. In Chapter 4, it was

demonstrated that PVA possesses adhesive force. Therefore, it was used as a based polymer to ensure adhesion to the buccal mucosa. Moreover, a set of micro-pyramids were printed on a patch surface to ensure membrane penetration and modify the release profile. Release properties of the drug from patch were analysed *ex-vivo* and through permeation study.

2. Material and Method

Clozapine was kindly provided from NHS Maudsley Hospital. RS PRO 1.75mm Natural PVA 3D Printer Filament, 500g and RS PRO 1.75mm green PLA 3D Printer Filament, 500g were purchased by RS UK. PBS with pH 7.4 and with pH 6.8, EtOH were purchased from Sigma-Aldrich. Water used was distilled.

2.1 Sample preparation

Eight different samples were printed by UP2 mini 3D printer and designed via software online TinkerCad producing a 3D CAD to be exported as STL file. Various models were designed to create the most efficient patch in term of prolonged-release and best permeation profile. Figure 46 showed the designed CAD file. At first, each patch was printed to obtain a 4cm ^{2} area with a dimension of 2cm x 2 cm x 0.5 cm (length, width, and thickness). As the aim of the device is to have a strong adhesiveness to buccal mucosa as well to ensure the release of clozapine just through the mucosa and not in oral cavity patches present different designs with a total of 4 different patches. Patches were provided with a set of micro-pyramids (0.2 cm x 0.1 cm width and radius) to control the release of drug towards to mucosa while to prevent release in oral cavities patches were provided with an impermeable backing membrane. PLA was used as a hydrophobic is not soluble in the aqueous environment for blacking layer creation.

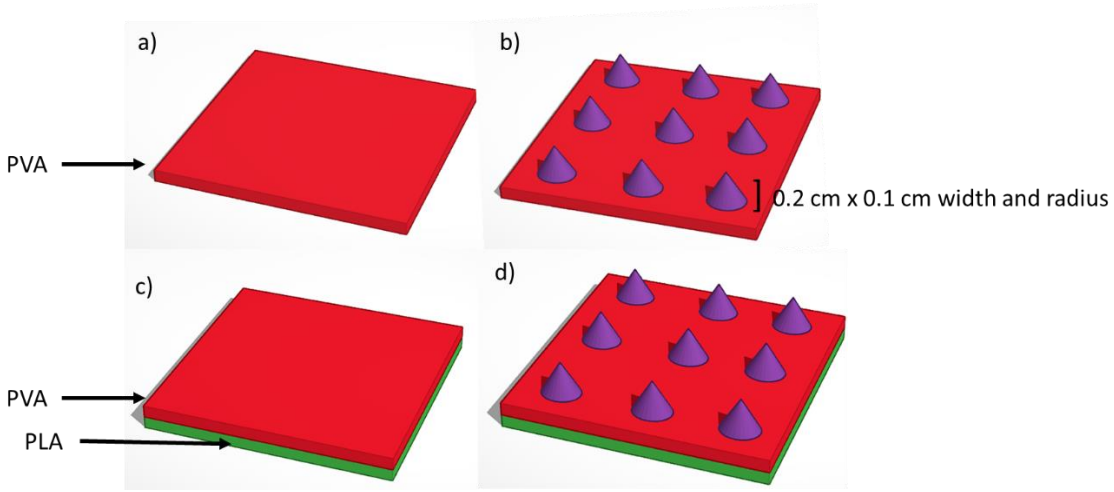
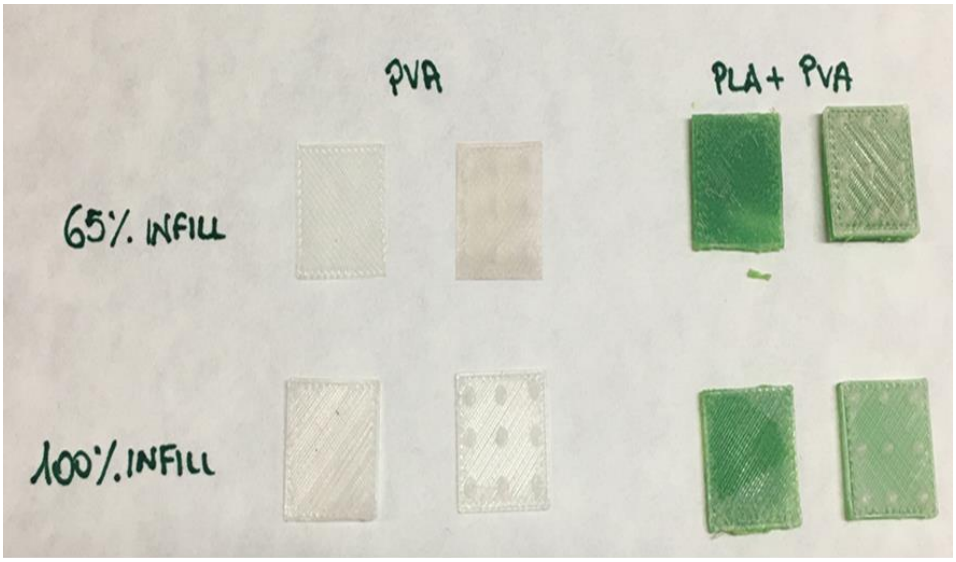


Figure 46. CAD design drafted via TinkerCad of four different patches with dimension 2cm x 2 cm x 0.5 cm: a) flat patch design to be made of PVA; b) PVA-based patch with nine micro-pyramids on top of surfaces with 0.2 cm x 0.1 cm width and radius; c) and d) corresponded to design a) and b) with an extra layer of PLA to ensure a one-way drug release.

The samples differ in infill used while printing 65% and 100%, creating a set of 8 different samples, as shown in Figure 47 and samples were named as reported in Table 22.

Table 22. 3D printed patches names according to their designs

Polymer/s	Infill Percentage 65%	100%
PVA	PVA Patch 65%	PVA Patch 100%
	PVA Patch with Micro-pyramid 65%	PVA Patch with Micro-pyramid 100%
PVA and PLA as a backing layer	PLA + PVA Patch 65%	PLA + PVA Patch 100%
	PLA +PVA Patch with Micro-pyramid 65%	PLA +PVA Patch with Micro-pyramid 100%



Transversal section of the 3D printed patch

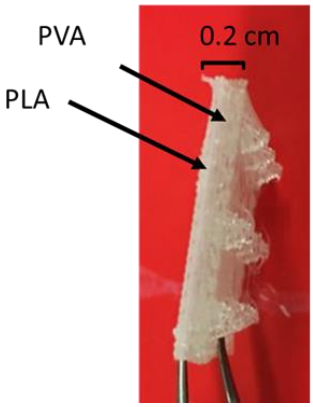


Figure 47. On top printed designs obtained with 65% infill and 100% infill. At the bottom, a transversal image of a 3D printed patch with PLA backing layer and micro-pyramids. Overall height of 0.2 cm.

The patches were printed using an UpMini 3D printer using nozzle size of 200µm and filament thickness of 200µm, 190°C as nozzle temperature and 40°C as bed temperature when printing with PVA filament. New polymer (PLA) was loaded and was printed using 230°C as nozzle temperature and 50°C as bed temperature.

2.2 Drug loading of 3D printed patches

A 10% w/w clozapine solution was prepared by dissolving clozapine in pre-heated EtOH at 50°C (ethanol boiling point 78.73°C). Clozapine is soluble in EtOH (30mg/mL), and according to with first law of thermodynamics once to a system is applied heat, it is transformed in kinetic energies improving drug aggregates detachment in solubilised molecules (Struchtrup; 2014). The solution was left under vigorous agitation at 800rpm, 50°C for two hours in a Thermo Mix with heating, provided by SciQuip UK. Then impregnation method(Chew; 2019) was modified, and 500ul of this 10% w/w clozapine solution (corresponding to 5 mg of clozapine) was soaked into the printed patch and left under a fume hood for EtOH complete evaporation overnight. In a conventional impregnation method, drug solution is left overnight, and the solvent evaporates in a second drying step(Goyanes; 2015; Goyanes et al.; 2017). This method is applied when the drug is expected to have a low drug loading (<2% w/w) in the polymer of selection (Tagami et al.; 2017; Tao et al.; 2016). In Chapter 4, it was that clozapine loading in PVA matrix (formulation A, F1 and F2) is 9.79 ± 0.48% w/w. Therefore, soaking and evaporating steps were conducted simultaneously in this work. Figure 48 shows a patch after soaking.

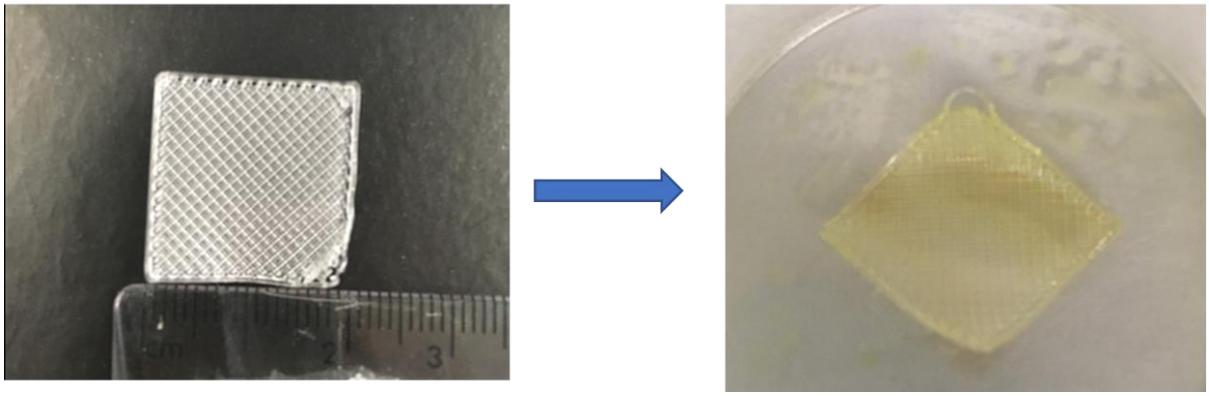


Figure 48. Image of a 4cm ^{2} patch before being soaked with clozapine solution and after soaking and drying process. Pure drug presents an intense yellow colour. Thus, drug impregnation could be visually detected. In the image, the patch was infused with 5mg of clozapine.

2.3 Physical Properties

The physical properties of each patch, in term of weight, dimensions, volume (V), surface area (Sa) and density were analysed to assess 3D printing consistency in formulations preparations. Analysis of this parameter is fundamental to understand the dissolution profile of each printed product as it is proved that geometry and dimension effect drug release(Goyanes; 2015). Information was obtained after optical support and analysed through software ImageJ. Each patch was aligned with a ruler; a camera was focused at 90°C (angle) and placed equidistant from the sample to avoid prospective alteration. Each reading was analysed in triplicates. Patches presented both a cuboid shape combined with cone shape for micro-pyramids formation. Therefore, V and Sa were calculated accordingly:

Cuboid

$$V = \text{length } (l) \times \text{width } (w) \times \text{height } (h) \qquad \text{Equation 9}$$

$$Sa = 2lw + 2lh + 2hw \qquad \text{Equation 10}$$

Cone

$$V = 1/3\pi r^2 h \qquad \text{Equation 11}$$

$$Sa = \pi ra + \pi r^2 \qquad \text{Equation 12}$$

When calculating Sa of the patch with micro-pyramids, micro-pyramids Sa was added to patch Sa. When PLA backing was applied, Sa available to drug release was reduced by subtraction of PLA Sa.

2.4 SEM

Hitachi S5000 Emission Gun (FEG) with Tungsten Tip (25 kV) was used to examine gold-coated (10 nm thickness) blank full-size PVA. Images were captured using a secondary electron detector from X70 to X10.9K magnification. The *d* of microparticles was determined from the mean value of 100 measurements using ImageJ (the USA, version 1.46 v).

2.5 ATR-FTIR

Studies carried out to identify main drug peaks after soaking patches. The analysis was conducted using a Perkin Elmer Frontier ATR-FTIR spectrometer, and samples were placed on crystal for characterisation. A total of 264 scans were taken at a resolution of 2 cm-1 and using a wavelength range of 4000-600cm-1. Each sample has been analysed in triplicate.

2.6 Drug content

The drug-loaded patches were dissolved in 10 mL of EtOH, filtered through a 0.45 mm syringe filters with cellulose acetate membrane (VWR International, USA) and drug content of each sample was determined spectrophotometrically. UV-VIS spectra were recorded by Perkin Elmer Lambda UV/VIS Spectrophotometer. A calibration curve was used for quantification of drug content. The calibration curve was performed in the concentration range of 0.1-0.0001 mg/mL of clozapine standard solutions in EtOH (R=0.999) and spectra recorded at 257 nm.

2.7 In-vitro Dissolution Studies

The studies were carried out in non-sink condition in 25 mL of PBS pH 7.4 to mimic in-vivo buccal environment as reported in European Pharmacopeia and literature evidence(European Pharmacopeia 7.6; 2012; Sun; 2016). In-vitro dissolutions studies were conducted using a shaking incubator (SciQuip Ltd) at 37°C, and 50 rpm. Samples were weighed and placed in 25 mL media to obtain 5 mg of clozapine. At predetermined intervals of 15, 30, 45, 60, 90, 120, 180, 360, 480 and 1440 minutes 500µL was withdrawn and diluted in 5mL and filtered through a 0.45 µm cellulose acetate syringe filter and replaced with the same amount of fresh buffer. Subsequently, the filtrate was analysed spectrophotometrically at UV with a wavelength of 257 nm. A calibration curve was performed in a concentration range of 0.1-0.0001 mg/mL of clozapine standard solutions in PBS pH 7.4 (R=0.999) and spectra recorded at 257 nm.

2.8 Statistical Analysis

The *f2* Equation, calculated according to Equation 1 (Chapter 3), was used to compare *in-vitro* dissolution performance between drug and corresponding formulations(Raimi-Abraham; 2015). When *f2* below 50, profiles are considered different, and when *f2* above50 compared release profiles are considered equivalent.

The two-way analysis of variance (ANOVA) was applied to compare different samples. Data were considered statistically significant with a value of $p < 0.05$.

3. Results and Discussion

At the first patch of $4cm^2$ were printed with four different designs and two different infill percentage (65 and 100%). Patches were characterised in term of physical properties to evaluate the reproducibility of the printing process and consistency with designed CAD files. Then, it was studied the influence of material properties and infill percentage on the dissolution profile of clozapine. Patches CAD files were created with a

surface area of 4cm^2, half size of conventional oral nicotine patch NiQuitin(Ltd.; 2018) (4cm x 4cm), with the aim of further investigation on inner cheek surface.

3.1 Physical properties of 3D printed patches

Physical properties of printed patches were analysed to identify printing reproducibility in terms of weight and dimensions. Further analysis of volume and surface area ratio was conducted to investigate the effect of those parameters on both dissolution and permeation. Physical properties investigations were conducted, as showed in Figure 49.

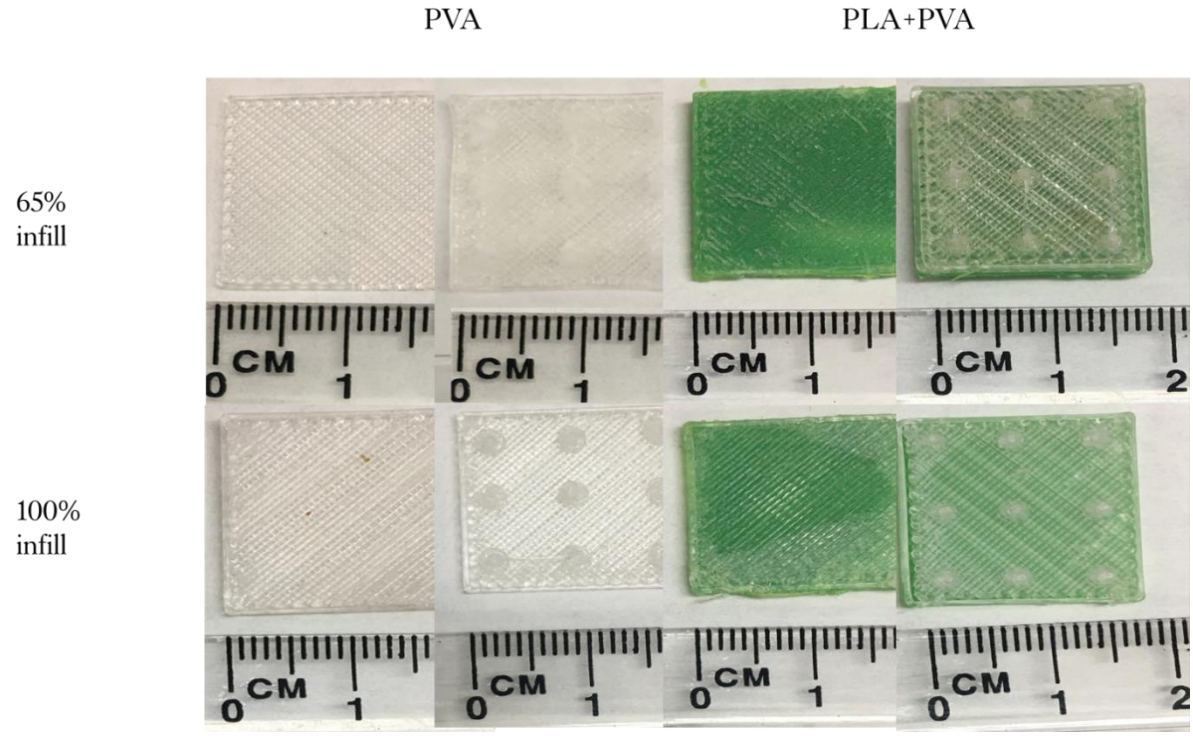


Figure 49. Visual representation of physical properties' assessment and accuracy. Each patch was aligned with a ruler starting at 0cm, and a camera was applied equidistance from each patch.

Table 23 summarises weight, V, Sa, Sa/V ratio and density for each patch design printed both using 65% and 100% infill. Analysis of physical properties was fundamental in the understanding of process reproducibility. Therefore, the calculation of the standard deviation was a key element. As reported in the Table 23, each weight measurement confirmed to have a relatively low standard deviation with a percentage error minimum of 0.05% ($p = 0.02$) in weight for PVA Patch 65%, and a maximum error of 6.67% ($p < 0.0001$) for patch PLA +PVA Patch with micro-pyramid 100%. FDM has the advantage to prepare 3D complex geometrical parts neatly, and such was used as a technology to develop a pancreatic patch in 2016(Yi et al.; 2016). In this study, weight consistency was fundamental to ensure encapsulation and release of the anticancer drug (5-Fluoruracil). Accordingly, FDM was recognised to provide high reproducibility in weight, and similar findings

were noted by Goyanes and co-workers (Goyanes et al.; 2016), and Skowyra and co-workers(Skowyra et al.; 2015). It was then observed that printing of an extra layer of PLA increased the overall weight of 1.65-fold (p=0.0012) in patches printed with 65% infill and of 1.70-fold (p <0.0001) increase when infill was 100%. Slight difference in weight increase depends on different infill used as it determines the amount of material packed into a product designed(L. K. Prasad and Smyth; 2016). Effect of infill in increasing overall weight was also evaluated when adding micro-pyramids on top patch. This caused an increase in weight of 1-fold (p=0.0013) in patches with 65% infill and of 1.20-fold (p=0.0034) with 100%. Overall, considering an average weight of 65% infill of 569.25 ± 15.21 mg and 100% infill of 620.25 ± 32.76 mg, it was assessed that using a high infill ratio there was an increase in weight of 1.08-fold with a statistical significance of p<0.0001.

Table 23. Summary of weight, V, Sa, Sa/V ratio and density for each patch design printed both using 65% and 100% infill.

	Weight mg	Length cm		V/Sa			
		Length	Thickness	V (cm^3)	Sa (cm^2)	Sa/V	Density (g/cm^3)
PVA Patch 65%	430.12 ± 0.05	1.54 ± 0.10	0.21 ± 0.002	0.51	6.06	11.85	0.83
PLA + PVA Patch 65%	706.30 ± 28.28	1.63 ± 0.10	0.25 ± 0.032	0.67	0.82	9.83	1.05
PVA Patch with micro-pyramid 65%	432.50 ± 4.33	1.60 ± 0.17	0.52 ± 0.171	0.18	12.87	71.44	2.4
PLA +PVA Patch with micro-pyramid 65%	706.21 ± 28.18	1.63 ± 0.10	0.35 ± 0.001	1	1.39	7.48	0.85
	Weights mg	Length cm		V/Sa			
		Length	Thickness	V (cm^3)	Sa (cm^2)	Sa/V	Density (g/cm^3)
PVA Patch 100%	439.62 ± 8.95	1.66 ± 0.10	0.10 ± 0.009	0.27	6.19	22.4	1.63
PLA + PVA Patch 100%	700.15 ± 42.43	1.62 ± 0.17	0.25 ± 0.021	0.66	0.81	9.84	1.05
PVA Patch with micro-pyramid 100%	496.11 ± 26.22	1.60 ± 0.10	0.17 ± 0.007	0.25	7.42	11.66	1.79
PLA +PVA Patch with micro-pyramid 100%	846.10 ± 53.47	1.79 ± 0.10	0.35 ± 0.068	1.13	1.89	7.38	0.74

The length of patches was constant for all eight different designs with an average value of 1.63 ± 0.07cm. The patch was designed to be of 4cm ^{2}, and CAD file was produced creating sides of 2cm. However, it is proved that the quality of printing depends on CAD file produced and on STL file exported definition. It was overall observed that best quality and reproducibility of drafted designed could be obtained by using software designed for specific printed(TS. Srivatsan, T.S. Sudarshan; 2018). However, in this study, the software was not user friendly for the design of complex shape as a bi-layered micro-pyramidal patch.

Regarding micro-pyramids, those were pyramidal-shaped structures printed on top of flat PVA patch. Micro-pyramids have a scope of penetrating the buccal mucosa to control the release of clozapine towards to mucosa. A different study on skin penetration showed that application of micro-needle between 70 µm and 200 mm in length improved permeation and adhesion in a patch like structure(Coulman et al.; 2009; Gomaa et al.; 2012). Micro-needles use to present a sharper design to penetrate thick layers of skin dermal(Al-Qallaf and Das; 2009). However, buccal mucosa is more delicate and permeable and less thick compared with skin cutaneous (Galey et al.; 1976; Turabelidze et al.; 2014); in this work, the design was modified to obtain a pyramidal shape proportioning both radius and width at 200mm. They were analysed by SEM to allow precise calculation in a small size range. Images obtained were analysed with Software ImageJ, and each measurement is taken a hundred times. Figure 50 showed micro-pyramids for both patches printed at 65 an 100% infill. In the image, it is possible to notice each layer deposition during the printing process and set from printing parameter to be of 200 µm.

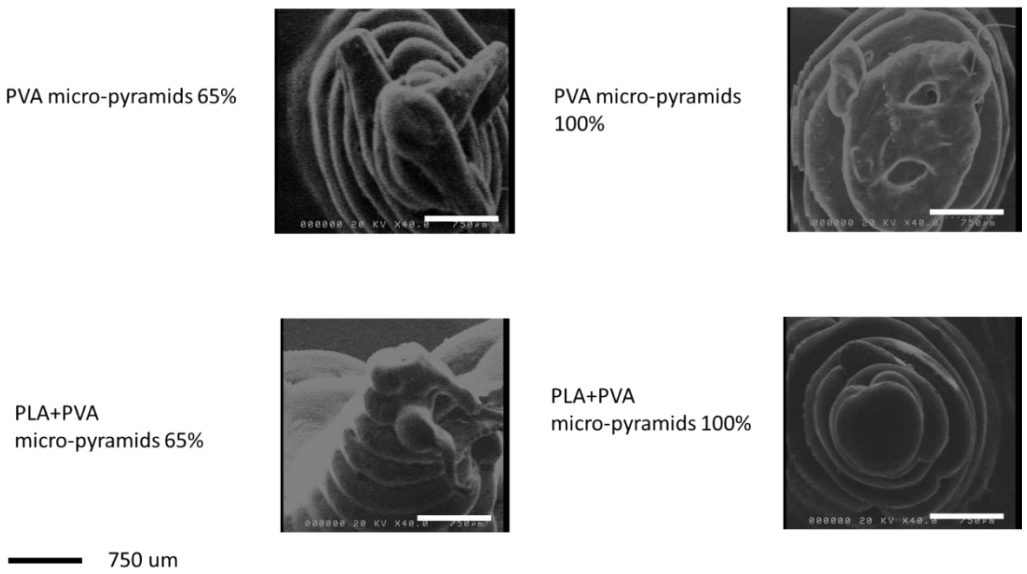


Figure 50. SEM images of micro-pyramids printed on top of flat PVA surface. Photos were taken at 750um and using 40x magnification. It is possible to notice layer thickness that was set at 200um.

The physical analysis assessed 173.76 ± 2.90 cm of pyramid width and 93.45 ± 7.42 cm of pyramid radius that results in reduction respect designed CAD of 13.87% ($p < 0.0001$) in width and of 10% ($p = 0.004$) in radius. Data reported in Table 24. It was proved that when exporting a CAD design as an STL file, it makes loose

resolution causing a reduction in length of initial design(Chen et al.; 2017). Smaller is design, and higher is lost in resolution(Sachs et al.; 1990). However, no change in the exterior aspect could be visually observed when printing at different infill.

Table 24. Summary of width, radius, V and Sa of micro-pyramids printed on top of the patch.

	Pyramid width mm	Pyramid radius mm	V Pyramid (mm^3)	Sa Pyramid (mm^2)
PVA Patch with micro-pyramid 65%	171.23 ± 19.98	195.09 ± 20.03	2.34 ± 0.01	1.23
PLA + PVA Patch with micro-pyramid 65%	213.87 ± 30.87	70.65 ± 10.45	2.67 ± 0.09	1.38
PVA Patch with micro-pyramid 100%	173.42 ± 0.44	80.12± 0.12	6.22 ± 0.02	1.89
PLA + PVA Patch with micro-pyramid 100%	149.67 ± 0.07	82.34 ± 2.54	1.24 ± 0.01	7.54

The problem of resolution in 3D printing is highly discussed as geometrical flexibility is restricted by typical layer thickness of 200 µm. Thin layers are preferred to achieve a relatively high resolution, but it depends on printer setting(Shirazi et al.; 2015; Will et al.; 2013). Therefore, layer thickness in each printed patch was investigated. At first, it was observed surface of printed patches and then calculated the average of each filament thickness and gap between each filament. Gaps represent distance needed by the printer to allow the creation of 65% and 100% infill(Farzadi et al.; 2014). Figure 51 are SEM images of each patch showing distance between two continuous filaments referred to as “gap”(Greenhall and Raeymaekers; 2017; Zhang et al.; 2016). From images, it is possible to visually observe that using a high infill reduces the gap between filaments. However, it was noticed that when patches were printed both at 65% and 100% with PLA backing, it was more challenging to distinguish between PVA and PLA layer that appears as a uniform solid block. This could be because PVA and PLA filaments were printed with two different temperatures 190 and 230°C, respectively. PLA backing needed a higher temperature of both nozzle and platform, respect PVA printing. This causes an overheating on PVA during printing. Even if it was considered to wait between two steps, it was seen that this induced detachment of two layers and it was therefore avoided. This effect was already discussed by Lopez and co-workers, in 2020, who proved that using PLA in multi-layered printing caused the formation of physical interaction that improved strength and flexibility of printed products(Baca Lopez and Ahmad; 2020). While Erokhin and co-workers, 2019, reported morphological changes as observed in showed images when combining PLA with other polymer printed with a lower nozzle temperature(Erokhin et al.; 2019).

As this part of work was focused on the evaluation of physical properties as tools to assess reproducibility and resolution of the printed patch, filament thickness was calculated by use of software ImageJ applied on SEM images, and results were reported in Table 25. Overall, patches printed

With 65% present filament thickness with an average value of 240.51 ± 53.23 µm, with an increment of 1.2-fold ($p < 0.0001$) respect pre-set parameter of 200 µm. Patches with 100% infill presented a size of 199.36 ± 52.99 µm. The difference with pre-set values is considered related to the impurity of CAD file produced at first instance(Park and Shin; 2018). High-resolution in 3D printing is an interplay between right printing parameters such as temperature, nozzle diameter and distance between pyramid and platform(Serra et al.; 2013).

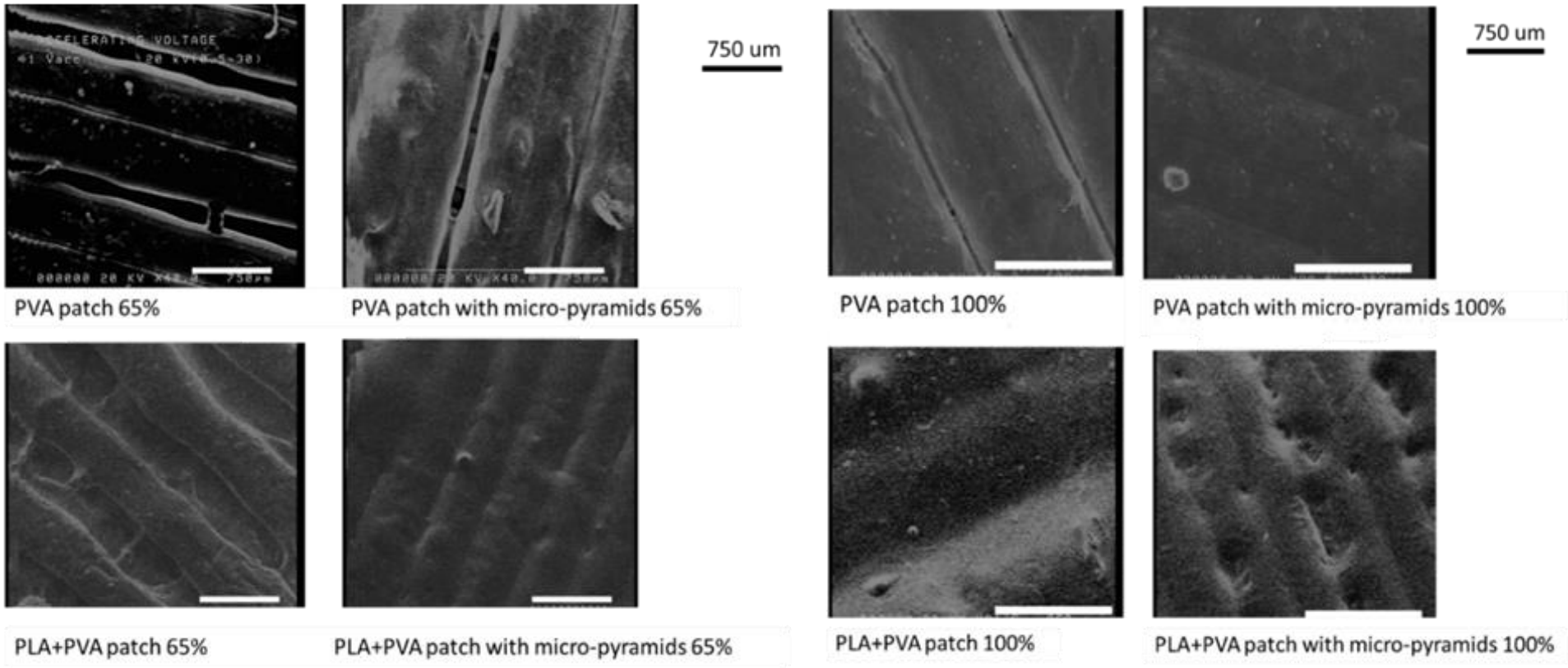


Figure 51. SEM images of patch PVA patch surface when printed with different infill. Images were obtained using 40x magnification and 750 µm as dimension range. The analysis showed that the application of PLA backing caused the formation of a solid molten state, while the use of 100% infill reduced the gap between filaments.

An interesting parameter that was analysed to identify accuracy in printing 65% and 100% of the total designed draft was gap dimension. It was observed that gap average in 65% patches was of 256.25 ± 5.12 µm, and 3.51 ± 1.29 µm in 100% infill patches. Ricotti and co-workers proved that when increasing printing infill, there is a difference in physical properties (as the gap between filaments) of 5% for each 20-percentile infill increment(Ricotti et al.; 2017). Nevertheless, this rule does not apply when comparing data with 100% infill as this printing style implies no cavities and formation of a compact(Goyanes; 2014; Patton et al.; 2019). Therefore, reduction in gap distance of 73% is considered consistent with pre-set printing option and statistically significant $p < 0.0001$.

Table 25. Physical analysis of patch thickness and the gap distance between filaments.

	Patch microscopic analysis µm	
	Patch thickness	Gap
PVA Patch 65%	192.23 ± 70.34	180.81 ± 20.02
PLA + PVA Patch 65%	283.98 ± 3.45	222.01 ± 17.64
PVA Patch with Micro-pyramid 65%	197.76 ± 64.52	303.65 ± 40.76
PLA + PVA Patch with Micro-pyramid 65%	290.23 ± 33.98	323.12 ± 10.32
	Patch thickness	Gap
PVA Patch 100%	212.65 ± 27.09	4.98 ± 13.86
PLA + PVA Patch 100%	237.81 ± 18.23	3.76 ± 0.56
PVA Patch with Micro-pyramid 100%	121.65 ± 11.12	2.82 ± 0.73
PLA + PVA Patch with Micro-pyramid 100%	226.19 ± 35.09	4.87 ± 0.86

3.2 Drug physical properties

ATR-FTIR analysis was used as a tool to identify the presence of hydrogen bonding between clozapine and PVA. Figure 52 shows the spectra overlay of 3D printed patch soaked with the drug compared with the pure drug, which physical-chemical characterisation was provided in Chapter 1. From spectra, it was possible to notice two of the main peaks of clozapine at 1025 and 1357 cm-1 both correlated with C-N stretching vibration. It was possible to see that no hydrogen bonds were created during the formulation process. ATR-FTIR analysis was essential to analyse the particle size of the drug. As shown in Figure 51, in precedent session, it is possible to notice particles on the surface of the patch. As ATR-FTIR is a surface analysis, it is reasonable to consider that clozapine particle cloud has deposited on patch surface during the soaking process. However, no chemical evaluation was conducted, and further analysis would be required.

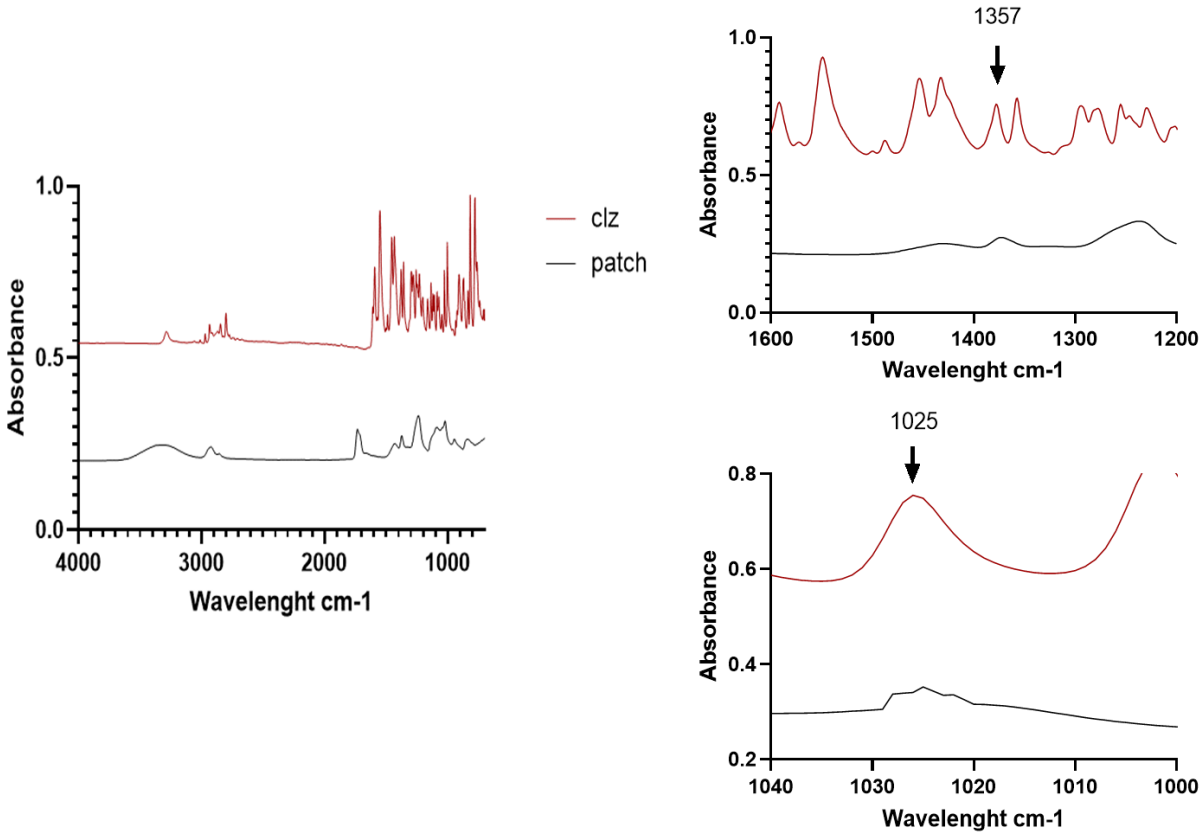


Figure 52. ATR-FTIR spectra recorded from 4000cm-1 to 700 cm-1 of clozapine in red and patch in black. Frequencies were then enlarged to identify main clozapine peaks that were found at 1025cm-1 and 1357cm-1.

Drug particle size was then evaluated using SEM and ImageJ to identify average diameter for each patch. Images reported in Figure 53 were obtained using different magnification due to small particle size in range < 100 µm. Detail of each measurement is reported in Table 26. particles resulted in having an average diameter of 6.93 nm ± 2.91, with a slight increase (doubled size) in particle obtained both in PVA Patch with micro-pyramid 65 and 100%. This small dimension was obtained as the drug was dissolved in EtOH where clozapine is highly soluble, and high agitation and a warm environment allowed total solubilisation of drug insolvent. Information about particle size is essential when designing a dosage form intended to release drug on buccal mucosa and to permeate through the mucosal membrane. Literature reports that that nanoparticle with a diameter lower than 200 nm can penetrate oral mucosa(Holpuch et al.; 2010; Teubl et al.; 2013). For a particle in this size range, it is considered possible a paracellular transport achieving a systemic absorption of the drug and smaller particle size, higher amount of drug permeated through the membrane(Evans et al.; 2011).

