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PREPARATION OF MACROMOLECULAR MATERIALS FOR BIOMEDICAL APPLICATIONS

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To my family

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INTRODUCTION

In last years, interest in synthetic polymers to be used in biomedical applications, such as controlled drug delivery and tissue engineering, has grown considerably. Such materials have to be characterised by several requirements but, first of all, they have to be compatible with tissues and blood. If the polymer, in addition to being biocompatible, is also biodegradable, it has to degrade to non-toxic products: the material achieves a specific therapeutic task and is subsequently, over time, degraded and removed harmlessly from the body.

The development of "green processes" to synthesize the polymers for biomedical applications is of fundamental importance, because this may avoid the necessity of purification of the obtained products. In this context, it could be interesting to consider the use of CO_2 as an alternative solvent for the polymerization reactions owing to its low-cost, large availability, excellent biocompatibility and mild critical parameters that make possible its utilization in the supercritical region with thermo labile compounds.

Thesis outline

The thesis is organized into seven chapters and it can be divided into two different sections.

The first section is focused on the preparation of drug-polymer composites for biomedical applications and in particular for controlled drug delivery, through the radical polymerization in supercritical carbon dioxide ($scCO_2$). The aim is to prepare a polymeric composite using a biocompatible matrix (poly(vinylpyrrolidone), PVP) in the presence of a low aqueous soluble drug (Piroxicam), in order to enhance its dissolution rate and thus improve its oral bioavailability. Chapters 1 and 2 are general background on oral drug delivery and of CO_2 as polymerization medium,

that provides the context and the objectives of the first research part. Successively, Chapter 3 describes the synthesis and characterization of drug-polymer composites in a single reactive step using $scCO_2$ as polymerization medium. Moreover, the research has been extended to the copolymerization of vivylpyrrolidone (VP) and acrylic acid (AA) to study the possibility of preparing a pH sensitive drug loaded macromolecular matrix using the non-toxic solvent.

The second section of this thesis concerns the ring opening polymerization (ROP) of cyclic esters working in the expanded liquid monomers. The aim is to synthesize biodegradable polymers that could be used in biomedical applications, carrying out the reactions in the presence of CO_2, thus avoiding the use of organic toxic solvents and exploiting its properties (in particular, its plasticizing effect) for improving the performances of the process. After the description of the general scenario on biodegradable polymers (Chapter 4), the reaction catalysed by a conventional catalyst (stannous octoate) is described in Chapter 5. This catalyst is accepted as a food additive by the US Food and Drug Agency (FDA) and thus no purification of the polymers is needed for applications such as packaging; but if the synthesised polymer has to be used in the pharmaceutical or biomedical field, due to tin toxicity, it has to be removed from the obtained product. Therefore, the ROP catalysed by enzymes, that are recyclable and non-toxic, is reported in Chapter 6. However, enzymes are characterised by high cost and thus their practical use could be justified only for specific applications of the synthesised polyesters. Due to this drawback, the possibility of using cyclodextrins, that may have an enzyme-like behaviour, as initiators for the ROP of cyclic esters is reported in Chapter 7.

CHAPTER 1

ORAL DRUG DELIVERY TECHNOLOGY

1.1 Introduction

The oral route is considered the best way of administration of drug molecules. However, for many drugs the oral route represents substantial gastrointestinal (GI) limitation in terms of optimal absorption and bioavailability characteristics, because of the physicochemical properties of the drug substance, the milieu within the GI tract and the barrier nature of the GI tract. These limitations are even more obvious with the ever-increasing number of new biotechnology-based products, such as peptides and proteins [1]. Together with the permeability, the solubility behaviour of a drug is a key determinant of its oral bioavailability. To make possible its utilization, the bioactive compound must exhibit high enough permeability and dissolution rate, which is dependent on its water solubility (Figure 1.1).

There are many well known drugs such as griseofulvin, digoxin, phenytoin, sulphathiazole and chloramphenicol, whose oral administration has been challenged by poor water solubility, that have required the development of suitable formulations. More recently, the availability of high throughput screening of potential therapeutic agents led to a significant enhancement of the number of poorly soluble drug candidates. Indeed, about 40% of new active compounds are characterized by a small water solubility resulting in poor oral bioavailability due to insufficient dissolution throughout the gastrointestinal tract [2]. This drawback risks to prevent their practical utilization and the development of new formulation of

poorly soluble compounds for oral delivery now presents one of the most frequent and greatest challenges to formulation scientists in the pharmaceutical industry [3].

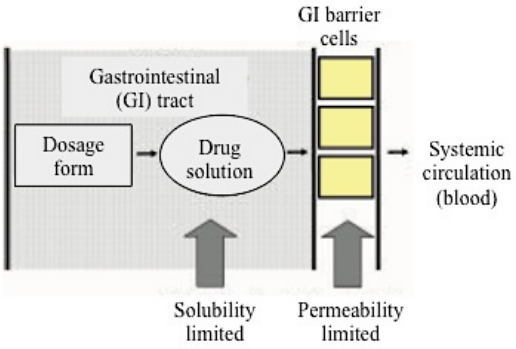

Figure 1.1. Solubility/permeability considerations for oral absorption of the dosage form [1].

1.2 Permeability-solubility drug classification

Gastrointestinal permeability and drug dissolution, which is influenced by its water solubility, are the fundamental parameters controlling rate and oral drug absorption. In order to correlate in vitro drug dissolution and in vivo bioavailability, a drug classification scheme has been proposed [4]. Drugs can be divided into high/low solubility-permeability classes: on the basis of this classification scheme, in vitro-in vivo correlations can be established and the absorption of drugs based on the fundamental dissolution and permeability properties of physiologic importance can be estimated. This classification scheme has been reported in Table 1.1.

Class	Solubility	Permeability	IVIV Correlation Expectation*
I	High	High	IVIV correlation if dissolution rate is slower that gastric emptying rate, otherwise limited or no correlation.
II	Low	High	IVIV correlation expected if *in vitro* dissolution rate is similar to *in vivo* dissolution rate, unless dose is very high (see discussion).
III	High	Low	Absorption (permeability) is rate determining and limited or no IVIV correlation with dissolution rate.
IV	Low	Low	Limited or no IVIV correlation expected

* A limited correlation means that the dissolution rate while not rate controlling may be similar to the absorption rate and the extent of correlation will depend on the relative rates.

Table 1.1. In vitro-in vivo (IVIV) correlation for immediate release products based on biopharmaceutics class [4].

- **Class I: high solubility-high permeability drugs**

This is the case where the drug dissolution is very rapid and it is well absorbed. For immediate release dosage forms that dissolve very rapidly, the absorption rate will be controlled by the gastric emptying rate and no correlation with dissolution rate is expected.

- **Class II: low solubility-high permeability drugs**

Absorption is high and dissolution is low; thus, drug dissolution in vivo is the rate controlling step in drug absorption. Since the intestinal luminal contents and the intestinal membrane change along the intestine, and much more of the intestine is exposed to the drug, the dissolution profile will determine the concentration profile along the intestine for a much greater time and absorption will occur over an extended period of time.

- **Class III: high solubility-low permeability drugs**

For this class of drugs, permeability is the rate controlling step in drug absorption. While the dissolution profile must be well defined, the simplification in dissolution specification as in Class I is applicable for immediate release dosage forms, where drug input to the intestine is gastric emptying rate controlled. Both the rate and extent of drug absorption may be highly variable for this class of drugs, but, if dissolution is fast, this variation will be due to the variable gastrointestinal transit, luminal contents and membrane permeability rather than dosage form factors.

- **Class IV: low solubility-low permeability drugs**

This class of drugs presents significant problems for effective oral delivery. The number of drugs that fall in this class will depend on the precise limits used for the permeability and solubility classification.

There are also several other factors that can influence the oral bioavailability and thus they will need further consideration: drugs with pH dependant solubility, drugs which exhibit complexation phenomena with gastrointestinal contents and drugs that are unstable in the gastrointestinal tract.

For drugs that exhibit pH dependant solubility, it is the drug solubility at the pH of the local point in the intestine that is the most relevant factor.

For drugs that are unstable or interact with gastrointestinal contents in such a manner to reduce their activity in solution, dissolution rates can have a profound effect on drug absorption. This can be the case even if the drug has high permeability (well absorbed from solution). For these drugs, where dissolution is not rapid, a multiple point dissolution profile at several pH in addition to gastric pH should be required.

1.3 Considerations about improvement of oral bioavailability

From the previous discussion, it is evident that the solubility behaviour of drugs is one of the most challenging aspects in formulation developments. In other words, we are talking about drugs that belong to the Class II of the Biopharmaceutics Classification System (BCS).

Considerations on the modified Noyes-Whitney equation [5] provide some hints to increase the dissolution rate of very poorly soluble compounds, in order to minimize their limitations to oral availability:

$$\frac{dm}{dt} = \frac{AD(C_S - C)}{h} \qquad 1.1$$

where *dm/dt* is the rate of dissolution, *A* is the surface area available for dissolution, *D* is the diffusion coefficient of the molecule, C_s is the apparent solubility of the compound in the dissolution medium, *C* is the instantaneous concentration of drug in the medium at time *t* and *h* is the thickness of the diffusion boundary layer adjacent to the surface of the dissolving compound.

From this simple mass balance equation, one can infer that to enhance the dissolution rate *dm/dt* it is possible to increase the total drug surface area available for dissolution *A* by decreasing the particle size of the solid compound and/or by optimizing its wetting characteristics; to promote perfect sink conditions for dissolution ($C \rightarrow 0$); to reduce the thickness of the boundary layer or to improve the apparent drug solubility C_s under physiologically relevant conditions. Among them, change in the fluodynamic regime to modify the value of *h* does not seem practical in vivo, while the attainment of sink conditions depends on the permeability of the drug across the gastrointestinal mucosa as well as on the composition and volume of the lumenal fluids. For these reasons, the easiest methods to enhance drug dissolution rate seem to be formulation approaches that can be classified as physical or chemical modifications [6]. Among the former, it can be mentioned particle size reduction by micronization or nanosuspension, modifications of the crystal habit, polymorphs, pseudopolymorphs (including solvates), complexation/solubilisation, use of surfactants or of cyclodextrines, drug dispersion in carriers, eutectic mixtures, solid dispersions both non-molecular and at the level of solid solutions.

In principle, the simplest route to reduce average size of a particulate drug is milling of the bioactive compound. Anyway the increase of the surface area amplifies the tendency of the polymer particle to agglomerate so that the final effect can be significantly decreased if coalescence of the particle is not prevented. The utilization of solid dispersion, particularly those obtained at molecular level (solid solutions), allows one to have the drug with the highest surface area possible and embedded in a carrier matrix that prevents recrystallization of the drug molecules.

For these reasons, solid dispersion, a concept firstly introduced by Sekiguchi and Obi in 1961 [7], has attracted considerable interest as a means of improving the dissolution rate and oral bioavailability of poorly water-soluble drugs. There are

mainly two methods to prepare solid dispersions, i.e. the melting method and the solvent method.

The former involves mixing of the drugs and carriers in their molten state and subsequent cooling and congealing at low temperatures to obtain solid dispersion slabs [8,9]. Cooling leads to supersaturation, but due to solidification the dispersed drug becomes trapped within the carrier matrix. Whether or not a molecular dispersion can be achieved depends on the degree of supersaturation and rate of cooling attained in the process. Thus, the process has an effect on the resultant dispersion and can be varied to optimize the product. An important prerequisite to the manufacture of solid dispersion by the melting method is the miscibility of the drug and the carrier in the molten form; when there are miscibility gaps in the phase diagram, this usually leads to a product that is not molecularly dispersed. Another important limitation to the hot melt method is the thermostability of the drug and the carrier; if a too high temperature is required, the drug may decompose or evaporate. Moreover, oxidative reactions can be avoided by processing in an inert atmosphere or under vacuum, while evaporation can be avoided by processing in a closed system.

Because of these limitations, the solvent method became more popular in the 1970s and 1980s. In the case of high-melting-point carriers like poly(vinylpyrrolidones) (PVP), coprecipitation of drugs and carriers is achieved by the alternative solvent method, which involves the solubilisation of drugs together with carriers in a suitable solvent, followed by the evaporation of the solvent under a reduced pressure to obtain coprecipitates [10,11]. An important prerequisite for the manufacture of a solid dispersion using the solvent method is that both the drug and the carrier are sufficiently soluble in the solvent; the solvent can be removed by any one of a number of methods. It must be remembered that, when an organic solvent has to be removed, small variations in the conditions used can lead to quite large changes in product performance; moreover, a very important point is to remove all the solvent, since most of the organic solvents used have toxicity issues.