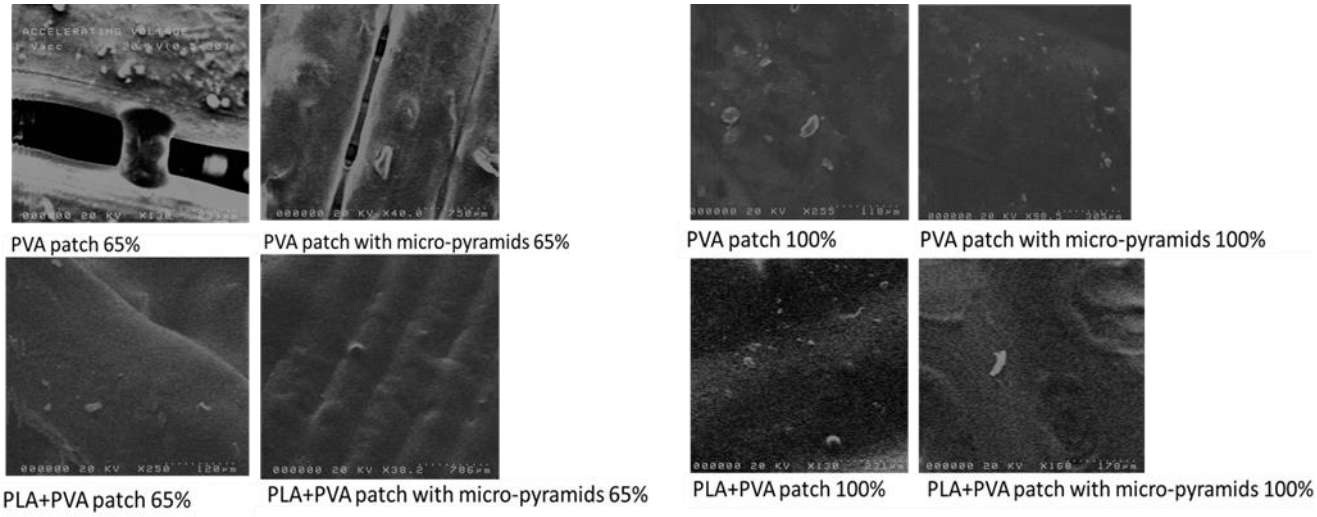


Figure 53. SEM images of all eight different designs focused on clozapine particles in the surface. Images were obtained using from 40x to 250x magnification, and the average particle size was detected to be lower than 20nm.

Table 26. Drug particle size expressed in nm. For each patch, the average particle size was lower than 20 nm.

	Drug Particle Size (nm)
PVA Patch 65%	4.01 ± 10.34
PLA + PVA Patch 65%	7.63 ± 6.08
PVA Patch with micro-pyramid 65%	10.04 ± 9.87
PLA + PVA Patch with micro-pyramid 65%	8.32 ± 2.65
	Drug Particle Size (nm)
PVA Patch 100%	4.06 ± 19.65
PLA + PVA Patch 100%	6.54 ± 5.23
PVA Patch with micro-pyramid 100%	12.01 ± 40.23
PLA + PVA Patch with micro-pyramid 100%	4.56 ± 1.24

3.3 In-vitro dissolution studies: Effect of infill percentage and geometry in clozapine dissolution profile in 4cm ^{2} patches

At first, it was analysed drug content in the patch with 4cm ^{2} surface area to calculate the amount of drug released during dissolution studies. Drug loading was obtained by soaking each sample with 500ul of clozapine-EtOH solution. Drug content analysis showed that each patch contained 5.04 ±.0.55 mg of clozapine, 100% of encapsulation efficacy. This amount of drug was used as proof of concept that different infills and shapes printing could affect the release profile.

4cm ^{2} patches loaded with 5 mg of clozapine were first analysed. In this study, different dissolution rate provided by patch once printed with varying percentages of infill and when PLA backing was applied was

observed. Figure 54 portrays release rate, expressed in mg/mL, of the drug from 65% infill patches. Release mg/mL was analysed and compared using value at time point 45 min, considered of importance in oral formulations for immediate release(Hermans; 2017; Rams-Baron; 2018). Notably, a dosage form is regarded as providing immediate release when more than 75% of the drug is released from the dosage form in 45 minutes, as discussed in Chapter 4(Mitrevska et al.; 2019). It was observed that in each patch within 45 minutes, 64% (3.23 mg ± 0.46) of medication was released and after 3 hours it was achieved 100% of drug release. Focusing on trend provided by 65% infill printed with PVA without micro-pyramids and no PLA backing layer, it was noticed drug was released with a rate of 0.0007 mg/min while application of micro-pyramids improved release to 0.0009 mg/min a 1.42-fold increase (p=0.0034). All patches release rate and *f2* values were reported in Table 27 for clarity. This increase in rate could be due to bigger, 2.12-fold (p<0.0001), surface area (Table 23) by PVA with micro-pyramids 65%, as expected by Fick's law (Frederic P. Miller, Agnes F. Vandome; 2017). Applying PLA backing layer on patch has the purpose of direct and control release in buccal mucosa, therefore in a dissolution study it would reduce surface area available for release reducing overall release profile. In fact in PVA patch with PLA backing layer and no micro-pyramids (sample name PLA + PVA Patch 65%), there is a reduction in release rate to 0.0003 mg/min that is consistent with increase 1.90-fold (p non-significant) in particle size observed compared to PVA patch and with expected reduced rate offered by PLA layer. Most interestingly, in patch PLA + PVA Patch with micro-pyramids 65%, application of PLA reduced release of 3-fold (p<0.0001) respect counterpart patch without micro-pyramids. Observing the *f2* value for this patch (PLA + PVA Patch with micro-pyramids 65%), it was registered the lowest data of 27.38 that represent the biggest difference in release profile.

At the contrary, it was noticed that in patches printed with 100% infill without PLA backing (PVA Patch 100% and PVA Patch with micro-pyramid 100%), reported in Figure 54b, it was achieved a 48% released in (2.35 mg ± 0.49) in 45 min, but a burst in the release is observed at 90 minutes to 75%. It is possible to notice that from minute 90th, the drug is released with a rate of approximately 0.11 mg/mL ± 0.019 that is equivalent to 0.0013 mg/min. Mainly, PVA patch 100%, releases drug with an average rate of 0.005 mg/min, that is 2.51-fold (p=0.003) faster than patch with micro-pyramids with a rate of 0.002 mg/min. Addition of PLA backing causes an overall decrease on the dissolution rate of 0.0007 mg/min for PVA+PLA patch 100% and 0.0002 mg/min observed in PLA+PVA with micro-pyramids 100%. This rate is coherent with the reduction in the surface area noted respectively of 2.39 (p=0.0043) and 3.40-fold (p <0.0001) respect to patches without PLA backing. However, observing the *f2* value, it was noticed that PLA+PVA 100% has a value above 50, considered equivalent to the unformulated clozapine behaviour. Overall, release profile observed for PVA patch 100% and PVA Patch with micro-pyramid 100% was too fast 7.88-fold (p=0.004) and 2.88-fold (p<0.0001), respectively, compared with its 65% counterpart. This effect could be due to higher surface area (Table 23) and smaller

particles size (Table 26) achieved in patch printed with 100% infill. However, after 200 min, all drug was released, and no constant rate was observed.

The objective of this Chapter was to determine the effect of infill on drug release profile to ensure a constant and prolonged release of the drug. Overall, it was observed that patch with 100% infill without PLA backing presented a faster release profile compared with 65% infill counterpart. Addition of PLA backing reduced release rate compared 100% patches without PLA backing, but it doubled release respect 65%. Previous studies, carried out using hydroxypropyl-methylcellulose and PVA filaments, suggested an opposite trend using soluble drugs as a model drug(Goyanes; 2015; Kadry et al.; 2018). However, it is well-known that drug-release could be drug-carried or polymer-carrier.

Moreover, in both patches at 65 and 100%, it was observed that the PLA backing layer reduced, as expected release profile, suggesting an advantage is driving delivery in one-way. Reason for the difference in release between 65% and 100% infill patches it is supported confronting particle dimension and surface area obtained. Overall, 100% infill patch presented a higher surface area and smaller particle size compared with 65% infill.

Finally, the influence of micro-pyramids on release profile was considered. When patches were printed with PLA backing layer, release becomes more persistent, but the addition of micro-pyramids showed a significant trend. Notably, the micro-pyramid improved rate just when added to PVA flat patch 65%, while in all other patches designs and infill percentages, they reduced profile controlling the release. Notably, patches PVA Patch 65%, PLA + PVA Patch 65%, PVA Patch with micro-pyramid 65 and PLA + PVA Patch with micro-pyramid 65% presented a most constant release and improved profile respect to clozapine unformulated and PLA backing layer offered advantage of a more controlled release. Therefore, those patches were reduced in size and loaded with different concentration of clozapine to understand if the release rate is drug-dependant.

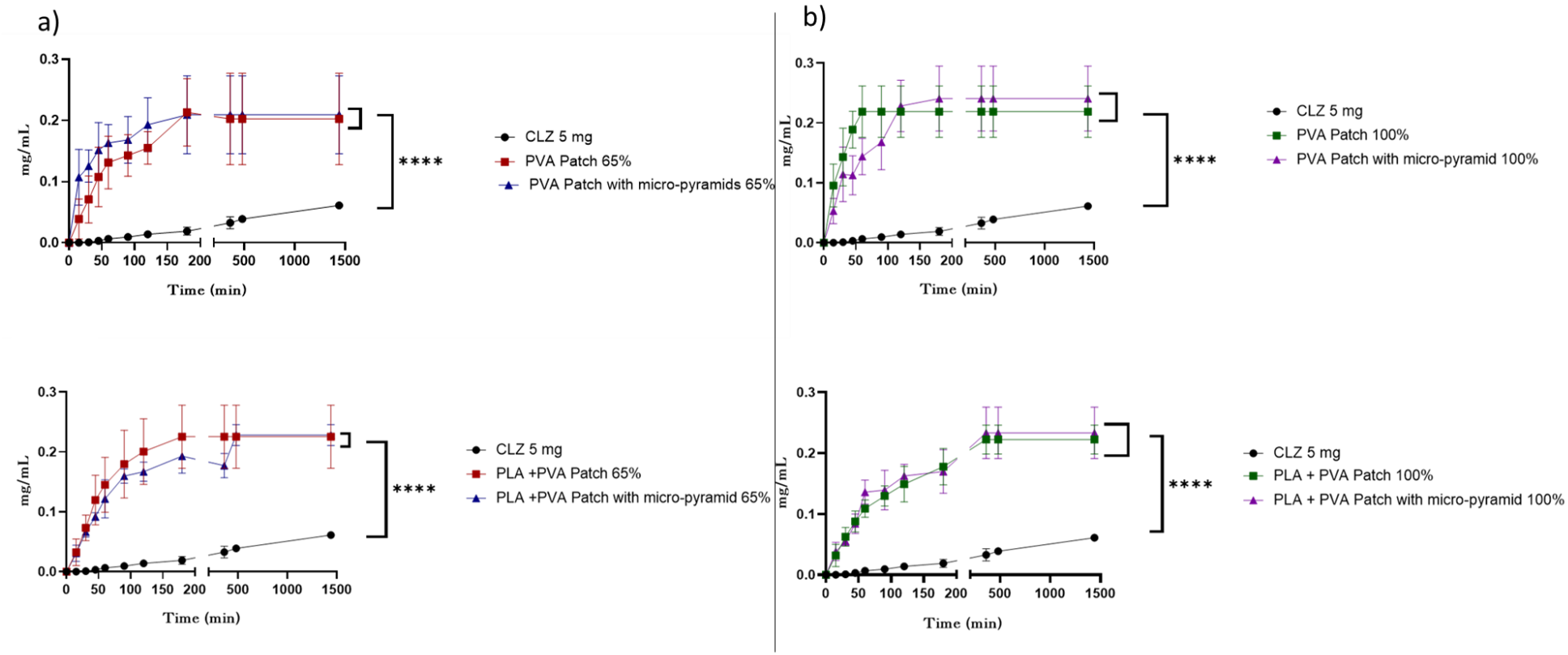


Figure 54. Patches 4cm^2 a) Dissolution profiles of patch printed with 65% infill, on the top patch without PLA backing, on bottom patch with PLA backing. Overall, patch printed with 65% infill showed a constant release and improved dissolution profile respect to clozapine pure drug. b) Dissolution profiles of patch printed with 100% infill, on the top patch without PLA backing, on bottom patch with PLA backing. Overall, patch printed with 100% infill showed an immediate-like release and not constant or prolonged release profile was assessed. All the release profiles compared with pure clozapine presented high statistical significance graphically represented as ** (high statistical relevance).**

Table 27. Dissolution rate expressed in average mg/min per each patch design and the respective f2 values that compare each patch with clozapine unformulated.

	PVA Patch 65%	PLA + PVA Patch 65%	PVA Patch with micro-pyramid 65%	PLA + PVA Patch with micro-pyramid 65%
Ratio	0.0007 mg/min	0.0003 mg/min	0.0009 mg/min	0.0001 mg/min
f2	27.64	34.51	49.81	27.38
	PVA Patch 100%	PLA + PVA Patch 100%	PVA Patch with micro-pyramid 100%	PLA + PVA Patch with micro-pyramid 100%
Ratio	0.005 mg/min	0.0007 mg/min	0.0026 mg/min	0.0002 mg/min
f2	48.89	52.54	47.37	32.95

5. Conclusion

This work aimed to propose 3D printed technology as a method to produce an oral transmucosal patch to prolong the release of clozapine by demonstrating 3D printing reliability and easy management. At first, it was evaluated consistency in physical properties of obtained products. Results show the difference between setting parameters, CAD designs, and final patch due to a reduction in quality of file produced in different software respect printed own software. Interestingly, it was observed that differences regularly occurred in each product and therefore, that 3D printer produced homogenous samples. Then, it was investigated the effect of different infill on clozapine dissolution profile, considering as final aim a prolonged release. Data showed that printing with 65% infill achieved an extended and constant release profile, increase solubility respect pure drug while using 100% profile was immediate and not consistent. Both 65% and 100% infill with PLA backing possessed a major constancy in the release, while the addition of micro-pyramids caused an increase of dissolution profile. Further investigation on clozapine release profile dependence on the drug itself or the carrier should be performed to evaluate the effect of increasing the dose for scale-up industrial manufacturing. Moreover, 3D printing advantage in product customisation should be investigated by monitoring different size effect on clozapine dissolution profile.

Chapter 6. 3D printing technology as technology to scale down the oral patch to 1cm ^{2} surface area: evaluation of clozapine dissolution profile and permeation through membranes.

1. Background

The purpose of this thesis was to tackle reasons for medication non-compliance, thus discontinuation, in a patient affected by treatment-resistant schizophrenia. So far, different methods such as solvent casting method, electrospinning, and 3D printing were proposed to produce alternative dosage form to reduce dose per day, recognised to be a significant issue of non-compliance. However, between all the techniques, 3D printing results flexible to customise and modify dosage form to be adaptable for different patients' needs or preferences (Aquino et al.; 2018). For example, the use of a 3D printer M3DIMAKER™ is on trial in a Central London hospital (name nondisclosed) to design patient-centric shape and size (FabRx; 2019). In the previous section, 4cm ^{2} patches were produced to conduct a broader analysis in term of infill percentage influence on the dissolution profile and in term of reproducibility of the 3D printing process.

Therefore, this thesis section aims to demonstrate the efficiency of 3D printing patch of improving both the dissolution profile and permeation of clozapine by designing a scaled-down version of the previous patch. New patches are designed with a surface area of 1cm ^{2} as dosage forms size is proved to affect patient acceptability toward a medication (Hsu; 2013; Lopez et al.; 2015; Ranmal et al.; 2018). 1cm ^{2} patches were loaded with varying concentrations of clozapine to evaluate at first the effect of drug strength on the release profile itself. It is well-recognised that the mechanism of release could be a carrier- or drug-dependent (Craig; 2002). This information is crucial when considering the scaling-up of a dosage form for commercial purposes. If the mechanism is mediated by the drug, thus by its concentration in the formulation, this would mean that increasing or decreasing the amount of drug affects the overall release profile modifying the therapeutic effect. At last, drug permeation through conventional cellulose acetate membrane and the biomimetic Permeapad™ was analysed. Permeapad^M presents two disks of cellulose acetate supporting a phospholipidic layer which allows evaluation of drug permeability through a lipidic component. This biomimetic membrane presents proved *in-vitro-in-vivo* correlation providing information on *in-vivo* absorption(Bibi et al.; 2015). It also reduces the use of animal tissue and in-vivo study ensuring accuracy in predicting *in-vivo* absorption improving laboratory animal welfare.

2. Materials and Method

Clozapine was kindly provided from NHS Maudsley Hospital. RS PRO 1.75mm Natural PVA 3D Printer Filament, 500g and RS PRO 1.75mm green PLA 3D Printer Filament, 500g were purchased by RS UK. PBS with pH 7.4, EtOH were purchased from Sigma-Aldrich. The water used was distilled.

2.1 3D printing

The patches were printed using an UpMini 3D printer using nozzle size of 200µm and filament thickness of 200µm, 190°C as nozzle temperature and 40°C as bed temperature when printing with PVA filament. PLA was loaded and was printed using 230°C as nozzle temperature and 50°C as bed temperature.

The patch CAD design size was reduced to 1cm ^{2} to evaluate printing efficacy and dissolution profile consistency. The 4cm ^{2} patches were printed once per time, while multiple 1cm ^{2} patches could be printed at once, as showed in Figure 55. The patches were 0.1 cm in thickness, and the micro-pyramids have 0.04 cm x 0.04 cm width and radius. These patches were printed with 65% infill due to the advantage showed in controlling the drug release profile, as discussed in Chapter 5.

Figure 55. 3D printed patch 1cm ^{2} printed with 65% infill. Each printing allows obtaining from three to nine samples per time, representing a good advantage in scalability.

2.2 Physical Properties

The physical properties of each patch, in term of V and Sa, were analysed to determine their effect on the release profile. The information was obtained after optical support and analysed through the software ImageJ. Each patch was aligned with a ruler; a camera was focused at 90°C (angle) and placed equidistant from the sample to avoid prospective alteration, as in Figure 49. Therefore, V and Sa were calculated accordingly with Equation 9 to 12 in Chapter 5.

2.3 Drug loading of 3D printed patches

A 10% w/w clozapine solution was prepared by dissolving clozapine in pre-heated EtOH at 50°C (ethanol boiling point 78.73°C) as described in Chapter 5. The solution was left under vigorous agitation at 800rpm, 50°C for two hours in a Thermo Mix with heating, provided by SciQuip UK. Then the modified impregnation method(Chew; 2019) used in the Chapter 5 was used, and 100ul of this 10% w/w clozapine solution (corresponding to 10 mg of clozapine) was soaked into the printed patch and left under a fume hood for EtOH complete evaporation overnight. In this section, it is investigated, whereas the drug release could be drug-carried or polymer-carried. Notably, it was searched for the influence of the drug on the release profile itself. Three different concentrations of clozapine were loaded reproducing per patch 0.1mg, 1 mg and 10 mg of clozapine.

2.4 In-vitro Dissolution Studies

The studies were carried out in non-sink condition in 25 mL of PBS pH 7.4 to mimic in-vivo buccal environment as reported in European Pharmacopeia and literature evidence(European Pharmacopeia 7.6; 2012; Sun; 2016). In-vitro dissolutions studies were conducted using a shaking incubator (SciQuip Ltd) at 37°C, and 50 rpm. Samples were weighed and placed in a beaker covered with parafilm to avoid evaporation. At predetermined intervals of 15, 30, 45, 60, 90, 120, 180, 360, 480 and 1440 minutes 500µL was withdrawn and diluted in 5mL and filtered through a 0.45 µm cellulose acetate syringe filter and replaced with the same amount of fresh buffer. Subsequently, the filtrate was analysed spectrophotometrically at the UV with the wavelength of 257 nm. The calibration curve was performed in the concentration range of 0.1-0.0001 mg/mL of clozapine standard solutions in PBS pH 7.4 (R=0.999) and spectra recorded at 257 nm.

2.5 Permeation study

Patches were weighed and placed in a PermeGear vertical glass diffusion Franz Cells kindly donated by SciQuipUK with a surface area of 1.77 cm2, upper chamber nominal volume of 3 mL and lower chamber volume of 12 mL. According to the European Pharmacopeia(European Pharmacopeia 7.6; 2012; Pharmacopoeia and 5.0; 2005) and literature evidence, each sample was placed in the donor compartment in a PBS solution at pH 7.4 to mimic the buccal environment. The two chambers were separated by a cellulose acetate filter disk with a dimension of 1.77 cm^2 area, as showed in the schematic Figure 56. Each sample was cut to allow 10 mg of clozapine to be analysed. The amount of

drug was chosen to mimic the dose of initiation of clozapine of 12.5 mg. At predetermined intervals of 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300minutes 1mL was withdrawn from the acceptor compartment and filtered through a 0.45 µm cellulose acetate syringe filter and replaced with the same amount of fresh buffer. Subsequently, the filtrate was analysed spectrophotometrically at the UV with the wavelength of 257 nm. The calibration curve was performed in the concentration range of 0.1-0.0001 mg/mL of clozapine standard solutions in PBS pH 7.4 (R=0.999) and spectra recorded at 257 nm.

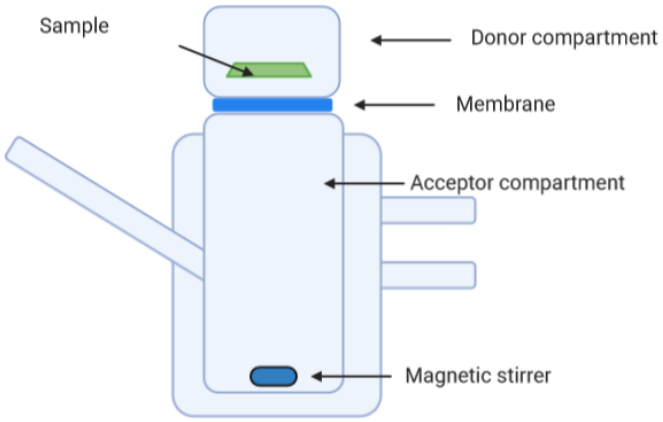


Figure 56. Design of the diffusion vertical Franz cell adapted via BioRender from Rouse and co-workers 2010(Ng et al.; 2010)**.**

For each permeation experiment, the (dQ) was calculated according to Equation 5 and the apparent solubility to Equation 6. (di Cagno; 2015).
Each experiment was replicated in triplicates.

The same studies parameters were used to conduct the permeation study by using the newest PermeaPad® membrane. It is a biomimetic membrane that simulates the passive transport through various barriers in the body (i.e. buccal) providing optimal in-vitro ex-vivo correlation.

The apparent permeability coefficient across the lipid component of Permeapad™ (P_{lip}) was estimated using Equation 13 (di Cagno; 2015):

$$\frac{1}{P_{app-permeapad}} = \left(2 \times \frac{1}{P_{app-cellulose}}\right) + \frac{1}{P_{lip}}$$ **Equation 13**

2.6 Statistical Analysis

The *f2* Equation, calculated according to Equation 1 in Chapter 3, was used to compare the dissolution performance between the drug and the corresponding formulations(Raimi-Abraham; 2015). When *f2* below 50, the profiles are considered different, and when *f2* above 50 the compared release profiles are considered equivalent.

The two-way analysis of variance (ANOVA) was applied to compare different samples. Data were considered statistically significant with a value of p below 0.05.

3. Result and Discussion

3.1 Physical Properties

At first, the physical properties of the two patch designs were investigated to evaluate consistency respect with the designed CAD file. The images were taken as described in the method section and analysed through ImageJ, as reported in Figure 57 and 58. Flat patch presented length and thickness compatible with the designed parameters as they were 1.02-fold ($p<0.0001$) and 1.26-fold (p non-significant), respectively, more prominent than expected. Values summarised in Table 28. It was achieved a total surface area of 1.25cm ^{2}, a 1.25-fold increase ($p=0.0035$). Changing in physical properties are expected when 3D printing in small scale as the present nozzle diameter of 0.2 cm as well as the filament thickness, parameters that could not be changed using UpMini printer and the related software(Trenfield; 2018). The small variations were coherent with the one observed when printing a higher surface patch area, as discussed in Chapter 5.

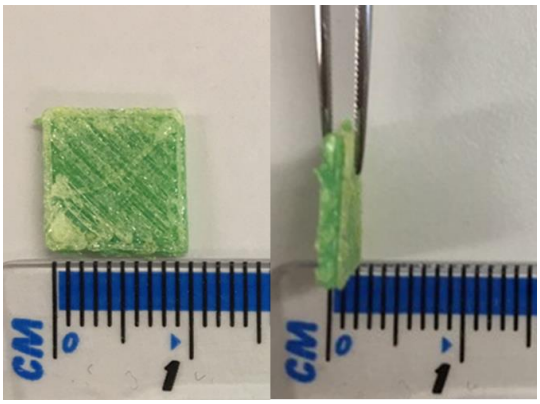


Figure 57. PLA+PVA patch 1cm ^{2} flat image acquisition.

Table 28. Physical properties for patch 1cm ^{2} flat.

PVA+PLA 1cm ^{2}

Length cm	Thickness cm	Volume cm^3	Surface Area cm^2	Sa/V
1.02 ± 0.02	0.12 ± 0.03	0.12	1.25	9.90

The physical analysis was conducted on the patches with micro-pyramids and demonstrated that their length was compatible with the printing parameter with just 0.003 cm reduction, while the thickness was 0.82-fold (p=0.0029) higher than designed. Data reported in Table 29. Interestingly, the width and radius of the cone presented both a size of 0.02 cm as designed. This relates to the filament thickness of 0.2 cm. The overall surface area was of 1.33cm^2, 1.33-fold increase (p<.0001), due to the application of the micro-pyramids on the patch surface.

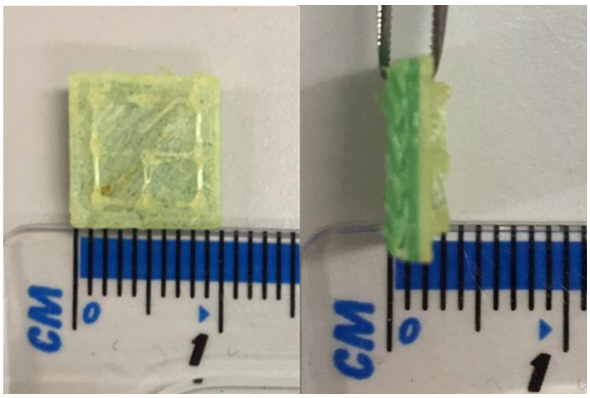


Figure 58. PLA+PVA patch 1cm^2 with micro-pyramids image acquisition.

Table 29. Physical properties of PLA+PVA patch 1cm^2 with micro-pyramids.

PLA +PVA patch with micro-pyramids							
Patch					Micro-pyramids		
Length cm	Thickness cm	Volume cm^3	Surface Area cm^2	Sa/V	Cone width cm	Cone Radius cm	Volume Cone cm^3
0.99 ± 0.01	0.12 ± 0.01	0.12	1.33	11.29	0.04 ± 0.01	0.02 ± 0.01	0.00025

3.2 Dissolution studies

Patches with 65% infill and PLA hydrophobic backing layer were printed in the scale-down size of 1 cm^2. The patches both flat and with micro-pyramids were loaded with three different strengths of clozapine 0.1, 1, 10 mg respectively and was analysed the drug dissolution profile. The drug loading study demonstrated that the soaking method was efficient and reproducible in obtaining 100%

efficacy. Therefore, the 1cm ^{2} patches presented respectively 0.1, 1 and 10 mg when produced with the intent to evaluate the release mechanism.

When the patches (with and without micro-pyramids) were loaded with 0.1 mg of clozapine, an immediate drug release was observed. A low concentration was obtained when in the dissolution media, 25 mL, that brought the clozapine concentration to 0.004 mg/mL, 4.5-fold ($p=0.0026$) less than the saturation solubility of the drug, previously calculated (Chapter 4). Therefore, clozapine was released immediately. Figure 59a shows the dissolution profile from the flat patches with PLA backing. It is possible to notice that when clozapine is in the formulation at 1 and 10 mg it release rate was 0.0001 mg/min achieving 72% ($p=0.002$) of drug release and 0.0004 mg/min reaching 23% ($p<0.0001$) of drug release, respectively. In both cases, the drug release percentage resulted in being low, and the total drug release was not achieved. On this data was to calculate the *f2* value, that expresses the similarity between the pure drug release profile and the respective formulation. The analysis concluded that when using each amount present a different release profile compared to the pure drug (*f2* below 50). This data showed a 5.46-fold ($p<0.0001$) increase in the dissolution rate when increasing the dose. A similar pathway could be observed in Figure 59b, where the dissolution rate was 0.0002 mg/min achieving 76% (p non-significant) when 1 mg of clozapine were loaded and 0.0005 mg/min achieving 48% of drug release ($p<0.0001$) when loaded with 10 mg of the drug. The evaluation of the similarity factor demonstrated for each drug loading a different profile compared with the pure drug.

Moreover, the results demonstrated that the presence of micro-pyramids improved the dissolution profile due to the higher Sa obtained compared with the patches without the pyramids. Interestingly, it was observed that the concentration of clozapine affects the dissolution profile demonstrating a mechanism drug-controlled and not carrier-controlled. Finally, it was noticed that the higher the drug loading, the faster the release profile. Such consideration is essential in the formulation of dosage form intended for systemic delivery as expected in the present work.

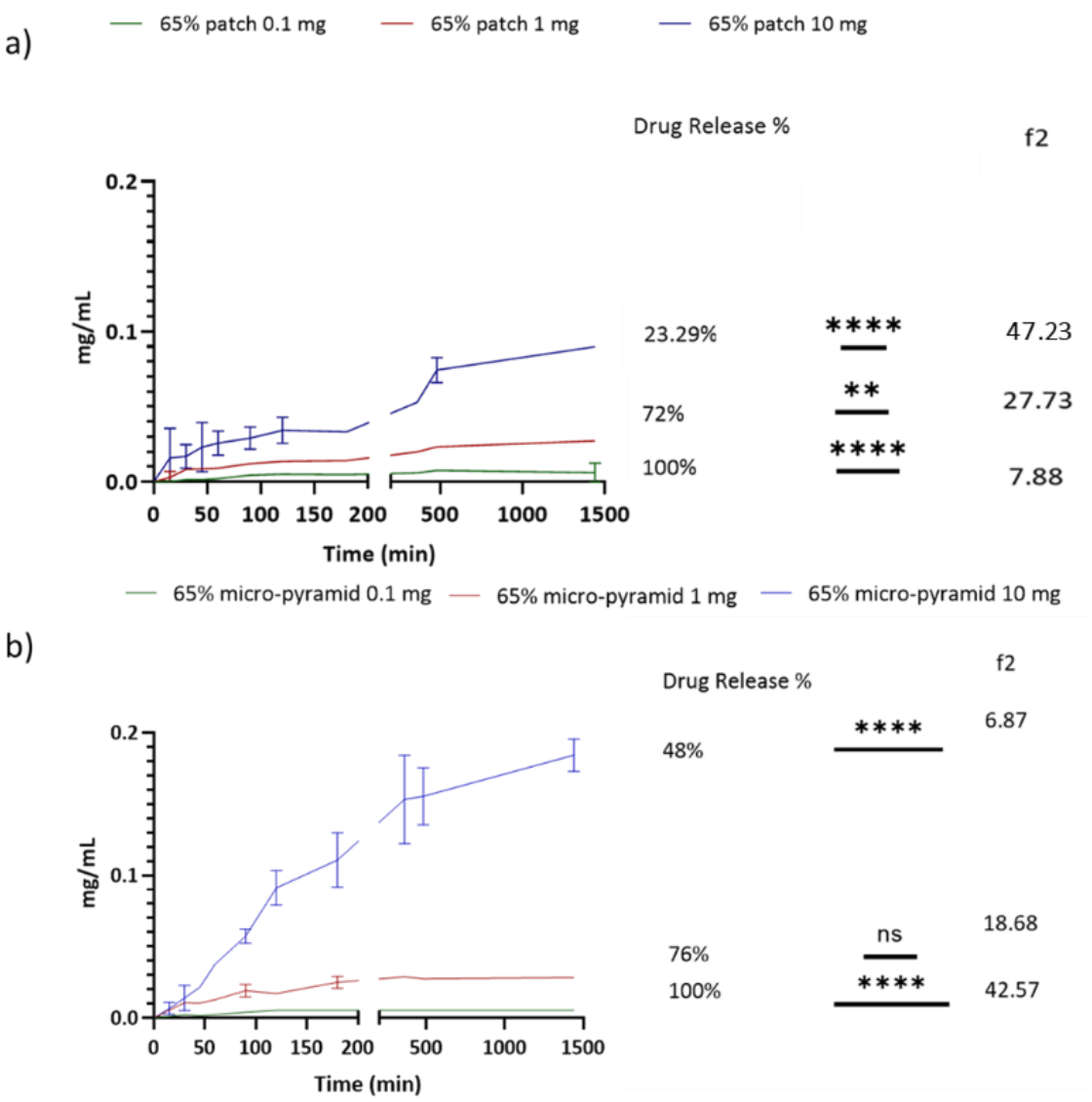


Figure 59. The dissolution profile of different clozapine loading at 0.1, 1 and 10 mg. a) 1cm ^{2} patches flat achieved an immediate dissolution for patch containing 0.1mg of clozapine, and respectively 72 and 23% of drug release for patch containing one and 10mg of clozapine. b) 1cm ^{2} patches with micro-pyramids achieved an immediate dissolution for patch containing 0.1mg of clozapine, and respectively 76 and 48% of drug release for patch containing 1 and 10mg of clozapine. The presence of micro-pyramids improved the release profile of the drug. The similarity factor, f2 value, was reported in correspondence of all the dissolution profile and was obtained comparing each pattern with the correspondent amount of pure drug. The asterisks indicate statistical significance where ns stands for non-significant while one to four asterisks suggest increasing significance.

3.3 Permeability studies using an biomimetic synthetic membrane

Subsequently, drug permeability when released from the patch was analysed using both cellulose acetate membrane and the biomimetic synthetic membrane, Permeapad™. Each patch was loaded with 10 mg of the drug. At first drug, permeation was investigated through cellulose acetate membrane, which has the advantages of low costs, high physical strength, and pore dimension of 0.20

µm. Cellulose acetate is the main component of Permeapad™ supporting layer. Therefore, the use of cellulose acetate membranes allows calculation of the permeability through the phospholipid bilayer (P_{lip}), which composed the biomimetic membrane(di Cagno; 2015).

The 3D printed patches permeation study in cellulose acetate membrane ($P_{app\text{-}cell}$) demonstrated that the patches increase permeability rate compared to unformulated clozapine, which presented a flux (J) of $6.96x10^{-7}$ and Papp $2x10^{-7}$ cm/sec. Data presented in Figure 60. Mainly, 65% PVA+PLA increase permeability of 4.5-fold ($p<0.0001$), due to the high surface area offered by the flat patch and to the fact that the drug is finely absorbed on patch surface. Interestingly, the application of micro-pyramids improved permeability from $18.01x10^{-7}$ cm/sec and $P_{app\text{-}cell}$ to $5.02x10^{-7}$ cm/sec a 2.5-fold increase ($p<0.0001$) respect to the unformulated drug. Data are also reported in term of permeation rate µg/cm^2/sec in Table 30 and Figure 61. Comparing the rate it was noted that flat patches increased rate of 3.35-fold ($p= 0.043$) respect with unformulated clozapine while patch with micro-pyramids of 2.37-fold ($p>0.05$), but non statistical significance was found. This result could be due to the radius of the micro-pyramids, which is 0.02 cm which might obstruct cellulose acetate pores with the same dimension. However, the surface area available for the drug to permeate is higher than the flat patches; therefore, it was noticed an increment compared to pure clozapine. Moreover, Etemadi and co-workers, demonstrated that when fine particles are dissolved in the donor compartment and permeate through the pore, they can deposit along the pore walls and cause blockage(Etemadi et al.; 2018). The higher dissolution in the dissolution media of the patch with micro-pyramids compared with the patch without, was demonstrated in the previous section, supporting this hypothesis.

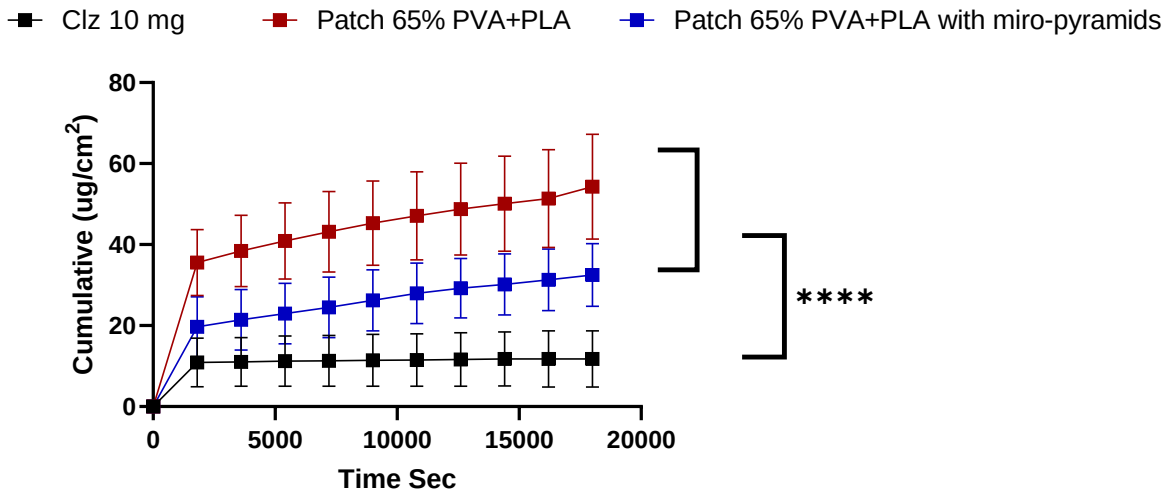


Figure 60. Permeation study through cellulose acetate membrane of unformulated clozapine, 65% patch PVA+PLA and 65% patch PVA+PLA with micro-pyramids. Both patches improved permeability compared with clozapine with a high statistically significance (****).

Table 30. Permeation rate expressed in µg/cm ^{2}/sec comparing rate obtained in cellulose acetate.

	Clozapine	Patch 65%	Patch 65% with micro-pyramids
Cellulose acetate	0.0079 ± 0.0044	0.031 ± 0.0073	0.018 ± 0.0052

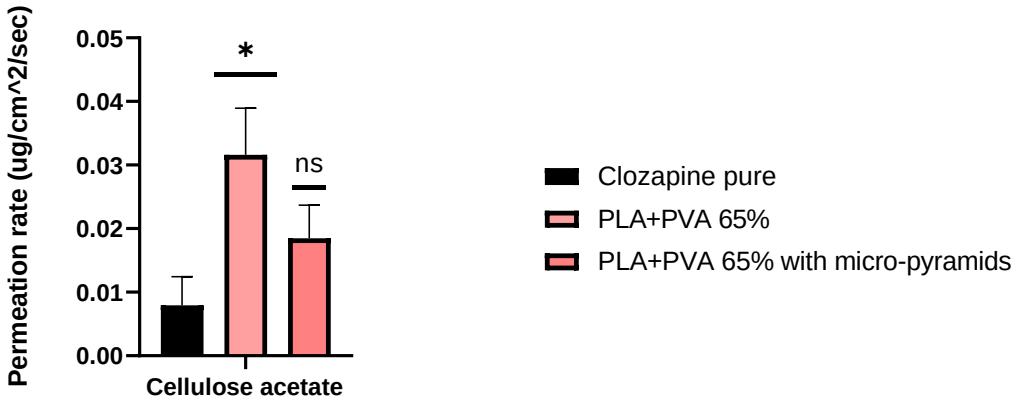


Figure 61. Permeation rate calculated as µg/cm ^{2}/sec in cellulose acetate. Data showed an increment in rate for flat patch, statically significant (*) of 0.0430 and a reduction in rate for patch with micro-pyramids, non-significant (ns).

Data were then investigated by using Permeapad™, which is a biomimetic membrane consisting of two hydrophilic cellulose hydrate membranes supports and a layer of soy phosphatidylcholine S-100. Permeapad™ possess a high *in-vivo-in-vitro* correlation which was proved for different drugs with the Mw between 100 to 600 g/mol(Bibi; 2015; Bibi et al.; 2016, ; 2017). Moreover, the permeation through Permeapad™ membrane was compared with porcine buccal mucosa and TR146 cell from human oral carcinoma, demonstrating being a tool to estimate absorption through the buccal mucosa(Bibi; 2016). Figure 62 displayed the permeation of clozapine unformulated compared with clozapine permeability from patch 65% PVA+PLA and patch 65% PVA+PLA with micro-pyramids. Overall, 65% PVA+PLA increase drug flux through the membrane of 1.15-fold (p=0.029) increase respect unformulated clozapine. Comparing the rate, displayed in Figure 63 and Table 31, it was noted that flat patches increased rate of 1.26-fold (p non-significant) respect with unformulated clozapine while patch with micro-pyramids of 2.52-fold (p=0.0145). This finding, relates with the previous data obtained in cellulose acetate and it is due to the high surface area available from the flat patch and for the solubilising effect of PVA, previously discussed. From the other hand side, the addition of the micro-pyramids reduced permeability rate of 2.58-fold compared with pure clozapine. This data is coherent with the previous finding where micro-pyramids provided a slower permeability profile through membrane compared with flat patches. This could be due to

blockage formation due to the improved solubility of clozapine in the donor compartment, as previously assessed.

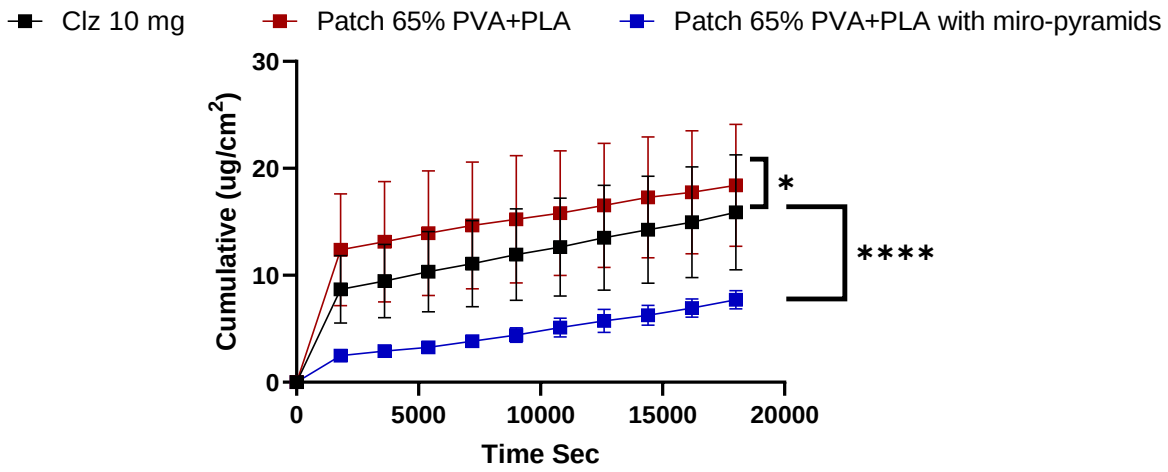


Figure 62. Permeability study using Permeapad ™ membrane. The study showed that flat patches 65% PVA+PLA increased clozapine permeation compared with both the unformulated drug, low statistical significance (*), and patches with micro-pyramids, high significance (****).

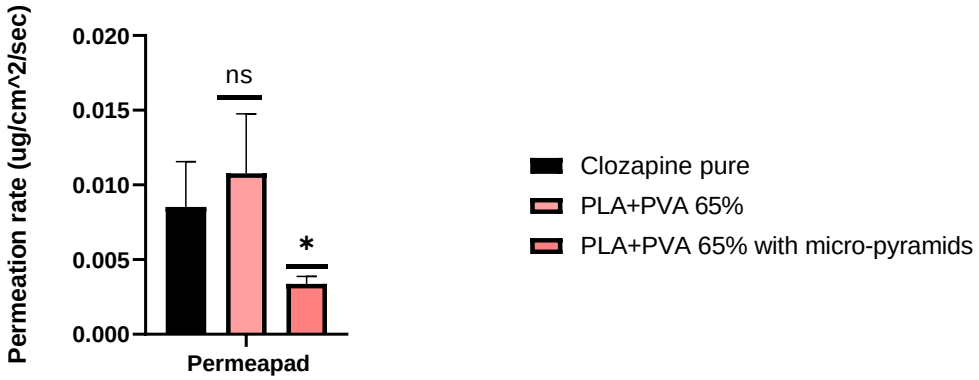


Figure 63. Permeation rate calculated as µg/cm ^{2}/sec in Permepad ™. Data showed an increment in rate for flat patch, statically non-significant (ns) and a reduction in rate for patch with micro-pyramids, with a low significance (*) of 0.0145.

Table 31. Permeation rate expressed in µg/cm ^{2}/sec comparing rate obtained in Permepad ™.

	Clozapine	Patch 65%	Patch 65% with micro-pyramids
Permeapad ™	0.0085± 0.003	0.010 ± 0.003	0.003 ± 0.0004

Permeation assessment through Permeapad ™ allows to detect the amount of drug that permeates through the lipidic membranes and to calculate $P_{lip,}$ data reported in Figure 64 and for

clarity in Table 32. Interestingly, clozapine does not permeate through the lipidic barrier but just through the cellulose acetate disks presented in Permeapad ™. A similar finding was reported by Shafaat and co-workers who conducted permeability study on mice abdominal skin showing a low permeability of 14.17 x 10^{-7} cm/sec(Shafaat et al.; 2013). The formulation of transmucosal patches improved drug permeability through the lipidic component due to the improved solubility in the donor compartment in the first place, where the membrane was wetted with dissolution media which mimicked the buccal mucosa surface. Mainly, patch 65% PVA+PLA provided a P_{lip} of 9.50 x 10^{-7} cm/sec a 5.58-fold (pbelow0.0001) increase respect with clozapine alone; while patch 65% PVA+PLA with micro-pyramids an increase of 1.41-fold (p=0.0041). The increase of clozapine permeability from patches with micro-pyramids could be due to the overall improved solubility of drug that which is best absorbed by a passive mechanism. Overall, flat patches provided an increase in both permeability flux and coefficient in both cellulose acetate and Permeapad ™ membrane, improving the partition of clozapine as well through its lipidic component.

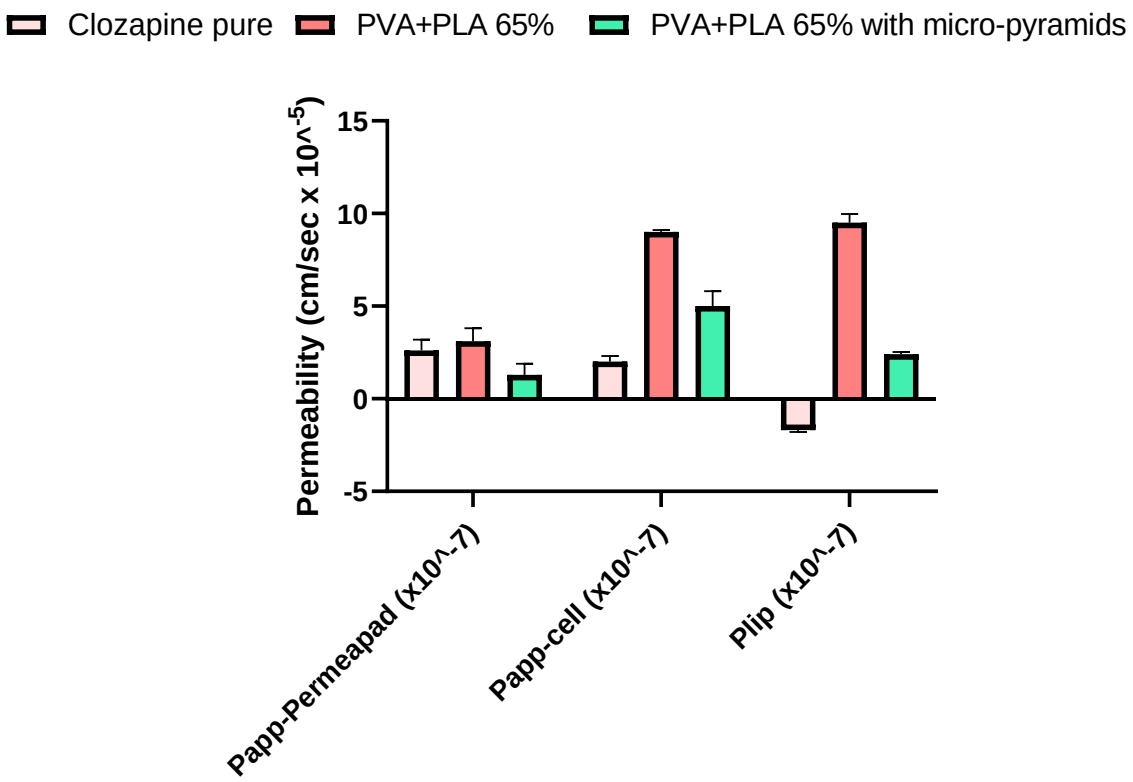


Figure 64. Comparison of permeability coefficient both Papp-Permeapad™, Papp-cell in the cellulose acetate membrane and Plip, which represents the permeation through the lipidic component of the Permeapad™ membrane.

Table 32. Permeability coefficient for clozapine, PVA+PLA 65% and PVA+PLA 65% with micro-pyramids. The coefficients were calculated according to Equation 5, 6 and 13, and allowed calculation of the amount of drug permeated through the lipidic component of the Permeapad™ membrane, which represent the *in-vivo* correlating barrier.

	Papp-Permeapad (x10 $^{-7}$) cm/sec	Papp-cell (x10 $^{-7}$) cm/sec	Plip (x10 $^{-7}$) cm/sec
Clozapine pure	26.01 ± 0.006	2.09 ± 0.003	-17.06
PVA+PLA 65%	31.04 ± 0.007	9.87 ± 0.001	9.50
PVA+PLA 65% with micro-pyramids	12.09 ± 0.007	5.43 ± 0.006	2.46

4. Conclusion

3D printing resulted in a flexible manufacturing process to produce unidirectional transmucosal patch provided with a hydrophobic backing layer with a surface area of 1cm ^{2}. Patches physical properties were analysed to establish 3D printing reproducibility respect to the CAD and STL files produced, resulting in being printed coherently in term of designed size. Clozapine was loaded with three different strengths in both patches, and it was evaluated it dissolution profile, determining that drug-controlled mechanism. This implicates that clozapine dosage forms present different release profile when using different drug concentration, fundamental information when formulating systemic and controlled release system like the one proposed in this work. Moreover, the release was improved by the application of the micro-pyramids, which increased the surface area available. Finally, the investigation of permeability was conducted both on cellulose acetate membrane and the biomimetic membrane Permeapad™. The analysis showed that the higher dissolution profile of the drug in the donor compartment caused membrane blockage in both membranes, reducing permeability in the patch with micro-pyramids. From the other hand side, flat patches PVA+PLA increased permeability. Results obtained using Permeapad™ allow to hypothesis a drug absorption *in-vivo* due to the membrane *in-vivo-in-vitro* correlation. Most importantly, both patches increase clozapine solubility through the lipidic layer, representing a promising absorption if the patch would be administered *in-vivo.*

Moving forward, the PVA+PLA patches generated a controlled-release profile improving drug permeability through the membrane, tackling the problem of medication acceptability related to dosing per day. However, the second concern refers to drug taste perception if a small amount of drug would be released in the oral cavity affecting medication acceptability. Therefore, a structured qualitative and quantitative investigation of clozapine taste is considered fundamental.

Chapter 7.Clozapine Taste Investigation

1. Background

In the management of treatment-resistant schizophrenia low compliance of clozapine is an issue related to the number of daily doses and taste. Chapters 4 to 6 have investigated the potential to develop modified and prolonged release of clozapine dosage forms to address the current issue of multiple daily drug dosing. Taste is one of five senses stimulated in the mouth by the compound's chemical characteristic and identified in brain, based on information provided by taste receptors(Bradbury; 2004). It is recognised by a family of receptor T2R, described in the Introduction, that is composed of 25 receptors, which gene expression creates the individual perception of taste(Shaw; 2018; Soares; 2013). In pharmaceutics, over 350 drugs are identified as aversive playing a crucial role in medication acceptability(Milne and Bruss; 2008). An aversive taste is defined as the avoidance of food or drug which is disliked. Literature references reported that clozapine taste is a cause of low compliance(Abeelen; 2013; S. Ramuth, J. Flanagan; 1996; Siva Prasad Reddy; 2011). In clinical practice, drug taste is considered to affect patient compliance toward antipsychotics medication(Bradshaw et al.; 2016; Brown and Bussell; 2011; Higashi et al.; 2013; Matsui; 2007). However, data about a quantitative and reliable assessment of clozapine taste or its formulation counterpart are missing. Therefore, it is fundamental to investigate claim about the aversive taste of clozapine and understand whereas or not the drug itself or formulation excipient causes low medication acceptability. Such is considered a leading cause of discontinuation in clozapine intake and risk of relapse(Shaker and Jones; 2018; D. M. Taylor et al.; 2009).

Taste is one of five senses which is perceived from gustatory receptors from T2R family in the tongue. In the pharmaceutical industry, more than 350 drugs are considered of possessing an aversive taste, and it is well understood that aversive medications are not considered palatable. Palatability is fundamental to endorse medication acceptability, thus compliance. Taste can be assessed using both *in-vitro* and *in-vivo* methods to determine taste threshold that is a minimum amount of compound that causes an aversive taste perception(Dale Purves, George J Augustine, David Fitzpatrick, Lawrence C Katz, Anthony-Samuel LaMantia, James O McNamara, and S Mark WilliamsDale Purves, George J Augustine, David Fitzpatrick, Lawrence C Katz, Anthony-Samuel LaMantia, James O McNamara; 2001). This value is fundamental to understand how much a drug is aversive or bitter and it is usually compared with most bitter known compound quinine and strychnine, which threshold is, respectively 0.008 mM, and 0.0001 mM(G. N. Martin; 2013). *In-vivo* methods are a human taste panel(Jessica Soto et al.; 2018) and animal preference studies(Mennella; 2013), while *in-vitro* strategies use

sensors(Latha RS; 2012), modified drug release studies(Jessica Soto; 2018) and cells(Ruiz-Avila; 2000). Human taste panel estimates gustatory perception responses in healthy human volunteers allowing a sensitive measure of taste designed to minimise bias and variable responses between volunteers, who rate sample according to an adjective scale. Human panel studies use limited due to potential toxicity and liability issues. From the other hand side, animal studies can be conducted obtained precise data, as it was proved that mice could detect a small quantity of bitter drug if diluted in sucrose solution(Contreras; 1995). As well novel drug release apparatus can be adapted to simulate buccal saliva and mimic the release of a drug from dosage forms to identify the concentration of drug that once release in mouth cause an aversive taste perception, named threshold(Yajima et al.; 2002). Another assessment method is electronic tongue which presents a sensor array and a detector, known as a sensor chip, capable to screen mainly bitterness when drugs are too toxic to be tested in humans(Podrażka; 2017).

Even if above mentioned, non-human techniques are advantageous if using toxic drugs. For ethical purposes, use of human taste panel is usually preferred to assess drug taste because it takes into consideration individuals perception mimicking most likely scenario of a patient taking prescribed medication(Anand; 2007). Moreover, this study can be standardised efficiently, reducing any operation error and improving the safety of candidates through the production of standard operation procedure (SOP)(Rui-xin Liu,Xiao-jie Gao,ming Wang, Li-ping Dai,4 Bing-ya Kang,Lu Zhang, Jun-han Shi, Xin-jing Gui, Pei Liu; 2017).

The present work aimed to both conduct a structured investigation of literature to investigate the extension of clozapine taste related compliance and to assess for the first-time clozapine human taste perception. Therefore, excipients used in clozapine marketed dosage forms, such as FazoClo®, Clozaril®, Clozapine Sandoz®, and oral solutions Versacloz ® and Denzapine ® were investigated to understand if reported aversive taste relates with drug or with excipients. Moreover, two pharmaceutical companies were reached to understand which method was used to describe drug of possessing an aversive taste. However, medicaments taste is considered a leading cause of discontinuation in clozapine intake and risk of relapse(Shaker; 2018; David M. Taylor; 2009). Therefore, a qualitative evaluation of its threshold once administered is fundamental and was pursued in this last thesis Chapter.

2.Materials and Methods

Clozapine Pharma grade was procured from Fagron (Newcastle, Tyne & Wear, UK). Transparent 30mL polypropylene universal tubes were purchased from Wheaton (Rochdale, Lancashire, UK). Buxton® Natural Mineral water was procured from Nestle Waters (Rickmansworth,

Hertfordshire, UK); unsalted, plain crackers (Rakusen® Traditional Matzos) were obtained from Waitrose (London, UK); and plastic tumbler was purchased from Office Depot (Raton, Florida, USA).

2.1 Structured literature review

It was searched Google Scholar, Google Books and PubMed using medical subject headings (MeSH) and free words. MeSH terms included "Clozapine" AND "taste", or "taste perception", or "taste disorders", or "bitter". Title and abstracts of obtained paper were reviewed for each paper considered potentially relevant from its title and then refined considering eligibility criteria. Eligibility criteria were papers and clinical reports referring to 1) taste of clozapine, 2) effect of clozapine taste on compliance 3) assessment of clozapine taste influence on compliance. Exclusion criteria were: 1) article dated before 01.01.2000, 2) literature referring to other antipsychotics, 3) reviews and PhD thesis.

2.2 Data Collection

BitterDB is a database that relates to the genome browser to predict the taste of the drug. Notably, it considers as aversive molecules with an Mw lower than 700 and hydrophobicity (AlogP) in a range between −3 and 7(Ayana Wiener, Marina Shudler, Anat Levit; 2011; Dagan-Wiener; 2017; Wiener; 2012). The BitterDB, database holds about 500 compounds that were reported as aversive or that activate at least one human bitter receptors(Dagan-Wiener; 2017; Wiener; 2012). It also contains data on bitter receptors and ligands from chicken, mouse, and cat.

The patient information leaflet (PIL) of all marketed dosage forms of clozapine reports aversive taste as one of medication side effects. Two medical information departments of pharmaceutical companies (i.e. Novartis-Mylan and Sandoz) were contacted to investigate how to taste alteration was assessed between October 2019 and January 2020 and information via email was received from both pharmaceutical companies contacted

2.3 Human Panel study design and methodology

This study was a single-blinded, randomised sensory evaluation (taste) of extemporaneously prepared aqueous solutions of clozapine. University College of London ethically approved this study of London Ethics Committee under reference REC 4612/024.

2.3.1 Sample preparation

Extemporaneous preparation of all samples was completed under the supervision of UK registered pharmacists, under strict quality measures, and according to pre-approved SOP. A copy of SOP created for solution preparation could be found in Appendix 2 of present work. A 0.018 mg/mL Clozapine stock solution was prepared by dissolving 36 mg of clozapine in 2 L of deionised water. This value of concentration represents Clozapine saturation solubility, and it was previously calculated in Chapter 4. Dilutions of stock were prepared to render clozapine solutions at 0.012, 0.00225 and 0.001125 mg/mL, according to Table 34. Previous taste assessment studies were conducted using the following dilution factor considering drug poorly water solubility and therefor these were proposed in the present work(J. Soto; 2016).

Table 33. Preparation of Clozapine dilutions.

No	Concentration (mg/mL)	Dilution factor	Volume of stock solution to prepare 1 L dilution (mL)
1	0.018	Stock	36 mg in 2 L
2	0.012	1.5	666.66 mL
3	0.00225	8	125 mL
4	0.001125	16	62.5

2.3.2 Participant selection

The study was advertised by internal email to all staff and students of University College London, where potential participants were invited to contact the research team to receive a detailed Participant Information Sheet. Potential participants were screened against inclusion and exclusion criteria, detailed in Table 35. Those who decided to participate and met inclusion/exclusion criteria signed an Informed Consent Form to participate. An equal number of male and female participants was maintained during recruitment.

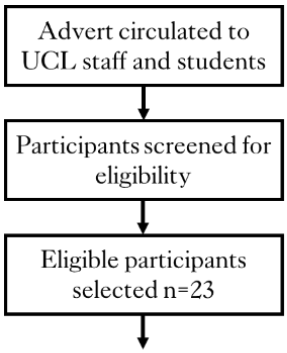
Table 34. Inclusion and exclusion criteria for participant recruitment.

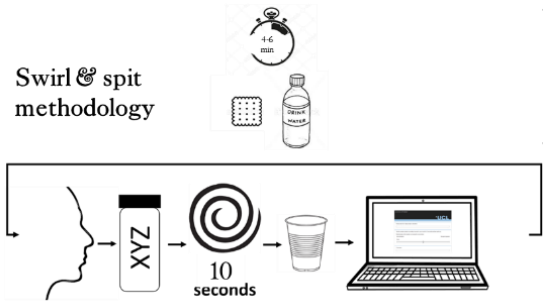
Inclusion criteria	- Healthy adult males and females aged 18-40 years - Able to understand speak English

Exclusion criteria	- Antecedent deterioration of taste or smell - Dental care is undertaken up to 15 days before the test - Local anaesthetics into mouth within 24 hours of study

2.3.3 Taste assessment protocol

The "swirl and spit" method was used as a means of taste assessment. Participants were instructed to swirl a 10 mL sample of test solution in their mouth for 5-10 seconds, ensuring that it was distributed across tongue and palate. After that, the sample was spit, and intensity of test stimuli was rated using a continuous measurement scale (Visual Analogue Scale, VAS, anchored from 0 "not aversive" to 100 "extremely aversive") and open-ended comments (Figure 66). samples were rated using an electronic data collection tool designed on Qualtrics. Each participant evaluated three samples of each concentration, to a total of 12 samples, which all samples blinded (coded) and randomised. Before and after assessment of each sample, participants were instructed to rinse their mouth with bottled water and to eat plain, wheat crackers to neutralise palate. An interval of 7.5 minutes was maintained between test samples to ensure the taste of the previous sample was no longer perceived. Each session lasted between 1.5 to 2.0 hours.





The 'swirl and spit' methodology will be employed in this study, whereby the participants are presented with 10 mL of sample, which they are then instructed to swirl around their mouth for 10 seconds, before spitting into a receptacle provided.
Each sample will be presented randomly in duplicate to each participant labelled with a random 3-digit code with a 4-6 minute break between each presentation to allow for rinsing with and consumption of water. During this inter-presentation interval, participants are also able to consume a plain cracker in order to neutralise their palate.

Figure 65. Graphical representation of swirl & spit methodology.

2.4 Human Panel Model Data treatment and analysis

Data normality was checked with a Shapiro-Wilk test, and Kruskal-Wallis test was performed to check if there were significant differences for the rating of positive control between different sessions and between volunteers. To compare clozapine taste intensities, a value which corresponds to the concentration of drug that produces half of the maximal rating (100) (EC_{50}) was calculated with the following Emax model:

$$E = \left({Emax\ X\ C^{Hill}} / {EC50^{Hill} + C^{Hill}} \right) + \varepsilon \quad \textbf{Equation 14}$$

where E is taste rating, Emax represents maximum taste rating fixed to 100, C refers to the concentration of the drug, Hill is slope factor affected by gradient of the curve, EC50 represents concentration which elicits a half-maximal taste rating, and ε is participant variability. EC50 values and 95% confidence interval were derived from this model. Variance values for each parameter were obtained as well as the error value, as a reflection of volunteer variability(Jessica Soto; 2018).

All statistical analyses, boxplots generation and some graphs were done with R (version 3.0.1). Microsoft Office Excel 2016 was also used to produce Figures. IC50 and EC50 values were estimated by using non-linear mixed-effects modelling performed using software NONMEM® (ICON, Ellicott City, Maryland, version 7.3) in conjunction with a Fortran (64-bit) compiler using Perl-Speaks NONMEM ® (PSN, version 4.2.0) as an interface to run NONMEM®.

3. Result and Discussion

3.1 Literature and excipients investigation

The structured literature review aimed to find evidence discussing the taste of clozapine with the overall objective to find an assessment of clozapine taste identifying if this possess an aversive taste or not. The screening of titles retrieved during the structured literature review provided 55 potentially relevant literatures: Google scholar 17, Google books 2, PubMed (MeSH) 4 and Web of Knowledge 32. After removing duplicates and reviewing both abstracts and title according to the inclusion criteria, a total of 15 papers were considered relevant. Table 35 reports the 15 papers in discussion.

Table 35. List of 15 papers and case reports obtained from the structured literature reviews. Those are displayed alphabetically.

Author	Title	Year	Reference
Ben-Aryeh et al.	Salivary Flow-Rate and Composition Schizophrenic Patients on Clozapine: Reports and Laboratory Data	1996	(Ben-Aryeh et al.; 1996)
Derman et al.	Effects of hM4Di activation in CamKII basolateral amygdala neurons and CNO treatment on sensory-specific vs. general PIT: refining PIT circuits and considerations for using CNO	2020	(Derman et al.; 2020)
Gaisler-Salomon et al.	Systemic administration of MK-801 produces an abnormally persistent latent inhibition which is reversed by clozapine but not haloperidol	2003	(Gaisler-Salomon & Weiner; 2003)
Gurok et al.	Treatment of burning mouth syndrome with low-dose clozapine: a case report	2019	(Mehmet Gürkan Güroka,Alaaddin Hekima; 2019)
Holger et al.	Haloperidol and clozapine antagonise amphetamine-induced disruption of latent inhibition of conditioned taste aversion	2003	(Holger Russig, Aneta Kovacevic et al.; 2003)
Kostakoglu et al.	Ketoacidosis as a side-effect of clozapine: a case report	1996	(Kostakoğlu et al.; 1996)
Man	Monitoring patients using clozapine	2018	(Man; 2018)
Masareddy et al.	Development of Mouth Dissolving Tablets of Clozapine Using Two Different Techniques	2008	(Masareddy et al.; 2008)
Oloyede et al.	Clozapine and Norclozapine Plasma Levels in Patients Switched Between Different Liquid Formulations	2019	(Oloyede; 2019)
Purohit et al.	Pharmacogenetic Manipulation of the Nucleus Accumbens Alters Binge-Like Alcohol Drinking in Mice	2018	(Purohit et al.; 2018)
Reddy et al.	Novel Approach in Designing of Mouth Dissolving Tablets for Bitter Drugs: Taking Clozapine as Model Drug	2011	(Siva Prasad Reddy; 2011)

Thrasher et al.	Clozapine's Effects on Ethanol's Motivational Properties	1999	(Thrasher et al.; 1999)
Van der Poorten et al.	Sublingual use of atropine for clozapine-induced sialorrhoea: literature review and two case reports	2019	(Poorten & Hert; 1996)
Vardigan et al.	MK-801 produces a deficit in sucrose preference that is reversed by clozapine, D-serine, and the metabotropic glutamate 5 receptor positive allosteric modulator CDPPB: Relevance to negative symptoms associated with schizophrenia?	2009	(Poorten; 1996)
Wang et al.	The coding of valence and identity in the mammalian taste system	2018	(Poorten; 1996)

References were analysed and sought relations between clozapine and taste. Interestingly, in each article, the taste of clozapine is deemed to be bitter; Douroumis(Douroumis; 2011) reported it as a known side effect of the drug whereas a case study from Gürkan et al., described the perception of a bitter taste in the mouth as a symptom of mental health condition(Mehmet Gürkan Güroka,Alaaddin Hekima; 2019; Melis et al.; 2019; Mullan; 2000; Pickering et al.; 2019). None of these carried strong evidence that the drug was bitter though.

The structured literature review conducted in the present work primarily aimed to find a quantitative assessment of clozapine taste i.e. threshold and/or any data (clinical or others) that could connect drug taste with compliance issues.

No papers measuring taste of clozapine were retrieved. However, only 26% of these papers (n=4 papers, p value < 0.0001) evaluated the effect of clozapine on the perception taste. While another 26% (n=4, p value < 0.0001) were case reports, respectively reported by Man(Man; 2018), Gurok et al.(Mehmet Gürkan Güroka,Alaaddin Hekima; 2019), Ben-Aryeh et al., and Kostakoglu et al.(Kostakoğlu; 1996). One case reports (33.3% of case reports, p value < 0.0001) discussed that after four weeks of treatment one patients complained a perception of "bad-taste" in the mouth(Kostakoğlu; 1996). The second case report said that addition of clozapine reduced the perception of a metallic taste that a postmenopausal lady was perceiving during the day(Mehmet Gürkan Güroka,Alaaddin Hekima; 2019). The third and fourth case reports, from Man(Mehmet Gürkan

Güroka,Alaaddin Hekima; 2019) and Ben-Aryeh et al.(Ben-Aryeh; 1996), representing 50% (p value < 0.0001) of all case report , monitored the perception of taste in patients taking clozapine. The study conducted by Man, in 2018, on 32 participants demonstrated that none of the participants perceived a taste which was different from the placebo (containing glycopyrrolate oral solution). While Ben-Aryeah et al, discussed that none of the 25 patients presented changes in the perception of taste. Overall, just the 6.66% (p value <0.0001) of the total refined search (one paper out of fifteen) reported a bitter taste for one patient after four weeks of administration.

Five papers (33.3%), investigated the effect of clozapine intake on taste perception, using mice models. The mice were deprived of water or food for 2 hours and then was evaluated the impact of clozapine on drinking behaviour of an appetitive 5% sucrose solutions. Notably, Thrasher et al., demonstrated that 1 mg/kg of clozapine dispersed in saline water plus 0.1 N HCl, administrated intraperitoneally to a mice model did not affect the intake of a sucrose solution (Thrasher; 1999). Same findings were proposed by Derman et al., (Rifka C. Derman, Caroline E. Bass; 2019), and Holger et al., (Holger Russig, Aneta Kovacevic; 2003) who dissolved clozapine in 0.1 N HCL in 0.9% or 100% DMSO saline solution and diluted to obtain a final concentration of 5.0 mg/kg and administered the solution intraperitoneally. They discovered that clozapine intake did not affect a 5% sucrose solution drinking. Purohit et al., in 2018, observed that an intraperitoneal administration of 0.5 mg/kg for four days and a dose of 1mh/Kg on day 6 of clozapine did not change drinking behaviour of water or a quinine solution, concluding that there is no post-administration metabolic effect of clozapine on the sense of taste(Purohit; 2018). Those data showed that clozapine did not affect sucrose intake but, no quantitative taste assessment of clozapine was reported in the aforementioned studies. Lastly, Vardigan et al., in 2009, demonstrated that 3 mg/Kg of clozapine by subcutaneous administration reverse the reduction MK-801-induced deficit in sucrose consumption. Overall, the 100% of papers discussed in this section demonstrated that intraperitoneal administration of clozapine did not alter the neuronal perception of taste.

Interestingly, six papers out of fifteen papers (40%) identified by the structured literature review, generically defined clozapine as a “bitter drug”, a drug with a bad-taste or an aversive taste. Particularly, the 33.3% (p values< 0.0001) of this category of papers published by Masareddy et al.(Masareddy; 2008), and Reddy et al.(Siva Prasad Reddy; 2011), proposed novel drug delivery systems to overcome clozapine bitterness. Reddy et al., in 2011, referred to a paper published by Ramuth et al., in 1996, where clozapine taste was not discussed. Finally, in 2019, Oloyede et al., highlighted a preference in taste of Denzapine® compared to clozapine suspension from crushed tablets. However, the authors highlighted an absence of evidence regarding clozapine aversive taste

and questioned the importance of the acquisition of this information to tackle medication acceptability in a patient with treatment-resistant schizophrenia(Oloyede; 2019).

Overall, this structured literature review showed that just the 6.66% of papers (n=1 out 15) reported a bad taste of clozapine after four weeks of oral clozapine administration. Moreover, 33.3% of papers (n=5 out of 15) demonstrated that intraperitoneally administration of clozapine solution did not affect sucrose or ethanol solution intake in mice models. The rest of the papers referred to an aversive taste of clozapine but without supporting evidence. Overall, none of the papers identified were able to provide a quantitative assessment of clozapine taste. Therefore, the data available from case reports suggest that clozapine does not alter patient taste perception (Ben-Aryeh et al.,(Ben-Aryeh; 1996)) and that the claimed bitterness of clozapine (supported by six papers) is not supported by sufficient evidence.

3.2 Database and PIL consultation

In recent years a new database has become available to predict compounds taste, BitterDB. This database is a predictive tool that uses compounds values as molecular weight and hydrophobicity to detect bitterness. Compounds with molecular weight lower than 700 g/mol and hydrophobicity between -3 and 7 (expressed in logarithmic scale) are considered as bitter, where values higher than 0 are considered very bitterly (Wang et al.; 2018). It cross-links information about molecular structures of compounds with a genomic server to predict drug taste. The use of this database to predict clozapine taste was carried out by using code Chemical Entities of Biological Interest (ChEBI) 3766, referred to clozapine molecule. BitterDB predicted drug taste to be aversive by generating a value of 3.39, as reported in Figure 66.

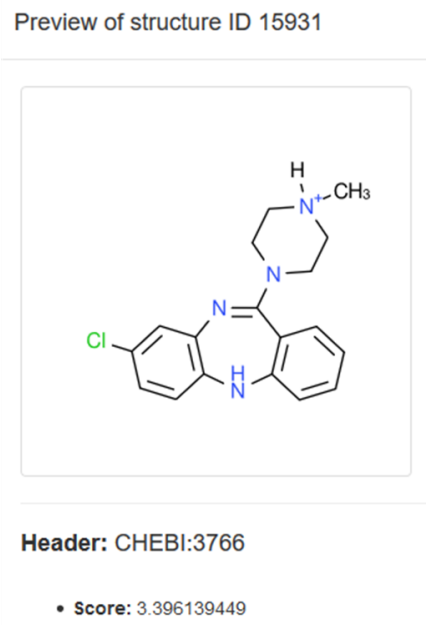


Figure 66. Taste score predicted by database BitterDB by using code ChEBI 3766

However, it does not create a taste threshold that is minimum concentration that activates taste perception. The tool does take into consideration that the molecule may bind taste receptor family T2R based on its chemical similarity. The main limitation of the database is that it does not take into account an individual's perception of taste that can differ depending on age, sex and co-current therapy or disease(Ayana Wiener, Marina Shudler, Anat Levit; 2011; Podrażka; 2017; Rudnitskaya et al.; 2013). For a more specific result that does not include in-vivo studies, a ligand-based approach would be able to recognise chemical family, as suggested by Levit and co-workers (Levit et al.; 2014). Even if BitterDB cross-links information about receptor physiology and chemical bond with reported receptor structure, it uses as a validation method literature evidence. Through structured literature review conducted here, it was showed that literature lacks in demonstrating clozapine taste and does not propose an assessment of bitterness threshold. Thus, we considered this tool as not reliable for taste prediction of clozapine(Behrens et al.; 2007; Shaw; 2018).

Clozapine taste was claimed both in literature and in all PILs as bitter. A taste aversion is considered in the literature to cause medication non-compliance. However, the structured literature review conducted here does not report evidence of clinical study or audit in which taste is considered as an issue. A PIL is the leaflet included in medicine boxes that it is written for patients and gives information about using the medicine, its composition, and side effects. PIL addition to dose box becomes a legal requirement since 1999; however, Raynor and co-workers , reported a lack of evidence that the patient refers leaflets(Holger Russig, Aneta Kovacevic; 2003). Usually, information about side effects reported in PIL is collected during step three of clinical trials when are conducted

animal study and have to be published with the highest accuracy(Holger Russig, Aneta Kovacevic; 2003). However, data on side effect could be reported post marketing through a pharmaco-vigilance reporting system that includes clinician, pharmacists, nurses, and patients themselves(Dal Pan et al.; 2019).

Consequently, two pharmaceutical companies were contacted to understand why "bitter taste" is reported in PILs. Both companies were not able to provide information on how this side effect was assessed and referred to main monography. The latest version of the monograph was revised in 2017 by Auro Pharma Inc., Canada, and presented bitterness as a side effect of medication(Inada and Ishigooka; 2013). Therefore, the reason for reporting aversive drug taste was sought in excipient of each marketed dosage forms: Clozaril® from Novartis, FazaClo® from Alamo Pharmaceuticals and Azur Pharma, Sandoz Clozapine tablets and in prescribing information provided by the FDA(Hardeman S.; n.d.; Novartis; n.d.; Oliver; 2013; Pharma; 2011; Sandoz; 2018). Moreover, it is well-known that in clinical practise clozapine dosage forms are crushed to be administered to patients with swallowing difficulties. Tablet crushing could cause a bad taste perception by exposing the oral cavity to different excipients. Therefore, it was investigated excipients listed in each of marketed medications and sought bitterness within them.

It was observed that each formulation presents at least one excipient that is considered bitter or having an unpleasant in taste. List of excipients per each medication is listed in Table 36. Interestingly, it was demonstrated that each formulation presents at least one aversive excipient. As in clinical practice, it is common to crush the tablet to administer medication; reported taste may refer to excipients themselves and not to drug.