Both these approaches are characterized by manufacturing difficulties and stability problems that complicate their applicative utilization. These could be the thermal

degradation of the bioactive compound, the lack of miscibility at the melt state or the difficulty of decreasing residual concentration of solvents, often toxic, to acceptable levels due to the mass transfer resistances induced by the gradual increase in the local viscosity of the coprecipitated coagulum.

In all these strategies, the preparation of the controlled release dosage form must be carried out in a two step process: first the polymer must be synthesized and then the drug must be dispersed in the matrix by hot melt or solvent method.

1.4 Stimuli-sensitive polymers

While studies on biomaterials based on "classical" polymers are still being continued, over the past 40 years the research interest has shifted towards stimuli-sensitive polymers.

An increasing number of publications in the field of drug delivery systems (DDS) describe DDS that simultaneously demonstrate more than one useful function by combining, for example, longevity, targetability and stimuli sensitivity. Ideally, DDS could be engineered, which can simultaneously or sequentially demonstrate the following set of properties:

- stay (circulate) long in the body;
- specifically target the site of the disease;
- respond to local stimuli characteristic of the pathological site, such as intrinsically abnormal pH values or temperature, externally applied heat, magnetic field or ultrasound, by releasing an entrapped drug or changing some other properties;
- provide an enhanced intracellular delivery of drugs and genes as required;
- carry a reporter (contrast) component supplying a real time information about the DDS biodistribution and target accumulation.

Living systems consisting of many biopolymers such as proteins, polysaccharides, and nucleic acid regulate their biological functions by precisely responding to the environmental stimuli. It is well known the human body ability to respond to its environment from the molecular to the macroscopic level. The

functions of living cells are regulated by macromolecules that respond to changes in local environment and these biopolymers form the basis around which all major natural processes are controlled. Stimuli response is crucial for maintaining normal function as well as fighting disease. At the molecular level, for example, the body releases insulin to initiate glycogen formation in response to higher glucose levels in the blood; molecular interactions also take place at cellular surfaces to stimulate a number of events, including cell signalling and endocytosis. At the macroscopic level, the body responds to external stimuli with a cascade of events, such as when nerve cells transmit signals to the brain in response to a pain-causing stimulus and subsequently cause muscle contraction [12,13].

These examples have inspired scientists to fabricate “smart” materials that respond to light, pH, temperature, mechanical stress or molecular stimuli. These responses are manifested as dramatic changes in shape, surface characteristics, solubility, etc. Nature serves as the model for stimuli-responsive polymers, but it remains a challenge to create materials that interact with, or respond to, biological environments.

Synthetic polymers that exhibit similar environmental responsive behaviour have been extensively investigated for use in intelligent and biomimetic systems. Of particular interest are these intelligent polymers which have found tremendous applications in the biomedical field, including drug delivery, bioseparation, biomolecular diagnostics, biosensor and tissue engineering. Common types of the stimuli-responsive polymers are temperature-responsive, pH-responsive, photo-responsive, electro-responsive, bio-responsive and multi-responsive polymers.

The ability of a material to recognize a stimulus and respond to it is derived from stimuli-sensitive processes on the molecular and/or supramolecular level, as well as on the level of phase morphology, which are translated and amplified to the macroscopic level. The response to a stimulus often involves several processes, which take place at different hierarchical levels; they can also occur on different time scales.

The field of stimuli-sensitive polymers is presently progressing rapidly. Current research topics involve extending the repertoire of suitable stimuli, realization of

multi-functionality in one material, exploring concepts for materials, which can continuously adapt their structural properties to the requirements of their environment by implementing self-regulating processes, and investigating the influence of specimen dimensions on the shape changing behaviour, e.g. on nano- and microparticles. On the other hand applications are being realized based on stimuli-sensitive polymers for various areas including aerospace, packaging, textiles, microfluidics, sensors and actuators as well as bioengineering [14,15].

The most important systems from a biomedical point of view are those sensitive to temperature and/or pH of the surroundings. The human body exhibits variations of pH along the gastrointestinal tract, and also in some specific areas like certain tissues (and tumoral areas) and subcellular compartments.

Polymer-polymer and polymer-solvent interactions show an abrupt readjustment in small ranges of pH or temperature: this is attributed to a chain transition between extended and compacted coil states. In the case of pH sensitive polymers, the key element of the system is the presence of ionizable weak acidic or basic moieties attached to a hydrophobic backbone. Upon ionization, the coiled chains extend dramatically, responding to the electrostatic repulsions of the generated charges (anions or cations).

Another type of responsive polymers results from surface modification of a polymer matrix by attachment of responsive chains to produce responsive interfaces showing different behaviour in response to small changes in environmental parameters. Surfaces may change from hydrophobic to hydrophilic or show a variation in pore size [16].

1.4.1 pH-sensitive polymers

Of particular concern for the research activity reported in this thesis are pH sensitive macromolecular matrixes.

A macromolecule that dissociates to give polymeric ions after dissolving in water or another ionizing solvent is termed polyelectrolyte. Because of the repulsion between charges on the polymeric chains, the chains are expanded when ionized in a suitable

solvent. However, if the solvent prevents ionization of the polyelectrolyte, the chains exist in a compact, folded state. If the polyelectrolyte's chains are hydrophobic when unionized, in a poor solvent they collapse into globules and precipitate from solution. The interplay between hydrophobic surface energy and electrostatic repulsion between charges dictates the behaviour of the polyelectrolytes. Since the degree of ionization of weak polyelectrolytes is controlled by the pH value and ionic composition of an aqueous medium, smart polymers dramatically change conformation in response to minute changes in the pH of aqueous environment [19]. By generating the charge along the polymer backbone, the electrostatic repulsion results in an increase in the hydrodynamic volume of the polymer. This transition between tightly coiled and expanded state is influenced by any condition that modify electrostatic repulsion, such as pH, ionic strength, and type of counterions. The transition from collapsed state to expanded state has been explained by changes in the osmotic pressure exerted by mobile counterions neutralizing the network charge. The pH range that a reversible phase transition occurs can be generally modulated by two strategies:

- selecting the ionizable moiety with a pK_a matching the desired pH range; therefore, the proper selection between polyacid or polybase should be considered for the desired application.
- incorporating hydrophobic moieties into the polymer backbone and controlling their nature, amount and distribution. When ionizable groups become neutral-non ionized and electrostatic repulsion forces disappear within the polymer network, hydrophobic interactions dominate. The introduction of a more hydrophobic moiety can offer a more compact conformation in the uncharged state and a more accused phase transition. The hydrophobicity of these polymers can be controlled by the copolymerization of hydrophilic ionisable monomers with more hydrophobic monomers with or without pH-sensitive moieties [20].

pH-sensitive polymers are materials that vary their dimensions with the changes in the pH of the surrounding media; these materials will swell or collapse depending on the pH of their environment. This behaviour is attributed to the

presence of certain functional groups in the polymer chains. They are normally produced by adding pendant acidic or basic functional groups to the polymer backbone; these either accept or release protons in response to appropriate pH and ionic strength changes in aqueous media.

In general, there are plenty of ionizable groups in the macromolecular networks of pH-responsive polymers. As the variation of environmental pH, these groups are ionized to cause differences of internal and external ionic strength and breakage of hydrogen bonds between the molecular segments. As a result, the decrease of crosslink densities and the increase of electrostatic repulsions lead to macroscopical swelling of the polymers. There are two kinds of pH-sensitive materials: one which have acidic group (-COOH, $-SO_3H$) and swell in basic pH (Figure 1.2), for example poly(acrylic acid), and another one which have basic groups ($-NH_2$) and swell in acidic pH, as chitosan.

The mechanism of response is the same for both, just the stimuli vary. Polyacidic polymers will be unswollen at low pH, since the acidic groups will be protonated and unionized; increasing the pH, a negatively charged polymer will swell. The opposite behaviour is found in polybasic polymers, since the ionization of the basic groups will increase decreasing the pH.

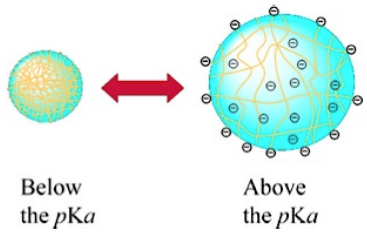

Figure 1.2. Schematic illustration of the pH-responsive behaviour (below and above the pK_a) of a particulate system constituted by a polymer with carboxylic acid segments [17].

Most anionic pH-sensitive polymers are based on polyacrylic acid (PAA) or its derivatives, such as poly(methacrylic acid) (Figure 1.3).

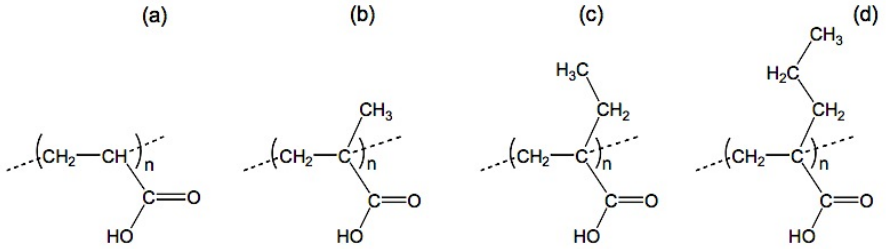

Figure 1.3. Chemical structure of pH-sensitive polyacids: (a) poly(acrylic acid), (b) poly(methacrylic acid), (c) poly(2-ethylacrylic acid), (d) poly(2-propylacrylic acid) [20].

A few examples of cationic polyelectrolytes are poly(N,N-diakyl aminoethyl methacrylates), poly(lysine), poly(ethylenimine) and chitosan [20].

pH-sensitive polymers have been used in several biomedical applications, the most important being their use as drug and gene delivery systems and glucose sensors. For the drug carriers made from pH-responsive polymers, the drug release is accelerated at a given pH in a controlled manner through the disassembly or dissolution of nanoparticles induced by the pH change. The range of physiological pH is from 1.2 to 7.4, and different body part may have a special pH surroundings: for example, the stomach possesses a pH of 1.2 while the pH of the intestine is 7.4. Moreover, it is known that the extracellular pH of tumours (6.8–6.9) is more acidic than both tumour intracellular pH (7.2) and normal extracellular tissues (7.4). In this sense, pH-sensitive polymers seem to have high potential application value as drug delivery agents. Besides, it is found that incorporation of a small amount of pH-sensitive ionisable groups, such as carboxyl and amino groups, into thermo-responsive polymers can offer a combination of different stimuli-responsive properties [17-21].

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CHAPTER 2

POLYMER SYNTHESIS USING SUPERCRITICAL CARBON DIOXIDE

2.1 Introduction

In last years, green chemistry has become an area of significant research interest. It is best defined as the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and applications of chemical products [1].

Currently, there is a strong interest on the development of more sustainable processes, particularly in the polymer industry. A key element of any chemical reaction is the solvent used and, therefore, the search for new environmentally benign solvents is of significant academic and industrial interest. Indeed, actually in the industrial processes there is the use of excessive amounts of organic solvents: about 20 million tonnes of volatile organic compounds (VOCs) are emitted into the atmosphere each year as a result of these industrial activities [2]. In particular, the polymer industry uses organic solvents either as reaction media in the polymerization process or as media for polymer processing. In each of these steps, most of the effort of the process is put into the solvent recovery, as schematically indicated in Figure 2.1 for the polymerization step.

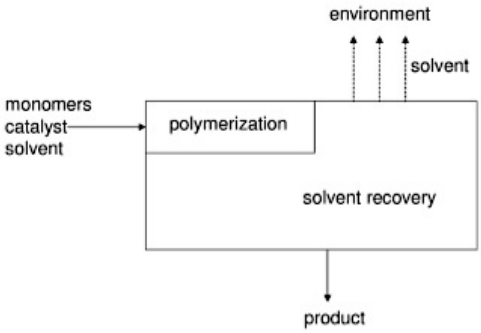

Figure 2.1. Visualisation of the relative effort required for polymerization and solvent recovery in conventional catalytic polymerization processes based on organic solvents [2].

Thus, it is highly desirable from an environmental, safety and economical point of view to develop alternative routes in order to reduce the use of organic solvents in polymer processes. There could be two solutions to the large use of organic solvents in these activities: the first is the development of solvent-free processes; the second consists in the replacement of solvents by environmentally benign products.

Solvent-free polymerizations generally suffer from processing difficulties as a result of increased viscosities and mass transfer limitations, for instance in melted phase polymerization.

Solvent replacement, although it prevents the loss of dangerous organic solvents, still necessitates a solvent removal step, that requires a high energy consumption. The simplest solution consists in the use of a volatile solvent, which makes the solvent removal step easier. An intermediate solution is using one of the reactants in excess: it partly acts as a solvent or plasticizer, but the excess of reactant still needs to be removed. Water may be used instead of organic solvents for conducting chemical reactions, but there is always the problem connected with the removal of hazardous aqueous waste that requires treatment.

Solvents that have interesting potential as environmentally benign alternatives to organic solvents include ionic liquids and supercritical fluids.

Ionic liquids are room-temperature molten organic salts, characterised by a very low vapour pressure. Because they are non-volatile, they can be easily used in existing

equipments, with the reduction of the VOCs emission, even if their separation from the process streams is difficult. Moreover, the cost of a room-temperature molten salt is substantial.

The critical properties of common substances used as supercritical fluids are shown in Table 2.1. Of these, water and carbon dioxide are the most frequently used in a wide range of applications.

Substance	T_c/°C	P_c/bar	ρ_c/g cm^{-3}
CH_4	−82.5	46.4	0.16
C_2H_4	10.0	51.2	0.22
C_2F_6	19.9	30.6	0.62
CHF_3	26.2	48.5	0.62
$CClF_3$	28.9	38.6	0.58
CO_2	**31.1**	**73.8**	**0.47**
C_2H_6	32.4	48.8	0.20
SF_6	45.6	37.2	0.73
Propylene	91.9	46.1	0.24
Propane	97.2	42.5	0.22
NH_3	132.5	112.8	0.24
Pentane	187.1	33.7	0.23
^{i}PrOH	235.4	47.6	0.27
MeOH	240.6	79.9	0.27
EtOH	243.5	63.8	0.28
^{i}BuOH	275.1	43.0	0.27
Benzene	289.0	48.9	0.30
Pyridine	347.1	56.3	0.31
H_2O	374.2	220.5	0.32

Table 2.1. Critical parameters for several substances. T_c=critical temperature, P_c=critical pressure, ρ_c=critical density (i.e. the density at the critical pressure and critical temperature) [3].

Supercritical water has been shown to be a very effective reaction medium for oxidation reactions. Supercritical water oxidation was first developed in the 1970s for the destruction of organic waste and has been extensively studied. However, there is a problem associated with its use, connected with the extreme operative conditions required as a consequence of the very high critical point of water. These severe conditions induce to problems connected with corrosion of materials, that cause an increase of the investment costs [2,4].

The interest in CO_2-based processes has strongly increased over the past decades. Supercritical carbon dioxide ($scCO_2$) is considered an interesting alternative to most

traditional organic solvents, because of its peculiar physical and chemical properties. Several of the advantages in using CO_2 include that it is inexpensive, non-flammable, environmentally benign and readily separated from products. Moreover, it is produced as by-product from the production of other chemicals, such as ammonia, ethanol, hydrogen and natural gases. As well as being environmentally friendly, CO_2-based processes can be more energy efficient that processes based on water and common organic solvents, owing to its low heat of vaporization [5].

2.2 Supercritical fluids

Historically it was Baron Charles Cagniard de la Tour who in 1822 first defined a supercritical fluid (SCF) as a state of matter where the liquid and vapour phases are indistinguishable [6]. The first technological application of SCF, however, was developed in 1970, when Kurt Zosel of Max Plank Institut Fur Kohlenforschung used supercritical carbon dioxide to extract caffeine from coffee beans and raisin from hops [7]. Nowadays, SCFs find application in many fields, the corresponding technologies are in different industrial sectors (Figure 2.2).

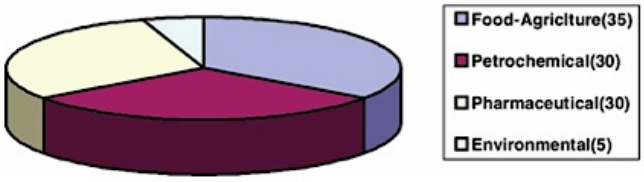

Figure 2.2. Industrial application of supercritical fluids [8].

A supercritical fluid is defined as the state of a compound or element above its critical temperature (T_c) and critical pressure (P_c). The phase behaviour of substances at various temperatures and pressures can be represented most clearly on a phase diagram, as seen in Figure 2.3.

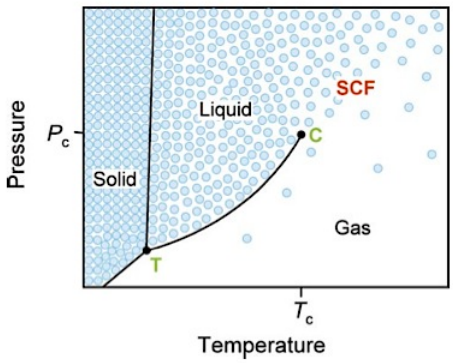

Figure 2.3. Schematic pressure-temperature phase diagram for a pure component showing the supercritical fluid (SCF) region. The triple point (T) and critical point (C) are marked; the blue circles represent the variation in density of the substance in the different regions of the phase diagram [3].

As both temperature and pressure increase, the gas-liquid coexistence curve moves upward. As the temperature increases, the liquid becomes less dense, due to thermal expansion, and as the pressure increases, the gas becomes more dense; once the densities become equal, the phase distinction between liquid and gas disappears and the critical point has been reached. The substance is now said to be supercritical and this blurring of phases can be observed visually in a view cell (Figure 2.4).

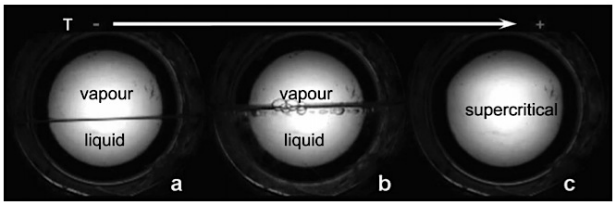

Figure 2.4. View cell showing the phase behaviour as a substance becomes supercritical. (a) Biphasic system is observed at lower temperatures with a distinct meniscus between liquid and gas phases. (b) Increasing the temperature, the meniscus between the two phases starts to become blurred. (c) At a higher temperature, a homogeneous supercritical fluid is observed. The process is reversed on decreasing the temperature [9].

Close to the critical density, supercritical fluids display properties that are to some

extent intermediate between those of a liquid and of a gas. For example, a supercritical fluid may be relatively dense and dissolve certain solids, while being miscible with permanent gases and exhibiting high diffusivity and low viscosity. In addition, supercritical fluids are highly compressible and the density (and therefore solvent properties) can be tuned over a wide range by varying the pressure.

2.2.1 Supercritical carbon dioxide

Carbon dioxide is an attractive solvent alternative for a variety of chemical and industrial processes. It is the most extensively studied supercritical fluid for free radical polymerization reactions: it is a solvent for monomers and a non-solvent for polymers and this leads to an easy separation. There are several important issues, such as drying, solubility and polymer plasticization, that are involved when scCO_2 is used as a polymerization solvent. Because CO_2 is an ambient gas, the polymers can be isolated from the reaction media by simple depressurization, resulting in a dry polymer product. This feature eliminates energy-intensive drying procedures required in polymer manufacturing to remove solvent and represents potential cost and energy savings for CO_2-based systems. This choice is also due to the mild critical parameters of CO_2, that allows to carry out the polymerization process at reaction temperature compatible with the utilization of conventional chemical initiators in a reaction medium characterized by high density, high diffusivity and low viscosity, thus decreasing the mass transfer limitations frequently found in liquid systems. These properties are coupled with CO_2 specific technical-economical features such as a low-cost, large availability and excellent biocompatibility; moreover, aforementioned mild critical parameters make possible its utilization in the supercritical region with thermo labile compounds. Furthermore, the produced polymeric materials can have a controlled morphology and are obtained as dry powders, with no organic solvents residues, avoiding further purification and drying steps. This is a major advantage when product purity is a key parameter, such as in pharmaceutical and biomedical applications [10-16].

At room temperature and above its vapour pressure, CO_2 exists as a liquid

with a density comparable to organic solvents, but with excellent wetting properties and a very low viscosity. Above its critical temperature and pressure (Table 2.1), CO_2 is in the supercritical state and has gas-like viscosities and liquid-like densities. Small changes in temperature or pressure cause large changes in density (Figure 2.5), viscosity and dielectric properties of supercritical CO_2, making it a versatile solvent.

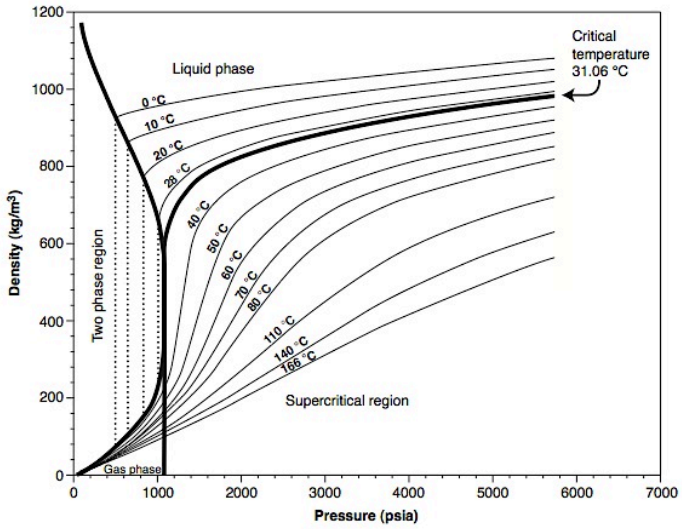

Figure 2.5. Density versus pressure isotherms for liquid and supercritical carbon dioxide [4].

Thus, in general, supercritical carbon dioxide can be regarded as an alternative solvent for polymer processes. Besides the environmental benefits, it has also desirable physical and chemical properties from a process point of view: in fact, it is characterised by chemical inertness, readily accessible critical point, excellent wetting characteristics, low viscosity and highly tunable solvent behaviour, facilitating easy separation. The use of such a "volatile" solvent makes the solvent removal step relatively easy; this allows for a closed-loop polymer process, in which the components like catalyst and monomers can be recycled. Figure 2.6 schematically illustrates the efficiency of a CO_2-based polymerization as compared to a conventional process shown in Figure 2.1.

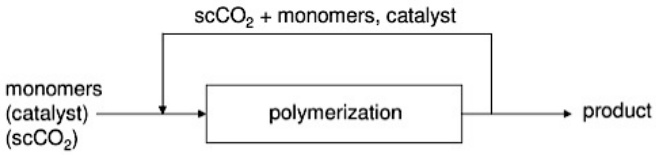

Figure 2.6. Schematic view of a catalytic polymerization based on CO_2 technology, in which the catalyst and monomers can be recycled in a closed-loop process [2].

2.3 Interactions of CO_2 with polymers and monomers

For application of $scCO_2$ as a medium in polymer processing, it is important to consider its interactions with polymers and monomers. In general, CO_2 is a good solvent for most nonpolar and some polar molecules of low molar mass and a poor solvent for most high molar mass polymers under mild conditions (<100°C, <350 bar), although there are some exceptions such as amorphous fluoropolymers and silicones [14]. The pressures and temperatures needed to dissolve a polymer in CO_2 depend on the intermolecular forces between solvent-solvent, solvent-polymer segment and polymer segment-segment pairs in solution, and on the free volume difference between the polymer and CO_2. Carbon dioxide is characterised by a low dielectric constant and its polarizability is close to that of gases such as methane, perfluoromethane and fluoroform. Due to its structural symmetry, CO_2 does not have a dipole moment, but it is characterised by a substantial quadrupole moment, that operates over a much shorter distance scale than dipolar interactions. It is for its quadrupole moment that CO_2 is a good solvent for nonpolar molecules with low molecular weight and slightly polar molecules, such as methanol and acetone [17-19].

Among of the various heterogeneous polymerization methods (i.e. precipitation, dispersion, emulsion and suspension polymerization), dispersion polymerization has been widely studied because most of low molecular weight monomers are soluble, but the formed polymers are rarely soluble in $scCO_2$, except a few amorphous fluorinated polymers and siloxane-based polymers. Thanks to their solubility in $scCO_2$, this kind of polymers have been used for dispersion polymerization as

polymeric surfactants, in order to prevent flocculation and aggregation of formed particles [3,13,14].