Table 36. Brand dosage forms and excipients that possess a bitter taste

Dosage Form	Brand Name	Excipients	Bitter or unpleasant taste
Tablet	FazoClo ®	Aspartame	Riera and co-workers , 2007(Riera et al.; 2007)
		Sodium Bicarbonate	Newbrun E., 1996(Newbrun; 1996)
		Citric Acid Monohydrate	Garden and co-workers , 2003(Garden, J., Roberts, K., Taylor, A., and Robinson; 2003)
	Clozaril ®	Silicon Dioxide	Shabir and co-workers , 2012(Shabir et al.; 2012)

Oral Solution	Clozapine Sandoz®	Sodium Lauryl Sulfate	CompoundChem 2014(COMPOUNDCHEM; 2014)
	Versacloz ®	Sodium Phosphate Monobasic Dihydrate	Shimokomaki and co-workers , 2003(Shimokomaki et al.; 2003)
	Denzapine ®	Sodium dihydrogen phosphate dihydrate	Shimokomaki and co-workers , 2003(Shimokomaki; 2003)

3.3 Human Taste Assessment

Assessment of taste aversiveness provides information about the acceptability of various taste stimuli, which perception depends on activation of receptor family T2R(Mennella; 2013). Recent studies hypothesised that diversity in gene sequences creates diversity and variation in the individual opinion of taste and therefore in medication adherence(Kim et al.; 2005; Mennella et al.; 2011) These differences could also be influenced by patient's personalities such as temperament and experience(Macht & Mueller; 2007; Mela et al.; 1992). In treatment-resistant schizophrenia clozapine taste is considered to affect patient medication acceptability, but literature evidence as lacking in the identification of a taste threshold. Therefore, this part of work aimed in threshold calculation as EC50, the concentration of drug that produces half of the maximal rating through a human panel study.

Volunteers were recruited according to inclusion and exclusion criteria defined in the method section, were asked to swirl four different concentrations of a clozapine solution and rate any aversive taste perception from 0 to 100%. Human taste panel study results are displayed in Figure 67 and showed an average of 7% of aversiveness for clozapine solutions, with a, and static Kruskal-Wallis test revealed concentration had a (just about) significant effect on human rating ($p=0.05$). However, it is usually considered that values under 50 are neutral. Therefore, no aversion against the taste of clozapine it was found. Moreover, no significant difference was found between four different concentration of clozapine solutions. Human taste panel aimed to calculate EC50, which give as information about taste threshold. However, results showed that no one of clozapine solutions at four different concentration values (0.018, 0.012, 0.0022 and 0.0011 mg/mL) affect volunteers taste perception; thus, EC50 could not be calculated, and clozapine taste perception has to be considered as not aversive. Low concentration of solution would not affect results as the use of a drug at its saturation solubility (clozapine saturation solubility 0.018 mg/mL) it is mimicked *in-vivo* condition. Due to clozapine low water solubility, saturation solubility represents the highest quantity of the drug that could be released in mouth and so capable of interacting with taste receptor(Colombo et al.; 2017).

Gao's post hoc analysis was used to look at differences between concentrations(Gao et al.; 2008). There was only one significant difference ($p < 0.05$) between 0.0022 and 0.0011 mg/mL ($p = 0.034$). All other concentrations were not rated in a significantly different way ($p > 0.05$).

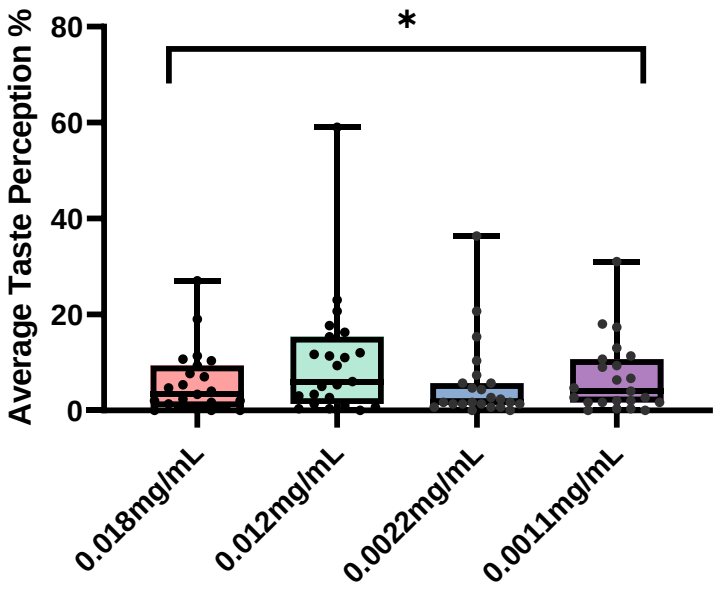


Figure 67. Human taste panel results from a cohort of 23 patients. Data showed an overall 7% of alteration of taste perception. This average value is not considered as aversive. Results are just about statistically significant (*) at static Kruskal-Wallis test.

Different in the perception of clozapine taste between gender and age groups were evaluated. Data showed in Figure 68. No significant differences observed between males and females, particularly, highest concentrations of 0.018 mg/mL showed an average value of averseness percentile of 11.12 ± 4.26 % ($p=0.013$) and 0.012 mg/mL of 19.87 ± 34.72 % ($p=0.016$). No difference was identified either for both lowest concentrations.

As well no difference between ages at all concentrations, data were analysed by searching for similarity using t-test obtaining all p values < 0.05, as displayed in Figure 69.

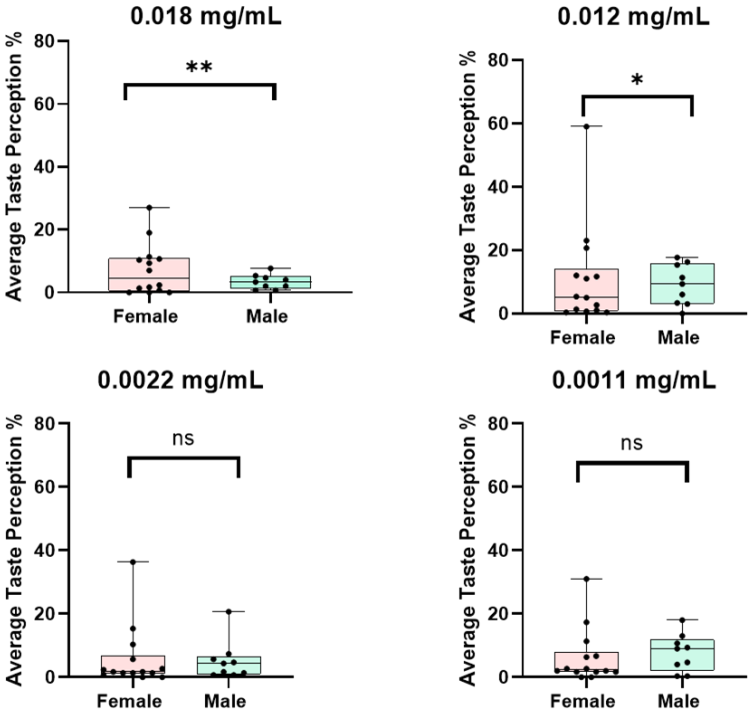


Figure 68. Displayed that no difference occurred between gender. With good statically significance (** and *) for higher concentrations (0.018 and 0.012 mg/mL), while not-significance (ns) was found for lower concentrations (0.0022 and 0.0011 mg/mL).

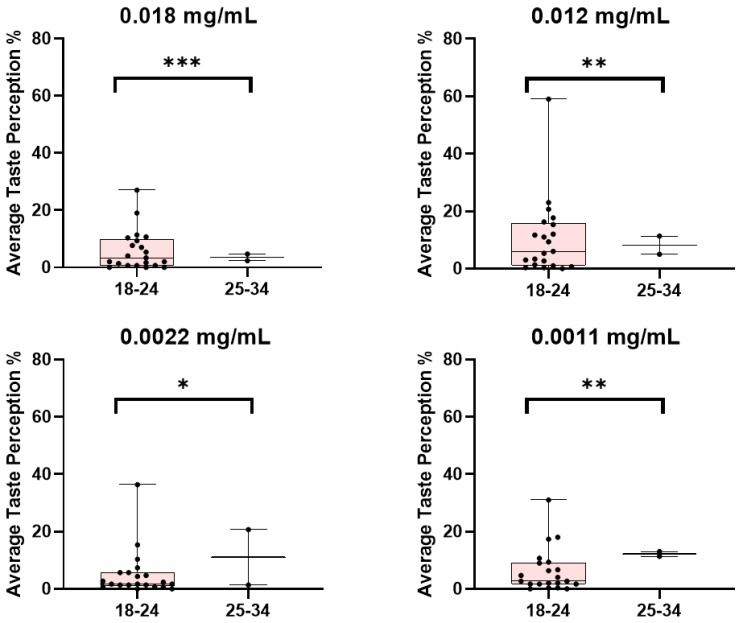


Figure 69. Displayed that no difference occurred between two age groups 18-24 and 25-34. High statistical significance were found comparing the highest concentration of 0.018 mg/mL (***). Good significance (**) were identified comparing groups age of concentrations 0.012 and 0.0011 mg/mL and low statically significance (*) was observed concentration 0.022 mg/mL.

This human panel study received ethics approval (REC 4612/024). Therefore, it was possible to analyse volunteer responses, as well as reporting all volunteer comments in Table 37. Comments were listed from the most concentrated solution to lowest focused solution of the drug. Overall, it was noticed that candidate did not perceive any taste from solution compared with "plain water", that was available to neutralise palate between each sample, as per guidance(Keeley; 2019). The taste was described as "not aversive" or "like water" from 22 candidates up to 23, where just one recognised an unpleasant feeling for 0.018 mg/mL. While for concentration 0.012 mg/mL, it was identified a slight aversive after taste from two candidates. However, most of the comments for all solutions reported a watery-taste, not aversive and quite acceptable, confirming rate provided.

Table 37. Comments provided by volunteers during the human panel study. This information can be disclosed as volunteer's privacy is respected and as allowed by ethical approval REC 4612/024.

0.018 mg/mL
There is no taste difference of sample compared to water
No difference to water
Slight powdery taste in the end but nothing aversive.
Also, I could not tell that I felt any flavour. Felt like water
less of a taste than the previous sample with similar viscosity and mouthfeel
taste like water
It tastes like tap water. Slightly bitter when it first came into the mouth, and feelings became stronger later
no taste at all
Seemingly tasteless, not aversive at all
The beginning taste was not aversive, but towards the end of 10 seconds, it became a lot more aversive. Taste it left in your mouth was not nice
It is a neutral flavour
not aversive at all, taste quite like water
plain as water
0.012 mg/mL
a slightly different taste when first swirled around back of the mouth which then disappears
The sample has no difference in taste compared to water.
Same taste as water.
A slight powdery taste again.
Feels like water. Subtle flavour but it is negligible
Slight taste upfront which fades
No taste at all
Bitter but not strong. taste last long after spitting it out. (compare to previous ones)
very slight taste but minimal
Seemingly tasteless, but a mild and subtle bitter aftertaste lingers around the mouth
Not aversive. Yet again beginning taste reminds me of a mushroom, but that went away very quickly.
0.0022 mg/mL

Like water
It is not bitter at all, not in aversive
No difference to the taste of water
Tastes neutral - like water.
No taste at all
No taste whatsoever
Water-like viscosity and mouthfeel; extremely mild almost bitter taste after several seconds but practically unremarkable
Palatable taste
no taste at all
slightly bitter
almost no taste to no taste at all
Very plain, water-like
quite like water
Is neutral, a little bit sweet at the end
Tasted like water with high pH
plain and smooth taste, not irritating and quite acceptable
No difference from water
taste like water
0.0011 mg/mL
The sample has little (nearly no difference) taste to water, probably to different pH of the sample compared to water given at the start of the project
Could not tell the difference with water
Slight bitter taste - not noticeable if you are not observing
Very subtle taste, can/t even describe it because it is barely noticeable. Just felt like water as well
The taste is acceptable
It is very bitter and a little astringent. Tastes like tap water.
Very slight medicinal taste at the end of 10 seconds
Seemingly tasteless, not aversive
No problems with this one. The taste reminded me off mushrooms. Did not leave an after taste in my mouth
It is neutral
Very neutral-tasting
taste acceptable

These findings suggest that none of the different concentrations of clozapine solution generate taste aversion. In Chapter 7 of this thesis, it was proved that literature lacks clarity regarding the claim that clozapine possesses an aversive taste. Moreover, it was suggested that unpleasant sensation related with marketed dosage forms could be due to use of at least one excipient recognised as bitter or aversive such as aspartame(Riera; 2007), sodium bicarbonate(Newbrun; 1996), silicon dioxide(Shabir; 2012) and sodium dihydrogen phosphate dihydrate(Shimokomaki; 2003). Therefore, it was recommended the use of produced data to improve existing formulations as a strategy that would improve patient acceptability of medication and thus reduce the cause of non-compliance.

4. Conclusion

Taste is recognised to be one of the causes of medication non-compliance. In treatment-resistant schizophrenia , non-compliance is considered to cause 70% of medication discontinuation that cause a relapse. In this section of present work, the problem of clozapine taste was addressed. At first, literature was revised extensively, and no reference regarding an assessment of clozapine taste was found. However, four papers reported that clozapine solution does not affect the drinking behaviour of a 5% sucrose solution and, therefore, was considered as not creating taste aversion. Then, the database BitterDB for taste prediction was used and predicted an aversive taste for the drug. Since the database relies on literature as a confirmation method and since no valuable documentation was found, this source was considered inconsistent with the research question. Investigation of the literature demonstrated that no evidence of human patient experiencing an aversive taste after clozapine intake.

Moreover, analysis of PIL showed that each formulation presents excipients known for its aversive taste. Lack of information was sought when contacting companies which were not able to provide evidence or guidance on how to taste aversion was assessed, but referred the monograph as main resource. Overall, due to the influence of taste on medication acceptability in patient affected by treatment-resistant schizophrenia patients being treated with clozapine, it is essential to investigate further its taste by a in-human taste assessment.

Therefore, the present work investigated taste of clozapine as literature reported that one of the leading causes of non-compliance toward clozapine market dosage forms could be due to its aversive taste. It was previously proved that in the literature, there is missing evidence reporting an assessment of this taste perception and its effect on medication acceptability. Therefore, in this definitive work, it was proposed a quantitative evaluation of clozapine taste, namely EC50, by performing a human panel study. The study was conducted on four different concentrations of clozapine and its saturation solubility. Data showed that no one of the solutions causes an unpleasant or aversive mouth perception and that EC50 was not calculable. Such results were confirmed by statistical analysis, demonstrating that no difference occurred between the different sample. No difference was also observed between gender or age groups. Moreover, volunteers commented that the solution tasted like water, and no aversiveness was overall found. These results are outstanding as for the first-time clozapine aversiveness was assessed proving that clozapine does not affect the mouth sense, suggesting that the excipient of the marketed dosage form is responsible for the claim reported in the literature.

Part 4. Conclusion and Recommendation of Future Work

This PhDfocused on different formulation strategies application to produce a patient-centric delivery system as a novel dosage form aimed to control clozapine release profile reducing dose per day and investigate medication acceptability challenge related to drug taste perception. Different methods were used and examined to obtain the optimal drug delivery system and propose a product that could potentially be introduced into the market.

To achieve this aim, different objectives were pursued to identify the best formulation process willing to both achieve clozapine controlled release profile and show process reproducibility and flexibility in designing a patient-centric dosage form.

At first, spray-drying was used to produce multi component solid dispersions able to increase solubility and permeability of the poorly-soluble drug, classified a BCS class II. By using optimised parameter, it was possible to improve both drug dissolution and release profile in the gastrointestinal as well as its permeability through colon tissue. Overall, the results suggested a potential of successive compression of the microparticles to obtain a conventional oral dosage form as a tablet. As discussed in the Introduction, even if the tablet represent the most common oral route, this is not suitable for poorly soluble drugs due to low solubility as well as the first past metabolism. Therefore, other common formulation strategies, solvent casting method and freeze-drying, were investigated and compared with spray-drying to detect the most suitable method of producing a controlled release dosage forms.

Three different poorly soluble drugs were used to increase the study reliability, such as olz, dsm, and trm. Mainly, were analysed the pharmaceutical advantages in achieving higher drug dissolution profile, surface homogeneity and drug encapsulation efficacy. Firstly, it was noticed that each of prepared product improved solubility of all drugs; however, through freeze-drying and the solvent-casting method was obtained a more homogenous and reproducible surface morphology and pore/particle size. Spray-drying caused mass loss and low drug encapsulation efficacy. In all formulation the drugs were maintained in the crystalline state in the micro-range, therefore, were produced solid micro-crystalline dispersion with high dissolution profile. Of all the methods, solvent-casting generated the fastest and most controlled drug release rate for each drug in the study, suggesting it as the most efficient and reproducible manufacturing strategy.

The advantage of the solvent casting method opened to the formulation of a novel oral transmucosal polymeric patch containing clozapine, the target drug of this thesis. Therefore, the

combination of the promising polymers PVA and PHEA was used in different concentrations to prepare transmucosal patch both via solvent casting method and wet electrospinning. Interestingly, it was concluded that 7.5% w/v of PVA and 5% w/v of PHEA achieved higher solubility enhancement potential. Moreover, PHEA showed a concentration-dependent action on adhesiveness providing, at the same polymer ratio, the highest adhesive forces. Electrospun nanofibress provided a controlled drug release profile while incrementing drug dissolution rate. However, this transmucosal patches if administered *in-vivo* would allow the multi-directional release of clozapine both through the mucosa and the oral cavity, causing drug clearance and low bioavailability.

Even if electrospinning and the combination of PVA-PHEA produced the advantage of controlling drug release profile, this technique did not allow the production of a complex structure such as the addition of a hydrophobic backing layer to the unidirectionally direct drug just through the buccal mucosa. Moreover, it did not allow product customisation in the creation of patient-centric formulation. Therefore, 3D printed technology was investigated as a manufacturing strategy to produce a unidirectional oral transmucosal patch. Eight different patches were printed with and without hydrophobic backing layer and with and without a set of 9 micro-pyramids to improve mucosa penetration and adhesion. The patches were realised with PVA as adhesive layer impregnated with drug and PLA as a hydrophobic backing layer. It was analysed the effect of printing infill on clozapine release profile. Data showed that patches printing with 65% infill presented a controlled-release profile while 100% patches an immediate-like release. The addition of the backing layer supported a controlled-release and was further investigated in the scaled-down of $1cm^2$.

The unidirectional transmucosal patch provided with a hydrophobic backing layer with a surface area of $1cm^2$ were loaded with three different strengths of clozapine to determine whereas the release mechanism was drug-controlled or carrier-controlled, resulting in being drug-controlled. In term of the potential of a market dosage form to control the release of clozapine, this information will be crucial as different strengths of clozapine will present different release profiles. Moreover, the application of micro-pyramids improved the dissolution profile respect both unformulated drug and flat patches. Permeation studies were conducted both on cellulose acetate membrane and the biomimetic membrane Permeapad™. The analysis showed that the higher dissolution profile of the drug in the donor compartment caused membrane blockage in both membranes, reducing permeability in the patch with micro-pyramids. From the other hand side, flat patches PVA+PLA increased permeability.

In conclusion, 3D printing technology represents the most convenient formulation strategy to generate PVA+PLA patches which controlled clozapine release profile and improve its permeability. Such dosage form could be further investigated through *in-vivo* study confirming an increase of drug

bioavailability. This formulation is the first dosage forms available to controlled clozapine release; therefore, further investigation *in-vivo* will be of advantage to bring the patch to a patentable and market product.

In this project, it was also analysed the issue related to drug taste, as if the patch would be applied in human in the oral cavity it could generate an aversive taste perception. A structured literature review showed that claim about clozapine taste was not based on scientific evidence or clinical report and that aversive taste perception could be due to excipient present in each marketed dosage forms. Moreover, it was conducted a human taste study to assess clozapine taste aversion, and it was found that even at the saturation solubility, the highest concentration of drug capable of interacting with mouth receptors, do not generate taste perception. Such results were confirmed by volunteers' free comments and statistical analysis, reconducting the reported taste issue to excipient used in the marketed dosage forms.

In conclusion, this thesis aimed to produce a patient-centric transmucosal dosage form to control clozapine release profile. To achieve this aim, different formulation strategies were proposed to tackle both clozapine dosing per day and taste. The analysis of the most common techniques brought to the identification of 3D printing as a flexible tool to produce complex structures as a unidirectional transmucosal patch. The patches printed with 65% infill and produced with PVA and PLA were able to provide a controlled release profile higher than the unformulated drug, improving both clozapine permeability rate and permeation through membrane lipidic component. Moreover, drug taste would not result in an aversive perception if the dosage form would be marketed and administered in human.

This patch represents the firstly produced clozapine dosage form with controlled-release; therefore, a future investigation of the *in-vivo* release of clozapine from the generated patch, thus its bioavailability, is considered fundamental. Further research and confirmation on the absence of taste aversion could be sought conducting BATA test using the dosage form instead of pure drug.

Appendix

Appendix 1. Influence of PVA) on PVA-PHEA microcrystalline solid dispersion films

This study was conducted to formulate buccal films consisting of PVA and PHEA, to improve the dissolution of the drug through the oral mucosa. Ibuprofen sodium salt was used as a model drug, and the buccal film was expected to enhance its dissolution rate. Two different concentrations of PVA (5% w/v and 7.5% w/v) were used. Solvent casting was used to prepare films, where a solution consisting of drug and polymer was cast and allowed to dry. ATR-FTIR, DSC, and scanning electron microscopy (SEM) were used to investigate the properties of films. *In-vitro* dissolution studies were also conducted to investigate drug release. SEM studies showed that films containing a higher concentration of PVA had larger particles in micro-range. FTIR studies confirmed the presence of the drug in films and indicated that ibuprofen sodium did not react with polymers. DSC studies confirmed the crystalline form of ibuprofen sodium when incorporated within films. *In-vitro* dissolution studies found that the dissolution percentage of ibuprofen sodium alone was increased when incorporated within the film from 59 to 74%. This study led to the development of solid microcrystalline dispersion as a buccal film with a faster dissolution rate than the drug alone overcoming problem of poor solubility.

Appendix 2. Human Taste Assessment SOP

Before the panel

1) **Check that you have all you need for the panel ~1 week before**
   order/buy what is missing

2) **Make sure the room is booked with CT/Susan Smyth**
   email Maria Buck to kindly remind her at what date /time you will be using the room

3) **Recruitment**

The recruitment process starts about one week before, and it usually takes half an hour to fill all the positions available. Then, the participants need to be contacted individually and informed about the session in which they have been allocated. A confirmation email has to be sent from the participants to avoid incomplete slots.

4) **Generate:**
   a. the randomisation orders
   b. and the coding of the samples
   c. and the anonymisation list of the participants
   d. The tiny URL link to the QUALTRICS questionnaire of the present panel

5) **Preparation of samples**
   Done in B21
   According to SOPs/BMRs
   Respect shelf life

What to do in PSL1 before the panel

NB you need access to PSL1 - arrange with CT and lodge
Two people are required to set up the room promptly but also:

- ✓ One to keep an eye on participants and
- ✓ The other one to prepare samples in between

One must be a pharmacist
Make sure the master file for the panel is in the room with all necessary information: one hard copy of the REC, REC approval, REC amendment if any, SoPS/BMRs, all H&S documents, the payment form, the anonymised randomisation sheets etc

Bring the trolley with all materials, samples etc. needed for the panel
Could you put it in the dispensary?
Clean the bench with alcoholic wipes

1) Switch on all computer needed for the session
   Leave 1 computer empty in-between each participant
   Pull the middle screen to create a separation between them
   (16 is good AND already a large number of participants in one go)

2) Log in
   PSLUSER
   PW: Pharma14

3) Open a web browser
4) Type in the URL linking to the questionnaires for the panel

5) Wipe all bench with the alcoholic wipes

Bring at each seat

- A bottle of water
- A paper plate
- A (big) plastic cup to spit in
- A box of tissue
- 2 consent forms
- 1 information sheet
- 1 pen
- 1 test tubes containing water

6) Put some crackers in the plate

During the panel

The participants arrive.

The PI or designated researcher gives a bit of background and introduces the taste testing methodology, and ask them to make sure that:

- they have read the Information leaflet
- ask if they have any question
- ask them fill in the consent form (one for UCL one for them) signed by both parties
- what the crackers and water are there for
- they do NOT refresh the link (as it erases all data and they would have to start again)

Monitor that they are not communicating about the samples/results.

It is good practice to start with a 'blank' sample eg 10ml of water (same as the one used to prepare sample if it is a water-based sample) and make sure they understand the swirl and spit and the scale.

Between the samples the researchers, prepare the tubes according the randomised codes and place them in a basket to distribute the sample to the participants. Before each new samples is provided the previous has been disposed from the researcher/PI to avoid any confusion with the codes.

At the end of the panel

Ask them to discard their spit in the sink

Make sure you give it a good clean after the last participants has finished

A big bin bag should be available for them to discard all disposable consumable

If specific bins are required, they need to be introduced to the participants

They get their payment and MUST sign the receipt form as well as the person who is giving them the money

At the end make sure you exit all applications on the computer Log out, switch of the computer
Put back the screen neatly as they were when you arrived

Wipe all spaces where panellists were sitting as well as the dispensary

PhD Publications

1. Engineering of Nanofibrous Amorphous and Crystalline Solid Dispersions for Oral Drug Delivery, **Laura Modica de Mohac**, Alison Veronica Keating, Maria de Fátima Pina, and Bahijja Tolulope Raimi-Abraham. doi.org/10.3390/pharmaceutics11010007. Pharmaceutics. 2018
2. Multicomponent solid dispersion as a formulation strategy to improve drug permeation: A case study on the anti-colorectal cancer irinotecan, **Laura Modica De Mohac**, Roberto Caruana, Francesco Carfì-Pavia, Gennara Cavallaro, Gaetano Giammona, Mariano Licciardi. doi.org/10.1016/j.jddst.2019.04.040. Journal of Drug Delivery Science and Technology. 2019
3. Chew SL, Modica de Mohac L, Tolulope Raimi-Abraham B. 3D-Printed Solid Dispersion Drug Products. Pharmaceutics. 2019;11(12):672. doi:10.3390/pharmaceutics11120672
4. Paolino M, Licciardi M, Savoca C, et al. Hyaluronan Graft Copolymers Bearing Fatty-Acid Residues as Self-Assembling Nanoparticles for Olanzapine Delivery. 2019;44.
5. De Mohac LM, Caruana R, Cavallaro G, Giammona G, Licciardi M. Spray-Drying, Solvent-Casting and Freeze-Drying Techniques: a Comparative Study on their Suitability for the Enhancement of Drug Dissolution Rates. Pharm Res. 2020;37(3):57. doi:10.1007/s11095-020-2778-1
6. Modica de Mohac LM, Raimi-Abraham B, Caruana R, Gaetano G, Licciardi M. Multicomponent solid dispersion a new generation of solid dispersion produced by spray-drying. J Drug Deliv Sci Technol. 2020;57(March):101750. doi:10.1016/j.jddst.2020.101750
7. Al-Sahaf Z, Raimi-Abraham B, Licciardi M, de Mohac LM. Influence of Polyvinyl Alcohol (PVA) on PVA-Poly-N-hydroxyethyl-aspartamide (PVA-PHEA) Microcrystalline Solid Dispersion Films. AAPS PharmSciTech. 2020;21(7):267. doi:10.1208/s12249-020-01811-z

PhD Conferences: Invited and awarded poster and podium presentations

1. *Global Congress & Expo on Biomaterials (Biomaterials-2019).* 13th and 14th May 2019. 16 hours. Kuala Lumpur, Malaysia. Invited Poster presentation**.** Title "Engineering Oral Mucosal Polymeric Patches
for Treatment of Childhood-Onset Schizophrenia".
2. *Cancer & Pharmaceutical Sciences Postgraduate Research Symposium.* 3rd June 2019. Invited Poster presentation. Title "Pediatric Oral Transmucosal Delivery of Clozapine for the Treatment of Childhood Schizophrenia".
3. *13th Excellence in Teaching Conference.* 20th June 2019. Invited Oral Presentation. Title "International experiences in early career academic development".
4. *Cancer & Pharmaceutical Sciences Postgraduate Research Symposium.* 2nd of September to 11th of September 2020. Awarded Oral presentation. Title "Clozapine Taste Assessment: the first in-human clozapine taste confirmation".

Bibliography

Abeelen, M. (2013). *GUIDELINE FOR THE USE OF CLOZAPINE*. 43.

Abruzzo, A., Bigucci, F., Cerchiara, T., Cruciani, F., Vitali, B., & Luppi, B. (2012). Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride. *Carbohydrate Polymers*, *87*(1), 581–588. https://doi.org/10.1016/j.carbpol.2011.08.024

Adams, L. V., Craig, S. R., Mmbaga, E. J., Naburi, H., Lahey, T., Nutt, C. T., Kisenge, R., Noel, G. J., & Spielberg, S. P. (2013). Children's Medicines in Tanzania: A National Survey of Administration Practices and Preferences. *PLoS ONE*, *8*(3), e58303. https://doi.org/10.1371/journal.pone.0058303

Agama-Acevedo, E., & Bello-Perez, L. A. (2017). Starch as an emulsions stability: the case of octenyl succinic anhydride (OSA) starch. *Current Opinion in Food Science*, *13*, 78–83. https://doi.org/10.1016/j.cofs.2017.02.014

Agid, O., Schulze, L., Arenovich, T., Sajeev, G., McDonald, K., Foussias, G., Fervaha, G., & Remington, G. (2013). Antipsychotic response in first-episode schizophrenia: efficacy of high doses and switching. *European Neuropsychopharmacology*, *23*(9), 1017–1022. https://doi.org/10.1016/j.euroneuro.2013.04.010

Ainurofiq, A., Choiri, S., Azhari, M. A., Siagian, C. R., Suryadi, B. B., Prihapsara, F., & Rohmani, S. (2016). Improvement of Meloxicam Solubility Using a β-Cyclodextrin Complex Prepared via the Kneading Method and Incorporated into an Orally Disintegrating Tablet. *Advanced Pharmaceutical Bulletin*, *6*(3), 399–406. https://doi.org/10.15171/apb.2016.052

Al-ghananeem, A. M. (2009). *Compositions and methods for transmucosal delivery oflofexidine* (Patent No. US12410114).

Al-Qallaf, B., & Das, D. B. (2009). Optimizing Microneedle Arrays to Increase Skin Permeability for Transdermal Drug Delivery. *Annals of the New York Academy of Sciences*, *1161*(1), 83–94. https://doi.org/10.1111/j.1749-6632.2009.04083.x

Aleman, A., Kahn, R. S., & Selten, J.-P. (2003). Sex Differences in the Risk of Schizophrenia. *Archives of General Psychiatry*, *60*(6), 565. https://doi.org/10.1001/archpsyc.60.6.565

Alessi-Severini, S., Le Dorze, J.-A., Nguyen, D., Honcharik, P., & Eleff, M. (2013). Clozapine Prescribing in a Canadian Outpatient Population. *PLoS ONE*, *8*(12), e83539. https://doi.org/10.1371/journal.pone.0083539

Ali, A. M. A. A. et al. A., Ali, A. M. A. A. et al. A., & Maghrabi, I. A. (2015). Clozapine-carboxylic acid plasticized co-amorphous dispersions: Preparation, characterization and solution stability evaluation. *Acta Pharmaceutica*, *65*(2), 133–146. https://doi.org/10.1515/acph-2015-0014

Ali, J., Chiang, M., Lee, J. B., Voronin, G. O., Bennett, J., Cram, A., Kagan, L., Garnett, M. C., Roberts, C. J., & Gershkovich, P. (2020). Is rat a good model for assessment of particulate-based taste-masked formulations? *European Journal of Pharmaceutics and Biopharmaceutics*, *146*, 1–9. https://doi.org/10.1016/j.ejpb.2019.11.001

Allen, L. V, Nicholas, G., & Ansel, H. C. (2013). Ansel's Pharmaceutical Dosage Forms. *Anticancer Research*, *33*(5), 2345. http://www.ncbi.nlm.nih.gov/pubmed/23645785

Alopaeus, J. F., Hagesæther, E., & Tho, I. (2019). Micellisation Mechanism and Behaviour of Soluplus®–Furosemide Micelles: Preformulation Studies of an Oral Nanocarrier-Based System. *Pharmaceuticals*, *12*(1), 15. https://doi.org/10.3390/ph12010015

Alopaeus, J. F., Hellfritzsch, M., Gutowski, T., Scherließ, R., Almeida, A., Sarmento, B., Škalko-Basnet, N., & Tho, I. (2020). Mucoadhesive buccal films based on a graft co-polymer – A mucin-retentive hydrogel scaffold. *European Journal of Pharmaceutical Sciences*, *142*, 105142. https://doi.org/10.1016/j.ejps.2019.105142

Altamimi, M. A., & Neau, S. H. (2017). Investigation of the in vitro performance difference of drug-Soluplus® and drug-PEG 6000 dispersions when prepared using spray drying or lyophilization. *Saudi Pharmaceutical Journal*, *25*(3), 419–439. https://doi.org/10.1016/j.jsps.2016.09.013

Anand, V., Kataria, M., Kukkar, V., Saharan, V., & Choudhury, P. K. (2007). The latest trends in the taste assessment of pharmaceuticals. *Drug Discovery Today*, *12*(5–6), 257–265. https://doi.org/10.1016/j.drudis.2007.01.010

Andreasen, N. C. (1982). Negative Symptoms in Schizophrenia. *Archives of General Psychiatry*, *39*(7), 784. https://doi.org/10.1001/archpsyc.1982.04290070020005

Andrews, G. P., & Jones, D. S. (2014). Hot melt extrusion - Processing solid solutions? *Journal of Pharmacy and Pharmacology*, *66*, 145–147. https://doi.org/10.1111/jphp.12202

Aquino, R. P., Barile, S., Grasso, A., & Saviano, M. (2018). Envisioning smart and sustainable healthcare: 3D Printing technologies for personalized medication. *Futures*, *103*, 35–50. https://doi.org/10.1016/j.futures.2018.03.002

Arias, M. J., Gines, J. M., Moyano, J. R., & Rabasco, A. M. (1994). The Application of Solid Dispersion Technique with D-mannitol to the Improvement in Oral Absorption of Triamterene. *Journal of Drug Targeting*, *2*(1), 45–51. https://doi.org/10.3109/10611869409015892

Arrighi, A., Marquette, S., Peerboom, C., Denis, L., Goole, J., & Amighi, K. (2019). Development of PLGA microparticles with high immunoglobulin G-loaded levels and sustained-release properties obtained by spray-drying a water-in-oil emulsion. *International Journal of Pharmaceutics*, *566*, 291–298. https://doi.org/10.1016/j.ijpharm.2019.05.070

Artursson, P., Neuhoff, S., Matsson, P., & Tavelin, S. (2007). Passive Permeability and Active Transport

Models for the Prediction of Oral Absorption. In *Comprehensive Medicinal Chemistry II* (pp. 259–278). Elsevier. https://doi.org/10.1016/B0-08-045044-X/00126-7

Asem, M., Nawawi, W. M. F. W., & Jimat, D. N. (2018). Evaluation of water absorption of polyvinyl alcohol-starch biocomposite reinforced with sugarcane bagasse nanofibre: Optimization using Two-Level Factorial Design. *IOP Conference Series: Materials Science and Engineering*, *368*, 012005. https://doi.org/10.1088/1757-899X/368/1/012005

Augustijns, P., & Brewster, M. E. (2012). Supersaturating Drug Delivery Systems: Fast is Not Necessarily Good Enough. *Journal of Pharmaceutical Sciences*, *101*(1), 7–9. https://doi.org/10.1002/jps.22750

Ault, J. T., Shin, S., & Stone, H. A. (2019). Characterization of surface–solute interactions by diffusioosmosis. *Soft Matter*, *15*(7), 1582–1596. https://doi.org/10.1039/C8SM01360H

Awad, A., Fina, F., Trenfield, S., Patel, P., Goyanes, A., Gaisford, S., & Basit, A. (2019). 3D Printed Pellets (Miniprintlets): A Novel, Multi-Drug, Controlled Release Platform Technology. *Pharmaceutics*, *11*(4), 148. https://doi.org/10.3390/pharmaceutics11040148

Ayana Wiener, Marina Shudler, Anat Levit, M. Y. N. (2011). BitterDB: a database of bitter compounds. *Nucleic Acids Research*. https://doi.org/10.1093/nar/gkr755

Aytac, Z., & Uyar, T. (2017). Core-shell nanofibers of curcumin/cyclodextrin inclusion complex and polylactic acid: Enhanced water solubility and slow release of curcumin. *International Journal of Pharmaceutics*, *518*(1–2), 177–184. https://doi.org/10.1016/j.ijpharm.2016.12.061

Baca Lopez, D. M., & Ahmad, R. (2020). Tensile Mechanical Behaviour of Multi-Polymer Sandwich Structures via Fused Deposition Modelling. *Polymers*, *12*(3), 651. https://doi.org/10.3390/polym12030651

Bachmann, C., Aagaard, L., Bernardo, M., Brandt, L., Cartabia, M., COma Fusté, A., Furu, K., Garuolienė, K., Hoffmann, F., Holligworth, S., Huybrechts, K., Kalverdijk, L., Kawakami, K., Kieler, H., Kinoshita, T., López, S., JE, M.-Al., Machado-Duque, M., Mahesri, M., … Clavenna, A. (2017). International trends in clozapine use : a study in 17 countries. *Acta Psychiatrica Scandinavica*, *136*, 37–51. https://doi.org/10.1111/acps.12742

Baghel, S., Cathcart, H., O'Reilly, N. J., & O'Reilly, N. J. (2018). Investigation into the Solid-State Properties and Dissolution Profile of Spray-Dried Ternary Amorphous Solid Dispersions: A Rational Step toward the Design and Development of a Multicomponent Amorphous System. *Molecular Pharmaceutics*, *15*(9), 3796–3812. https://doi.org/10.1021/acs.molpharmaceut.8b00306

Ball, C., & Woodrow, K. A. (2014). Electrospun solid dispersions of maraviroc for rapid intravaginal preexposure prophylaxis of HIV. *Antimicrobial Agents and Chemotherapy*, *58*(8), 4855–4865.

https://doi.org/10.1128/AAC.02564-14

Balogh, A., Farkas, B., Domokos, A., Farkas, A., Démuth, B., Borbás, E., Nagy, B., Marosi, G., & Nagy, Z. K. (2017). Controlled-release solid dispersions of Eudragit® FS 100 and poorly soluble spironolactone prepared by electrospinning and melt extrusion. *European Polymer Journal*, *95*, 406–417. https://doi.org/10.1016/j.eurpolymj.2017.08.032

Barltrop, D., & Meek, F. (2013). Effect of Particle Size on Lead Absorption from the Gut. *Archives of Environmental Health: An International Journal*, *34*(4), 280–285. https://doi.org/10.1080/00039896.1979.10667414

Barnes T R E; McEvedy, C. J. B. (n.d.). *Pharmacological_treatment_strategies_in_the.11.pdf*.