Although the solubility of polymers in CO_2 is typically very low, the solubility of carbon dioxide in many polymers is substantial. The sorption of carbon dioxide by the polymers and the resulting swelling of the polymer influence the mechanical and physical properties of the polymer [20]. Thus, working under supercritical conditions is of great interest, due to the possibilities of modifying the morphological and functional properties of polymers by swelling in $scCO_2$.

The sorption of CO_2 by the polymers leads to a dramatic decrease in the glass transition temperature (i.e. plasticization). It is reported in the literature that polymers are highly plasticized by CO_2 [14]. The plasticization allows important effects, such as the removal of residual monomer from the polymer, incorporation of additives and formation of foams. Thus, this phenomenon facilitates mass transfer properties of solutes into and out of the polymer phase, which leads to many applications: increased monomer diffusion for polymer synthesis, enhanced diffusion of small components in polymers for impregnation and extraction purposes, polymer fractionation and polymer extrusion.

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CHAPTER 3

A ONE-POT METHOD FOR THE PREPARATION OF DRUG-POLYMER COMPOSITES

3.1 Introduction

In this part of the research activity we have studied the preparation in a single step of drug-polymer composites using dispersion polymerization in supercritical carbon dioxide.

First we have studied a one-pot method to prepare a polymeric composite able to enhance the rate of dissolution of Piroxicam, selected as a model of low aqueous soluble drugs. The dissolution rate of a "class-II" drug (characterized by low solubility and high permeability) can be enhanced by dispersing it in a water-soluble biocompatible carrier, such as polyvinylpyrrolidone (PVP), which inhibits the crystallization of the drug. It was studied the possibility of taking advantage of the particular properties of $scCO_2$, such as biocompatibility, large availability, low-cost, mild critical parameters and its intense plasticizing effect towards amorphous polymers, to prepare the drug-polymer composites in a single step. The experimental work was opportunely planned in order to understand the effect of various variables on the release kinetics of the drug.

On the basis of the results obtained from this initial study, then we have attempted to develop a pH sensitive poly(vinylpyrrolidone)-co-acrylic acid (poly(VP-co-AA)) copolymer in a single step process, in order to have a release devise sensitive toward a stimulus that could cause a variation in the release rate of the drug. We have studied the copolymerization process in the presence of Piroxicam or Caffeine, two pharmaceutical compounds selected as representatives of "Class II" (low solubility,

high permeability) and "Class I" (high solubility, high permeability) drugs respectively, in order to assess the generality of the process. Furthermore, we have performed the dissolution experiments from tablets, in order to understand how the specific formulation could influence the release kinetics, evaluating differences between drug release from powders, obtained directly after venting the reactor, and from tablets, opportunely prepared starting from these powders. The idea was to create a better release system, able to control the drug dissolution as a function of the specific formulation.

Moreover, the morphology of the synthesised composites was investigated by SEM, XRD, Raman, FTIR and ^{1}H-NMR analysis.

3.2 Materials

1-vinyl-2-pyrrolidone 99+% (VP) and acrylic acid 99% (AA) monomers were purchased from Aldrich and used as received; CO_2 (99.998% pure) was supplied by Rivoira. For the homopolymerization reactions we used 2,2'-azobis(isobutyronitrile) (AIBN, Fluka) as initiator and the reactive macromonomer polydimethylsiloxane Sb1784 (kindly donated by Degussa), with double methacrylic chain ends, as surfactant. For the copolymerization reactions, we used as initiator diethylperoxidicarbonate (DEPDC), that was synthesized with a procedure that was already described in the literature [1], using water as solvent and extracting the peroxydicarbonate into Freon 113 (HPLC grade). The concentration of active peroxide in the solution was determined by the iodine titration technique, ASTM method E 298-91; all chemicals used in the synthesis of the DEPDC solution were obtained from Aldrich and used as received. The manipulations of the initiator solution were performed at 0°C and the final product was stored under dark at -22°C. The reactive macromonomer polydimethylsiloxane monomethacryloxypropyl terminated AB146684 was used as surfactant (kindly donated by ABCR GmbH & Co.) and used as received. Piroxicam and Caffeine anhydrous were purchased from Aldrich (assay higher than 98%). Cyclohexane was Riedel de Han HPLC grade.

NaCl, Na_2HPO_4, KH_2PO_4 and H_3PO_4 were Aldrich ACS grade and used as received; bidistilled water was used to prepare buffer solutions.

3.3 Phase behaviour investigation apparatus

The visual investigation of the phase behaviour of the CO_2/VP/Piroxicam and CO_2/VP/AA/Piroxicam or CO_2/VP/AA/Caffeine mixtures was performed in a stainless steel fixed volume view cell with a volume of 54 mL from Thar Technologies (designed to operate up to 50.0 MPa and 423 K), stirred by a magnetic stir bar. The temperature control of the system was ensured by a heating tape, controlled by a PID controller with an accuracy of ± 0.3 K. The pressure vessel was equipped with a pressure transducer Barksdale UPA3 (estimated accuracy by calibration with an high precision manometer ± 0.05 MPa) and a termocouple (estimated accuracy ± 0.3 K).

In the case of homopolymerization, Piroxicam was loaded in the view cell dissolved in the liquid monomer. After purging of the air with a controlled flow rate of gaseous CO_2, the vessel was sealed and loaded with liquid CO_2 at room temperature by using an ISCO syringe pump, up to reach the desired value of density. The total amount of solvent introduced was measured weighing the vessel with an electronic scale. Then the cell was heated to the reaction temperature (65°C) with steps of 5°C, acquiring the pressure and the temperature values inside the cell and, through the visual observation, determining the number of phases.

In the case of copolymerization, mixtures were prepared loading the proper amount of all solid and liquid reagents, at the different concentration conditions, and then purging from air with a controlled flow rate of gaseous CO_2. After venting, the cell was heated to the reaction temperature (50°C) and, after reaching thermal equilibrium, CO_2 was loaded using an air-driven Maximator pump. The amount of carbon dioxide loaded was controlled measuring the pressure inside the view cell. Pressurization was performed step wisely with pressure increments all of the same amplitude of 3.0 MPa. After each addition of cold CO_2, the fluid system was thermally equilibrated at 50°C: the attainment of equilibrium was waited for the

acquisition of pressure and temperature values inside the cell and for to count, by visual observation, the number of phases inside the closed windowed vessel.

3.4 Polymerization apparatus and reaction procedure

Polymerization experiments were carried out in an AISI 316 batch reactor (Figure 3.1), having a volume of about 27 mL, stirred by a magnetic bar and inserted in an automated temperature control system (Figure 3.2). The reactor was equipped with high-pressure valve (Parker), pressure transducer (Barksdale UPA3, estimated accuracy by calibration with a high-precision manometer of ± 0.05 MPa), and Pt 100 thermal sensor (estimated accuracy of ± 0.3 K). The proper amounts of monomer, surfactant, drug and, for copolymerization reactions, comonomer were charged into the reactor; in the case of homopolymerization reactions, the initiator was inserted into the reactor at the beginning together with the other condensed reagents, while, in the case of copolymerization reactions, it was added with CO_2 only after the system was homogeneous. The vessel was then deoxygenated by a controlled flow rate of gaseous CO_2 maintained for at least 10 min.

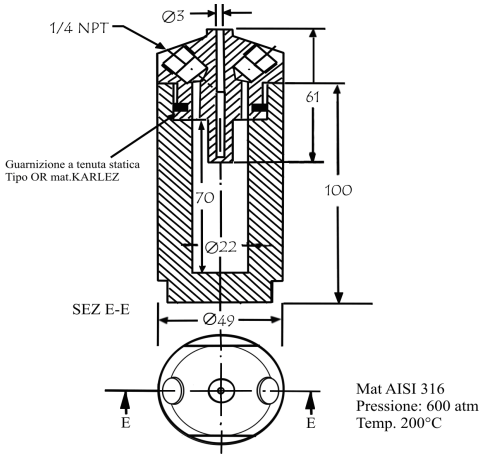

Figure 3.1. Schematic representation of the stainless steel batch reactor.

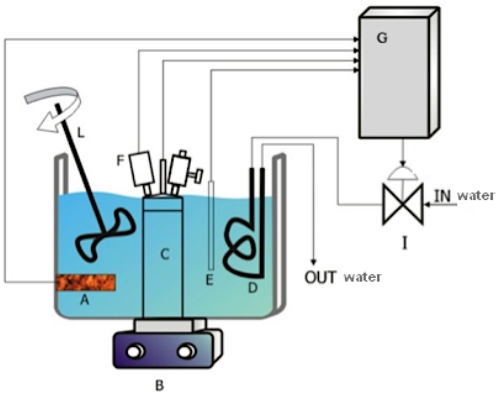

Figure 3.2. Schematic representation of the polymerization apparatus: heating electric resistance (A), magnetic stirrer (B), reactor (C), cooling coil (D), Pt100 temperature sensor (E), pressure transducer (F), control system (G), electro-valve (I), mechanical stirrer of the thermostatic bath (L).

For homopolymerization reactions, after sealing the reactor, liquid CO_2 was added at room temperature by using an ISCO syringe pump; the total amount of solvent introduced was measured weighing the vessel with an electronic scale up to reach the desired value of density of the polymerization mixture (Figure 3.3).
The reactor was then inserted in the control system and heated at the reaction temperature (65°C), while the acquisition of the temperature and pressure of the polymerization mixture was started. The completion of the polymerization process was determined from the observation of pressure and temperature profiles recorded by the control system, its end considered corresponding to the stabilization of the pressure. At the end of the polymerization the reactor was cooled down to room temperature and depressurized by bubbling the gas in cyclohexane to trap solid polymer entrained by the fluid stream. The reactor was opened and the collected polymer was washed with cyclohexane to remove the unreacted monomer and the solute superficially present; the polymer product was recovered through centrifugation and dried in a vacuum oven at 50°C for 5 h and stored in a dry atmosphere for further characterization.

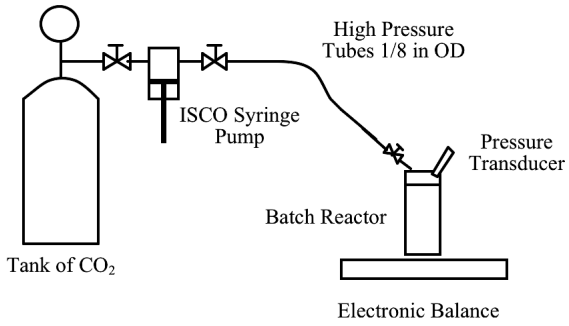

Figure 3.3. Experimental set-up to charge the liquid CO_2 at room temperature.

For copolymerization reactions, after sealing, the reaction vessel was put into the temperature-controlled bath and heated at the reaction temperature (50°C). When the set-point temperature was reached, liquid CO_2 was pumped inside the reactor by an air-driven Maximator pump up to reach a pressure of about 20.0 MPa, in order to have a single phase system before starting the polymerization. Then the initiator solution was loaded in a sample loop of suitable volume and delivered inside the reactor by a stream of liquid CO_2 that was fed to the reactor until the pressure increased up to about 32.0 MPa. The apparatus used to pump CO_2 and DEPDC into the reactor is shown in Figure 3.4. The loading time of initiator was about 2-4 min, after which the reaction time was set to zero. The completion of the polymerization process was determined from the observation of the pressure and temperature profiles recorded by the control system, its end considered corresponding to the stabilization of the pressure. At the end of the polymerization, the reactor was cooled down to room temperature and slowly depressurized until venting of CO_2 was completed. Finally, the amount of formed copolymer was determined gravimetrically.

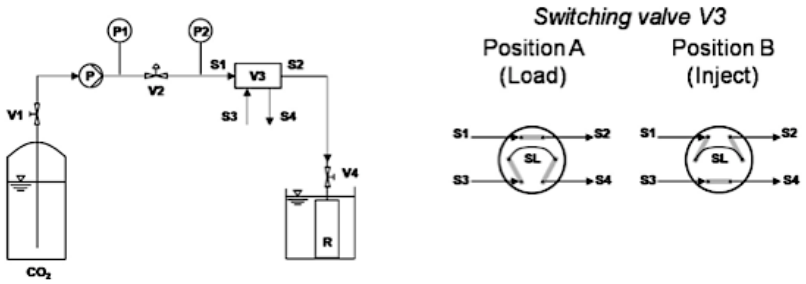

Figure 3.4. (left) Sketch of the experimental apparatus used to feed CO_2 and DEPDC into the batch reactor and (right) schematic representation of the switching valve V3 [2]. V1, V4: valves; V2: reducing pressure regulator; V3: switching valve; P: air-driven pump; P1, P2: manometers; R: reactor; S1, S2: CO_2 stream line; S3, S4: stream line of DEPDC solution; SL: sampling loop. With V4 closed and V3 in position A, the CO_2 feed line is pressurized and the DEPDC solution is fed into the SL; then V3 is switched to B, V4 is opened and the CO_2 pumped into R through SL; when the desired pressure is reached, V4 is closed.

3.5 Polymer characterization

Polymer yields were determined gravimetrically. Particle morphologies were analysed with a Philips scanning electron microscope (SEM). Samples were sputter-coated with gold to a thickness of 200 Å. The particle size distributions were evaluated by measuring the diameters (D_i) of at least 100 individual particles through a software for image analysis of micrographs; then the number average particle size (D_n) and particle size distribution (PSD=D_w/D_n) were determined according to equations reported elsewhere [3].

X-ray powder diffractograms were obtained by Philips PW 1130 diffractometer (θ/2θ geometry) using Cu Kα radiation produced by an X-ray sealed tube powered by 40 kV × 30 mA.

Raman studies were performed using a Renishaw Labram micro-Raman spectrometer equipped with a 532 nm He/Ne laser source, 1800 L/nm grating and a Leica microscope system. Spectra collection was performed at room temperature under the following conditions: 50× microscope objective, 50 μm pin-hole size, 300 μm slit width and 5 s exposure time. Each spectrum represents the average of two

measurements. Sample profiling (2D mapping) was performed under the same conditions taken randomly at least 5 points in the x and y plane to verify that collected spectra can be considered representative of the sample as a whole. The samples were spread flat on a glass microscope slide.

Infrared spectra were recorded on a Perkin-Elmer Spectrum 2000 Explorer FTIR with an averaging of 30 scans at a resolution of 1 cm−1 using a near-IR fast recovery deuterated tryglicine sulphate detector. Analyses were performed on the polymer powder mixed with anhydrous KBr and then compressed to produce solid pellet.

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded with a Bruker AN 400 spectrometer, using chloroform as the solvent, at 25°C. Chemical shifts were given in parts per million with tetramethylsilane as an internal reference.

Piroxicam or Caffeine release kinetics were studied in vitro at 37 ± 0.2 °C dispersing 50 mg of polymer in 50 mL of an aqueous buffer solution at pH 6.8 or 2.5, prepared mixing suitable amounts of sodium chloride, sodium phosphate bibasic, potassium phosphate monobasic or phosphoric acid in water. The solution was stirred by a magnetic stir bar and the drug release was monitored using a PC controlled Avantes fibre optic UV–vis spectrophotometer equipped with a DH2000 light source and a reflection dip probe (optical path 1 cm) that was immersed in the aqueous phase. All connections between components and optical fibres were made with SMA 905 connectors. The signal from each spectrometer was recorded as transmitted intensity and converted in absorbance spectra by the spectrometer manufacturer's software (AVA-Soft version 5, Avantes) running on the PC, using a previously recorded blank spectrum of the buffer solution at the typical operating conditions. Piroxicam concentration was estimated, after calibration with solutions of known concentration, by integrating the absorbance in the range of λ from 354 to 359 nm, while Caffeine concentration in the range of λ from 270 to 274 nm, where are located the maximum absorbance of the drugs.

Drug loading of polymers was estimated by dissolving about 10 mg of the composite in 40 g of methanol. A sample of the solution was analysed by UV-vis spectroscopy. Also in this case a calibration with samples of known concentration

was performed. Reported values are the average of three determinations with an uncertainty estimated within ±7% and are expressed as weight concentration with respect to the amount of synthesized polymer.

In the case of copolymers, release kinetics were studied loading the polymer in the buffer solutions under the form of powder or tablet. The powder was taken from the solid recovered from the reactor after its venting, the tablets were obtained by cold compression of the powder with a pressure of about 10 tons/cm^2 for 2 minutes.

3.6 PVP/Piroxicam composites

The idea of preparing a drug-polymer solid dispersion by polymerization of VP in the presence of the pharmaceutical compound was prompted by some considerations on what it is known about the synthesis of PVP in dense carbon dioxide. The polymerization of VP in scCO_2 is a heterogeneous process since the monomer is soluble in the compressible solvent, while the polymer is not; thus the most probable technique is the dispersion polymerization. In this case the polymerization starts in a homogeneous mixture and a short time interval is postulated where pure homogeneous polymerization kinetics is operative, leading to the formation of oligomers whose solubility depends on their size. As soon as a critical chain length is reached, oligomeric free radicals undergo homogeneous nucleation to eventually form precursor particles. They grow through homocoagulation and propagation, the former being the main mechanism as they imbibe a relatively low amount of monomer as a consequence of the high curvature of their surface. This coagulation of primary particles continues until the interfacial surface is covered by the minimum amount of steric stabilizer that imparts colloidal stability to the particles. From this point on (critical point) the particle count is fixed and they growth by heterocoagulation and/or propagation. According to such a theory a crucial factor for the successful performance of the polymerization is the availability of a surfactant effective in the stabilization of the growing polymer particles preventing their coalescence and allowing the progression of the process up to high yields. Under these conditions, all the components of the polymerization

system are partitioned between the continuous and the dispersed phase. The main locus of polymerization gradually shifts from the continuous medium to the dispersed one, since radicals generated in the continuous medium are trapped by the polymer particles before they are terminated owing to their high specific area. If this event occurs, high molecular weight polymer is obtained because macroradicals growth in an environment with high local viscosity where termination is slower.

In this conceptual scenario it seems reasonable to think that, if drug molecules have a good affinity towards the monomer and the polymer matrix, they could accumulate inside the polymer particles with high concentration and they could be found dispersed at the end of the process under nanocrystalline or even molecular form. The latter possibility should be more probable if the drug is soluble in the monomer and the vinyl compound is preferentially partitioned in the polymer particles. In this case the drug molecularly dissolved in the monomer swollen polymer particles could precipitate under non-structured form, while the monomer concentration is depleted.

Sekikawa et al. [4] observed that PVP might impede the crystallization of drug molecules when they are coprecipitated with the polymer from supersaturated solution and this effect was attributed to the presence of specific interactions between the two components that suppress or retard the crystallization of the low molecular weight compound.

When chemical processes are carried out in supercritical fluids, the solubility behaviour of involved species can significantly affect the performances of the process. For the process under consideration, in principle, the success of the methodology does not require a complete miscibility of the drug with the polymerization mixture. To obtain high drug loading in the polymer, it should be in fact enough a high solubility of the pharmaceutical compound in the dispersed phase, since the good transport properties of the supercritical continuous medium allows a fast transport rate of the drug inside the particles. In this case, if part of the drug is present as a solid dispersion, it would act as a reservoir phase.

3.6.1 Phase behaviour of the VP/CO_2/Piroxicam system

The phase behaviour of the ternary system constituted by the monomer, the drug and the compressible solvent was studied by visual observation in a fixed volume view cell. The experiments were performed with a total density ≥0.93 g/mL, using a concentration of VP fixed at 20% (w/w) and changing the concentration of the drug (Table 3.1).

ρ, g/mL	VP, %(w/w)	Drug, %(w/w)[a]
0.93	20	5
0.93	20	10
0.95	20	15

[a] Based on the monomer.

Table 3.1. Composition of the ternary systems studied in the view cell.

All systems were initially characterized by the coexistence of two fluid phases: an heavy one richer in VP and yellow coloured, reasonably because the drug was mainly dissolved in it, and a light one transparent and richer in CO_2. While the temperature was increased, the volume of the monomer rich phase gradually decreased and in the temperature interval 45-50°C we observed the disappearance of the meniscus between the two phases accompanied by the precipitation of the drug under the form of a solid powder.

This behaviour was attributed to the solubilisation of VP in scCO_2 accompanied by precipitation of the drug, which must have a limited solubility in the polymerization medium also in the presence of the monomer that, increasing the polarity of the fluid phase, should act as a cosolvent. In the case of the lowest investigated concentration of Piroxicam, the powder dissolved at about 62°C when the pressure inside the view cell reached a value of about 26 MPa and the system appeared to be constituted by a single phase to the visual observation. When the concentration of the drug was augmented, the system never became homogeneous and was always constituted by a solid dispersion of part of the drug in a fluid phase constituted by CO_2 and VP.

3.6.2 Preparation of PVP/Piroxicam composite by dispersion polymerization of VP in the presence of the drug in $scCO_2$

To test the validity of the previously discussed one-pot route in the preparation of drug-polymer composites to enhance the rate of dissolution of low solubility drugs, we performed polymerizations of VP in $scCO_2$, at 0.93-0.94 g/mL density values, in the presence of different initial concentrations of Piroxicam. The results are summarized in Table 3.2 (entries 2-4) together with data obtained in a control test performed in the absence of any drug (entry 1). In all the experiments monomer converted almost quantitatively (yield equal or higher than 90%) in a free flowing powder substantially constituted by sub-micron spherical particles (Figure 3.5, a-d). The product collected from the drug free run was white; when the anti-inflammatory was present, the powder was yellow coloured and characterized by particles with broader particle size distribution.

Entry	ρ, g/mL	Drug, %(w/w)[a]	P^0, MPa	Yield, %	Drug loading, %(w/w)	Product	D_n, μm	PSD
1	0.94	0	35	90	-	Powder	0.23	1.09
2	0.93	5	34	95	4.5	Powder	0.26	1.33
3	0.93	10	33	98	9.3	Powder	0.21	1.29
4	0.94	15	38	95	12.3	Powder	0.24	1.30
5	0.90	5	24	82	4.6	Powder	0.25	1.16
6	0.88	5	21	84	4.0	Powder	0.31	1.50

VP 20% (w/w); AIBN and Sb1784 concentration respectively 0.33% and 5% both (w/w) based on the monomer; T=65°C; CO_2 added in such amount to reach the desired density. Reaction time 320-430 min, but for drug free experiment 90 min. P^0: initial pressure. D_n: number average diameter; PSD: D_w/D_n: particle size distribution; D_w: weight average diameter.

[a] Based on the monomer.

Table 3.2. Polymerization of VP in the presence of Piroxicam: effect of the initial drug concentration and of the density of the polymerization medium.

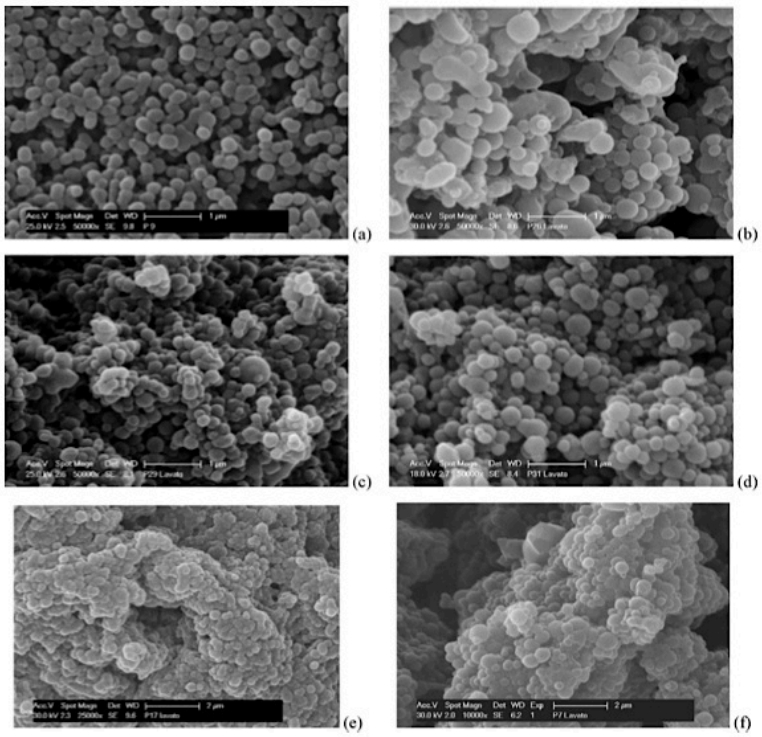

Figure 3.5. PVP particles synthesised in $scCO_2$ in the presence of different Piroxicam concentrations and density of the polymerization medium. Initial drug concentration (%, w/w with respect to the monomer) and density (g/mL) respectively are: 0 and 0.94 (a), 5 and 0.93 (b), 10 and 0.93 (c), 15 and 0.94 (d), 5 and 0.90 (e), 5 and 0.88 (f). All other experimental conditions are reported in Table 3.2.