Basf. (2010). Technical Information Soluplus. *BASF, Pharma Ingredients & Services*, *July*, 1–8.

Baysan, U., Elmas, F., & Koç, M. (2019). The effect of spray drying conditions on physicochemical properties of encapsulated propolis powder. *Journal of Food Process Engineering*, *42*(4), e13024. https://doi.org/10.1111/jfpe.13024

Beach, E. R., Tormoen, G. W., Drelich, J., & Han, R. (2002). Pull-off Force Measurements between Rough Surfaces by Atomic Force Microscopy. *Journal of Colloid and Interface Science*, *247*(1), 84–99. https://doi.org/10.1006/jcis.2001.8126

Beak, I.-H., & Kim, M.-S. (2012). Improved Supersaturation and Oral Absorption of Dutasteride by Amorphous Solid Dispersions. *Chemical and Pharmaceutical Bulletin*, *60*(11), 1468–1473. https://doi.org/10.1248/cpb.c12-00563

Beerpoot, L. J., Lipska, B. K., & Weinberger, D. R. (1996). Neurobiology of treatment-resistant schizophrenia: new insights and new models. *European Neuropsychopharmacology*, *6*(2), S27-34. https://doi.org/10.1016/0924-977X(96)00008-9

Behrens, M., Foerster, S., Staehler, F., Raguse, J.-D., & Meyerhof, W. (2007). Gustatory Expression Pattern of the Human TAS2R Bitter Receptor Gene Family Reveals a Heterogenous Population of Bitter Responsive Taste Receptor Cells. *Journal of Neuroscience*, *27*(46), 12630–12640. https://doi.org/10.1523/JNEUROSCI.1168-07.2007

Bell, R., McLaren, A., Gaianos, J., & Copolov, D. (1998). The Clinical Use of Plasma Clozapine Levels. *Australian & New Zealand Journal of Psychiatry*, *32*(4), 567–574. https://doi.org/10.3109/00048679809068332

Ben-Aryeh, H., Jungerman, T., Szargel, R., Klein, E., & Laufer, D. (1996). Salivary flow-rate and composition in schizophrenic patients on clozapine: Subjective reports and laboratory data. *Biological Psychiatry*, *39*(11), 946–949. https://doi.org/10.1016/0006-3223(95)00296-0

Benson, H. A. E., Grice, J. E., Mohammed, Y., Namjoshi, S., & Roberts, M. S. (2019). Topical and Transdermal Drug Delivery: From Simple Potions to Smart Technologies. *Current Drug Delivery*,

16(5), 444–460. https://doi.org/10.2174/1567201816666190201143457

Berman, J. A., Talmage, D. A., & Role, L. W. (2007). *Cholinergic Circuits and Signaling in the Pathophysiology of Schizophrenia* (pp. 193–223). https://doi.org/10.1016/S0074-7742(06)78007-2

Bhat, S. V., Sultana, T., Körnig, A., McGrath, S., Shahina, Z., & Dahms, T. E. S. (2018). Correlative atomic force microscopy quantitative imaging-laser scanning confocal microscopy quantifies the impact of stressors on live cells in real-time. *Scientific Reports*, *8*(1), 8305. https://doi.org/10.1038/s41598-018-26433-1

Bhugra, C., & Pikal, M. J. (2008). Role of Thermodynamic, Molecular, and Kinetic Factors in Crystallization from the Amorphous State. *Journal of Pharmaceutical Sciences*, *97*(4), 1329–1349. https://doi.org/10.1002/jps.21138

Bibi, H. A., di Cagno, M., Holm, R., & Bauer-Brandl, A. (2015). Permeapad™ for investigation of passive drug permeability: The effect of surfactants, co-solvents and simulated intestinal fluids (FaSSIF and FeSSIF). *International Journal of Pharmaceutics*, *493*(1–2), 192–197. https://doi.org/10.1016/j.ijpharm.2015.07.028

Bibi, H. A., Holm, R., & Bauer-Brandl, A. (2016). Use of Permeapad® for prediction of buccal absorption: A comparison to in vitro, ex vivo and in vivo method. *European Journal of Pharmaceutical Sciences*, *93*, 399–404. https://doi.org/10.1016/j.ejps.2016.08.041

Bibi, H. A., Holm, R., & Bauer-Brandl, A. (2017). Simultaneous lipolysis/permeation in vitro model, for the estimation of bioavailability of lipid based drug delivery systems. *European Journal of Pharmaceutics and Biopharmaceutics*, *117*, 300–307. https://doi.org/10.1016/j.ejpb.2017.05.001

Boateng, J. S., Stevens, H. N. E., Eccleston, G. M., Auffret, A. D., Humphrey, M. J., & Matthews, K. H. (2009). Development and mechanical characterization of solvent-cast polymeric films as potential drug delivery systems to mucosal surfaces. *Drug Development and Industrial Pharmacy*, *35*(8), 986–996. https://doi.org/10.1080/03639040902744704

Borbás, E., Balogh, A., Bocz, K., Müller, J., Kiserdei, É., Vigh, T., Sinkó, B., Marosi, A., Halász, A., Dohányos, Z., Szente, L., Balogh, G. T., & Nagy, Z. K. (2015). In vitro dissolution-permeation evaluation of an electrospun cyclodextrin-based formulation of aripiprazole using µFlux™. *International Journal of Pharmaceutics*, *491*(1–2), 180–189. https://doi.org/10.1016/j.ijpharm.2015.06.019

Bradbury, J. (2004). Taste Perception: Cracking the Code. *PLoS Biology*, *2*(3), e64. https://doi.org/10.1371/journal.pbio.0020064

Bradshaw, H., Mitchell, M. J., Edwards, C. J., Stolz, U., Naser, O., Peck, A., & Patanwala, A. E. (2016).

Medication Palatability Affects Physician Prescribing Preferences for Common Pediatric Conditions. *Academic Emergency Medicine*, *23*(11), 1243–1247. https://doi.org/10.1111/acem.13020

Brako, F., Raimi-Abraham, B., Mahalingam, S., Craig, D. Q. M., & Edirisinghe, M. (2015). Making nanofibres of mucoadhesive polymer blends for vaginal therapies. *European Polymer Journal*, *70*, 186–196. https://doi.org/10.1016/j.eurpolymj.2015.07.006

Brako, F., Raimi-Abraham, B. T., Mahalingam, S., Craig, D. Q. M., & Edirisinghe, M. (2018). The development of progesterone-loaded nanofibers using pressurized gyration: A novel approach to vaginal delivery for the prevention of pre-term birth. *International Journal of Pharmaceutics*, *540*(1–2), 31–39. https://doi.org/10.1016/j.ijpharm.2018.01.043

Brenman, M., & Lerchenthal, C. H. (1976). Increase of adhesive bond strength through the mechanochemical creation of free radicals: I. *Polymer Engineering and Science*, *16*(11), 747–759. https://doi.org/10.1002/pen.760161107

Brisch, R., Saniotis, A., Wolf, R., Bielau, H., Bernstein, H.-G., Steiner, J., Bogerts, B., Braun, A. K., Jankowski, Z., Kumaritlake, J., Henneberg, M., & Gos, T. (2014). The Role of Dopamine in Schizophrenia from a Neurobiological and Evolutionary Perspective: Old Fashioned, but Still in Vogue. *Frontiers in Psychiatry*, *5*. https://doi.org/10.3389/fpsyt.2014.00047

Brough, C., Miller, D. A., Keen, J. M., Kucera, S. A., Lubda, D., & Williams, R. O. (2016). Use of Polyvinyl Alcohol as a Solubility-Enhancing Polymer for Poorly Water Soluble Drug Delivery (Part 1). *AAPS PharmSciTech*, *17*(1), 167–179. https://doi.org/10.1208/s12249-015-0458-y

Brown, M. T., & Bussell, J. K. (2011). Medication Adherence: WHO Cares? *Mayo Clinic Proceedings*, *86*(4), 304–314. https://doi.org/10.4065/mcp.2010.0575

Bruni, G., Berbenni, V., Maggi, L., Mustarelli, P., Friuli, V., Ferrara, C., Pardi, F., Castagna, F., Girella, A., Milanese, C., & Marini, A. (2018). Multicomponent crystals of gliclazide and tromethamine: preparation, physico-chemical, and pharmaceutical characterization*. *Drug Development and Industrial Pharmacy*, *44*(2), 243–250. https://doi.org/10.1080/03639045.2017.1386208

Buchanan, R. W., Kreyenbuhl, J., Kelly, D. L., Noel, J. M., Boggs, D. L., Fischer, B. A., Himelhoch, S., Fang, B., Peterson, E., Aquino, P. R., & Keller, W. (2010). The 2009 Schizophrenia PORT Psychopharmacological Treatment Recommendations and Summary Statements. *Schizophrenia Bulletin*, *36*(1), 71–93. https://doi.org/10.1093/schbul/sbp116

Buckley, P. F., Chiles, J. A., Conley, R. R., Crismon, M. L., Pharm, D., Essock, S. M., Ph, D., Finnerty, M., Marder, S. R., Miller, D. D., Mcevoy, J. P., Robinson, D. G., Schooler, N. R., Shon, S. P., Stroup, T. S., & Miller, A. L. (2007). Antipsychotic Algorithm for Schizophrenia : 2006 Update. *Psychiatry: Interpersonal and Biological Processes*, *November*, 1752–1763.

Buscemi, S., Damiano, G., Fazzotta, S., Maffongelli, A., Palumbo, V. D., Ficarella, S., Fiorica, C., Cassata, G., Licciardi, M., Palumbo, F. S., Gulotta, L., Buscemi, G., & lo Monte, A. I. (2017). Electrospun Polyhydroxyethyl-Aspartamide–Polylactic Acid Scaffold for Biliary Duct Repair: A Preliminary In Vivo Evaluation. *Transplantation Proceedings*, *49*(4), 711–715. https://doi.org/10.1016/j.transproceed.2017.02.016

Buscemi, S., Palumbo, V. D., Maffongelli, A., Fazzotta, S., Palumbo, F. S., Licciardi, M., Fiorica, C., Puleio, R., Cassata, G., Fiorello, L., Buscemi, G., & lo Monte, A. I. (2017). Electrospun PHEA-PLA/PCL Scaffold for Vascular Regeneration: A Preliminary in Vivo Evaluation. *Transplantation Proceedings*, *49*(4), 716–721. https://doi.org/10.1016/j.transproceed.2017.02.017

Butnarasu, C., Barbero, N., Pacheco, D., Petrini, P., & Visentin, S. (2019). Mucin binding to therapeutic molecules: The case of antimicrobial agents used in cystic fibrosis. *International Journal of Pharmaceutics*, *564*, 136–144. https://doi.org/10.1016/j.ijpharm.2019.04.032

Campardelli, R., Oleandro, E., Scognamiglio, M., Della Porta, G., & Reverchon, E. (2017). Palmitoylethanolamide sub-micronization using fast precipitation followed by supercritical fluids extraction. *Powder Technology*, *305*, 217–225. https://doi.org/10.1016/j.powtec.2016.09.084

Campbell RJ. (2005). Adherence to Medication. *New England Journal of Medicine*, *353*(18), 1972–1974. https://doi.org/10.1056/NEJM200511033531819

Canadian Pharmacists Association: Compendium of Pharmaceuticals and Specialities (CPS). Ottawa, Canadian Pharmacists Association, 2012. (n.d.).

Capra, R. H., Baruzzi, A. M., Quinzani, L. M., & Strumia, M. C. (2007). Rheological, dielectric and diffusion analysis of mucin/carbopol matrices used in amperometric biosensors. *Sensors and Actuators B: Chemical*, *124*(2), 466–476. https://doi.org/10.1016/j.snb.2007.01.022

Carfì Pavia, F. et al. (2014). Synthesis, characterization and foaming of PHEA–PLLA, a new graft copolymer for biomedical engineering. *Materials Science and Engineering: C*, *41*, 301–308. https://doi.org/10.1016/j.msec.2014.04.045

Carlsson, A., Waters, N., Holm-Waters, S., Tedroff, J., Nilsson, M., & Carlsson, M. L. (2001). Schizophrenia: New Evidence. *Annual Review of Pharmacology and Toxicology*, *41*(1), 237–260. https://doi.org/10.1146/annurev.pharmtox.41.1.237

Caron, V., Hu, Y., Tajber, L., Erxleben, A., Corrigan, O. I., McArdle, P., & Healy, A. M. (2013). Amorphous Solid Dispersions of Sulfonamide/Soluplus® and Sulfonamide/PVP Prepared by Ball Milling. *AAPS PharmSciTech*, *14*(1), 464–474. https://doi.org/10.1208/s12249-013-9931-7

Cavallaro, G. et al. (2004). Tamoxifen-Loaded Polymeric Micelles: Preparation, Physico-Chemical Characterization and In Vitro Evaluation Studies. *Macromolecular Bioscience*, *4*(11), 1028–1038. https://doi.org/10.1002/mabi.200400089

Cavallaro, Gennara, Craparo, E. F., Sardo, C., Lamberti, G., Barba, A. A., & Dalmoro, A. (2015). PHEA–PLA biocompatible nanoparticles by technique of solvent evaporation from multiple emulsions. *International Journal of Pharmaceutics*, *495*(2), 719–727. https://doi.org/10.1016/j.ijpharm.2015.09.050

Cavallaro, Gennara, Licciardi, M., Giammona, G., Caliceti, P., Semenzato, A., & Salmaso, S. (2003). Poly(hydroxyethylaspartamide) derivatives as colloidal drug carrier systems. *Journal of Controlled Release*, *89*(2), 285–295. https://doi.org/10.1016/S0168-3659(03)00121-4

Cespi, M., Bonacucina, G., Mencarelli, G., Giorgioni, G., & Palmieri, G. F. (2010). Evaluation of Polymer Mucoadhesiveness by the Use of Acoustic Spectroscopy. *AAPS PharmSciTech*, *11*(3), 1232–1236. https://doi.org/10.1208/s12249-010-9490-0

Chakos, M., Lieberman, J., Hoffman, E., Bradford, D., & Sheitman, B. (2004). Effectiveness of Second-Generation Antipsychotics in Patients With Treatment-Resistant Schizophrenia: A Review and Meta-Analysis of Randomized Trials. *FOCUS*, *2*(1), 111–121. https://doi.org/10.1176/foc.2.1.111

Chakrabarti, S. (2014). What's in a name? Compliance, adherence and concordance in chronic psychiatric disorders. *World Journal of Psychiatry*, *4*(2), 30. https://doi.org/10.5498/wjp.v4.i2.30

Channer, K. S., & Virjee, J. P. (1986). The Effect of Size and Shape of Tablets on Their Esophageal Transit. *The Journal of Clinical Pharmacology*, *26*(2), 141–146. https://doi.org/10.1002/j.1552-4604.1986.tb02922.x

Charlson, F. J., Ferrari, A. J., Santomauro, D. F., Diminic, S., Stockings, E., Scott, J. G., McGrath, J. J., & Whiteford, H. A. (2018). Global Epidemiology and Burden of Schizophrenia: Findings From the Global Burden of Disease Study 2016. *Schizophrenia Bulletin*, *44*(6), 1195–1203. https://doi.org/10.1093/schbul/sby058

Chen, F., Mac, G., & Gupta, N. (2017). Security features embedded in computer aided design (CAD) solid models for additive manufacturing. *Materials & Design*, *128*, 182–194. https://doi.org/10.1016/j.matdes.2017.04.078

Cheng, H., Yang, X., Che, X., Yang, M., & Zhai, G. (2018). Biomedical application and controlled drug release of electrospun fibrous materials. *Materials Science and Engineering C*, *90*(2017), 750–763. https://doi.org/10.1016/j.msec.2018.05.007

Chew, S. L., Modica de Mohac, L., & Tolulope Raimi-Abraham, B. (2019). 3D-Printed Solid Dispersion Drug Products. *Pharmaceutics*, *11*(12), 672. https://doi.org/10.3390/pharmaceutics11120672

Chinna Babu, P., Sundaraganesan, N., Sudha, S., Aroulmoji, V., & Murano, E. (2012). Molecular structure and vibrational spectra of Irinotecan: A density functional theoretical study. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *98*, 1–6. https://doi.org/10.1016/j.saa.2012.08.005

Chinna Reddy, P., Chaitanya, K. S. C., & Madhusudan Rao, Y. (2011). A review on bioadhesive buccal drug delivery systems: current status of formulation and evaluation methods. *Daru : Journal of Faculty of Pharmacy, Tehran University of Medical Sciences*, *19*(6), 385–403. http://www.ncbi.nlm.nih.gov/pubmed/23008684

Cho, H.-J., Lee, J.-Y., Kang, W.-S., Piao, J., Yoon, I.-S., & Kim, D.-D. (2015). Soluplus/TPGS-based solid dispersions prepared by hot-melt extrusion equipped with twin-screw systems for enhancing oral bioavailability of valsartan. *Drug Design, Development and Therapy*, 2745. https://doi.org/10.2147/DDDT.S84070

Cho, S. Y., Choung, R. S., Saito, Y. A., Schleck, C. D., Zinsmeister, A. R., Locke, G. R., & Talley, N. J. (2015). Prevalence and risk factors for dysphagia: a USA community study. *Neurogastroenterology & Motility*, *27*(2), 212–219. https://doi.org/10.1111/nmo.12467

Choi, J.-Y., Park, C. H., & Lee, J. (2008). Effect of Polymer Molecular Weight on Nanocomminution of Poorly Soluble Drug. *Drug Delivery*, *15*(5), 347–353. https://doi.org/10.1080/10717540802039113

Christison, G. W., Kirch, D. G., & Wyatt, R. J. (1991). When symptoms persist: choosing among alternative somatic treatments for schizophrenia. *Schizophrenia Bulletin*, *17*(2), 217–245. https://doi.org/10.1093/schbul/17.2.217

Civiale, C. et al. (2009). Polyhydroxyethylaspartamide-based micelles for ocular drug delivery. *International Journal of Pharmaceutics*, *378*(1–2), 177–186. https://doi.org/10.1016/j.ijpharm.2009.05.028

Clinical, N. (2019). *The Top 300 of 2019 Provided by the ClinCalc DrugStats Database*. https://clincalc.com/DrugStats/Top300Drugs.aspx

Cohen, S. M., Tsien, R. W., Goff, D. C., & Halassa, M. M. (2015). The impact of NMDA receptor hypofunction on GABAergic neurons in the pathophysiology of schizophrenia. *Schizophrenia Research*, *167*(1–3), 98–107. https://doi.org/10.1016/j.schres.2014.12.026

Colombo, M., Staufenbiel, S., Rühl, E., & Bodmeier, R. (2017). In situ determination of the saturation solubility of nanocrystals of poorly soluble drugs for dermal application. *International Journal of Pharmaceutics*, *521*(1–2), 156–166. https://doi.org/10.1016/j.ijpharm.2017.02.030

COMPOUNDCHEM. (2014). *EVERYDAY COMPOUNDS: SODIUM LAURYL SULFATE*. Compound Interest.

Conley, R. R., & Buchanan, R. W. (1997). Evaluation of Treatment-Resistant Schizophrenia. *Schizophrenia Bulletin*, *23*(4), 663–674. https://doi.org/10.1093/schbul/23.4.663

Conley, Robert R., & Kelly, D. L. (2001). Management of treatment resistance in schizophrenia. *Biological Psychiatry*, *50*(11), 898–911. https://doi.org/10.1016/S0006-3223(01)01271-9

Contreras, R. J., Carson, C. A., & Pierce, C. E. (1995). A Novel Psychophysical Procedure for Bitter Taste

Assessment in Rats. *Chemical Senses*, *20*(3), 305–312. https://doi.org/10.1093/chemse/20.3.305

Cooper, P. D., Barclay, T. G., Ginic-Markovic, M., & Petrovsky, N. (2013). The polysaccharide inulin is characterized by an extensive series of periodic isoforms with varying biological actions. *Glycobiology*, *23*(10), 1164–1174. https://doi.org/10.1093/glycob/cwt053

Corrigan, Owen I. "Mechanisms of dissolution of fast release solid dispersions." Drug development and industrial pharmacy 11.2-3 (1985): 697-724. (n.d.).

Coulman, S. A., Anstey, A., Gateley, C., Morrissey, A., McLoughlin, P., Allender, C., & Birchall, J. C. (2009). Microneedle mediated delivery of nanoparticles into human skin. *International Journal of Pharmaceutics*, *366*(1–2), 190–200. https://doi.org/10.1016/j.ijpharm.2008.08.040

Craig, D. Q. M. (2002). The mechanisms of drug release from solid dispersions in water-soluble polymers. *International Journal of Pharmaceutics*, *231*(2), 131–144. https://doi.org/10.1016/S0378-5173(01)00891-2

Craparo, E. F., Teresi, G., Ognibene, M. C., Casaletto, M. P., Bondì, M. L., & Cavallaro, G. (2010). Nanoparticles based on novel amphiphilic polyaspartamide copolymers. *Journal of Nanoparticle Research*, *12*(7), 2629–2644. https://doi.org/10.1007/s11051-009-9842-4

Cui, F., Qian, F., & Yin, C. (2006). Preparation and characterization of mucoadhesive polymer-coated nanoparticles. *International Journal of Pharmaceutics*, *316*(1–2), 154–161. https://doi.org/10.1016/j.ijpharm.2006.02.031

Cui, H., Gensini, M., Kataria, R., Twaddle, T., Zhang, J., Wadsworth, S., Petrilli, J., Rodgers, K., DiZerega, G., & Cooper, K. (2009). Reducing post-surgical adhesions utilizing a drug-enhanced device: sodium carboxymethylcellulose aqueous gel/poly(p-dioxanone) and Tranilast. *Biomedical Materials*, *4*(1), 015001. https://doi.org/10.1088/1748-6041/4/1/015001

da Silva dos Passos, A. P., Scaramal Madrona, G., Marcolino, V. A., Baesso, M. L., & Matioli, G. (2015). The Use of Thermal Analysis and Photoacoustic Spectroscopy in the Evaluation of Maltodextrin Microencapsulation of Anthocyanins from Juçara Palm Fruit (Euterpe edulis Mart.) and Its Application in Food. *Food Technology and Biotechnology*, *53*. https://doi.org/10.17113/ftb.53.04.15.3726

Dagan-Wiener, A., Nissim, I., Ben Abu, N., Borgonovo, G., Bassoli, A., & Niv, M. Y. (2017). Bitter or not? BitterPredict, a tool for predicting taste from chemical structure. *Scientific Reports*, *7*(1), 12074. https://doi.org/10.1038/s41598-017-12359-7

Dahan, A., Miller, J. M., & Amidon, G. L. (2009). Prediction of solubility and permeability class membership: provisional BCS classification of the world's top oral drugs. *The AAPS Journal*, *11*(4), 740–746. https://doi.org/10.1208/s12248-009-9144-x

Dal Pan, G. J., Lindquist, M., & Gelperin, K. (2019). Postmarketing Spontaneous Pharmacovigilance

Reporting Systems. In *Pharmacoepidemiology* (pp. 165–201). Wiley. https://doi.org/10.1002/9781119413431.ch10

Dale Purves, George J Augustine, David Fitzpatrick, Lawrence C Katz, Anthony-Samuel LaMantia, James O McNamara, and S Mark WilliamsDale Purves, George J Augustine, David Fitzpatrick, Lawrence C Katz, Anthony-Samuel LaMantia, James O McNamara, and S. M. W. (2001). *Neuroscience, 2nd edition*.

Das, A., Nayak, A. K., Mohanty, B., & Panda, S. (2011). Solubility and Dissolution Enhancement of Etoricoxib by Solid Dispersion Technique Using Sugar Carriers. *ISRN Pharmaceutics*, *2011*, 1–8. https://doi.org/10.5402/2011/819765

David Jones. (2008). *Pharmaceutics - Dosage Form and Design*.

David M. Taylor; Thomas R.E. Barnes, A. H. Y. (2018). The Maudsley Prescribing Guidelines in Psychiatry 13th Edition. *Royal Marsden Hospital*, 0.

Davidson, N., Tong, H.-J., Kalberer, M., Seville, P. C., Ward, A. D., Kuimova, M. K., & Pope, F. D. (2017). Measurement of the Raman spectra and hygroscopicity of four pharmaceutical aerosols as they travel from pressurised metered dose inhalers (pMDI) to a model lung. *International Journal of Pharmaceutics*, *520*(1–2), 59–69. https://doi.org/10.1016/j.ijpharm.2017.01.051

Davis, M. C., Fuller, M. A., Strauss, M. E., Konicki, P. E., & Jaskiw, G. E. (2014). Discontinuation of clozapine: a 15-year naturalistic retrospective study of 320 patients. *Acta Psychiatrica Scandinavica*, *130*(1), 30–39. https://doi.org/10.1111/acps.12233

Davis, M. T., Potter, C. B., Mohammadpour, M., Albadarin, A. B., & Walker, G. M. (2017). Design of spray dried ternary solid dispersions comprising itraconazole, soluplus and HPMCP: Effect of constituent compositions. *International Journal of Pharmaceutics*, *519*(1–2), 365–372. https://doi.org/10.1016/j.ijpharm.2017.01.043

de Melo Ramos, F., Ubbink, J., Silveira Júnior, V., & Prata, A. S. (2019). Drying of Maltodextrin solution in a vacuum spray dryer. *Chemical Engineering Research and Design*, *146*, 78–86. https://doi.org/10.1016/j.cherd.2019.03.036

De Mohac, L. M., Caruana, R., Cavallaro, G., Giammona, G., & Licciardi, M. (2020). Spray-Drying, Solvent-Casting and Freeze-Drying Techniques: a Comparative Study on their Suitability for the Enhancement of Drug Dissolution Rates. *Pharmaceutical Research*, *37*(3), 57. https://doi.org/10.1007/s11095-020-2778-1

Deitzel, J. ., Kleinmeyer, J., Harris, D., & Beck Tan, N. . (2001). The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*, *42*(1), 261–272. https://doi.org/10.1016/S0032-3861(00)00250-0

Deng, L., Kang, X., Liu, Y., Feng, F., & Zhang, H. (2018). Characterization of gelatin/zein films fabricated

by electrospinning vs solvent casting. *Food Hydrocolloids*, *74*, 324–332. https://doi.org/10.1016/j.foodhyd.2017.08.023

Derman, R. C., Bass, C. E., & Ferrario, C. R. (2020). Effects of hM4Di activation in CamKII basolateral amygdala neurons and CNO treatment on sensory-specific vs. general PIT: refining PIT circuits and considerations for using CNO. *Psychopharmacology*, *237*(5), 1249–1266. https://doi.org/10.1007/s00213-020-05453-8

di Cagno, M., Bibi, H. A., & Bauer-Brandl, A. (2015). New biomimetic barrier Permeapad™ for efficient investigation of passive permeability of drugs. *European Journal of Pharmaceutical Sciences*, *73*, 29–34. https://doi.org/10.1016/j.ejps.2015.03.019

Dias, S. B. T., Nascimento, T. G., Santos, A. F. O., Nicácio Viana, I. M. M., Almeida, R. M., Júnior, I. D. B., Macêdo, R. O., & de Araújo-Júnior, J. X. (2015). Polymorphic characterization and compatibility study of clozapine: implications on its stability and some biopharmaceutics properties. *Journal of Thermal Analysis and Calorimetry*, *120*(1), 795–805. https://doi.org/10.1007/s10973-014-4142-3

DILWORTH, S., BUCKTON, G., GAISFORD, S., & RAMOS, R. (2004). Approaches to determine the enthalpy of crystallisation, and amorphous content, of lactose from isothermal calorimetric data. *International Journal of Pharmaceutics*, *284*(1–2), 83–94. https://doi.org/10.1016/j.ijpharm.2004.07.016

DiNunzio, J. C., Miller, D. A., Yang, W., McGinity, J. W., & Williams, R. O. (2008). Amorphous Compositions Using Concentration Enhancing Polymers for Improved Bioavailability of Itraconazole. *Molecular Pharmaceutics*, *5*(6), 968–980. https://doi.org/10.1021/mp800042d

Dobos, A. M., Onofrei, M.-D., Stoica, I., Olaru, N., Olaru, L., & Ioan, S. (2012). Rheological properties and microstructures of cellulose acetate phthalate/hydroxypropyl cellulose blends. *Polymer Composites*, *33*(11), 2072–2083. https://doi.org/10.1002/pc.22350

Dodou, D., Breedveld, P., & Wieringa, P. A. (2005). Mucoadhesives in the gastrointestinal tract: revisiting the literature for novel applications. *European Journal of Pharmaceutics and Biopharmaceutics*, *60*(1), 1–16. https://doi.org/10.1016/j.ejpb.2005.01.007

Dokoumetzidis, A., & Macheras, P. (2006). A century of dissolution research: From Noyes and Whitney to the Biopharmaceutics Classification System. *International Journal of Pharmaceutics*, *321*(1–2), 1–11. https://doi.org/10.1016/j.ijpharm.2006.07.011

Dold, M., & Leucht, S. (2014). Pharmacotherapy of treatment-resistant schizophrenia: a clinical perspective. *Evidence-Based Mental Health*, *17*(2), 33–37. https://doi.org/10.1136/eb-2014-101813

Dong, L., & Johnson, D. (2003). Surface Tension of Charge-Stabilized Colloidal Suspensions at the

Water−Air Interface. *Langmuir*, *19*(24), 10205–10209. https://doi.org/10.1021/la035128j

Douglas Collins. (1990). *The Story of Kodak*.

Douroumis, D. (2011). *Orally disintegrating dosage forms and taste-masking technologies; 2010.* https://doi.org/10.1517/17425247.2011.566553

Dressman, J. B., Amidon, G. L., Reppas, C., & Shah, V. P. (1998). Dissolution testing as a prognostic tool for oral drug absorption: Immediate release dosage forms. In *Pharmaceutical Research* (Vol. 15, Issue 1, pp. 11–22). https://doi.org/10.1023/A:1011984216775

DrugBank. (2020). *Dexamethasone DrugBank*. https://www.drugbank.ca/drugs/DB01234

Du, J., Wang, Y., Lang, X., & Fan, S. (2011). Effects of Polyvinyl Alcohol on the Adhesion Force of Tetrahydrofuran Hydrate Particles. *Energy & Fuels*, *25*(7), 3204–3211. https://doi.org/10.1021/ef200131y

Duan, R., Sun, X., Liu, J., Gong, T., & Zhang, Z. (2011). Mixed micelles loaded with silybin-polyene phosphatidylcholine complex improve drug solubility. *Acta Pharmacologica Sinica*, *32*(1), 108–115. https://doi.org/10.1038/aps.2010.192

Dubois, J.-L., & Ford, J. L. (1985). Similarities in the release rates of different drugs from polyethylene glycol 6000 solid dispersions. *Journal of Pharmacy and Pharmacology*, *37*(7), 494–495. https://doi.org/10.1111/j.2042-7158.1985.tb03048.x

Dukali, R., Radovic, I., Stojanovic, D., Sevic, D., Radojevic, V., Jocic, D., & Aleksic, R. (2014). Electrospinning of laser dye Rhodamine B-doped poly(methyl methacrylate) nanofibers. *Journal of the Serbian Chemical Society*, *79*(7), 867–880. https://doi.org/10.2298/JSC131014011D

Eddleston, M. D., Patel, B., Day, G. M., & Jones, W. (2013). Cocrystallization by freeze-drying: Preparation of novel multicomponent crystal forms. *Crystal Growth and Design*, *13*(10), 4599–4606. https://doi.org/10.1021/cg401179s

Ekdahl, A., Mudie, D., Malewski, D., Amidon, G., & Goodwin, A. (2019). Effect of Spray-Dried Particle Morphology on Mechanical and Flow Properties of Felodipine in PVP VA Amorphous Solid Dispersions. *Journal of Pharmaceutical Sciences*, *108*(11), 3657–3666. https://doi.org/10.1016/j.xphs.2019.08.008

Elkis, H. (2007). Treatment-Resistant Schizophrenia. *Psychiatric Clinics of North America*, *30*(3), 511–533. https://doi.org/10.1016/j.psc.2007.04.001

Emami, F., Vatanara, A., Park, E. J., & Na, D. H. (2018). Drying technologies for the stability and bioavailability of biopharmaceuticals. *Pharmaceutics*, *10*(3), 1–22. https://doi.org/10.3390/pharmaceutics10030131

Encina, C., Márquez-Ruiz, G., Holgado, F., Giménez, B., Vergara, C., & Robert, P. (2018). Effect of spray-drying with organic solvents on the encapsulation, release and stability of fish oil. *Food*

Chemistry, *263*, 283–291. https://doi.org/10.1016/j.foodchem.2018.05.026

Erokhin, K. S., Gordeev, E. G., & Ananikov, V. P. (2019). Revealing interactions of layered polymeric materials at solid-liquid interface for building solvent compatibility charts for 3D printing applications. *Scientific Reports*, *9*(1), 20177. https://doi.org/10.1038/s41598-019-56350-w

Etemadi, H., Yegani, R., Seyfollahi, M., & Rabiee, M. (2018). Synthesis, characterization, and anti-fouling properties of cellulose acetate/polyethylene glycol-grafted nanodiamond nanocomposite membranes for humic acid removal from contaminated water. *Iranian Polymer Journal*, *27*(6), 381–393. https://doi.org/10.1007/s13726-018-0616-7

European Pharmacopeia 7.6. (2012). 20903E_Dissolution Test for Solid Dosage Forms. *The European Pharmacopoeia*, *1*, 3–9.

Evans, A., Song, Peddie, & Evans, A. (2011). Particle size reduction to the nanometer range: a promising approach to improve buccal absorption of poorly water-soluble drugs. *International Journal of Nanomedicine*, 1245. https://doi.org/10.2147/IJN.S19151

FabRx. (2019). *FabRx's pharmaceutical 3D printer for personalised medicines, M3DIMAKER™*. https://www.fabrx.co.uk/2020/04/06/fabrxs-pharmaceutical-3d-printer-for-personalised-medicines-m3dimaker-is-now-available/

FabRx. (2020). *PERSONALISING DRUG PRODUCTSUSING 3D PRINTING*.

Farooq, S., Choudry, A., Cohen, D., Naeem, F., & Ayub, M. (2019). Barriers to using clozapine in treatment-resistant schizophrenia: systematic review. *BJPsych Bulletin*, *43*(1), 8–16. https://doi.org/10.1192/bjb.2018.67

Farzadi, A., Solati-Hashjin, M., Asadi-Eydivand, M., & Abu Osman, N. A. (2014). Effect of Layer Thickness and Printing Orientation on Mechanical Properties and Dimensional Accuracy of 3D Printed Porous Samples for Bone Tissue Engineering. *PLoS ONE*, *9*(9), e108252. https://doi.org/10.1371/journal.pone.0108252

Fenton, W. S., Blyler, C. R., & Heinssen, R. K. (1997). Determinants of Medication Compliance in Schizophrenia: Empirical and Clinical Findings. *Schizophrenia Bulletin*, *23*(4), 637–651. https://doi.org/10.1093/schbul/23.4.637

Fischbach, M. A., & Sonnenburg, J. L. (2011). Eating For Two: How Metabolism Establishes Interspecies Interactions in the Gut. *Cell Host & Microbe*, *10*(4), 336–347. https://doi.org/10.1016/j.chom.2011.10.002

Franks Felix. Tony Auffret. (2007). *Freeze-drying of Pharmaceuticals and Biopharmaceuticals : Principles and Practice.* Royal Society of Chemistry.

Frederic P. Miller, Agnes F. Vandome, M. J. (2017). *Fick's Laws of Diffusion*.

Friston, K. J. (1995). *Schizophrenia a disconnection syndrome.pdf*.