The reaction time was decided according to the evolution of the pressure profile inside the reactor. In all the experiments the P gradually increased during the process of about 3-4 MPa above P^0 up to reach a stable value. The attainment of this value was much faster when no drug was added to the polymerization mixture.

Lepilleur and Beckman [5] have shown that the variation of the pressure during the synthesis of macromolecules in $scCO_2$ can be related to the polymerization rate, the degree of compressibility of the polymerization mixture, the difference between molar volumes of the polymer and the monomer and the variation of the volume changes upon mixing terms for each phase. This means that, provided that

temperature, pressure and initial monomer concentration are similar, one can use the change in pressure with time to compare the rate of polymerization at different drug concentrations. According to these considerations, one can conclude that the presence of the pharmaceutical compound decreased the rate of the polymerization.

When we estimated drug loadings (Table 3.2, entries 2-4), we found that, under adopted conditions, the higher the amount of Piroxicam initially loaded in the reactor the higher its concentration entrapped in the polymer matrix. The XRD patterns of pure Piroxicam and PVP, of PVP/Piroxicam composites prepared with 10 and 15% (w/w) initial concentration of the anti-inflammatory agent with respect to the monomer and of selected physical mixtures of the polymer and the drug at different compositions are depicted in Figure 3.6. The polymer product is an amorphous powder having no crystalline structure (Figure 3.6, f), while the Piroxicam has well defined crystalline peaks (Figure 3.6, a). The XRD peaks of crystalline Piroxicam in all physical mixtures (Figure 3.6, d-e) were similar to those in the pure drug, indicating no modification in the crystallinity of the pharmaceutical compound. In the case of the diffraction pattern of the composites prepared by polymerization in $scCO_2$, no peak was displayed (Figure 3.6, b-c), thus suggesting that an amorphous dispersion was obtained.

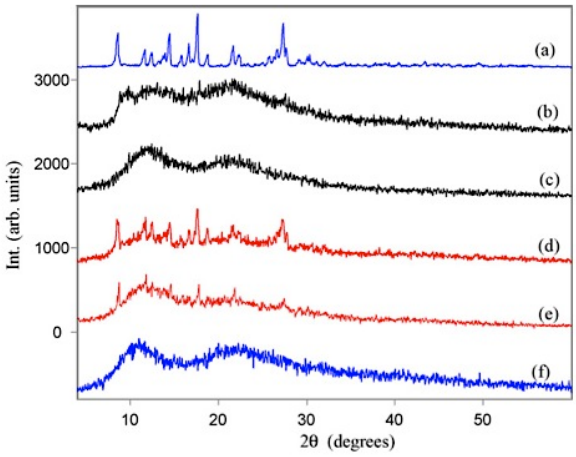

Figure 3.6. XRD patterns of pure Piroxicam (a); PVP sample synthesised in $scCO_2$ in the absence of the drug (entry 1, Table 3.2) (f); physical mixtures prepared mixing PVP and Piroxicam at different drug concentrations: 15% (w/w) (e) and 25% (w/w) (d); drug-PVP composites prepared in $scCO_2$ with different initial drug loadings in the polymerization mixture: 10% (w/w) (entry 3, Table 3.2) (c); 15% (w/w) (entry 4, Table 3.2) (b).

Drugs in amorphous dispersions may be present in two forms [6]: molecularly dispersed, a condition ensuring the highest dissolution rates, or nanodispersed with particle sizes that in our study must be smaller than the average diameter of the polymer particles (about 250 nm).

To further investigate the state of the drug in the polymer composite we used micro-Raman spectroscopy. When FT-Raman spectra of the pure drug were collected (Figure 3.7, a) we observed the presence of a band at 1523 cm^{-1}, that is reported to be characteristic of the β form Piroxicam [7,8]. No relevant peak was detected in the spectrum of the polymer synthesized in $scCO_2$ in the region 1510-1590 cm^{-1} (Figure 3.7, b). When physical mixture was analysed, the peak at 1523 cm^{-1} could be detected with a shape quite similar to that of the pure drug (Figure 3.7, c). On the other hand, in the case of the composites, the peak shifted to 1529 cm^{-1} and broadened (Figure 3.7, d-e). This can be considered a further indication that the crystalline structure of the pharmaceutical compound was altered presumably as a

consequence of the interactions between the drug molecules and the macromolecular network.

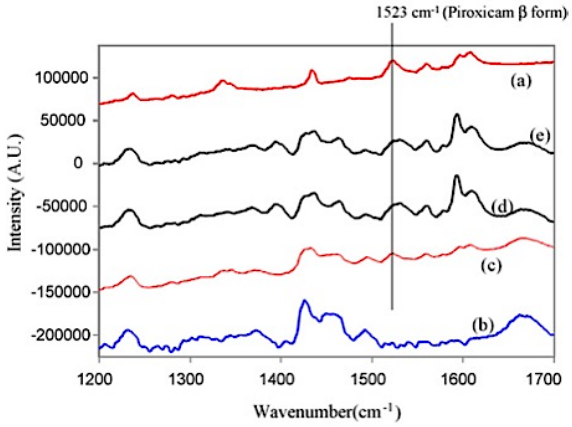

Figure 3.7. Raman spectra of pure Piroxicam (a); PVP sample synthesized in $scCO_2$ in the absence of the drug (entry 1, Table 3.2) (b); physical mixture prepared mixing PVP and Piroxicam at 5% (w/w) drug concentration (c); drug-PVP composites prepared in $scCO_2$ with different initial drug loadings in the polymerization mixture: 5% (w/w) (entry 2, Table 3.2) (d); 10% (w/w) (entry 3, Table 3.2) (e).

The dissolution profiles of Piroxicam from the composites prepared in $scCO_2$ are reported in Figure 3.8, where is also shown the dissolution of 2.5 mg of the pure compound dispersed in the same volume of buffer solution used for the dissolution tests. We can clearly see that the release of the drug from the composites is considerably faster than the dissolution of the pure pharmaceutical compound. Moreover, the higher the initial amount of the drug loaded in the reactor the faster its dissolution rate. This effect can be highlighted by comparing the values of time necessary to dissolve 20% of the total amount of drug released (Table 3.3). We observed a 65% reduction of this parameter when mixing the drug with the polymer (physical mixture); quite interestingly it was further reduced of almost one order of magnitude when the pharmaceutical compound dissolved from the composites.

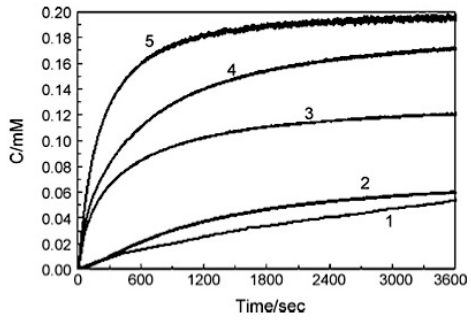

Figure 3.8. Release profiles of Piroxicam from composites prepared in experiments of Table 3.2. Initial drug concentration % (w/w) with respect to the monomer: 5 (curve 3), 10 (curve 4), 15 (curve 5). The dissolution profiles of 2.5 mg of the pure drug (curve 1) and of a physical mixture PVP-Piroxicam at 5% (w/w) drug concentration (curve 2) are added for comparison.

Profile	System	Drug, %(w/w)	$t_{20\%}$, s	*n* (t<45s) R^2	*n* (t>60s) R^2
1	Piroxicam	100	1190	-	-
2	Physical Mixture 5%	5	430	-	-
3	Composite 5%	4.8	56	1.40	0.53
				0.98	0.99
4	Composite 10%	9.3	65	1.23	0.52
				0.99	0.99
5	Composite 15%	12.3	51	1.40	0.68
				0.99	0.99

Table 3.3. Time necessary to the dissolution of 20% of the drug, values of *n* exponent and corresponding correlation coefficients R^2 obtained from the fitting of experimental release profiles for systems studied in Figure 3.9.

In the case of polymeric controlled drug delivery systems, it was proposed to classify the release mechanisms of the pharmaceutical compound using three main typologies of system, differentiated on the basis of the chemico-physical characteristics of the macromolecular matrix. According to this classification, one can distinguish: (a) drug diffusion from non-degraded polymer (diffusion-controlled system); (b) enhanced drug diffusion due to polymer swelling (swelling-controlled system); (c) drug release due to polymer degradation and erosion (erosion-controlled system).

Water is a good solvent of PVP, but it was observed that the polymer synthesized in

$scCO_2$ with a monofunctional polysiloxane macromonomer surfactant was characterized by a significant gel fraction [3,9]. This result was attributed to the formation of a polymer with a very high molecular weight and to the presence of a hydrophobic shell constituted by the CO_2-philic tails of the stabilizer, which is grafted or even crosslinked with the polymer chains.

During all dissolution tests performed in this study, part of the composite added to the buffer solution was collect undissolved at the end of the experiment, so that the drug dissolution is only partially accompanied by the dissolution of the polymer matrix. According to aforementioned considerations, it seems reasonable that the release of Piroxicam from the composites should be mainly regulated by a mechanism of the type b.

The release kinetics of a drug from a swelling-controlled polymer matrix depends on several factors, such as the rate of molecular transport of the drug molecules inside the polymer matrix, the specific surface area of the composite, the concentration and the microstructure of the drug encapsulated in the polymer particles. The matrix is penetrated by water molecules that induce swelling of the material, decreasing polymer chain concentration and changing the level of their disentanglement [10,11]. This process results in the transformation of part of the polymer from the glassy to the rubbery state, with consequent increase of its volume, and leads to the generation of a rubbery region (also termed gel layer) in which the drug mobility increases. The polymer will also dissolve at the polymer-solvent interface, that moves owing to the swelling of the matrix, because at this site concentration of chains is very low and their entanglement is weak. In this system a deviation from Fickian model is observed when the drug release is controlled not only by the diffusion of the drug inside the matrix, but also by the polymer disentanglement and dissolution process. In fact, when the matrix is contacted with water, the drug dissolves owing to a concentration difference at the glassy-rubbery front and diffuses out through the rubbery-solvent front under the driving force of the concentration gradient established between the two interfaces. If water penetration can be neglected no polymer relaxation occurs and the drug dissolution is controlled by Fickian diffusion (Case I) inside the glassy polymer matrix.

Differently if the water mobility is relevant, the rate of the solute release is controlled by the kinetics of the structural modifications induced by the solvent uptake and we are in the case of a relaxation controlled release generally identified by the expression "non-Fickian Case II-transport". Between these limiting situations an "anomalous transport" can be operative if both diffusion and relaxation contemporary occurs in a quite undistinguishable manner.

A rigorous mathematical modelling of the release profiles for swellable polymeric systems is rather complex. The problem is a special case of moving-boundary diffusion problems and requires the utilization of highly non-linear constitutive equations that must be solved using numerical methods. Anyway a simple semi-empirical equation based on a power-law expression was proposed by Korsmeyer et al. [12,13] to describe the drug release from swelling-controlled systems:

$$\frac{M_t}{M_\infty} = X = kt^n \qquad 3.1$$

where k is a constant incorporating structural and geometric characteristics of the drug dosage form, n is the release exponent, indicative of the drug release mechanism and $X = M_t/M_\infty$ is the fractional release of drug. In the case of drug release from monodisperse spheres, $n = 0.43$ and $n = 0.83$ for Fickian and Case II-transport respectively, while intermediate values are indicative of anomalous transport.

Values of $lnX = ln(M_t/M_\infty)$ vs. $ln(time)$ for Piroxicam dissolution were plotted at low release time ($t < 45$ s) and for time higher than 60 s, keeping $X \leq 0.60$ (Figure 3.9). We observed that both these set of data can be fitted linearly with a good agreement ($R^2 \geq 0.98$) and much higher value of the exponent n were obtained in the initial part of the release process (Table 3.3). This behaviour was already reported by other researchers and defined as super Case II release kinetics [11]. After this initial period, n reaches values typical of an anomalous transport in which the role of polymer relaxation and drug diffusion cannot be easily decoupled.

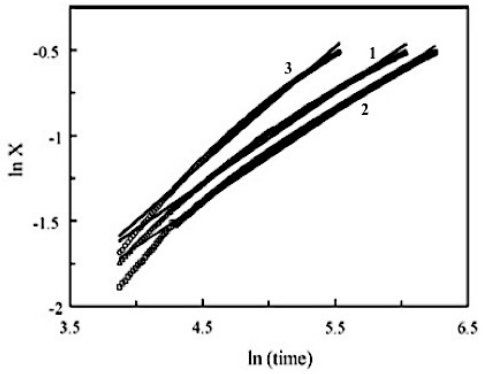

Figure 3.9. Logarithmic plot of M_t/M_∞ vs. release time computed at t > 60 s for composites prepared in $scCO_2$ with different initial drug concentrations (Table 3.2): (curve 1) entry 2, (curve 2) entry 3, (curve 3) entry 4.

Since the preparation of the drug-polymer composites was performed using $scCO_2$ as a dispersing medium, an operative parameter that we decided to investigate was the density of the polymerization mixture. To this purpose we performed a set of polymerizations at fixed composition of the reaction system, but changing the total amount of components loaded in the reactor. These experiments were performed at 20% (w/w) VP concentration (a value that was kept unaltered for the whole investigation) with 5% (w/w) Piroxicam and 0.33% (w/w) AIBN concentrations with respect to the vinyl monomer (entries 5-6, Table 3.2). In these experiments the composite was obtained with yields slightly higher than 80% under the form of a powder constituted by substantially spherical particles with D_n in the range 0.25-0.31 μm. PVP particles generated at lower density (Figure 3.5, e-f) resulted more agglomerated than those obtained at higher density at the same drug concentration (Figure 3.5, b).

The amount of Piroxicam entrapped inside the matrixes does not seem significantly affected by the medium density, drug loadings ranging in the interval 4.0-4.6% (w/w) for all investigated samples (Table 3.2, entries 2, 5 and 6). Also the drug dissolution rate, always much faster than that of the pure anti-inflammatory agent, was found substantially not affected by the density of the polymerization

medium. When composites were analysed by Raman spectroscopy, we observed the same broadening and shift of the characteristic peak at 1529 cm^{-1} previously commented in the discussion of the effect of the initial drug concentration. This result suggests that, at all investigated densities, the crystalline structure of the drug is altered during the polymerization process. We want to underline that in these experiments the drug was completely dissolved in the polymerization mixture only when its density was 0.93 g/mL. Anyway, also in lower density systems, the drug was significantly loaded under non-crystalline form inside the polymer, thus giving a further element supporting the hypothesis that, owing to a favourable partitioning equilibrium of the drug inside PVP, the undissolved bioactive compound can act as a reservoir phase, supplying the drug to the growing polymer particles thanks to the intermediation of the continuous fluid phase. This aspect increases the generality of the method since it is not a priori necessary to start with an homogeneous reaction system that is a condition markedly dependent on the nature of the considered drug.

3.6.3 Homopolymerization of VP in the presence of Piroxicam: effect of the concentration of the initiator and of the stabilizer

The release kinetics of a swelling-controlled DDS can be markedly affected by the rate of molecular transport of the water molecules inside the polymer matrix, its relaxation kinetics and the osmotic pressure occurring during the swelling process [11]. These phenomena should be modified changing the molecular weight distribution of the polymer support; to investigate such possibility, we performed the polymerization of VP in the presence of Piroxicam at two different density levels (0.90-0.92 g/mL and 0.93-0.94 g/mL), modifying the initial concentration of AIBN (Table 3.4, entries 1-6). Higher values of initiator accelerate the rate of free radical generation, making easier the formation of shorter dead chains. In all experiments the composites were obtained with yields higher than 80% under the form of spherical particles with D_n =0.2-0.3 μm (Figure 3.10, a-f) and drug loadings between 3.7 and 4.8% (w/w).

Entry	ρ, g/mL	P^0, MPa	AIBN, %(w/w)[a]	Sb1784, %(w/w)[a]	Yield, %	Drug loading, %(w/w)	Time, min	D_n, µm	PSD
1	0.90	24	0.33	5	82	4.6	430	0.25	1.16
2	0.92	30	1.00	5	88	3.8	290	0.28	1.09
3	0.91	25	2.99	5	98	4.0	150	0.18	1.47
4	0.93	33	0.33	5	95	4.8	350	0.26	1.33
5	0.94	36	1.00	5	97	4.4	260	0.28	1.13
6	0.94	34	2.98	5	93	3.7	180	0.29	1.09
7	0.93	32	0.33	10	96	4.5	310	0.19	1.19
8	0.92	31	2.96	10	99	3.7	150	0.17	1.23

VP 20% (w/w); T=65°C; CO_2 added in such amount to reach the desired density. Piroxicam 5% (w/w) with respect to the monomer. P^0: initial pressure. D_n: number average diameter; PSD: D_w/D_n: particle size distribution; D_w: weight average diameter.

[a] Based on the monomer.

Table 3.4. Polymerization of VP in the presence of Piroxicam. Effect of the initial concentration of the initiator and of the stabilizer.

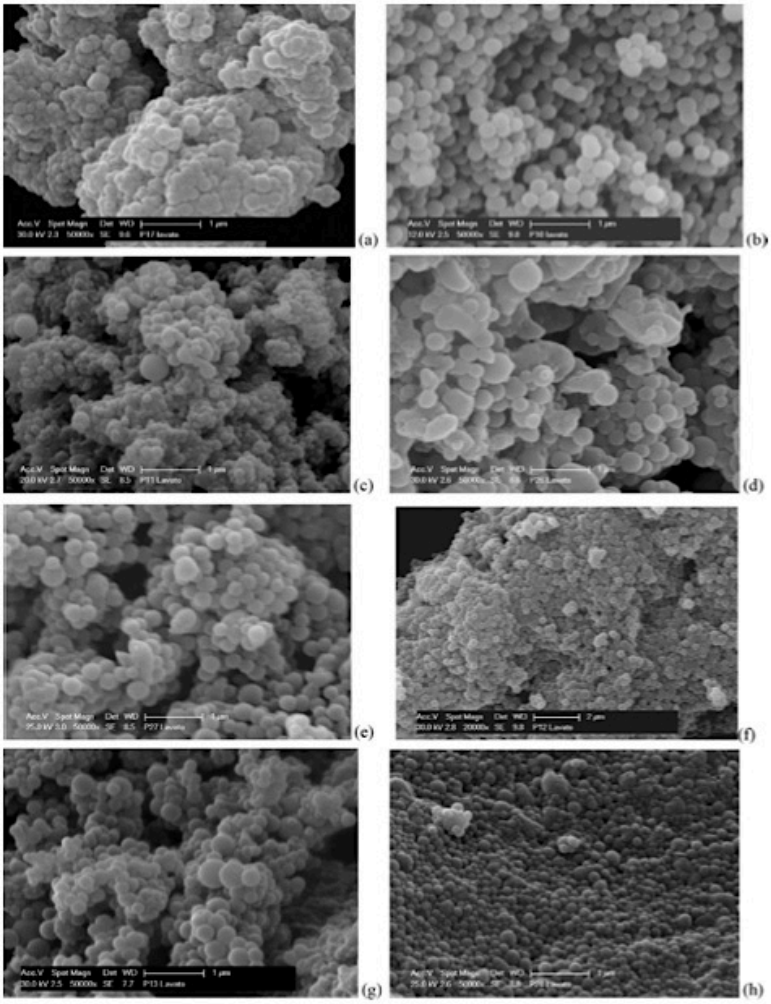

Figure 3.10. PVP-Piroxicam composites synthesised in $scCO_2$ at different initial concentrations of initiator and stabilizer (%, w/w with respect to the monomer). Experimental conditions are reported in Table 3.4, entries 1 (a), 2 (b), 3 (c), 4 (d), 5 (e), 6 (f), 7 (g) and 8 (h).

Also for these materials, Raman spectroscopy indicated that, under adopted conditions, the drug is dispersed in the polymer matrix under non-crystalline form independently on the investigated initial concentration of AIBN.

As previously reported, the density seemed to have no effect on the

dissolution kinetics of the drug when its role was studied at the lowest initiator concentration adopted in this study. On the other hand, when we recorded the release profiles from composites obtained at different AIBN loadings, we observed different behaviours at the two different levels of densities adopted. Composites prepared in reaction media with density equal or lower than 0.92 g/mL gave dissolution kinetics of the drug that does not significantly change with the initiator concentration used to synthesize the polymer matrix. When we studied the dissolution rate of Piroxicam from composites prepared in systems with initial density of 0.93-0.94 g/mL, we observed a slight acceleration of the release rate when the initiator concentration was increased (Figure 3.11).

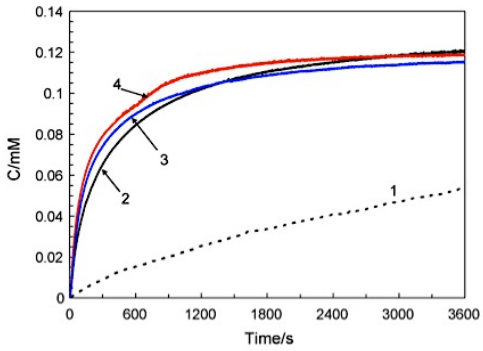

Figure 3.11. Release profiles of Piroxicam from composites prepared in experiments of Table 3.4 (entries 4-6). AIBN (%, w/w with respect to the monomer): 0.33 (curve 2), 1.00 (curve 3) and 2.99 (curve 4). The dissolution of 2.5 mg of the pure drug (curve 1) is added for comparison.

Such different behaviour brought us to reconsider the effect of the density on the solvent power of the continuous medium. In the investigated system, the polymerization occurs heterogeneously and, after polymer nucleation, two phases are present inside the reactor. Given the presence of the surfactant, that leads to the formation of a dispersed phase with high interfacial area, it seems reasonable to assume that low molecular weight species are equilibrium partitioned between the two phases. The initiator dissolved in the highly local viscous polymer phase should

give a limited contribution to the rate of generation of free radicals in the reactor, owing to a small efficiency factor in the decomposition step arising from the significant cage effect. Then free radicals should be substantially generated in the supercritical phase, whose solvent power increases with the density, thus biasing the equilibrium partitioning of the initiator towards the continuous medium. When high concentrations of initiator are used, this solvent effect can increase the rate of generation of the radicals to such a level that the modification of the average molecular weight of the polymer becomes so relevant to affect the dissolution rate of the entrapped Piroxicam. These considerations could explain why the acceleration of the dissolution of the drug with an enhancement of the initiator concentration was observed only at the highest investigated values of density.

Another important parameter that can change the rate of dissolution of a low water solubility compound dispersed in a polymer matrix is the interfacial area of the composite. The higher is its value the faster should be the rate of accumulation of the bioactive molecule in the release medium. On the basis of this consideration, we studied the effect of such parameter in the dissolution of Piroxicam from composites prepared in $scCO_2$.

It is reported in the literature that, in the case of the polymerization of VP in $scCO_2$ in the presence of siloxane reactive surfactants, the interfacial area of the polymer matrix can be increased by increasing the concentration of the stabilizer [3]. To check if this behaviour can be reproduced also in the presence of the drug, we performed additional experiments at high densities of the polymerization mixture changing the AIBN loading, while the stabilizer concentration was augmented to 10% (w/w) (Table 3.4, entries 7 and 8).

At both initiator concentrations, when the surfactant concentration was augmented from 5 to 10% (w/w), the average diameter of the polymer particles decreased (Table 3.4 entries 4-7 and 6-8 and Figure 3.10, d-g and f-h), thus leading to a polymer dispersion with higher interfacial area as already occurred in the case of drug free polymerization of VP. In all cases we found that more than 70% of the drug initially loaded in the reactor was entrapped inside the polymer particles and spectroscopic analyses confirmed once more a modification of its crystalline

structure with respect to that of the pure compound.

In spite of this similarity of behaviours, when we recorded the dissolution profiles, we observed different effects of the stabilizer concentration depending on the AIBN concentration. When its initial concentration was 0.33% (w/w), the higher interfacial area of the macromolecular matrix was coupled with an acceleration of the dissolution rate of the drug (curves 1 and 2, Figure 3.12). Differently when the Sb1784 concentration was augmented at initial AIBN loading of about 3% (w/w), we observed that the reduction of the average diameters of the particles was accompanied by a decrease in the dissolution rate of the drug (curves 3 and 4, Figure 3.12) that, in any cases, was significantly higher than that of the pure Piroxicam (curve 5, Figure 3.12).