Frogley, C., Taylor, D., Dickens, G., & Picchioni, M. (2012). A systematic review of the evidence of clozapine's anti-aggressive effects. *The International Journal of Neuropsychopharmacology*, *15*(09), 1351–1371. https://doi.org/10.1017/S146114571100201X

Fuentes, A., Pineda, M., & Venkata, K. (2018). Comprehension of Top 200 Prescribed Drugs in the US as a Resource for Pharmacy Teaching, Training and Practice. *Pharmacy*, *6*(2), 43. https://doi.org/10.3390/pharmacy6020043

Gaebel, W. (2014). *A landmark of schizophrenia practice guidelines."* https://www.nice.org.uk/guidance/cg178/evidence/full-guideline-490503565

Gaisler-Salomon, I., & Weiner, I. (2003). Systemic administration of MK-801 produces an abnormally persistent latent inhibition which is reversed by clozapine but not haloperidol. *Psychopharmacology*, *166*(4), 333–342. https://doi.org/10.1007/s00213-002-1311-z

Galey, W. R., Lonsdale, H. K., & Nacht, S. (1976). The In Vitro Permeability Of Skin And Buccal Mucosa To Selected Drugs And Tritiated Water. *Journal of Investigative Dermatology*, *67*(6), 713–717. https://doi.org/10.1111/1523-1747.ep12598596

Ganesan, P., Soundararajan, R., Shanmugam, U., & Ramu, V. (2015). Development, characterization and solubility enhancement of comparative dissolution study of second generation of solid dispersions and microspheres for poorly water soluble drug. *Asian Journal of Pharmaceutical Sciences*, *10*(5), 433–441. https://doi.org/10.1016/j.ajps.2015.05.001

Gao, X., Alvo, M., Chen, J., & Li, G. (2008). Nonparametric multiple comparison procedures for unbalanced one-way factorial designs. *Journal of Statistical Planning and Inference*, *138*(8), 2574–2591. https://doi.org/10.1016/j.jspi.2007.10.015

Garden, J., Roberts, K., Taylor, A., and Robinson, D. (2003). "Evaluation of the Provision of Single Use Citric Acid Sachets to Injecting Drug Users. *Scottish Center for Infection and Environmental Health.*

Gareth R. Williams, B. T. R.-A. and C. J. L. (2018). *Nanofibres in Drug Delivery*. UCL Press. https://doi.org/10.14324/111.9781787350182

Gaumet, M., Vargas, A., Gurny, R., & Delie, F. (2008). Nanoparticles for drug delivery: The need for precision in reporting particle size parameters. *European Journal of Pharmaceutics and Biopharmaceutics*, *69*(1), 1–9. https://doi.org/10.1016/j.ejpb.2007.08.001

Gavhane, Y. N., & Yadav, A. V. (2012). Loss of orally administered drugs in GI tract. *Saudi Pharmaceutical Journal*, *20*(4), 331–344. https://doi.org/10.1016/j.jsps.2012.03.005

Gavini, E., Chetoni, P., Cossu, M., Alvarez, M. G., Saettone, M. F., & Giunchedi, P. (2004). PLGA microspheres for the ocular delivery of a peptide drug, vancomycin using emulsification/spray-drying as the preparation method: In vitro/in vivo studies. *European Journal of Pharmaceutics*

and Biopharmaceutics, *57*(2), 207–212. https://doi.org/10.1016/j.ejpb.2003.10.018

Gee, S. H., Shergill, S. S., & Taylor, D. M. (2017). Patient attitudes to clozapine initiation. *International Clinical Psychopharmacology*, *32*(6), 337–342. https://doi.org/10.1097/YIC.0000000000000188

Gee, S., Vergunst, F., Howes, O., & Taylor, D. (2014). Practitioner attitudes to clozapine initiation. *Acta Psychiatrica Scandinavica*, *130*(1), 16–24. https://doi.org/10.1111/acps.12193

Gibson, M., Walters, K., & Brain, K. (2001). Topical and Transdermal Delivery. In *Pharmaceutical Preformulation and Formulation*. https://doi.org/10.1201/b14413-18

Gibson, S., Brand, S. L., Burt, S., Boden, Z. V. R., & Benson, O. (2013). Understanding treatment non-adherence in schizophrenia and bipolar disorder: A survey of what service users do and why. *BMC Psychiatry*, *13*. https://doi.org/10.1186/1471-244X-13-153

Gilani, K., Najafabadi, A. R., Barghi, M., & Rafiee-Tehrani, M. (2005). The Effect of Water to Ethanol Feed Ratio on Physical Properties and Aerosolization Behavior of Spray Dried Cromolyn Sodium Particles. *Journal of Pharmaceutical Sciences*, *94*(5), 1048–1059. https://doi.org/10.1002/jps.20315

Gilmer, T. P., Dolder, C. R., Lacro, J. P., Folsom, D. P., Lindamer, L., Garcia, P., & Jeste, D. V. (2004). Adherence to Treatment with Antipsychotic Medication and Health Care Costs among Medicaid Beneficiaries with Schizophrenia. *American Journal of Psychiatry*, *161*(4), 692–699. https://doi.org/10.1176/appi.ajp.161.4.692

Girgis, R. R., Phillips, M. R., Li, X., Li, K., Jiang, H., Wu, C., Duan, N., Niu, Y., & Lieberman, J. A. (2011). Clozapine v. chlorpromazine in treatment-naive, first-episode schizophrenia: 9-year outcomes of a randomised clinical trial. *British Journal of Psychiatry*, *199*(04), 281–288. https://doi.org/10.1192/bjp.bp.110.081471

Golafshan, N., Kharaziha, M., & Fathi, M. (2017). Tough and conductive hybrid graphene-PVA: Alginate fibrous scaffolds for engineering neural construct. *Carbon*, *111*, 752–763. https://doi.org/10.1016/j.carbon.2016.10.042

Goldberg, a H., Gibaldi, M., Kanig, J. L., & Mayersohn, M. (1966). Increasing dissolution rates and gastrointestinal absorption of drugs via solid solutions and eutectic mixtures. IV. Chloramphenicol--urea system. *Journal of Pharmaceutical Sciences*, *55*(6), 581–583. https://doi.org/10.1002/jps.2600540810

Gomaa, Y. A., El-Khordagui, L. K., Garland, M. J., Donnelly, R. F., McInnes, F., & Meidan, V. M. (2012). Effect of microneedle treatment on the skin permeation of a nanoencapsulated dye. *Journal of Pharmacy and Pharmacology*, *64*(11), 1592–1602. https://doi.org/10.1111/j.2042-7158.2012.01557.x

Goodman and Gilman. (2013). Goodman and Gilman's The Pharmacological Basis of Therapeutics. In

Journal of Chemical Information and Modeling (Vol. 53, Issue 9). https://doi.org/10.1017/CBO9781107415324.004

Goswami, T., Li, X., & Jasti, B. R. (2017). Effect of Lipophilicity and Drug Ionization on Permeation Across Porcine Sublingual Mucosa. *AAPS PharmSciTech*, *18*(1), 175–181. https://doi.org/10.1208/s12249-016-0479-1

Goyanes, A., Buanz, A. B. M., Basit, A. W., & Gaisford, S. (2014). Fused-filament 3D printing (3DP) for fabrication of tablets. *International Journal of Pharmaceutics*, *476*(1–2), 88–92. https://doi.org/10.1016/j.ijpharm.2014.09.044

Goyanes, A., Buanz, A. B. M., Hatton, G. B., Gaisford, S., & Basit, A. W. (2015). 3D printing of modified-release aminosalicylate (4-ASA and 5-ASA) tablets. *European Journal of Pharmaceutics and Biopharmaceutics*, *89*, 157–162. https://doi.org/10.1016/j.ejpb.2014.12.003

Goyanes, A., Fina, F., Martorana, A., Sedough, D., Gaisford, S., & Basit, A. W. (2017). Development of modified release 3D printed tablets (printlets) with pharmaceutical excipients using additive manufacturing. *International Journal of Pharmaceutics*, *527*(1–2), 21–30. https://doi.org/10.1016/j.ijpharm.2017.05.021

Goyanes, A., Kobayashi, M., Martínez-Pacheco, R., Gaisford, S., & Basit, A. W. (2016). Fused-filament 3D printing of drug products: Microstructure analysis and drug release characteristics of PVA-based caplets. *International Journal of Pharmaceutics*, *514*(1), 290–295. https://doi.org/10.1016/j.ijpharm.2016.06.021

Goyanes, A., Robles Martinez, P., Buanz, A., Basit, A. W., & Gaisford, S. (2015). Effect of geometry on drug release from 3D printed tablets. *International Journal of Pharmaceutics*, *494*(2), 657–663. https://doi.org/10.1016/j.ijpharm.2015.04.069

Goyanes, A., Wang, J., Buanz, A., Martínez-Pacheco, R., Telford, R., Gaisford, S., & Basit, A. W. (2015). 3D Printing of Medicines: Engineering Novel Oral Devices with Unique Design and Drug Release Characteristics. *Molecular Pharmaceutics*, *12*(11), 4077–4084. https://doi.org/10.1021/acs.molpharmaceut.5b00510

Greenhall, J., & Raeymaekers, B. (2017). 3D Printing Macroscale Engineered Materials Using Ultrasound Directed Self-Assembly and Stereolithography. *Advanced Materials Technologies*, *2*(9), 1700122. https://doi.org/10.1002/admt.201700122

Grover, S., Hazari, N., Chakrabarti, S., & Avasthi, A. (2015). Delay in initiation of clozapine: A retrospective study from a tertiary care hospital in North India. *Psychiatry Research*, *226*(1), 181–185. https://doi.org/10.1016/j.psychres.2014.12.046

Gulati, N., & Gupta, H. (2011). Parenteral Drug Delivery: A Review. *Recent Patents on Drug Delivery & Formulation*, *5*(2), 133–145. https://doi.org/10.2174/187221111795471391

Haddad, P., Brain, C., & Scott, J. (2014). Nonadherence with antipsychotic medication in schizophrenia: challenges and management strategies. *Patient Related Outcome Measures*, 43. https://doi.org/10.2147/PROM.S42735

Haider, A., Haider, S., & Kang, I.-K. (2018). A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. *Arabian Journal of Chemistry*, *11*(8), 1165–1188. https://doi.org/10.1016/j.arabjc.2015.11.015

Hall, W., & Degenhardt, L. (2000). Cannabis Use and Psychosis: A Review of Clinical and Epidemiological Evidence. *Australian & New Zealand Journal of Psychiatry*, *34*(1), 26–34. https://doi.org/10.1046/j.1440-1614.2000.00685.x

Hallouard, F., Mehenni, L., Lahiani-Skiba, M., Anouar, Y., & Skiba, M. (2016). Solid Dispersions for Oral Administration: An Overview of the Methods for their Preparation. *Current Pharmaceutical Design*, *22*(32), 4942–4958. https://doi.org/10.2174/1381612822666160726095916

Haneef, J., & Chadha, R. (2017). Drug-Drug Multicomponent Solid Forms: Cocrystal, Coamorphous and Eutectic of Three Poorly Soluble Antihypertensive Drugs Using Mechanochemical Approach. *AAPS PharmSciTech*, *18*(6), 2279–2290. https://doi.org/10.1208/s12249-016-0701-1

Hao, J., & Heng, P. W. S. (2003). Buccal Delivery Systems. *Drug Development and Industrial Pharmacy*, *29*(8), 821–832. https://doi.org/10.1081/DDC-120024178

Hardeman S. (n.d.). FAZALCO (Clozaoine, USP) Orally Disintegrating Tablet. *FDA*, 1–21. http://s3-us-west-2.amazonaws.com/drugbank/fda_labels/DB00363.pdf?1265922812

Hasan, A., Falkai, P., Wobrock, T., Lieberman, J., Glenthoj, B., Gattaz, W. F., Thibaut, F., & Möller, H. J. (2012). World Federation of Societies of Biological Psychiatry (WFSBP) Guidelines for Biological Treatment of Schizophrenia, Part 1: Update 2012 on the acute treatment of schizophrenia and the management of treatment resistance. *World Journal of Biological Psychiatry*, *13*(5), 318–378. https://doi.org/10.1109/HICSS.2014.210

Hashib, S. A., Rahman, N. A., Suzihaque, M. U. ., Ibrahim, U. K., & Hanif, N. E. (2015). Effect of Slurry Concentration and Inlet Temperature Towards Glass Temperature of Spray Dried Pineapple Powder. *Procedia - Social and Behavioral Sciences*, *195*, 2660–2667. https://doi.org/10.1016/j.sbspro.2015.06.472

Hatta, K., Sugiyama, N., & Ito, H. (2018). Switching and augmentation strategies for antipsychotic medications in acute-phase schizophrenia: latest evidence and place in therapy. *Therapeutic Advances in Psychopharmacology*, *8*(6), 173–183. https://doi.org/10.1177/2045125318754472

Hawton, K., Sutton, L., Haw, C., Sinclair, J., & Deeks, J. J. (2005). Schizophrenia and suicide: Systematic review of risk factors. *British Journal of Psychiatry*, *187*(1), 9–20.

https://doi.org/10.1192/bjp.187.1.9

Haywood, A., & Glass, B. D. (2013). Liquid Dosage Forms Extemporaneously Prepared from Commercially Available Products – Considering New Evidence on Stability. *Journal of Pharmacy & Pharmaceutical Sciences*, *16*(3), 441. https://doi.org/10.18433/J38887

Hedaya, M. A. (2012). *Basic Pharmacokinetics*.

Hermann, R. B. (1972). Theory of hydrophobic bonding. II. The correlation of hydrocarbon solubility in water with solvent cavity surface area. *Journal of Physical Chemistry*, *76*(19), 2754–2759. https://doi.org/10.1021/j100663a023

Hermans, A., Abend, A. M., Kesisoglou, F., Flanagan, T., Cohen, M. J., Diaz, D. A., Mao, Y., Zhang, L., Webster, G. K., Lin, Y., Hahn, D. A., Coutant, C. A., & Grady, H. (2017). Approaches for Establishing Clinically Relevant Dissolution Specifications for Immediate Release Solid Oral Dosage Forms. *The AAPS Journal*, *19*(6), 1537–1549. https://doi.org/10.1208/s12248-017-0117-1

Higashi, K., Medic, G., Littlewood, K. J., Diez, T., Granström, O., & De Hert, M. (2013). Medication adherence in schizophrenia: factors influencing adherence and consequences of nonadherence, a systematic literature review. *Therapeutic Advances in Psychopharmacology*, *3*(4), 200–218. https://doi.org/10.1177/2045125312474019

Hillery, A. . L. A. . S. J. (2013). Drug Delivery and Targeting. In *Journal of Chemical Information and Modeling* (Vol. 53, Issue 9). https://doi.org/10.1017/CBO9781107415324.004

Hiriyanna, S. H., Basavaiah, K., Goud, P., Dhayanithi, V., Raju, K., & Pati, H. P. (2008). Identification and characterization of olanzapine degradation products under oxidative stress conditions. *Acta Chromatographica*, *20*(1), 81–93. https://doi.org/10.1556/AChrom.20.2008.1.7

Hofman, D. L., van Buul, V. J., & Brouns, F. J. P. H. (2016). Nutrition, Health, and Regulatory Aspects of Digestible Maltodextrins. *Critical Reviews in Food Science and Nutrition*, *56*(12), 2091–2100. https://doi.org/10.1080/10408398.2014.940415

Holger Russig, Aneta Kovacevic, C. A. M. & J. F. (2003). Haloperidol and clozapine antagonise amphetamine-induced disruption of latent inhibition of conditioned taste aversion. *Psychopharmacology Volume*. https://doi.org/10.1007/s00213-003-1544-5

Holger Russig, Aneta Kovacevic, C. A. M. & J. F., Russig, H., Kovacevic, A., Murphy, C. A., & Feldon, J. (2003). Haloperidol and clozapine antagonise amphetamine-induced disruption of latent inhibition of conditioned taste aversion. *Psychopharmacology Volume*, *170*(3), 263–270. https://doi.org/10.1007/s00213-003-1544-5

Holpuch, A. S., Hummel, G. J., Tong, M., Seghi, G. A., Pei, P., Ma, P., Mumper, R. J., & Mallery, S. R. (2010). Nanoparticles for Local Drug Delivery to the Oral Mucosa: Proof of Principle Studies. *Pharmaceutical Research*, *27*(7), 1224–1236. https://doi.org/10.1007/s11095-010-0121-y

Homayun, B., Lin, X., & Choi, H.-J. (2019). Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. *Pharmaceutics*, *11*(3), 129. https://doi.org/10.3390/pharmaceutics11030129

Hong, Wen; Kinam, P. (2011). *Oral Controlled Release Formulation Design and Drug Delivery: Theory to Practice*.

Hooda, R., Tripathi, M., & Kapoor, K. (2012). A Review on Oral Mucosal Drug Delivery System. *The Pharma Innovation*, *1*(1), 14–21. www.thepharmajournal.com

Horne, E. (2018). *Clozapine Guidelines*. *December*, 1–54.

Horne, R., Weinman, J., Barber, N., Elliott, R., & Morgan, M. (2005). *Concordance, adherence and compliance in medicine taking: a conceptual map and research priorities.* 40–46. http://www.medslearning.leeds.ac.uk/pages/documents/useful_docs/76-final-report[1].pdf

Howes, O. D., & Kapur, S. (2009). The Dopamine Hypothesis of Schizophrenia: Version III--The Final Common Pathway. *Schizophrenia Bulletin*, *35*(3), 549–562. https://doi.org/10.1093/schbul/sbp006

Howes, Oliver D., Kambeitz, J., Kim, E., Stahl, D., Slifstein, M., Abi-Dargham, A., & Kapur, S. (2012). The Nature of Dopamine Dysfunction in Schizophrenia and What This Means for Treatment. *Archives of General Psychiatry*, *69*(8). https://doi.org/10.1001/archgenpsychiatry.2012.169

Howes, Oliver D., Vergunst, F., Gee, S., McGuire, P., Kapur, S., & Taylor, D. (2012). Adherence to treatment guidelines in clinical practice: study of antipsychotic treatment prior to clozapine initiation. *British Journal of Psychiatry*, *201*(06), 481–485. https://doi.org/10.1192/bjp.bp.111.105833

Hsu, N.-C., Lin, Y.-F., Shu, C.-C., Yang, M.-C., & Ko, W.-J. (2013). ADHERENCE TO LONG-TERM THERAPIES. *WHO Report*, *30*(4), 334–338. https://doi.org/10.1177/1049909112449068

Huhtinenet, M. (1993). *Transmucosal veterinary composition comprising detomidine* (Patent No. US1667100).

Hunter, J., & Hirst, B. H. (1997). Intestinal secretion of drugs. The role of P-glycoprotein and related drug efflux systems in limiting oral drug absorption. *Advanced Drug Delivery Reviews*, *25*(2–3), 129–157. https://doi.org/10.1016/S0169-409X(97)00497-3

Hussain, A., Smith, G., Khan, K. A., Bukhari, N. I., Pedge, N. I., & Ermolina, I. (2018). Solubility and dissolution rate enhancement of ibuprofen by co-milling with polymeric excipients. *European Journal of Pharmaceutical Sciences*, *123*, 395–403. https://doi.org/10.1016/j.ejps.2018.08.001

Ignatious, F., Sun, L., Lee, C.-P., & Baldoni, J. (2010). Electrospun Nanofibers in Oral Drug Delivery. *Pharmaceutical Research*, *27*(4), 576–588. https://doi.org/10.1007/s11095-010-0061-6

Ii, B. A. B., & Pustaka, S. (2009). *FDA triamcinolone acetonide report*. 4–18.

Ikeuchi-Takahashi, Y., Ishihara, C., & Onishi, H. (2017). Evaluation of polyvinyl alcohols as mucoadhesive polymers for mucoadhesive buccal tablets prepared by direct compression. *Drug Development and Industrial Pharmacy*, *43*(9), 1489–1500. https://doi.org/10.1080/03639045.2017.1321657

Inada, K., & Ishigooka, J. (2013). [Clozapine]. *Nihon Rinsho. Japanese Journal of Clinical Medicine*, *71*(4), 678–683.

Ishtikhar, M., Khan, M. V., Khan, S., Chaturvedi, S. K., Badr, G., Mahmoud, M. H., & Khan, R. H. (2016). Biophysical and molecular docking insight into interaction mechanism and thermal stability of human serum albumin isoforms with a semi-synthetic water-soluble camptothecin analog irinotecan hydrochloride. *Journal of Biomolecular Structure and Dynamics*, *34*(7), 1545–1560. https://doi.org/10.1080/07391102.2015.1082504

Ismar Borges De Lima and Walter Leal Filho. (2016). *Rare Earths Industry*. Elsevier. https://doi.org/10.1016/C2014-0-01863-1

Items, R., Rose, W., Rose, W., If, T., & Rose, W. (2018). *The design and scale-up of spray dried particle delivery systems.*

Jaipakdee, N., Pongjanyakul, T., & Limpongsa, E. (2018). PREPARATION AND CHARACTERIZATION OF POLY (VINYL ALCOHOL)–POLY (VINYL PYRROLIDONE) MUCOADHESIVE BUCCAL PATCHES FOR DELIVERY OF LIDOCAINE HCL. *International Journal of Applied Pharmaceutics*, *10*(1), 115. https://doi.org/10.22159/ijap.2018v10i1.23208

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, Z., Abera, S. F., Abil, O. Z., Abraha, H. N., Abu-Raddad, L. J., Abu-Rmeileh, N. M. E., Accrombessi, M. M. K., … Murray, C. J. L. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, *392*(10159), 1789–1858. https://doi.org/10.1016/S0140-6736(18)32279-7

Jenkins, P. G., Howard, K. A., Blackball, N. W., Thomas, N. W., Davis, S. S., & O'Hagan, D. T. (1994). Microparticulate absorption from the rat intestine. *Journal of Controlled Release*, *29*(3), 339–350. https://doi.org/10.1016/0168-3659(94)90079-5

Ji, W., Yang, F., van den Beucken, J. J. J. P., Bian, Z., Fan, M., Chen, Z., & Jansen, J. A. (2010). Fibrous scaffolds loaded with protein prepared by blend or coaxial electrospinning. *Acta Biomaterialia*, *6*(11), 4199–4207. https://doi.org/10.1016/j.actbio.2010.05.025

Jiang, Yawen, & Ni, W. (2015). Estimating the Impact of Adherence to and Persistence with Atypical Antipsychotic Therapy on Health Care Costs and Risk of Hospitalization. *Pharmacotherapy: The*

Journal of Human Pharmacology and Drug Therapy, *35*(9), 813–822. https://doi.org/10.1002/phar.1634

Jiang, Yijie, & Turner, K. T. (2016). Measurement of the strength and range of adhesion using atomic force microscopy. *Extreme Mechanics Letters*, *9*, 119–126. https://doi.org/10.1016/j.eml.2016.05.013

Jimmy, B., & Jose, J. (2011). Patient Medication Adherence: Measures in Daily Practice. *Oman Medical Journal*, *26*(3), 155–159. https://doi.org/10.5001/omj.2011.38

Kadry, H., Al-Hilal, T. A., Keshavarz, A., Alam, F., Xu, C., Joy, A., & Ahsan, F. (2018). Multi-purposable filaments of HPMC for 3D printing of medications with tailored drug release and timed-absorption. *International Journal of Pharmaceutics*, *544*(1), 285–296. https://doi.org/10.1016/j.ijpharm.2018.04.010

Kahn, R. S., Winter van Rossum, I., Leucht, S., McGuire, P., Lewis, S. W., Leboyer, M., Arango, C., Dazzan, P., Drake, R., Heres, S., Díaz-Caneja, C. M., Rujescu, D., Weiser, M., Galderisi, S., Glenthøj, B., Eijkemans, M. J. C., Fleischhacker, W. W., Kapur, S., Sommer, I. E., … Eijkemans, M. J. C. (2018). Amisulpride and olanzapine followed by open-label treatment with clozapine in first-episode schizophrenia and schizophreniform disorder (OPTiMiSE): a three-phase switching study. *The Lancet Psychiatry*, *5*(10), 797–807. https://doi.org/10.1016/S2215-0366(18)30252-9

Kalepu, S., & Nekkanti, V. (2015). Insoluble drug delivery strategies: review of recent advances and business prospects. *Acta Pharmaceutica Sinica B*, *5*(5), 442–453. https://doi.org/10.1016/j.apsb.2015.07.003

Kamalakkannan, V., Puratchikody, a, Masilamani, K., & Senthilnathan, B. (2010). Solubility Enhancement of Poorly Soluble Drugs By Solid Dispersion Technique - Review. *Journal of Pharmacy Research*, *3*(9), 2314–2321.

Kane, J. (1988). Clozapine for the Treatment-Resistant Schizophrenic. *Archives of General Psychiatry*, *45*(9), 789. https://doi.org/10.1001/archpsyc.1988.01800330013001

Karlsson, A., & Singh, S. K. (1998). Thermal and Mechanical Characterization of Cellulose Acetate Phthalate Films for Pharmaceutical Tablet Coating: Effect of Humidity During Measurements. *Drug Development and Industrial Pharmacy*, *24*(9), 827–834. https://doi.org/10.3109/03639049809088527

Kasap, S., Málek, J., & Svoboda, R. (2017). Thermal Properties and Thermal Analysis: Fundamentals, Experimental Techniques and Applications. In *Springer Handbook of Electronic and Photonic Materials* (pp. 1–1). Springer International Publishing. https://doi.org/10.1007/978-3-319-48933-9_19

Kawakami, K., Suzuki, K., Fukiage, M., Matsuda, M., Nishida, Y., Oikawa, M., & Fujita, T. (2020). Impact

of degree of supersaturation on the dissolution and oral absorption behaviors of griseofulvin amorphous solid dispersions. *Journal of Drug Delivery Science and Technology*, *56*, 101172. https://doi.org/10.1016/j.jddst.2019.101172

Keeley, A., Teo, M., Ali, Z., Frost, J., Ghimire, M., Rajabi-Siahboomi, A., Orlu, M., & Tuleu, C. (2019). In Vitro Dissolution Model Can Predict the in Vivo Taste Masking Performance of Coated Multiparticulates. *Molecular Pharmaceutics*, *16*(5), 2095–2105. https://doi.org/10.1021/acs.molpharmaceut.9b00060

Keiji Sekiguchi And Noberu Obi. (1961). *Studies on absorption of eutectic mixture I. A comparison of the behaviour of eutectic mixture of sulfathiazole and that of ordinary sulfathiazole in man. Chem Pharm Bull 1961; 9: 866–872. 152*.

Khaled, S. A., Burley, J. C., Alexander, M. R., Yang, J., & Roberts, C. J. (2015a). 3D printing of tablets containing multiple drugs with defined release profiles. *International Journal of Pharmaceutics*, *494*(2), 643–650. https://doi.org/10.1016/j.ijpharm.2015.07.067

Khaled, S. A., Burley, J. C., Alexander, M. R., Yang, J., & Roberts, C. J. (2015b). 3D printing of five-in-one dose combination polypill with defined immediate and sustained release profiles. *Journal of Controlled Release*, *217*, 308–314. https://doi.org/10.1016/j.jconrel.2015.09.028

Kianfar, F., Antonijevic, M. D., Chowdhry, B. Z., & Boateng, J. S. (2011). *Formulation development of a carrageenan based delivery system for buccal drug delivery using ibuprofen as a model drug*. *2011*(December), 582–595. https://doi.org/10.4236/jbnb.2011.225070

Kim, U., Wooding, S., Ricci, D., Jorde, L. B., & Drayna, D. (2005). Worldwide haplotype diversity and coding sequence variation at human bitter taste receptor loci. *Human Mutation*, *26*(3), 199–204. https://doi.org/10.1002/humu.20203

Klingmann, V., Spomer, N., Lerch, C., Stoltenberg, I., Frömke, C., Bosse, H. M., Breitkreutz, J., & Meissner, T. (2013). Favorable Acceptance of Mini-Tablets Compared with Syrup: A Randomized Controlled Trial in Infants and Preschool Children. *The Journal of Pediatrics*, *163*(6), 1728-1732.e1. https://doi.org/10.1016/j.jpeds.2013.07.014

Koland, M., Sandeep, V., & Charyulu, N. (2010). Fast Dissolving Sublingual Films of Ondansetron Hydrochloride: Effect of Additives on in vitro Drug Release and Mucosal Permeation. *Journal of Young Pharmacists : JYP*, *2*(3), 216–222. https://doi.org/10.4103/0975-1483.66790

Kolter K, Karl M, G. a. (2012). Hot-Melt Extrusion with BASF Pharma Polymers. *BASF. The Chemical Company*, 192.

Kostakoğlu, A. E., Yazici, K. M., Erbas, T., & Güvener, N. (1996). Ketoacidosis as a side-effect of clozapine: A case report. *Acta Psychiatrica Scandinavica*, *93*(3), 217–218. https://doi.org/10.1111/j.1600-0447.1996.tb10635.x

Kraan, H., Vrieling, H., Czerkinsky, C., Jiskoot, W., Kersten, G., & Amorij, J.-P. (2014a). Buccal and sublingual vaccine delivery. *Journal of Controlled Release*, *190*, 580–592. https://doi.org/10.1016/j.jconrel.2014.05.060

Kraan, H., Vrieling, H., Czerkinsky, C., Jiskoot, W., Kersten, G., & Amorij, J. (2014b). Buccal and sublingual vaccine delivery. *Journal of Controlled Release*, *190*, 580–592. https://doi.org/10.1016/j.jconrel.2014.05.060

Ku, M. S. (2008). Use of the Biopharmaceutical Classification System in Early Drug Development. *The AAPS Journal*, *10*(1), 208–212. https://doi.org/10.1208/s12248-008-9020-0

Ku, M. S., & Dulin, W. (2012). A biopharmaceutical classification-based Right-First-Time formulation approach to reduce human pharmacokinetic variability and project cycle time from First-In-Human to clinical Proof-Of-Concept. *Pharmaceutical Development and Technology*, *17*(3), 285–302. https://doi.org/10.3109/10837450.2010.535826

Kumar, L., Verma, S., Vaidya, B., & Gupta, V. (2017). Bioadhesive Polymers for Targeted Drug Delivery. In *Nanotechnology-Based Approaches for Targeting and Delivery of Drugs and Genes* (Issue October 2018). https://doi.org/10.1016/B978-0-12-809717-5.00012-9

Lallemant, M. (2018). *Acceptability*. https://www.who.int/hiv/pub/5.pdf

Lam, J. K. W., Xu, Y., Worsley, A., & Wong, I. C. K. (2014). Oral transmucosal drug delivery for pediatric use. *Advanced Drug Delivery Reviews*, *73*, 50–62. https://doi.org/10.1016/j.addr.2013.08.011

Landry, P., Rousseau, A. M., & Skalli, L. (2010). Adverse Effects of Antipsychotics. *Clinical Trials in Psychopharmacology: A Better Brain: Second Edition*, *81*(5), 337–380. https://doi.org/10.1002/9780470749180.ch17

Langoth, N., & Kalbe, J. (2003). Development of buccal drug delivery systems based on a thiolated polymer. *International Journal of Pharmaceutics*, *252*(1–2), 141–148. https://doi.org/10.1016/S0378-5173(02)00638-5

Latha RS, L. P. (2012). Electronic tongue: An analytical gustatory tool. *J Adv Pharm Technol Res*. https://doi.org/10.4103/2231-4040.93556.

Laulicht, B., Mancini, A., Geman, N., Cho, D., Estrellas, K., Furtado, S., Hopson, R., Tripathi, A., & Mathiowitz, E. (2012). Bioinspired Bioadhesive Polymers: Dopa-Modified Poly(acrylic acid) Derivatives. *Macromolecular Bioscience*, *12*(11), 1555–1565. https://doi.org/10.1002/mabi.201200179

Lavra, Z. M. M., Pereira de Santana, D., & Ré, M. I. (2017). Solubility and dissolution performances of spray-dried solid dispersion of Efavirenz in Soluplus. *Drug Development and Industrial Pharmacy*, *43*(1), 42–54. https://doi.org/10.1080/03639045.2016.1205598

Le, J. (2019). *Drug Absorption*. https://www.msdmanuals.com/professional/clinical-

pharmacology/pharmacokinetics/drug-absorption

Lee, J. (2005). Intrinsic Adhesion Properties of Poly(vinyl pyrrolidone) to Pharmaceutical Materials: Humidity Effect. *Macromolecular Bioscience*, *5*(11), 1085–1093. https://doi.org/10.1002/mabi.200500146

Lee, J. W., Park, J. H., & Robinson, J. R. (2000). Bioadhesive-Based Dosage Forms: The Next Generation. *Journal of Pharmaceutical Sciences*, *89*(7), 850–866. https://doi.org/10.1002/1520-6017(200007)89:7<850::AID-JPS2>3.0.CO;2-G

Lee, Y., Moon, H. T., & Byun, Y. (1998). Preparation of Slightly Hydrophobic Heparin Derivatives which Can Be Used for Solvent Casting in Polymeric Formulation. *Thrombosis Research*, *92*(4), 149–156. https://doi.org/10.1016/S0049-3848(98)00124-8

Legge, S. E., Hamshere, M., Hayes, R. D., Downs, J., O'Donovan, M. C., Owen, M. J., Walters, J. T. R., & MacCabe, J. H. (2016). Reasons for discontinuing clozapine: A cohort study of patients commencing treatment. *Schizophrenia Research*, *174*(1–3), 113–119. https://doi.org/10.1016/j.schres.2016.05.002

Leucht, S., Komossa, K., Rummel-Kluge, C., Corves, C., Hunger, H., Schmid, F., Asenjo Lobos, C., Schwarz, S., & Davis, J. M. (2009). A Meta-Analysis of Head-to-Head Comparisons of Second-Generation Antipsychotics in the Treatment of Schizophrenia. *American Journal of Psychiatry*, *166*(2), 152–163. https://doi.org/10.1176/appi.ajp.2008.08030368

Levit, A., Nowak, S., Peters, M., Wiener, A., Meyerhof, W., Behrens, M., & Niv, M. Y. (2014). The bitter pill: clinical drugs that activate the human bitter taste receptor TAS2R14. *The FASEB Journal*, *28*(3), 1181–1197. https://doi.org/10.1096/fj.13-242594

Lewis, A., Gibbs, A., & Hope-Gill, B. (2012). Probable occupational pneumonitis caused by inhalation of crushed clozapine. *Occupational Medicine*, *62*(5), 385–387. https://doi.org/10.1093/occmed/kqs041

Li, D., & Xia, Y. (2004). Electrospinning of Nanofibers: Reinventing the Wheel? *Advanced Materials*, *16*(14), 1151–1170. https://doi.org/10.1002/adma.200400719

Licciardi, M., Di Stefano, M., Craparo, E. F., Amato, G., Fontana, G., Cavallaro, G., & Giammona, G. (2012). PHEA-graft-polybutylmethacrylate copolymer microparticles for delivery of hydrophobic drugs. *International Journal of Pharmaceutics*, *433*(1–2), 16–24. https://doi.org/10.1016/j.ijpharm.2012.04.052

Liddle, P. F. (1987). The Symptoms of Chronic Schizophrenia. *British Journal of Psychiatry*, *151*(2), 145–151. https://doi.org/10.1192/bjp.151.2.145

Lieberman, J. A., Phillips, M., Gu, H., Stroup, S., Zhang, P., Kong, L., Ji, Z., Koch, G., & Hamer, R. M. (2003). Atypical and conventional antipsychotic drugs in treatmentnaive first-episode

schizophrenia: A 52-week randomized trial of clozapine vs chlorpromazine. *Neuropsychopharmacology*, *28*(5), 995–1003. https://doi.org/10.1038/sj.npp.1300157

Lin, W. C., Yeh, I. T., Niyama, E., Huang, W. R., Ebara, M., & Wu, C. S. (2018). Electrospun poly(ε-caprolactone) nanofibrous mesh for imiquimod delivery in melanoma therapy. *Polymers*, *10*(3). https://doi.org/10.3390/polym10030231

Liu, P., De Wulf, O., Laru, J., Heikkilä, T., van Veen, B., Kiesvaara, J., Hirvonen, J., Peltonen, L., & Laaksonen, T. (2013). Dissolution Studies of Poorly Soluble Drug Nanosuspensions in Non-sink Conditions. *AAPS PharmSciTech*, *14*(2), 748–756. https://doi.org/10.1208/s12249-013-9960-2

Lo Monte A. et al. (2012). Biocompatibility and Biodegradability of Electrospun Phea-Pla Scaffolds: Our Preliminary Experience in a Murine Animal Model. *Digest Journal of Nanomaterials and Biostructures*, *7*(2), 841–851.

London, N. C., & Committee, M. O. (n.d.). *Guidance for Pharmacy Staff Managing Clozapine For Patients Admitted to Acute Hospitals*. *June 2018*, 1–11.

Lopez, F. L., Ernest, T. B., Tuleu, C., & Gul, M. O. (2015). *Formulation approaches to pediatric oral drug delivery : benefits and limitations of current platforms*. 1727–1740.

Lowe, P., Krivoy, A., Porffy, L., Henriksdottir, E., Eromona, W., & Shergill, S. S. (2018). When the drugs don't work: treatment-resistant schizophrenia, serotonin and serendipity. *Therapeutic Advances in Psychopharmacology*, *8*(1), 63–70. https://doi.org/10.1177/2045125317737003

Lowman, P. D., & Montgomery, B. C. (1998). *The Effectiveness of Olanzapine in Treatment-Refractory Schizophrenia When Patients Are Nonresponsive to or Unable to Tolerate Clozapine*. *9*, 1021–1034. http://denali.gsfc.nasa.gov/dtam/seismic/

Ltd., O. P. (2018). *NiQuitin*. Medicines.Org. https://www.medicines.org.uk/emc/product/5592/smpc

Lu, J., Wang, T., & Drzal, L. T. (2008). Preparation and properties of microfibrillated cellulose polyvinyl alcohol composite materials. *Composites Part A: Applied Science and Manufacturing*, *39*(5), 738–746. https://doi.org/10.1016/j.compositesa.2008.02.003

Lu, L., Dong, M., Zhang, L., Zhu, X.-M., Ungvari, G. S., Ng, C. H., Wang, G., & Xiang, Y.-T. (2020). Prevalence of suicide attempts in individuals with schizophrenia: a meta-analysis of observational studies. *Epidemiology and Psychiatric Sciences*, *29*, e39. https://doi.org/10.1017/S2045796019000313

Lu, T., Sun, Y., Ding, D., Zhang, Q., Fan, R., He, Z., & Wang, J. (2017). Study on Enhanced Dissolution of Azilsartan-Loaded Solid Dispersion, Prepared by Combining Wet Milling and Spray-Drying Technologies. *AAPS PharmSciTech*, *18*(2), 473–480. https://doi.org/10.1208/s12249-016-0531-1

Maa, Y. F., Costantino, H. R., Nguyen, P. A., & Hsu, C. C. (1997). The effect of operating and formulation variables on the morphology of spray-dried protein particles. *Pharmaceutical Development and*

Technology, *2*(3), 213–223. https://doi.org/10.3109/10837459709031441

Maas, S. G., Schaldach, G., Littringer, E. M., Mescher, A., Griesser, U. J., Braun, D. E., Walzel, P. E., & Urbanetz, N. A. (2011). The impact of spray drying outlet temperature on the particle morphology of mannitol. *Powder Technology*, *213*(1), 27–35. https://doi.org/10.1016/j.powtec.2011.06.024

Macht, M., & Mueller, J. (2007). Increased negative emotional responses in PROP supertasters. *Physiology & Behavior*, *90*(2–3), 466–472. https://doi.org/10.1016/j.physbeh.2006.10.011

Madani, F., Lindberg, S., Langel, Ü., Futaki, S., & Gräslund, A. (2011). Mechanisms of Cellular Uptake of Cell-Penetrating Peptides. *Journal of Biophysics*, *2011*, 1–10. https://doi.org/10.1155/2011/414729

Mahmah, O., Tabbakh, R., Kelly, A., & Paradkar, A. (2014). A comparative study of the effect of spray drying and hot-melt extrusion on the properties of amorphous solid dispersions containing felodipine. *Journal of Pharmacy and Pharmacology*, *66*(2), 275–284. https://doi.org/10.1111/jphp.12099

Man, W. H. (2018). *Monitoring patients using clozapine*.

Marano, S., Barker, S. A., Raimi-Abraham, B. T., Missaghi, S., Rajabi-Siahboomi, A., Aliev, A. E., & Craig, D. Q. M. (2017). Microfibrous Solid Dispersions of Poorly Water-Soluble Drugs Produced via Centrifugal Spinning: Unexpected Dissolution Behavior on Recrystallization. *Molecular Pharmaceutics*, *14*(5), 1666–1680. https://doi.org/10.1021/acs.molpharmaceut.6b01126

Marano, S., Barker, S. A., Raimi-Abraham, B. T., Missaghi, S., Rajabi-Siahboomi, A., & Craig, D. Q. M. (2016). Development of micro-fibrous solid dispersions of poorly water-soluble drugs in sucrose using temperature-controlled centrifugal spinning. *European Journal of Pharmaceutics and Biopharmaceutics*, *103*, 84–94. https://doi.org/10.1016/j.ejpb.2016.03.021

Marroum, P. J. (2012). Clinically Relevant Dissolution Methods and Specifications. *American Pharmaceutical Review*.

Martin, G. N. (2013). *The Neuropsychology of Smell and Taste*.

Martin, L. (2003). Sustained buccal delivery of the hydrophobic drug denbufylline using physically cross-linked palmitoyl glycol chitosan hydrogels. *European Journal of Pharmaceutics and Biopharmaceutics*, *55*(1), 35–45. https://doi.org/10.1016/S0939-6411(02)00118-2

Martinez, M. N., & Amidon, G. L. (2002). A Mechanistic Approach to Understanding the Factors Affecting Drug Absorption: A Review of Fundamentals. *The Journal of Clinical Pharmacology*, *42*(6), 620–643. https://doi.org/10.1177/00970002042006005

Masareddy, R., Kadia, R., & Manvi, F. (2008). Development of mouth dissolving tablets of clozapine using two different techniques. *Indian Journal of Pharmaceutical Sciences*, *70*(4), 526–528.

https://doi.org/10.4103/0250-474X.44611

Matsui, D. (2007). Current Issues in Pediatric Medication Adherence. *Pediatric Drugs*, *9*(5), 283–288. https://doi.org/10.2165/00148581-200709050-00001

McGrath, J., Saha, S., Chant, D., & Welham, J. (2008). Schizophrenia: A Concise Overview of Incidence, Prevalence, and Mortality. *Epidemiologic Reviews*, *30*(1), 67–76. https://doi.org/10.1093/epirev/mxn001

McGrath, John, Saha, S., Welham, J., El Saadi, O., MacCauley, C., & Chant, D. (2004). A systematic review of the incidence of schizophrenia: the distribution of rates and the influence of sex, urbanicity, migrant status and methodology. *BMC Medicine*, *2*(1), 13. https://doi.org/10.1186/1741-7015-2-13

Mehmet Gürkan Güroka,Alaaddin Hekima, A. N. G. G. (2019). *Treatment of burning mouth syndrome with low-dose clozapine: a case report*. https://search.proquest.com/docview/2291474994?pq-origsite=gscholar

Mela, D. J., Mattes, R. D., Tanimura, S., & García-Medina, M. R. (1992). Relationships between ingestion and gustatory perception of caffeine. *Pharmacology Biochemistry and Behavior*, *43*(2), 513–521. https://doi.org/10.1016/0091-3057(92)90186-J

Meliana, Y., Utami, D., Septiyanti, M., Wulandari, E. T., Ghozali, M., Restu, W. K., Fahmiati, S., & Lelono, R. A. A. (2020). Characterization of Artemisinin Solid Dispersion in Maltodextrin and Gum Arabic by Freeze Dried and High Energy Milling Methods. *Macromolecular Symposia*, *391*(1), 1900186. https://doi.org/10.1002/masy.201900186

Melis, M., Errigo, A., Crnjar, R., Pes, G. M., & Tomassini Barbarossa, I. (2019). TAS2R38 bitter taste receptor and attainment of exceptional longevity. *Scientific Reports*, *9*(1), 18047. https://doi.org/10.1038/s41598-019-54604-1

Meltzer, H. Y. (2003). Clozapine Treatment for Suicidality in Schizophrenia. *Archives of General Psychiatry*, *60*(1), 82. https://doi.org/10.1001/archpsyc.60.1.82

Meng, F., Gala, U., & Chauhan, H. (2015). Classification of solid dispersions: correlation to (i) stability and solubility (ii) preparation and characterization techniques. *Drug Development and Industrial Pharmacy*, *41*(9), 1401–1415. https://doi.org/10.3109/03639045.2015.1018274

Mennella, J. A., Lukasewycz, L. D., Castor, S. M., & Beauchamp, G. K. (2011). The timing and duration of a sensitive period in human flavor learning: a randomized trial. *The American Journal of Clinical Nutrition*, *93*(5), 1019–1024. https://doi.org/10.3945/ajcn.110.003541

Mennella, J. A., Spector, A. C., Reed, D. R., & Coldwell, S. E. (2013). The Bad Taste of Medicines: Overview of Basic Research on Bitter Taste. *Clinical Therapeutics*, *35*(8), 1225–1246. https://doi.org/10.1016/j.clinthera.2013.06.007

Mensink, M. A., Frijlink, H. W., Van Der Voort Maarschalk, K., & Hinrichs, W. L. J. (2015). Inulin, a flexible oligosaccharide I: Review of its physicochemical characteristics. *Carbohydrate Polymers*, *130*, 405–419. https://doi.org/10.1016/j.carbpol.2015.05.026

Messias, E. L., Chen, C.-Y., & Eaton, W. W. (2007). Epidemiology of Schizophrenia: Review of Findings and Myths. *Psychiatric Clinics of North America*, *30*(3), 323–338. https://doi.org/10.1016/j.psc.2007.04.007

Michael, E. (2007). *Aulton Pharmaceutics : The Design and Manufacture of Medicines*.

Miller, T. A. (2016). Health literacy and adherence to medical treatment in chronic and acute illness: A meta-analysis. *Patient Education and Counseling*, *99*(7), 1079–1086. https://doi.org/10.1016/j.pec.2016.01.020

Milne, C.-P., & Bruss, J. B. (2008). The economics of pediatric formulation development for off-patent drugs. *Clinical Therapeutics*, *30*(11), 2133–2145. https://doi.org/10.1016/j.clinthera.2008.11.019

Minnes, R., Nissinmann, M., Maizels, Y., Gerlitz, G., Katzir, A., & Raichlin, Y. (2017). Using Attenuated Total Reflection–Fourier Transform Infra-Red (ATR-FTIR) spectroscopy to distinguish between melanoma cells with a different metastatic potential. *Scientific Reports*, *7*(1), 4381. https://doi.org/10.1038/s41598-017-04678-6

Mirza, H. (2018). Child and Adolescent Mental Health Services in Oman. *London Journal of Primary Care*, *10*(4), 121–122. https://doi.org/10.1080/17571472.2018.1482661

Mistry, P., & Batchelor, H. (2017). Evidence of acceptability of oral paediatric medicines: a review. *Journal of Pharmacy and Pharmacology*, *69*(4), 361–376. https://doi.org/10.1111/jphp.12610

Mitelman, S. A., Brickman, A. M., Shihabuddin, L., Newmark, R. E., Hazlett, E. A., Haznedar, M. M., & Buchsbaum, M. S. (2007). A comprehensive assessment of gray and white matter volumes and their relationship to outcome and severity in schizophrenia. *NeuroImage*, *37*(2), 449–462. https://doi.org/10.1016/j.neuroimage.2007.04.070

Mitrevska, I., Achkoska, T., Brezovska, K., Toshev, K., Dimitrovska, A., & Ugarkovic, S. (2019). Development and Validation of Discriminative Dissolution Method for Metformin Immediate-Release Film-Coated Tablets. *Journal of Analytical Methods in Chemistry*, *2019*, 1–8. https://doi.org/10.1155/2019/4296321

MJ Goodman and A Gilman. (n.d.). *Goodman and Gilman's the pharmacological basis of therapeutics*.

Modica De Mohac, L., Caruana, R., Pavia, F. C., Cavallaro, G., Giammona, G., & Licciardi, M. (2019). Multicomponent solid dispersion as a formulation strategy to improve drug permeation: A case study on the anti-colorectal cancer irinotecan. *Journal of Drug Delivery Science and Technology*, *52*, 346–354. https://doi.org/10.1016/j.jddst.2019.04.040

Modica de Mohac, L., de Fátima Pina, M., & Raimi-Abraham, B. T. (2016). Solid microcrystalline dispersion films as a new strategy to improve the dissolution rate of poorly water soluble drugs: A case study using olanzapine. *International Journal of Pharmaceutics*, *508*(1–2), 42–50. https://doi.org/10.1016/j.ijpharm.2016.05.012

Modica de Mohac, L., Keating, A., de Fátima Pina, M., & Raimi-Abraham, B. (2018). Engineering of Nanofibrous Amorphous and Crystalline Solid Dispersions for Oral Drug Delivery. *Pharmaceutics*, *11*(1), 7. https://doi.org/10.3390/pharmaceutics11010007

Modica de Mohac, L. M., Raimi-Abraham, B., Caruana, R., Gaetano, G., & Licciardi, M. (2020). Multicomponent solid dispersion a new generation of solid dispersion produced by spray-drying. *Journal of Drug Delivery Science and Technology*, *57*(March), 101750. https://doi.org/10.1016/j.jddst.2020.101750

Mohamed-Ahmed, A. H. A., Soto, J., Ernest, T., & Tuleu, C. (2016). Non-human tools for the evaluation of bitter taste in the design and development of medicines: a systematic review. *Drug Discovery Today*, *21*(7), 1170–1180. https://doi.org/10.1016/j.drudis.2016.05.014

Mohanty, S., Larsen, L. B., Trifol, J., Szabo, P., Burri, H. V. R., Canali, C., Dufva, M., Emnéus, J., & Wolff, A. (2015). Fabrication of scalable and structured tissue engineering scaffolds using water dissolvable sacrificial 3D printed moulds. *Materials Science and Engineering: C*, *55*, 569–578. https://doi.org/10.1016/j.msec.2015.06.002

Mondal, R., Das, A., Sen, D., Satapathy, D. K., & Basavaraj, M. G. (2019). Spray drying of colloidal dispersions containing ellipsoids. *Journal of Colloid and Interface Science*, *551*, 242–250. https://doi.org/10.1016/j.jcis.2019.05.012

Morales, J. O., & McConville, J. T. (2011). Manufacture and characterization of mucoadhesive buccal films. *European Journal of Pharmaceutics and Biopharmaceutics*, *77*(2), 187–199. https://doi.org/10.1016/j.ejpb.2010.11.023

Mortimer, A. M. (2011). Using clozapine in clinical practice. *Advances in Psychiatric Treatment*, *17*(4), 256–265. https://doi.org/10.1192/apt.bp.110.008136

Mullan, Z. (2000). Identification of taste-receptor family may improve patients' compliance. *The Lancet*, *355*(9211), 1246. https://doi.org/10.1016/S0140-6736(05)74680-8

Muwaffak, Z., Goyanes, A., Clark, V., Basit, A. W., Hilton, S. T., & Gaisford, S. (2017). Patient-specific 3D scanned and 3D printed antimicrobial polycaprolactone wound dressings. *International Journal of Pharmaceutics*, *527*(1–2), 161–170. https://doi.org/10.1016/j.ijpharm.2017.04.077

N.Jobdevairakkam, C. (2005). *Composition of fentanyl citrate oral solid transmucosal dosageform* (Patent No. US11271767).

Nagy, Z. K., Balogh, A., Vajna, B., Farkas, A., Patyi, G. G., Kramarics, Á., & Marosi, G. (2012). Comparison

of electrospun and extruded soluplus®-based solid dosage forms of improved dissolution. *Journal of Pharmaceutical Sciences*, *101*(1), 322–332. https://doi.org/10.1002/jps.22731

Naik, J. B., & Mokale, V. J. (2014). *Preparation of Freeze-dried Solid Dispersion Powder using Mannitol to Enhance Solubility of Lovastatin and Development of Sustained Release Tablet Dosage Form*. *1*(1), 11–26.

National Institute of Mental Health. (1998). National Institute of Mental Health. In *Depression* (Vol. 36, Issue 3). https://doi.org/10.1001/archinternmed.2010.261

Newbrun. (1996). The use of sodium bicarbonate in oral hygiene products and practice. *Compend Contin Educ Dent Suppl*, *17*, 19. https://www.ncbi.nlm.nih.gov/pubmed/11524863

Ng, S.-F., Rouse, J. J., Sanderson, F. D., Meidan, V., & Eccleston, G. M. (2010). Validation of a Static Franz Diffusion Cell System for In Vitro Permeation Studies. *AAPS PharmSciTech*, *11*(3), 1432–1441. https://doi.org/10.1208/s12249-010-9522-9

NHS. (2016). *NHS-Schizoprenia*.

Nireesha, G. R., Divya, L., Sowmya, C., Venkateshan, N., Babu, M. N., & Lavakumar, V. (2013). *Lyophilization / Freeze Drying*. *3*(4), 87–98.

Nolden, A. A., & Feeney, E. L. (2020). Genetic Differences in Taste Receptors: Implications for the Food Industry. *Annual Review of Food Science and Technology*, *11*(1), 183–204. https://doi.org/10.1146/annurev-food-032519-051653

Novartis. (n.d.). *Drug information Clozaril® (clozapine) Tablets*.

Nune, S. K., Rama, K. S., Dirisala, V. R., & Chavali, M. Y. (2017). Electrospinning of collagen nanofiber scaffolds for tissue repair and regeneration. In *Nanostructures for Novel Therapy* (pp. 281–311). Elsevier. https://doi.org/10.1016/B978-0-323-46142-9.00011-6

Ogawa, N., Hiramatsu, T., Suzuki, R., Okamoto, R., Shibagaki, K., Fujita, K., Takahashi, C., Kawashima, Y., & Yamamoto, H. (2018). Improvement in the water solubility of drugs with a solid dispersion system by spray drying and hot-melt extrusion with using the amphiphilic polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer and D-mannitol. *European Journal of Pharmaceutical Sciences*, *111*(June 2017), 205–214. https://doi.org/10.1016/j.ejps.2017.09.014

Oka, Y. (2018). Opening a “Wide” Window onto Taste Signal Transmission. *Neuron*, *98*(3), 456–458. https://doi.org/10.1016/j.neuron.2018.04.020

Okhuijsen-Pfeifer, C., Huijsman, E. A. H., Hasan, A., Sommer, I. E. C., Leucht, S., Kahn, R. S., & Luykx, J. J. (2018). Clozapine as a first- or second-line treatment in schizophrenia: a systematic review and meta-analysis. *Acta Psychiatrica Scandinavica*, *138*(4), 281–288. https://doi.org/10.1111/acps.12954

Oktavia, D. A., Fithriani, D., & Martosuyono, P. (2020). Physical Characteristics of Probiotic

Effervescent Tablets with Various Concentration of Maltodextrin as Coating Materials. *E3S Web of Conferences*, *147*, 03023. https://doi.org/10.1051/e3sconf/202014703023

Oldenhof, H., Wolkers, W. F., Fonseca, F., Passot, S., & Marin, M. (2008). Effect of Sucrose and Maltodextrin on the Physical Properties and Survival of Air-Dried Lactobacillus bulgaricus: An in Situ Fourier Transform Infrared Spectroscopy Study. *Biotechnology Progress*, *21*(3), 885–892. https://doi.org/10.1021/bp049559j

Oliveira, R. P. de S., Perego, P., Oliveira, M. N. de, & Converti, A. (2011). Effect of inulin as prebiotic and synbiotic interactions between probiotics to improve fermented milk firmness. *Journal of Food Engineering*, *107*(1), 36–40. https://doi.org/10.1016/j.jfoodeng.2011.06.005

Oliver, J. (2013). FazaClo® (clozapine, USP) Orally Disintegrating Tablets. *Journal of Chemical Information and Modeling*, *53*(9), 1689–1699. https://doi.org/10.1017/CBO9781107415324.004

Oloyede, E., Dzahini, O., Whiskey, E., & Taylor, D. (2019). Clozapine and Norclozapine Plasma Levels in Patients Switched Between Different Liquid Formulations. *Therapeutic Drug Monitoring*. https://doi.org/10.1097/FTD.0000000000000711

Oscarson, S., & Sehgelmeble, F. W. (2002). Chemical Syntheses of Inulin and Levan Structures. *The Journal of Organic Chemistry*, *67*(24), 8457–8462. https://doi.org/10.1021/jo020341q

Ottong, S. E., & Garver, D. L. (1997). A biomodal distribution of plasma in the psychoses. *Psychiatry Research*, *69*(2–3), 97–103. https://doi.org/10.1016/S0165-1781(96)03061-2

Overgaard, A. B. A., Møller-Sonnergaard, J., Christrup, L. L., Højsted, J., & Hansen, R. (2001). Patients' evaluation of shape, size and colour of solid dosage forms. *Pharmacy World and Science*, *23*(5), 185–188. https://doi.org/10.1023/A:1012050931018

Owen, F., Cross, A. J., Waddington, J. L., Poulter, M., Gamble, S. J., & Crow, T. J. (1980). Dopamine-mediated behaviour and 3H-spiperone binding to striatal membranes in rats after nine months haloperidol administration. *Life Sciences*, *26*(1), 55–59. https://doi.org/10.1016/0024-3205(79)90188-7

Paaver, U., Tamm, I., Laidmäe, I., Lust, A., Kirsimäe, K., Veski, P., Kogermann, K., & Heinämäki, J. (2014). Soluplus Graft Copolymer: Potential Novel Carrier Polymer in Electrospinning of Nanofibrous Drug Delivery Systems for Wound Therapy. *BioMed Research International*, *2014*, 1–7. https://doi.org/10.1155/2014/789765

Padday, J. F., & Uffindell, N. D. (1968). The calculation of cohesive and adhesive energies from intermolecular forces at a surface. *The Journal of Physical Chemistry*, *72*(5), 1407–1414. https://doi.org/10.1021/j100851a002

Pai, N. B., & Vella, S. C. (2012). Reason for clozapine cessation. *Acta Psychiatrica Scandinavica*, *125*(1), 39–44. https://doi.org/10.1111/j.1600-0447.2011.01776.x

Pal, K., Banthia, A. K., & Majumdar, D. K. (2007). Preparation and characterization of polyvinyl alcohol-gelatin hydrogel membranes for biomedical applications. *AAPS PharmSciTech*, *8*(1), E142–E146. https://doi.org/10.1208/pt080121

Palmieri, G. F., Bonacucina, G., Di Martino, P., & Martelli, S. (2001). Spray-Drying as a Method for Microparticulate Controlled Release Systems Preparation: Advantages and Limits. I. Water-Soluble Drugs. *Drug Development and Industrial Pharmacy*, *27*(3), 195–204. https://doi.org/10.1081/DDC-100000237

Park, M.-E., & Shin, S.-Y. (2018). Three-dimensional comparative study on the accuracy and reproducibility of dental casts fabricated by 3D printers. *The Journal of Prosthetic Dentistry*, *119*(5), 861.e1-861.e7. https://doi.org/10.1016/j.prosdent.2017.08.020

Patel, D., Smith, J. R., Smith, A. W., Grist, N., Barnett, P., & Smart, J. D. (2000). An atomic force microscopy investigation of bioadhesive polymer adsorption onto human buccal cells. *International Journal of Pharmaceutics*, *200*(2), 271–277. https://doi.org/10.1016/S0378-5173(00)00396-3

Patel, V. F., Liu, F., & Brown, M. B. (2011). Advances in oral transmucosal drug delivery. *Journal of Controlled Release*, *153*(2), 106–116. https://doi.org/10.1016/j.jconrel.2011.01.027

Paton, C., Barnes, T. R. E., Cavanagh, M.-R., Taylor, D., & Lelliott, P. (2008). High-dose and combination antipsychotic prescribing in acute adult wards in the UK: The challenges posed by p.r.n. prescribing. *British Journal of Psychiatry*, *192*(06), 435–439. https://doi.org/10.1192/bjp.bp.107.042895

Patton, M. V., Ryan, P., Calascione, T., Fischer, N., Morgenstern, A., Stenger, N., & Nelson-Cheeseman, B. B. (2019). Manipulating magnetic anisotropy in fused filament fabricated parts via macroscopic shape, mesoscopic infill orientation, and infill percentage. *Additive Manufacturing*, *27*, 482–488. https://doi.org/10.1016/j.addma.2019.03.026

Paudel, A., & Van Den Mooter, G. (2012). Influence of solvent composition on the miscibility and physical stability of naproxen/PVP K 25 solid dispersions prepared by cosolvent spray-drying. *Pharmaceutical Research*, *29*(1), 251–270. https://doi.org/10.1007/s11095-011-0539-x

Paudel, A., Worku, Z. A., Meeus, J., Guns, S., & Van Den Mooter, G. (2013). Manufacturing of solid dispersions of poorly water soluble drugs by spray drying: Formulation and process considerations. *International Journal of Pharmaceutics*, *453*(1), 253–284. https://doi.org/10.1016/j.ijpharm.2012.07.015

Peabody, J., Muttil, P., Chackerian, B., & Tumban, E. (2017). Characterization of a spray-dried candidate HPV L2-VLP vaccine stored for multiple years at room temperature. *Papillomavirus Research*, *3*, 116–120. https://doi.org/10.1016/j.pvr.2017.03.004

Peppas, N. A., & Mongia, N. K. (1997). Ultrapure poly(vinyl alcohol) hydrogels with mucoadhesive drug delivery characteristics. *European Journal of Pharmaceutics and Biopharmaceutics*, *43*(1), 51–58. https://doi.org/10.1016/S0939-6411(96)00010-0

Persano, L., Camposeo, A., Tekmen, C., & Pisignano, D. (2013). Industrial Upscaling of Electrospinning and Applications of Polymer Nanofibers: A Review. *Macromolecular Materials and Engineering*, *298*(5), 504–520. https://doi.org/10.1002/mame.201200290

Peter B. Jones, MD, PhD; Thomas R. E. Barnes, MD, DSc; Linda Davies, MSc; Graham Dunn, PhD; Helen Lloyd, B., & Karen P. Hayhurst, MSc; Robin M. Murray, MD, DSc; Alison Markwick, BA; Shoˆn W. Lewis, M. (2006). Randomized Controlled Trial of the Effect on Quality of Life of Second- vs First-Generation Antipsychotic Drugs in Schizophrenia. *Methods*, *63*(CUtLASS 1).

Petrovsky, N. (2010). Inulin - a versatile polysaccharide: use as food chemical and pharmaceutical agent. *Journal of Excipients and Food Chemicals*, *1*(3), 27–50. https://doi.org/10.1021/jf030383v

Pharma, A. (2011). FazaClo (clozapine, USP) Orally Disintegrating Tablets. *NDA Prior Approval Labeling Supllement*, *024*(November), 1–37. https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/021590s024lbl.pdf

Pharmacopoeia, E., & 5.0. (2005). *Buffer solutions*. 3–8.

Pickering, A. J., Crider, Y., Sultana, S., Swarthout, J., Goddard, F. G., Anjerul Islam, S., Sen, S., Ayyagari, R., & Luby, S. P. (2019). Effect of in-line drinking water chlorination at the point of collection on child diarrhoea in urban Bangladesh: a double-blind, cluster-randomised controlled trial. *The Lancet Global Health*, *7*(9), e1247–e1256. https://doi.org/10.1016/S2214-109X(19)30315-8

Pina, M. F. F., Zhao, M., Pinto, J. F. J. F., Sousa, J. J. J., & Craig, D. Q. M. (2014). The influence of drug physical state on the dissolution enhancement of solid dispersions prepared via hot-melt extrusion: A case study using olanzapine. *Journal of Pharmaceutical Sciences*, *103*(4), 1214–1223. https://doi.org/10.1002/jps.23894

Podrażka, M., Bączyńska, E., Kundys, M., Jeleń, P., & Witkowska Nery, E. (2017). Electronic Tongue—A Tool for All Tastes? *Biosensors*, *8*(1), 3. https://doi.org/10.3390/bios8010003

Pond, S. M., & Tozer, T. N. (1984). First-Pass Elimination. *Clinical Pharmacokinetics*, *9*(1), 1–25. https://doi.org/10.2165/00003088-198409010-00001

Pongpeerapat, A., Higashi, K., Tozuka, Y., Moribe, K., & Yamamoto, K. (2006). Molecular Interaction among Probucol/PVP/SDS Multicomponent System Investigated by Solid-State NMR. *Pharmaceutical Research*, *23*(11), 2566–2574. https://doi.org/10.1007/s11095-006-9089-z

Poomalai, P., Ramaraj, B., & Siddaramaiah. (2007). Thermal and mechanical properties of poly(methyl methacrylate) and ethylene vinyl acetate copolymer blends. *Journal of Applied Polymer Science*, *106*(1), 684–691. https://doi.org/10.1002/app.26696

Poorten, T., & Hert, M. (1996). *Sublingual use of atropine for clozapine-induced sialorrhoea: literature review and two case reports*.

Poozesh, S., Setiawan, N., Arce, F., Sundararajan, P., Rocca, J. Della, Rumondor, A., Wei, D., Wenslow, R., Xi, H., Zhang, S., Stellabott, J., Su, Y., Moser, J., & Marsac, P. J. (2017). Understanding the process-product-performance interplay of spray dried drug-polymer systems through complete structural and chemical characterization of single spray dried particles. *Powder Technology*, *320*, 685–695. https://doi.org/10.1016/j.powtec.2017.07.042

Porsio, B., Cusimano, M. G., Schillaci, D., Craparo, E. F., Giammona, G., & Cavallaro, G. (2017). Nano into Micro Formulations of Tobramycin for the Treatment of Pseudomonas aeruginosa Infections in Cystic Fibrosis. *Biomacromolecules*, *18*(12), 3924–3935. https://doi.org/10.1021/acs.biomac.7b00945

Pourfarzad, A., Habibi Najafi, M. B., Haddad Khodaparast, M. H., & Khayyat, M. H. (2015). Serish inulin and wheat biopolymers interactions in model systems as a basis for understanding the impact of inulin on bread properties: a FTIR investigation. *Journal of Food Science and Technology*, *52*(12), 7964–7973. https://doi.org/10.1007/s13197-015-1939-4

Pouton, C. W. (2006). Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. *European Journal of Pharmaceutical Sciences*, *29*(3–4), 278–287. https://doi.org/10.1016/j.ejps.2006.04.016

Practice, P. (2017). *Protocol for the use of intramuscular (IM) clozapine injection* (Issue Im). papers3://publication/uuid/636335A6-3E3F-4C74-B21A-5A989652F723

Prasad, A., Sankar, M. R., & Katiyar, V. (2017). State of Art on Solvent Casting Particulate Leaching Method for Orthopedic ScaffoldsFabrication. *Materials Today: Proceedings*, *4*(2), 898–907. https://doi.org/10.1016/j.matpr.2017.01.101

Prasad, L. K., & Smyth, H. (2016a). 3D Printing technologies for drug delivery: a review. *Drug Development and Industrial Pharmacy*, *42*(7), 1019–1031. https://doi.org/10.3109/03639045.2015.1120743

Prasad, L. K., & Smyth, H. (2016b). 3D Printing technologies for drug delivery: a review. *Drug Development and Industrial Pharmacy*, *42*(7), 1019–1031. https://doi.org/10.3109/03639045.2015.1120743

Purohit, K., Parekh, P. K., Kern, J., Logan, R. W., Liu, Z., Huang, Y., McClung, C. A., Crabbe, J. C., & Ozburn, A. R. (2018). Pharmacogenetic Manipulation of the Nucleus Accumbens Alters Binge-Like Alcohol Drinking in Mice. *Alcoholism: Clinical and Experimental Research*, *42*(5), 879–888. https://doi.org/10.1111/acer.13626

Qi, S., & Craig, D. (2016). Recent developments in micro- and nanofabrication techniques for the preparation of amorphous pharmaceutical dosage forms. *Advanced Drug Delivery Reviews*, *100*, 67–84. https://doi.org/10.1016/j.addr.2016.01.003

Raedler, T. J., Bymaster, F. P., Tandon, R., Copolov, D., & Dean, B. (2007). Towards a muscarinic hypothesis of schizophrenia. *Molecular Psychiatry*, *12*(3), 232–246. https://doi.org/10.1038/sj.mp.4001924

Raimi-Abraham, B. T., Mahalingam, S., Davies, P. J., Edirisinghe, M., & Craig, D. Q. M. (2015). Development and Characterization of Amorphous Nanofiber Drug Dispersions Prepared Using Pressurized Gyration. *Molecular Pharmaceutics*, acs.molpharmaceut.5b00127. https://doi.org/10.1021/acs.molpharmaceut.5b00127

Rams-Baron, M., Jachowicz, R., Boldyreva, E., Zhou, D., Jamroz, W., & Paluch, M. (2018). *Amorphous Drugs*. Springer International Publishing. https://doi.org/10.1007/978-3-319-72002-9

Ranmal, S. R., O'Brien, F., Lopez, F., Ruiz, F., Orlu, M., Tuleu, C., Walsh, J., & Liu, F. (2018). Methodologies for assessing the acceptability of oral formulations among children and older adults: a systematic review. *Drug Discovery Today*, *23*(4), 830–847. https://doi.org/10.1016/j.drudis.2018.01.038

Rao, V., Ashokan, P. ., & Shridhar, M. . (1999). Studies on the compatibility and specific interaction in cellulose acetate hydrogen phthalate (CAP) and poly methyl methacrylate (PMMA) blend. *Polymer*, *40*(25), 7167–7171. https://doi.org/10.1016/S0032-3861(99)00311-0

Ray, L. (2016). Guidelines for the management of weight gain and metabolic disturbances linked with psychosis and antipsychotic drug treatment. *NHS Sussex Partnership NHS Foundation Trust*.

Raymond C R, Paul J S, M. E. Q. (2009). *Handbook Pharmaceutical Excipients,Sixth Edition*.

Reguieg, F., Ricci, L., Bouyacoub, N., Belbachir, M., & Bertoldo, M. (2020). Thermal characterization by DSC and TGA analyses of PVA hydrogels with organic and sodium MMT. *Polymer Bulletin*, *77*(2), 929–948. https://doi.org/10.1007/s00289-019-02782-3

Reineke, J. J., Cho, D. Y., Dingle, Y.-T., Morello, A. P., Jacob, J., Thanos, C. G., & Mathiowitz, E. (2013). Unique insights into the intestinal absorption, transit, and subsequent biodistribution of polymer-derived microspheres. *Proceedings of the National Academy of Sciences*, *110*(34), 13803–13808. https://doi.org/10.1073/pnas.1305882110

Reintjes, T. (2011). 10. Kolliphor™ P grades (Poloxamers). *Solubility Enhancement with BASF Pharma Polymers. Solubilizer Compendium*, 103–111.

Report, P. A., Board, M. E., & Lesvi, L. (2009). *of the Medicines Evaluation Board in the Netherlands Olanzapine Niolib 15 mg and 20 mg film-coated tablets Laboratorios Lesvi , Spain*. 1–11.

Report, P. A., Board, M. E., Lesvi, L., Petters, M. D., Kreidenweis, S. M., Ii, B. A. B., & Pustaka, S. (2009).

A single parameter representation of hygroscopic growth and cloud condensation nucleus activity. *Atmospheric Chemistry and Physics*, *7*(8), 1961–1971. https://doi.org/10.5194/acp-7-1961-2007

Reynolds, G. P., & Beasley, C. L. (2001). GABAergic neuronal subtypes in the human frontal cortex — development and deficits in schizophrenia. *Journal of Chemical Neuroanatomy*, *22*(1–2), 95–100. https://doi.org/10.1016/S0891-0618(01)00113-2

Richard A. Storey, I. Y. (2011). *Solid State Characterization of Pharmaceuticals*.

Ricotti, R., Ciardo, D., Pansini, F., Bazani, A., Comi, S., Spoto, R., Noris, S., Cattani, F., Baroni, G., Orecchia, R., Vavassori, A., & Alicja Jereczek-Fossa, B. (2017). Dosimetric characterization of 3D printed bolus at different infill percentage for external photon beam radiotherapy. *Physica Medica*, *39*, 25–32. https://doi.org/10.1016/j.ejmp.2017.06.004

Riera, C. E., Vogel, H., Simon, S. A., & Coutre, J. le. (2007). Artificial sweeteners and salts producing a metallic taste sensation activate TRPV1 receptors. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *293*(2), R626–R634. https://doi.org/10.1152/ajpregu.00286.2007

Rifka C. Derman, Caroline E. Bass, C. R. F. (2019). *Effects of hM4Di activation in CamKII basolateral amygdala neurons and CNO treatment on Sensory-Specific vs. General-PIT; refining PIT circuits and considerations for using CNO*. https://doi.org/10.1101/700120

Robinson, R. L., Long, S. R., Chang, S., Able, S., Baser, O., Obenchain, R., & Swindle, R. W. (2006). Higher Costs and Therapeutic Factors Associated With Adherence to NCQA HEDIS Antidepressant Medication Management Measures: Analysis of Administrative Claims. *Journal of Managed Care Pharmacy*, *12*(1), 43–54. https://doi.org/10.18553/jmcp.2006.12.1.43

Rodrigues, L. B., Leite, H. F., Yoshida, M. I., Saliba, J. B., Junior, A. S. C., & Faraco, A. A. G. (2009). In vitro release and characterization of chitosan films as dexamethasone carrier. *International Journal of Pharmaceutics*, *368*(1–2), 1–6. https://doi.org/10.1016/j.ijpharm.2008.09.047

Roni Shiloh, Rafael Stryjer, Abraham Weizman, D. J. N. (1999). *Atlas of Psychiatric Pharmacotherapy 2nd Edition*.

Rosenheck, R., Cramer, J., Xu, W., Thomas, J., Henderson, W., Frisman, L., Fye, C., & Charney, D. (1997). A Comparison of Clozapine and Haloperidol in Hospitalized Patients with Refractory Schizophrenia. *New England Journal of Medicine*, *337*(12), 809–815. https://doi.org/10.1056/NEJM199709183371202

Rowland, M., & Tozer, T. (2005). *Clinical pharmacokinetics/pharmacodynamics*.

Rozin, P. (1982). "Taste-smell confusions" and the duality of the olfactory sense. *Perception & Psychophysics*, *31*(4), 397–401. https://doi.org/10.3758/BF03202667

Rudnitskaya, A., Kirsanov, D., Blinova, Y., Legin, E., Seleznev, B., Clapham, D., Ives, R. S., Saunders, K. A., & Legin, A. (2013). Assessment of bitter taste of pharmaceuticals with multisensor system employing 3 way PLS regression. *Analytica Chimica Acta*, *770*, 45–52. https://doi.org/10.1016/j.aca.2013.02.006

Rui-xin Liu,Xiao-jie Gao,ming Wang, Li-ping Dai,4 Bing-ya Kang,Lu Zhang, Jun-han Shi, Xin-jing Gui, Pei Liu, and X. L. (2017). Traditional Human Taste Panel and Taste Sensors Methods for Bitter Taste Masking Research on Combined Bitterness Suppressants of Berberine Hydrochloride. *Sensors and Materials*, 105. https://doi.org/10.18494/SAM.2017.1432

Rui, Y., Gang, X., Shuang-Shuang, M., Hua-Yu, Y., Xin-Ting, S., Wei, S., & Yi-Lei, M. (2016). Three-dimensional printing: review of application in medicine and hepatic surgery. *Cancer Biology & Medicine*, *13*(4), 443. https://doi.org/10.20892/j.issn.2095-3941.2016.0075

Ruiz-Avila, L. (2000). An In Vitro Assay Useful to Determine the Potency of Several Bitter Compounds. *Chemical Senses*, *25*(4), 361–368. https://doi.org/10.1093/chemse/25.4.361

S. Gaisford R. Ramos. (2007). Calorimetry for amorphous content quantification. *European Pharmaceutical Review*.

S. Ramuth, J. Flanagan, D. T. (1996). A liqui clozapine preparation for oral administration in hospital. *The Pharmacueticla Journal*, *257*, 190–192.

Sachs, E., Cima, M., & Cornie, J. (1990). Three-Dimensional Printing: Rapid Tooling and Prototypes Directly from a CAD Model. *CIRP Annals*, *39*(1), 201–204. https://doi.org/10.1016/S0007-8506(07)61035-X

Šagud, M. (2015). Treatment-resistant schizophrenia: Challenges and implications for clinical practice. *Psychiatria Danubina*, *27*(3), 319–326.

Sahoo, N. G., Abbas, A., Judeh, Z., Li, C. M., & Yuen, K.-H. (2009). Solubility Enhancement of a Poorly Water-Soluble Anti-Malarial Drug: Experimental Design and Use of a Modified Multifluid Nozzle Pilot Spray Drier. *Journal of Pharmaceutical Sciences*, *98*(1), 281–296. https://doi.org/10.1002/jps.21399

Sahoo, S., Ang, L. T., Goh, J. C.-H., & Toh, S.-L. (2009). Growth factor delivery through electrospun nanofibers in scaffolds for tissue engineering applications. *Journal of Biomedical Materials Research Part A*, n/a-n/a. https://doi.org/10.1002/jbm.a.32645

Saiano, F. et al. (2002). Evaluation of mucoadhesive properties of α,β-poly(N-hydroxyethyl)-dl-aspartamide and α,β-poly(aspartylhydrazide) using ATR–FTIR spectroscopy. *Polymer*, *43*(23), 6281–6286. https://doi.org/10.1016/S0032-3861(02)00504-9

Sajatovic, M., Velligan, D. I., Weiden, P. J., Valenstein, M. A., & Ogedegbe, G. (2010). Measurement of psychiatric treatment adherence. *Journal of Psychosomatic Research*, *69*(6), 591–599.

https://doi.org/10.1016/j.jpsychores.2009.05.007

Salawi, A., & Nazzal, S. (2018). The physiochemical, mechanical, and adhesive properties of solvent-cast vitamin E/Soluplus® films. *International Journal of Pharmaceutics*, *552*(1–2), 378–387. https://doi.org/10.1016/j.ijpharm.2018.10.018

Saldanha, J. M., & Kyu, T. (1987). Influence of Solvent Casting on the Evolution of Phase Morphology of PC/PMMA Blends. *Macromolecules*, *20*(11), 2840–2847. https://doi.org/10.1021/ma00177a033

Samie, A., Desiraju, G. R., & Banik, M. (2017). Salts and Cocrystals of the Antidiabetic Drugs Gliclazide, Tolbutamide, and Glipizide: Solubility Enhancements through Drug–Coformer Interactions. *Crystal Growth & Design*, *17*(5), 2406–2417. https://doi.org/10.1021/acs.cgd.6b01804

Sandoz. (2018). *Sandoz clozapine*. 1–52.

Schiffman, S. S. (2015). Influence of Drugs on Taste Function. In *Handbook of Olfaction and Gustation* (pp. 911–926). John Wiley & Sons, Inc. https://doi.org/10.1002/9781118971758.ch40

Schiffman, S. S. (2018). Influence of medications on taste and smell. *World Journal of Otorhinolaryngology - Head and Neck Surgery*, *4*(1), 84–91. https://doi.org/10.1016/j.wjorl.2018.02.005

Seligmann, E. B., & Farber, J. F. (1971). Freeze drying and residual moisture. *Cryobiology*, *8*(2), 138–144. https://doi.org/10.1016/0011-2240(71)90021-6

Serajuddln, A. T. M. (1999). Solid dispersion of poorly water-soluble drugs: Early promises, subsequent problems, and recent breakthroughs. *Journal of Pharmaceutical Sciences*, *88*(10), 1058–1066. https://doi.org/10.1021/js980403l

Serra, T., Planell, J. A., & Navarro, M. (2013). High-resolution PLA-based composite scaffolds via 3-D printing technology. *Acta Biomaterialia*, *9*(3), 5521–5530. https://doi.org/10.1016/j.actbio.2012.10.041

Shabir, Q., Skaria, C., Brien, H. O., Loni, A., Barnett, C., & Canham, L. (2012). Taste and mouthfeel assessment of porous and non-porous silicon microparticles. *Nanoscale Research Letters*, *7*(1), 407. https://doi.org/10.1186/1556-276X-7-407

Shafaat, K., Kumar, B., Das, S. K., Ul Hasan, R., & Prajapati, S. K. (2013). Novel nanoemulsion as vehicles for transdermal delivery of Clozapine: In vitro and in vivo studies. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(SUPPL 3), 126–134.

Shah, N.; Sandhu, H.; Choi, D.S.; Cholkshu, H.; Malick, A. W. (2014). *Amorphous Solid Dispersions Theory and Practice*.

Shah, B., Kakumanu, V. K., & Bansal, A. K. (2006). Analytical techniques for quantification of amorphous/crystalline phases in pharmaceutical solids. *Journal of Pharmaceutical Sciences*,

95(8), 1641–1665. https://doi.org/10.1002/jps.20644

Shah, V. P., Lesko, L. J., Fan, J., Fleischer, N., Handerson, J., Malinowski, H., Makary, M., Ouderkirk, L., Bay, S., Sathe, P., Singh, G. J. P., Iillman, L., Tsong, Y., & Williams, R. I. (1997). FDA guidance for industry 1 dissolution testing of immediate release solid oral dosage forms. *Dissolution Technologies*, *4*(4), 15–22. https://doi.org/10.14227/DT040497P15

Shah, V. P., Tsong, Y., Sathe, P., & Williams, R. L. (1999). Dissolution Profile Comparison Using Similarity Factor, f2. *Dissolution Technologies*, *6*(3), 15–15. https://doi.org/10.14227/DT060399P15

Shaker, A., & Jones, R. (2018). Clozapine discontinuation in early schizophrenia: a retrospective case note review of patients under an early intervention service. *Therapeutic Advances in Psychopharmacology*, *8*(1), 3–11. https://doi.org/10.1177/2045125317741449

Shalini Mishra, G. Kumar, P. K. (2012). Formulation and Evaluation of Buccal Patches of Simvastatin by Using Different Polymers. *The Pharma Innovation*, *3*(12), 1–7.

Shamma, R. N., & Basha, M. (2013). Soluplus: A novel polymeric solubilizer for optimization of Carvedilol solid dispersions: Formulation design and effect of method of preparation. *Powder Technology*, *237*, 406–414. https://doi.org/10.1016/j.powtec.2012.12.038

Shaw, L., Mansfield, C., Colquitt, L., Lin, C., Ferreira, J., Emmetsberger, J., & Reed, D. R. (2018). Personalized expression of bitter 'taste' receptors in human skin. *PLOS ONE*, *13*(10), e0205322. https://doi.org/10.1371/journal.pone.0205322

Shchodryi, V., Obernikhina, N., Shaydyk, Y., Kachkovsky, O., Yu., S., & Tkachuk, Z. (2019). Fluorescent Probe for Investigation of Influence of Ribonucleosides With D–Mannitol. *2019 IEEE 39th International Conference on Electronics and Nanotechnology (ELNANO)*, 385–389. https://doi.org/10.1109/ELNANO.2019.8783955

Shen, X., Yu, D., Zhu, L., Branford-White, C., White, K., & Chatterton, N. P. (2011). Electrospun diclofenac sodium loaded Eudragit® L 100-55 nanofibers for colon-targeted drug delivery. *International Journal of Pharmaceutics*, *408*(1–2), 200–207. https://doi.org/10.1016/j.ijpharm.2011.01.058

Shi, Q., Moinuddin, S. M., & Cai, T. (2019). Advances in coamorphous drug delivery systems. *Acta Pharmaceutica Sinica B*, *9*(1), 19–35. https://doi.org/10.1016/j.apsb.2018.08.002

Shimokomaki, M., Youssef Youssef, E., & Terra, N. N. (2003). CURING. In *Encyclopedia of Food Sciences and Nutrition* (pp. 1702–1708). Elsevier. https://doi.org/10.1016/B0-12-227055-X/00316-3

Shin, S.-C., Kim, J.-Y., & Oh, I.-J. (2000). Mucoadhesive and Physicochemical Characterization of Carbopol-Poloxamer Gels Containing Triamcinolone Acetonide. *Drug Development and Industrial Pharmacy*, *26*(3), 307–312. https://doi.org/10.1081/DDC-100100358

Shirazi, S. F. S., Gharehkhani, S., Mehrali, M., Yarmand, H., Metselaar, H. S. C., Adib Kadri, N., & Osman,

N. A. A. (2015). A review on powder-based additive manufacturing for tissue engineering: selective laser sintering and inkjet 3D printing. *Science and Technology of Advanced Materials*, *16*(3), 033502. https://doi.org/10.1088/1468-6996/16/3/033502

Shivanand, A., & Mukhopadhayaya, S. (2017). *A Review on Lyophilization : A Technique to Improve Stability of Hygroscopic , Thermolabile Substances*. *5*(11).

Siemann, U. (2005). Solvent cast technology - A versatile tool for thin film production. *Progress in Colloid and Polymer Science*, *130*(June), 1–14. https://doi.org/10.1007/b107336

Sigurdsson, H. H., Loftsson, T., & Lehr, C.-M. (2006). Assessment of mucoadhesion by a resonant mirror biosensor. *International Journal of Pharmaceutics*, *325*(1–2), 75–81. https://doi.org/10.1016/j.ijpharm.2006.06.027

Silverstein M.Robert, Webster X. Francis, K. J. D. (2005). Spectrometric Identification of Organic Compounds. In *Organic Chemistry* (pp. 1–550).

Singh, A., & Van den Mooter, G. (2016). Spray drying formulation of amorphous solid dispersions. *Advanced Drug Delivery Reviews*, *100*, 27–50. https://doi.org/10.1016/j.addr.2015.12.010

Sinha, V. R., & Kumria, R. (2003). Coating polymers for colon specific drug delivery: a comparative in vitro evaluation. *Acta Pharmaceutica (Zagreb, Croatia)*, *53*, 41–47. http://www.ncbi.nlm.nih.gov/pubmed/14769251

Sinko, P. J. (2011). Martin's physical pharmacy and pharmaceutical sciences Physical Chemical and Biopharmaceutical Principles in the Pharmaceutical sciences. *Martin's Physical Pharmacy and Pharmaceutical Sciences*, 182–196.

Siskind, D., McCartney, L., Goldschlager, R., & Kisely, S. (2016). Clozapine v. first- and second-generation antipsychotics in treatment-refractory schizophrenia: systematic review and meta-analysis. *British Journal of Psychiatry*, *209*(05), 385–392. https://doi.org/10.1192/bjp.bp.115.177261

Siva Prasad Reddy, S., Ahad, H. A., Sreenivasulu, R., Kishore Kumar Reddy, B., Krishna Mahesh, C. H., Kranthi, G., & Chandrasekhar, A. (2011). Novel approach in designing of mouth dissolving tablets for bitter drugs: Taking clozapine as model drug. *Der Pharmacia Lettre*, *3*(1), 113–120.

Skowyra, J., Pietrzak, K., & Alhnan, M. A. (2015). Fabrication of extended-release patient-tailored prednisolone tablets via fused deposition modelling (FDM) 3D printing. *European Journal of Pharmaceutical Sciences*, *68*, 11–17. https://doi.org/10.1016/j.ejps.2014.11.009

Slatter, J. G., Schaaf, L. J., Sams, J. P., Feenstra, K. L., Johnson, M. G., Bombardt, P. A., Cathcart, K. S., Verburg, M. T., Pearson, L. K., Compton, L. D., Miller, L. L., Baker, D. S., & Pesheck, C. V. (2000). Pharmacokinetics, Metabolism, and Excretion of Irinotecan (Cpt-11) following I. V. Infusion of [14C] Cpt-11 in Cancer Patients. *Drug Metabolism and Disposition*, *28*(4), 423–433.

Smith, A. H., & Mendel, L. B. (1920). THE ADJUSTMENT OF BLOOD VOLUME AFTER INJECTION OF ISOTONIC SOLUTIONS OF VARIED COMPOSITION. *American Journal of Physiology-Legacy Content*, *53*(2), 323–344. https://doi.org/10.1152/ajplegacy.1920.53.2.323

Soares, S., Kohl, S., Thalmann, S., Mateus, N., Meyerhof, W., & De Freitas, V. (2013). Different Phenolic Compounds Activate Distinct Human Bitter Taste Receptors. *Journal of Agricultural and Food Chemistry*, *61*(7), 1525–1533. https://doi.org/10.1021/jf304198k

Soto, J., Winzenburg, G., Turner, R., Desset-Brèthes, S., Sheng, Y., Orlu-Gul, M., & Tuleu, C. (2016). Assessing the bitter taste of medicines: A comparison between rat taste panels (via the brief-access taste aversion (BATA) model) and human taste panels. *International Journal of Pharmaceutics*, *511*(2), 1127–1128. https://doi.org/10.1016/j.ijpharm.2016.06.063

Soto, Jessica, Keeley, A., Keating, A. V., Mohamed-Ahmed, A. H. A., Sheng, Y., Winzenburg, G., Turner, R., Desset-Brèthes, S., Orlu, M., & Tuleu, C. (2018). Rats can predict aversiveness of Active Pharmaceutical Ingredients. *European Journal of Pharmaceutics and Biopharmaceutics*, *133*, 77–84. https://doi.org/10.1016/j.ejpb.2018.09.027

Sperelakis, N., & Kaneshiro, and E. (2012). *Cell Physiology Source Book*.

Spina, E., Avenoso, A., Facciolà, G., Scordo, M. G., Ancione, M., Madia, A. G., Ventimiglia, A., & Perucca, E. (2000). Relationship between plasma concentrations of clozapine and norclozapine and therapeutic response in patients with schizophrenia resistant to conventional neuroleptics. *Psychopharmacology*, *148*(1), 83–89. https://doi.org/10.1007/s002130050028

Sritham, E., & Gunasekaran, S. (2017). FTIR spectroscopic evaluation of sucrose-maltodextrin-sodium citrate bioglass. *Food Hydrocolloids*, *70*, 371–382. https://doi.org/10.1016/j.foodhyd.2017.04.023

Steen, R. G., Mull, C., Mcclure, R., Hamer, R. M., & Lieberman, J. A. (2006). Brain volume in first-episode schizophrenia. *British Journal of Psychiatry*, *188*(06), 510–518. https://doi.org/10.1192/bjp.188.6.510

Struchtrup, H. (2014). The First Law of Thermodynamics. In *Thermodynamics and Energy Conversion* (pp. 33–53). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-43715-5_3

Sudhakar, Y., Kuotsu, K., & Bandyopadhyay, A. K. (2006). Buccal bioadhesive drug delivery — A promising option for orally less efficient drugs. *Journal of Controlled Release*, *114*(1), 15–40. https://doi.org/10.1016/j.jconrel.2006.04.012

Sumarokova, M., Iturri, J., Weber, A., Maares, M., Keil, C., Haase, H., & Toca-Herrera, J. L. (2018). Influencing the adhesion properties and wettability of mucin protein films by variation of the environmental pH. *Scientific Reports*, *8*(1), 9660. https://doi.org/10.1038/s41598-018-28047-z

Sun, D. D., Wen, H., & Taylor, L. S. (2016). Non-Sink Dissolution Conditions for Predicting Product

Quality and In Vivo Performance of Supersaturating Drug Delivery Systems. *Journal of Pharmaceutical Sciences*, *105*(9), 2477–2488. https://doi.org/10.1016/j.xphs.2016.03.024

Surini, S., Evangelista, C. N., & Iswandana, R. (2018). Development of Glimepiride Solid Dispersion using The Coprocessed Excipients of Polyvinylpyrrolidone, Maltodextrin, and Polyethylene Glycol. *Journal of Young Pharmacists*, *10*(2s), S45–S50. https://doi.org/10.5530/jyp.2018.2s.9

Sussex, N. (2008). Guidance on the use of Antipsychotics. *Icao*, *October 2015*, 1–60. www.icao.int

Suzuki, T., Remington, G., Mulsant, B. H., Rajji, T. K., Uchida, H., Graff-Guerrero, A., & Mamo, D. C. (2011). Treatment resistant schizophrenia and response to antipsychotics: A review. *Schizophrenia Research*, *133*(1–3), 54–62. https://doi.org/10.1016/j.schres.2011.09.016

Sweeney, Y. M. S. J. shire; T. (1998). *Effect of spray drying and subsequent processing on residual moisture content and physical/biochemical stability of protein inhalation powders*. *October 2002*, 193–194.

Tagami, T., Fukushige, K., Ogawa, E., Hayashi, N., & Ozeki, T. (2017). 3D Printing Factors Important for the Fabrication of Polyvinylalcohol Filament-Based Tablets. *Biological & Pharmaceutical Bulletin*, *40*(3), 357–364. https://doi.org/10.1248/bpb.b16-00878

Takeuchi, I., Taniguchi, Y., Tamura, Y., Ochiai, K., & Makino, K. (2018). Effects of l -leucine on PLGA microparticles for pulmonary administration prepared using spray drying: Fine particle fraction and phagocytotic ratio of alveolar macrophages. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *537*(October 2017), 411–417. https://doi.org/10.1016/j.colsurfa.2017.10.047

Talukder, R., Reed, C., Dürig, T., & Hussain, M. (2011). Dissolution and Solid-State Characterization of Poorly Water-Soluble Drugs in the Presence of a Hydrophilic Carrier. *AAPS PharmSciTech*, *12*(4), 1227–1233. https://doi.org/10.1208/s12249-011-9697-8

Tandon, R., Belmaker, R. H., Gattaz, W. F., Lopez-Ibor, J. J., Okasha, A., Singh, B., Stein, D. J., Olie, J.-P., Fleischhacker, W. W., & Moeller, H.-J. (2008). World Psychiatric Association Pharmacopsychiatry Section statement on comparative effectiveness of antipsychotics in the treatment of schizophrenia. *Schizophrenia Research*, *100*(1–3), 20–38. https://doi.org/10.1016/j.schres.2007.11.033

Tao, M., Meng, X., Lv, Y., Bian, Z., & Xin, Z. (2016). Effect of impregnation solvent on Ni dispersion and catalytic properties of Ni/SBA-15 for CO methanation reaction. *Fuel*, *165*, 289–297. https://doi.org/10.1016/j.fuel.2015.10.023

Taylor, D. M., & Duncan-McConnell, D. (2000). Refractory schizophrenia and atypical antipsychotics. *Journal of Psychopharmacology*, *14*(4), 409–418. https://doi.org/10.1177/026988110001400411

Taylor, D. M., Smith, L., Gee, S. H., & Nielsen, J. (2012). Augmentation of clozapine with a second antipsychotic - a meta-analysis. *Acta Psychiatrica Scandinavica*, *125*(1), 15–24. https://doi.org/10.1111/j.1600-0447.2011.01792.x

Taylor, David M. (2017). Clozapine for Treatment-Resistant Schizophrenia: Still the Gold Standard? *CNS Drugs*, *31*(3), 177–180. https://doi.org/10.1007/s40263-017-0411-6

Taylor, David M., Douglas-Hall, P., Olofinjana, B., Whiskey, E., & Thomas, A. (2009). Reasons for discontinuing clozapine: matched, case–control comparison with risperidone long-acting injection. *British Journal of Psychiatry*, *194*(2), 165–167. https://doi.org/10.1192/bjp.bp.108.051979

Taylor, David M., Taylor, D. M., & Duncan-McConnell, D. (2000). Refractory schizophrenia and atypical antipsychotics. *Journal of Psychopharmacology*, *14*(4), 409–418. https://doi.org/10.1177/026988110001400411

Taylor, David M., Young, C., & Paton, C. (2003). Prior antipsychotic prescribing in patients currently receiving clozapine: A case note review. *Journal of Clinical Psychiatry*, *64*(1), 30–34. https://doi.org/10.4088/JCP.v64n0107

Taylor, M. J., Tanna, S., & Sahota, T. (2010). In vivo study of a polymeric glucose-sensitive insulin delivery system using a rat model. *Journal of Pharmaceutical Sciences*, *99*(10), 4215–4227. https://doi.org/10.1002/jps

Taylor, P., Boateng, J. S., Stevens, H. N. E., Eccleston, G. M., Auffret, A. D., Humphrey, M. J., Matthews, K. H., Boateng, J. S., Stevens, H. N. E., Eccleston, G. M., & Auffret, A. D. (n.d.). *solvent-cast polymeric films as potential drug delivery systems to mucosal surfaces Development and mechanical characterization of solvent-cast polymeric films as potential drug delivery systems to mucosal surfaces*. *July 2015*. https://doi.org/10.1080/03639040902744704

Teo, W. E., Inai, R., & Ramakrishna, S. (2011). Technological advances in electrospinning of nanofibers. *Science and Technology of Advanced Materials*, *12*(1). https://doi.org/10.1088/1468-6996/12/1/013002

Teubl, B. J., Meindl, C., Eitzlmayr, A., Zimmer, A., Fröhlich, E., & Roblegg, E. (2013). In-Vitro Permeability of Neutral Polystyrene Particles via Buccal Mucosa. *Small*, *9*(3), 457–466. https://doi.org/10.1002/smll.201201789

Thakral, N. K., Ray, A. R., Bar-Shalom, D., Eriksson, A. H., & Majumdar, D. K. (2012). Soluplus-Solubilized Citrated Camptothecin—A Potential Drug Delivery Strategy in Colon Cancer. *AAPS PharmSciTech*, *13*(1), 59–66. https://doi.org/10.1208/s12249-011-9720-0

The National Institute for Health and Care Excellence. (2019). *Clinical Protocol for the Safe Use of Clozapine*. *October*, 1–41.

Thomas, C. J., Rahier, N. J., & Hecht, S. M. (2004). Camptothecin: Current perspectives. *Bioorganic and Medicinal Chemistry*, *12*(7), 1585–1604. https://doi.org/10.1016/j.bmc.2003.11.036

Thompson, M. T. (2014). Review of Diode Physics and the Ideal (and Later, Nonideal) Diode. In *Intuitive Analog Circuit Design* (pp. 53–86). Elsevier. https://doi.org/10.1016/B978-0-12-405866-8.00003-6

Thrasher, M. J., Freeman, P. A., & Risinger, F. (1999). *Clozapine ' s Effects on Ethanol ' s Motivational Properties*. 1377–1385.

Tiihonen, J., Lönnqvist, J., Wahlbeck, K., Klaukka, T., Niskanen, L., Tanskanen, A., & Haukka, J. (2009). 11-year follow-up of mortality in patients with schizophrenia: a population-based cohort study (FIN11 study). *The Lancet*, *374*(9690), 620–627. https://doi.org/10.1016/S0140-6736(09)60742-X

Till, A., Selwood, J., & Silva, E. (2019). The assertive approach to clozapine: nasogastric administration. *BJPsych Bulletin*, *43*(1), 21–26. https://doi.org/10.1192/bjb.2018.61

Tilson, H. H. (2004). Adherence or Compliance? Changes in Terminology. *Annals of Pharmacotherapy*, *38*(1), 161–162. https://doi.org/10.1345/aph.1D207

Torchilin, V. P. (2006). Micellar Nanocarriers: Pharmaceutical Perspectives. *Pharmaceutical Research*, *24*(1), 1–16. https://doi.org/10.1007/s11095-006-9132-0

Tordoff, M. G. (2003). Mouse Taste Preference Tests: Why Only Two Bottles? *Chemical Senses*, *28*(4), 315–324. https://doi.org/10.1093/chemse/28.4.315

Torres-Lugo, M., & Peppas, N. A. (2000). Transmucosal delivery systems for calcitonin: a review. *Biomaterials*, *21*(12), 1191–1196. https://doi.org/10.1016/S0142-9612(00)00011-9

Trenfield, S. J., Awad, A., Goyanes, A., Gaisford, S., & Basit, A. W. (2018). 3D Printing Pharmaceuticals: Drug Development to Frontline Care. *Trends in Pharmacological Sciences*, *39*(5), 440–451. https://doi.org/10.1016/j.tips.2018.02.006

Trevor, A. (2015). Emotional Well-Being in Anorexia Nervosa: Negative Affect, Sleeping Problems, Use of Mood-enhancing Drugs and Exercise Frequency. *Archives of Depression and Anxiety*, 001–005. https://doi.org/10.17352/2455-5460.000001

Triolo, D., Craparo, E. F., Porsio, B., Fiorica, C., Giammona, G., & Cavallaro, G. (2017). Polymeric drug delivery micelle-like nanocarriers for pulmonary administration of beclomethasone dipropionate. *Colloids and Surfaces B: Biointerfaces*, *151*, 206–214. https://doi.org/10.1016/j.colsurfb.2016.11.025

TS. Srivatsan, T.S. Sudarshan, K. (2018). *Manufacturing Techniques for Materials: Engineering and Engineered*.

Tungaraza, T. E., & Farooq, S. (2015). Clozapine prescribing in the UK: Views and experience of

consultant psychiatrists. *Therapeutic Advances in Psychopharmacology*, *5*(2), 88–96. https://doi.org/10.1177/2045125314566808

Turabelidze, A., Guo, S., Chung, A. Y., Chen, L., Dai, Y., Marucha, P. T., & DiPietro, L. A. (2014). Intrinsic Differences between Oral and Skin Keratinocytes. *PLoS ONE*, *9*(9), e101480. https://doi.org/10.1371/journal.pone.0101480

U.S. Department of Health and Human Services Food and Drug Administration (CDER). (2013). Guidance for Industry: Size, Shape and Other Physical Attributes of Generic Tablets and Capsules. *Pharmaceutical Quality/CMC*, *December*, 1–11. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

Van de Waterbeemd, H., Smith, D. A., Beaumont, K., & Walker, D. K. (2001). Property-Based Design: Optimization of Drug Absorption and Pharmacokinetics. *Journal of Medicinal Chemistry*, *44*(9), 1313–1333. https://doi.org/10.1021/jm000407e

van der Beek, C. M., Canfora, E. E., Kip, A. M., Gorissen, S. H. M., Olde Damink, S. W. M., van Eijk, H. M., Holst, J. J., Blaak, E. E., Dejong, C. H. C., & Lenaerts, K. (2018). The prebiotic inulin improves substrate metabolism and promotes short-chain fatty acid production in overweight to obese men. *Metabolism*, *87*, 25–35. https://doi.org/10.1016/j.metabol.2018.06.009

van Drooge, D.-J., Hinrichs, W. L. J., Dickhoff, B. H. J., Elli, M. N. A., Visser, M. R., Zijlstra, G. S., & Frijlink, H. W. (2005). Spray freeze drying to produce a stable Δ9-tetrahydrocannabinol containing inulin-based solid dispersion powder suitable for inhalation. *European Journal of Pharmaceutical Sciences*, *26*(2), 231–240. https://doi.org/10.1016/j.ejps.2005.06.007

van Drooge, D. J., Braeckmans, K., Hinrichs, W. L. J., Remaut, K., De Smedt, S. C., & Frijlink, H. W. (2006). Characterization of the Mode of Incorporation of Lipophilic Compounds in Solid Dispersions at the Nanoscale Using Fluorescence Resonance Energy Transfer (FRET). *Macromolecular Rapid Communications*, *27*(14), 1149–1155. https://doi.org/10.1002/marc.200600177

Ventriglio, A., Gentile, A., Bonfitto, I., Stella, E., Mari, M., Steardo, L., & Bellomo, A. (2016). Suicide in the Early Stage of Schizophrenia. *Frontiers in Psychiatry*, *7*. https://doi.org/10.3389/fpsyt.2016.00116

Verreck, G., Chun, I., Rosenblatt, J., Peeters, J., Van Dijck, A., Mensch, J., Noppe, M., & Brewster, M. E. (2003). Incorporation of drugs in an amorphous state into electrospun nanofibers composed of a water-insoluble, nonbiodegradable polymer. *Journal of Controlled Release*, *92*(3), 349–360. https://doi.org/10.1016/S0168-3659(03)00342-0

Vieira, M. G. A., da Silva, M. A., dos Santos, L. O., & Beppu, M. M. (2011). Natural-based plasticizers and biopolymer films: A review. *European Polymer Journal*, *47*(3), 254–263.

https://doi.org/10.1016/j.eurpolymj.2010.12.011

Visser, M. R., Baert, L., Klooster, G. van 't, Schueller, L., Geldof, M., Vanwelkenhuysen, I., de Kock, H., De Meyer, S., Frijlink, H. W., & Rosier, J. (2010). Inulin solid dispersion technology to improve the absorption of the BCS Class IV drug TMC240. *European Journal of Pharmaceutics and Biopharmaceutics*, *74*(2), 233–238. https://doi.org/10.1016/j.ejpb.2009.10.004

Volavka, J. (2012). Clozapine is gold standard, but questions remain. *The International Journal of Neuropsychopharmacology*, *15*(09), 1201–1204. https://doi.org/10.1017/S1461145712000284

W.M. GROENEWOUD. (2001). *Characterisation of Polymers by Thermal Analysis*. Elsevier. https://doi.org/10.1016/B978-0-444-50604-7.X5000-6

Waddington, J. L., Youssef, H. A., & Kinsella, A. (1998). Mortality in schizophrenia. *British Journal of Psychiatry*, *173*(04), 325–329. https://doi.org/10.1192/bjp.173.4.325

Wali, A., Zhang, Y., Sengupta, P., Higaki, Y., Takahara, A., & Badiger, M. V. (2018). Electrospinning of non-ionic cellulose ethers/polyvinyl alcohol nanofibers: Characterization and applications. *Carbohydrate Polymers*, *181*, 175–182. https://doi.org/10.1016/j.carbpol.2017.10.070

Wang, L., Gillis-Smith, S., Peng, Y., Zhang, J., Chen, X., Salzman, C. D., Ryba, N. J. P., & Zuker, C. S. (2018). The coding of valence and identity in the mammalian taste system. *Nature*, *558*(7708), 127–131. https://doi.org/10.1038/s41586-018-0165-4

Warnez, S., & Alessi-Severini, S. (2014). Clozapine: a review of clinical practice guidelines and prescribing trends. *BMC Psychiatry*, *14*(1), 102. https://doi.org/10.1186/1471-244X-14-102

Weinberger, D. R. (1987). Implications of Normal Brain Development for the Pathogenesis of Schizophrenia. *Archives of General Psychiatry*, *44*(7), 660. https://doi.org/10.1001/archpsyc.1987.01800190080012

Weiss, S. C., Skerra, A., & Schiefner, A. (2015). Structural Basis for the Interconversion of Maltodextrins by MalQ, the Amylomaltase of Escherichia coli. *Journal of Biological Chemistry*, *290*(35), 21352–21364. https://doi.org/10.1074/jbc.M115.667337

Wheeler, A. J., Feetam, C. L., & Harrison, J. (2014). Pathway to Clozapine Use: A Comparison Between a Patient Cohort from New Zealand and a Cohort from the United Kingdom. *Clinical Drug Investigation*, *34*(3), 203–211. https://doi.org/10.1007/s40261-013-0166-x

Wiener, A., Shudler, M., Levit, A., & Niv, M. Y. (2012). BitterDB: a database of bitter compounds. *Nucleic Acids Research*, *40*(D1), D413–D419. https://doi.org/10.1093/nar/gkr755

Will, J., Detsch, R., & Boccaccini, A. R. (2013). Structural and Biological Characterization of Scaffolds. In *Characterization of Biomaterials* (pp. 299–310). Elsevier. https://doi.org/10.1016/B978-0-12-415800-9.00008-5

Williams, G. R., Chatterton, N. P., Nazir, T., Yu, D.-G., Zhu, L.-M., & Branford-White, C. J. (2012).

Electrospun nanofibers in drug delivery: recent developments and perspectives. *Therapeutic Delivery*, *3*(4), 515–533. https://doi.org/10.4155/tde.12.17

Williams, H., Trevaskis, N., Charman, S., Shanker, R., Charman, W., Pouton, C., & Porter, C. (2013). Strategies to address low drug solubility in discovery and development. *Pharmacological Reviews*, *65*(1), 315–499. https://doi.org/10.1124/pr.112.005660

Wilson WH, C. A. (1995). 18-month outcome of clozapine treatment for 100 patients in a state psychiatric hospital. *Psychiatric Services*, *46*(4), 386–389. https://doi.org/10.1176/ps.46.4.386

Wlodarski, K., Sawicki, W., Haber, K., Knapik, J., Wojnarowska, Z., Paluch, M., Lepek, P., Hawelek, L., & Tajber, L. (2015). Physicochemical properties of tadalafil solid dispersions - Impact of polymer on the apparent solubility and dissolution rate of tadalafil. *European Journal of Pharmaceutics and Biopharmaceutics*, *94*(May), 106–115. https://doi.org/10.1016/j.ejpb.2015.04.031

Woertz, K., Tissen, C., Kleinebudde, P., & Breitkreutz, J. (2011a). A comparative study on two electronic tongues for pharmaceutical formulation development. *Journal of Pharmaceutical and Biomedical Analysis*, *55*(2), 272–281. https://doi.org/10.1016/j.jpba.2011.02.002

Woertz, K., Tissen, C., Kleinebudde, P., & Breitkreutz, J. (2011b). Taste sensing systems (electronic tongues) for pharmaceutical applications. *International Journal of Pharmaceutics*, *417*(1–2), 256–271. https://doi.org/10.1016/j.ijpharm.2010.11.028

Wolff, I. A., Davis, H. A., Cluskey, J. E., Gundrum, L. J., & Rist, C. E. (1951). Preparation of Films from Amylose. *Industrial & Engineering Chemistry*, *43*(4), 915–919. https://doi.org/10.1021/ie50496a039

Wood, C. D., Ajdari, A., Burkhart, C. W., Putz, K. W., & Brinson, L. C. (2016). Understanding competing mechanisms for glass transition changes in filled elastomers. *Composites Science and Technology*, *127*, 88–94. https://doi.org/10.1016/j.compscitech.2016.02.027

Wroe, A. L. (2002). Intentional and Unintentional Nonadherence: A Study of Decision Making. *Journal of Behavioral Medicine*, *25*(4), 355–372. https://doi.org/10.1023/A:1015866415552

Wu, J. X., Yang, M., Berg, F. Van Den, Pajander, J., Rades, T., & Rantanen, J. (2011). Influence of solvent evaporation rate and formulation factors on solid dispersion physical stability. *European Journal of Pharmaceutical Sciences*, *44*(5), 610–620. https://doi.org/10.1016/j.ejps.2011.10.008

Xie, Q., Han, L., Shan, G., Bao, Y., & Pan, P. (2016). Polymorphic Crystalline Structure and Crystal Morphology of Enantiomeric Poly(lactic acid) Blends Tailored by a Self-Assemblable Aryl Amide Nucleator. *ACS Sustainable Chemistry & Engineering*, *4*(5), 2680–2688. https://doi.org/10.1021/acssuschemeng.6b00191

Yajima, T., Fukushima, Y., Itai, S., & Kawashima, Y. (2002). Method of Evaluation of the Bitterness of Clarithromycin Dry Syrup. *CHEMICAL & PHARMACEUTICAL BULLETIN*, *50*(2), 147–152.

https://doi.org/10.1248/cpb.50.147

Yamsani, V., Gannu, R., Kolli, C., Rao, M., & Yamsani, M. (2007). Development and in vitro evaluation of buccoadhesive carvedilol tablets. *Acta Pharmaceutica*, *57*(2), 185–197. https://doi.org/10.2478/v10007-007-0015-7

Yan, H.-X., Zhang, S.-S., He, J.-H., & Liu, J.-P. (2016). Application of ethyl cellulose, microcrystalline cellulose and octadecanol for wax based floating solid dispersion pellets. *Carbohydrate Polymers*, *148*, 143–152. https://doi.org/10.1016/j.carbpol.2016.04.050

Yang, G., Li, J., Yu, D., He, M., Yang, J., & Williams, G. R. (2017). Acta Biomaterialia Nanosized sustained-release drug depots fabricated using modified tri-axial electrospinning. *Acta Biomaterialia*, 1–9. https://doi.org/10.1016/j.actbio.2017.01.069

Ye, P., & Byron, T. (2008). Characterization of d-mannitol by thermal analysis, FTIR, and raman spectroscopy. *American Laboratory*, *40*(14), 24–27.

Yi, H.-G., Choi, Y.-J., Kang, K. S., Hong, J. M., Pati, R. G., Park, M. N., Shim, I. K., Lee, C. M., Kim, S. C., & Cho, D.-W. (2016). A 3D-printed local drug delivery patch for pancreatic cancer growth suppression. *Journal of Controlled Release*, *238*, 231–241. https://doi.org/10.1016/j.jconrel.2016.06.015

Yilmaz, Z., Zai, C. C., Hwang, R., Mann, S., Arenovich, T., Remington, G., & Daskalakis, Z. J. (2012). Antipsychotics, dopamine D2 receptor occupancy and clinical improvement in schizophrenia: A meta-analysis. *Schizophrenia Research*, *140*(1–3), 214–220. https://doi.org/10.1016/j.schres.2012.06.027

Yolken, R. H., & Torrey, E. F. (1995). Viruses, schizophrenia, and bipolar disorder. *Clinical Microbiology Reviews*, *8*(1), 131–145. http://www.ncbi.nlm.nih.gov/pubmed/172852

Yoo, S., Krill, S. L., Wang, Z., & Telang, C. (2009). Miscibility/stability considerations in binary solid dispersion systems composed of functional excipients towards the design of multi-component amorphous systems. *Journal of Pharmaceutical Sciences*, *98*(12), 4711–4723. https://doi.org/10.1002/jps.21779

Yu, D.G., Wang, X., Li, X. Y., Chian, W., Li, Y., & Liao, Y. Z. (2013). Electrospun biphasic drug release polyvinylpyrrolidone/ethyl cellulose core/sheath nanofibers. *Acta Biomaterialia*, *9*(3), 5665–5672. https://doi.org/10.1016/j.actbio.2012.10.021

Yu, Deng Guang, Zhu, L. M., Branford-White, C. J., Yang, J. H., Wang, X., Li, Y., & Qian, W. (2011). Solid dispersions in the form of electrospun core-sheath nanofibers. *International Journal of Nanomedicine*, *6*, 3271–3280. https://doi.org/10.2147/IJN.S27468

Zammit, S., Allebeck, P., Dalman, C., Lundberg, I., Hemmingson, T., Owen, M. J., & Lewis, G. (2003). Paternal age and risk for schizophrenia. *British Journal of Psychiatry*, *183*(5), 405–408.

https://doi.org/10.1192/bjp.183.5.405

Zelkó, R., And, D. A. L., & Sebe, I. (2020). *Recent Development of Electrospinning for Drug Delivery*. https://doi.org/10.3390/books978-3-03928-141-1

Zhang, D., Chi, B., Li, B., Gao, Z., Du, Y., Guo, J., & Wei, J. (2016). Fabrication of highly conductive graphene flexible circuits by 3D printing. *Synthetic Metals*, *217*, 79–86. https://doi.org/10.1016/j.synthmet.2016.03.014

Zhang, H., Zhang, J., & Streisand, J. B. (2002). Oral Mucosal Drug Delivery. *Clinical Pharmacokinetics*, *41*(9), 661–680. https://doi.org/10.2165/00003088-200241090-00003

Zhong, H., Chan, G., Hu, Y., Hu, H., & Ouyang, D. (2018). A Comprehensive Map of FDA-Approved Pharmaceutical Products. *Pharmaceutics*, *10*(4), 263. https://doi.org/10.3390/pharmaceutics10040263

Zhu, Y., Wang, A., Shen, W., Patel, S., Zhang, R., Young, W. L., & Li, S. (2010). Nanofibrous Patches for Spinal Cord Regeneration. *Advanced Functional Materials*, *20*(9), 1433–1440. https://doi.org/10.1002/adfm.200901889

Zhu, Z., Fu, S., & Lucia, L. A. (2019). Tuning the Morphology of Microparticles from Spray Drying of Cellulose Nanocrystal Suspensions by Hydrophobic Lignin. *ACS Sustainable Chemistry & Engineering*, *7*(5), 5376–5384. https://doi.org/10.1021/acssuschemeng.8b06399

Zink, M., & Englisch, S. (2012). Treatment-resistant schizophrenia: Evidence-based strategies. *Mens Sana Monographs*, *10*(1), 20. https://doi.org/10.4103/0973-1229.91588

Zornberg, G. L. (2000). Hypoxic-Ischemia-Related Fetal/Neonatal Complications and Risk of Schizophrenia and Other Nonaffective Psychoses: A 19-Year Longitudinal Study. *American Journal of Psychiatry*, *157*(2), 196–202. https://doi.org/10.1176/appi.ajp.157.2.196

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Abbreviation List

The abbreviation are listed in order of appearance along the text

Molecular Weight (Mw)

Area Under Curve (AUC)

Biopharmaceutical Classification Systems (BCS)

Polyvinylpyrrolidone (PVP)

Polyvinylalcohol (PVA)

α-β,Poly (N-2-Hydroxyethyl)-D, L-Aspartammide (PHEA)

Soluplus (SLP)

Nano Newton (Nn)

Atomic Force Microscope (AFM) In Quantitative Mode (AFM-Qi)

Apparent Permeability (P_{app})

Permeated Drug (dq)

Time Interval (dt)

Drug Flux (J)

Membrane Surface Area (A)

Concentration of The Drug in The Donor Compartment (C_0)

Food and Drug Administration Agency (FDA)

Multicomponent Solid Dispersion (multi component solid dispersion)

Cellulose Acetate Phthalate (CAP)

Poly (Methyl Methacrylate-Co-Methacrylic) Acid (PMMA)

Poly-Lactic Acid Filament (PLA)

Three-Dimensional (3D)

Hydroxypropyl-Methylcellulose (HPMC)

Computer-Aided Design (CAD)

Stereolithography (STL)

Fused Deposition Modelling (FDM)

Melting Point (T_m)

Glass Transition (T_g)

World Health Organization (WHO)

Neurotransmitters (NT)

Amino-Gamma-Butyric Acid (GABA)

N-Methyl-D-Aspartate (NMDA)

G-Protein-Coupled Receptors (T2R)

Adenosine Triphosphate (ATP)

Brief-Access Taste Aversion (BATA)

Differential Scanning Calorimetry (DSC)

Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Thermogravimetric Analysis (TGA)

Irinotecan (Irn)

Olanzapine (Olz)

Dexamethasone (Dsm)

Triamcinolone (Trm)

Department of Biological, Chemical, And Pharmaceutical Sciences and Technologies (STEBICEF)

Enthalpy of Fusion (Δ_{cp})

Maltodextrin (MDX)

Dulbecco Buffer Phosphate (DPBS)

Buffer Phosphate (PBS)

Chloride Acid (HCl)

Potassium Bromide (KBr)

Dimethyl Sulfoxide (DMSO)

Polymers Dispersion (PD)

Inlet Temperature (T_{inl})

Outlet Temperature (T_{out})

Average Diameter (*d)*

Polydispersity Index (PDI)

A One-Way Analysis of Variance (ANOVA)

Physical Mixture (PM)

Ultraviolet (UV)

Volume (V)

Surface Area (Sa)

Length (l)

Width (w)

Height (h)

Permeability Coefficient Across the Lipid Component (P_{lip})

Permeation Study in Cellulose Acetate Membrane ($P_{app\text{-}Cell}$)

Patient Information Leaflet (PIL)

Standard Operation Procedure (SOP)