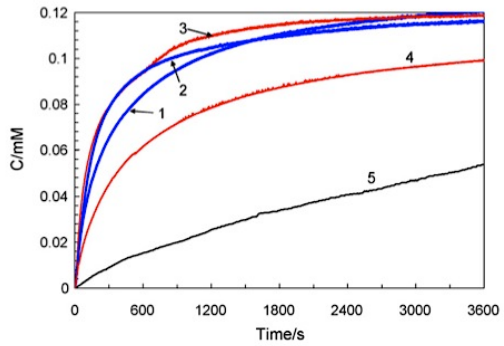

Figure 3.12. Release profiles of Piroxicam from composites prepared with different Sb 1784 concentrations in experiments of Table 3.4: entry 4 (curve 1), entry 7 (curve 2), entry 6 (curve 3) and entry 8 (curve 4). The dissolution of 2.5 mg of the pure drug (curve 5) is added for comparison.

The lower dissolution rate from composites prepared at the highest adopted concentrations of initiator and stabilizer was first tentative attributed to differences in the surface concentration of adsorbed silicone surfactant, that is not eliminable by washing with liquid cyclohexane [9]. Given its hydrophobic nature, an increase in the surface density of such compound should decrease the wettability of the matrix thus adversely affecting the rate of dissolution of the drug.

To verify this hypothesis, we estimated the surfactant content of the synthesized polymers by FT-IR spectroscopy. In the spectra it can be detected a band at 1660 cm^{-1}, corresponding to the stretching of the carbonyl of the VP repeat union, and two absorption bands at about 1000-1100 cm^{-1} (Si-O-Si bonds) and 810 cm^{-1} (Si-CH_3 bonds), related to the PDMS present in the polymer. In this estimation it was neglected any contribution of the amide I resonance band of Piroxicam (located at 1640-1630 cm^{-1} depending on its crystalline form) [14] to the carbonyl peak of PVP, given the low drug concentration adopted in this set of experiments and the marked difference in the response factors of the two different functionalities. When we compared the ratios of the absorbance of the Si bands to that of the band at 1660 cm^{-1} normalized with respect to the average diameter of the particles, we did not find significant differences in the surface concentration of surfactant among the different composites.

From a purely speculative point of view, it could be considered that the surfactant adopted in this study is a bifunctional reactive stabilizer, characterized by an high solubility in the polymerization mixture, that must generate in situ the real surface active agent by graft copolymerization with the monomer in the continuous medium. If the rate of generation of the graft copolymers becomes too high in comparison of the rate of growth of the interfacial area, the amount in excess with respect to that necessary to cover particle surfaces can be buried inside them during their growth, making possible the generation of crosslinks in the matrix. This event, whose probability should increase with the concentration of initiator and reactive surfactant, should decrease the swelling of the matrix, thus limiting the diffusion rate of the drug.

3.7 Drug/poly(VP-co-AA) composites

Once obtained PVP/Piroxicam composites in a single-step process, able to enhance the dissolution rate of the pharmaceutical compound, we thought to adopt the same reaction process to prepare pH sensitive polymers, performing the dispersion copolymerization of vinylpyrrolidone and acrylic acid in the presence of

the drug. But, when we tried to perform this kind of reactions, we realized that experiments interrupted at almost zero reaction times (for example making a quench during the heating transitory) were characterized by high conversions. This may be due to the high reactivity of the AA that, being still an expanded liquid during the heating, is subject to quasi-bulk polymerization as soon as it comes into contact with the initiator AIBN. This means that the homogenization time of monomers with CO_2 can be comparable with the characteristic polymerization time of the liquid AA. This is a very important point for the study of the reaction, because it may lead problems in the control of the reaction kinetics: for example, the AA could react during the first part of the reaction, while the VP later. This may lead to the formation of a polymer with non homogeneous properties, due to the uncontrolled reaction.

Thus, due to the different reactivity of the two monomers, we decided to use a different reaction system, putting the liquid initiator (DEPDC in Freon) inside the reactor only after the system was homogeneous, in order to have a better control of the reaction kinetics.

3.7.1 Phase behaviour of the VP/AA/CO_2/Drug systems

The phase behaviour of the quaternary systems constituted by the monomers, the drug and the compressible solvent was investigated by visual observation in a fixed volume view cell. The experiments were performed with a total concentration of monomers fixed at 1 mol/L, with four different mole fractions of AA in the monomer mixture, namely 0.15, 0.25, 0.40 and 0.60 and in the presence of Piroxicam or Caffeine concentration fixed at 3 mg/mL.

At 50°C (i.e. the reaction temperature adopted in this study), without CO_2, all the investigated systems were constituted by one liquid phase, in which the drug powder was dissolved. After loading of CO_2 and thermal equilibration at 50°C and pressure higher than 18.0 MPa, for all studied systems the view cell was observed to be completely filled with a single fluid phase in which the drug resulted dispersed as powder. This behaviour is an indication that at high enough nominal density of the

system AA, VP and CO_2 are mixed in single phase in which the drug is only partially soluble. As said before, for the process under consideration, in principle, the success of the methodology does not require a complete miscibility of the drug with the polymerization mixture. To reach high drug loading in the synthesized polymer, it should be enough a high solubility of the pharmaceutical compound in the polymer-rich dispersed phase generated during the particle forming polymerization of the selected vinyl monomers since, differently from the monomers, both PVP [24,25] and PAA [26] are not soluble in $scCO_2$.

3.7.2 Synthesis and characterization of drug/poly(VP-co-AA) composites

Initially we tested the validity of the previously discussed one-pot route for the preparation of drug/poly(VP-co-AA) composites, performing the copolymerization of VP and AA in $scCO_2$, in the presence of Caffeine or Piroxicam, chosen as model drugs of Class I and Class II of the BCS (Biopharmaceutical Classification System) [15]. The results are summarized in Table 3.5 (entries 2-7) together with data obtained in a control test performed in the absence of any drug (entry 1).

The reaction time required for the completion of the polymerization was estimated from the recorded profile of the pressure inside the reactor. As seen before, the variation of the pressure during dispersion polymerization can be related to the polymerization rate, the degree of compressibility of the polymerization mixture, the difference between molar volumes of the polymer and the monomer and the variation of the volume changes upon mixing terms for each phase [5].

The free-radical copolymerization of VP and AA in $scCO_2$ by dispersion technique assisted by a silicone steric stabiliser appeared very fast since almost quantitative polymer yield (99%) was reached in less than 40 min. The time for the completion of the polymerization decreased when the initial concentration of AA in the feed

was increased. The product was collected as a free flowing and dry powder, substantially constituted by particles having sub-micron size (Figure 3.13, a-e).

In precipitation polymerization, instead, the reaction time was longer (higher than 8 hours); the product was obtained with high yield, even if the conversion of monomers was not complete.

Entry	Surfactant, %(w/w)[a]	AA, %(mol)	Drug	Yield, %	Product
1	15	40	-	≥99	White Powder
2	-	40	Piroxicam	95	Yellow Powder
3	15	40	Piroxicam	≥99	Yellow Powder
4	15	60	Piroxicam	≥99	Yellow Powder
5	-	40	Caffeine	94	White Powder
6	15	40	Caffeine	≥99	White Powder
7	15	60	Caffeine	≥99	White Powder

Operating conditions: T=50°C, P^0: initial pressure=32.0 MPa; monomers feed concentration: [VP]+[AA]=1 M; [DEPDC]=3 mM; Drug=3% (w/w) based on the monomers.

[a] Based on the monomers.

Table 3.5. Copolymerization of VP and AA in the presence of Piroxicam or Caffeine.

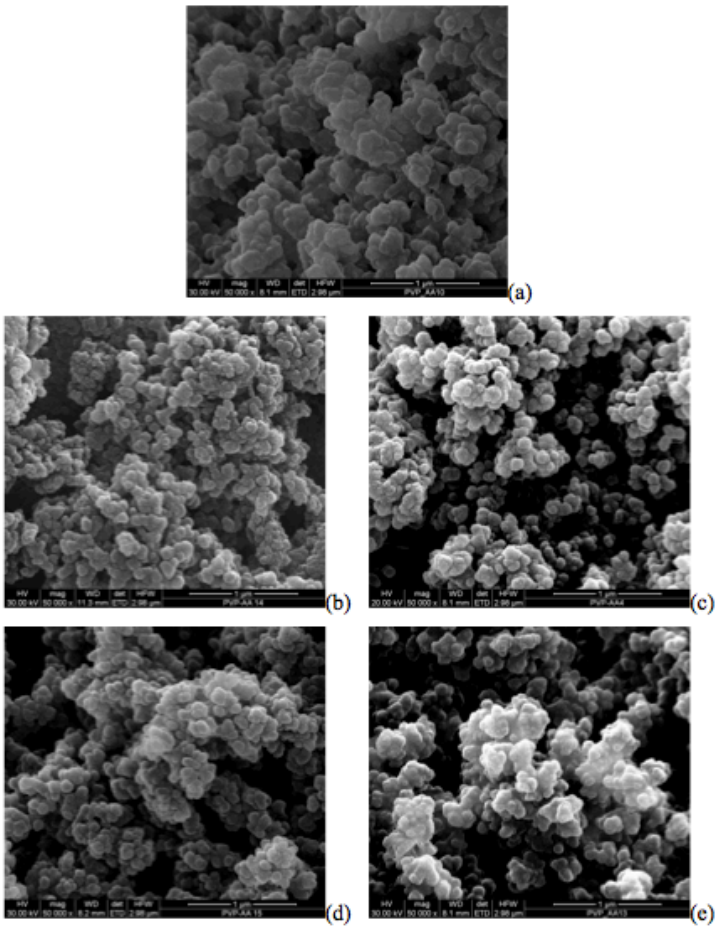

Figure 3.13. Poly(VP-co-AA) particles synthesised in $scCO_2$ in the presence of Piroxicam or Caffeine as model drugs. Copolymer prepared through dispersion polymerization in the absence of any drug (a); copolymer prepared through precipitation (b) or dispersion (c) polymerization in the presence of Piroxicam; copolymer prepared through precipitation (d) or dispersion (e) polymerization in the presence of Caffeine.

In all the experiments, after the loading of the initiator, pressure increased up to a maximum located at about 2.0-3.0 MPa above its initial value P_0 and then decreased reaching a constant value that was always higher than P_0 (Figure 3.14).

The time when the stable pressure was reached was considered to be corresponding to the end of the copolymerization process.

Drug loading of polymers was estimated after dissolution of about 10 mg of each composite in 40 g of methanol. Estimated drug loading ranged from 2.5 to 3% w/w with respect to the amount of synthesized polymer with an uncertainty estimated within ±10%.

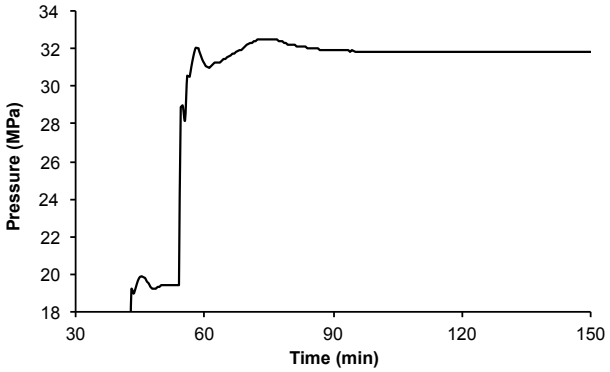

Figure 3.14. Typical pressure profile recorded during dispersion copolymerization experiments.

To investigate the state of the drug in the polymer composite, we used micro-Raman spectroscopy. When FT-Raman spectra of the pure Piroxicam were recorded, we observed the presence of a band at 1523 cm^{-1} (Figure 3.15, a), that was reported to be characteristic of the β form [7,8]. No relevant peak in the region 1510-1600 cm^{-1} was detected in the spectra of the copolymers synthesised in $scCO_2$ in the absence of any drug (Figure 3.15, d); instead, in the case of the composites prepared in the presence of Piroxicam, the aforementioned characteristic peak resulted more broadened and shifted to 1529 cm^{-1} (Figure 3.15, b-c). The spectrum of pure Caffeine exhibits a characteristic peak at 555 cm^{-1} (Figure 3.16, a) [16-18] in a range of wavelengths where no resonance band can be detected in the spectra of drug-free copolymers. As previously observed in the case of Piroxicam, in the Caffeine/poly(VP-co-AA) composites prepared in $scCO_2$ this peak resulted more

broadened, even if there was not a marked difference in the wavelength value of the peak position (Figure 3.16, b-c).

The shift and the different shape of characteristic peaks of drugs entrapped in the polymer matrix can be considered an indication that the crystalline structure of the pharmaceutical compounds was altered presumably because of the presence of specific interactions between the drugs molecules and the macromolecular network.

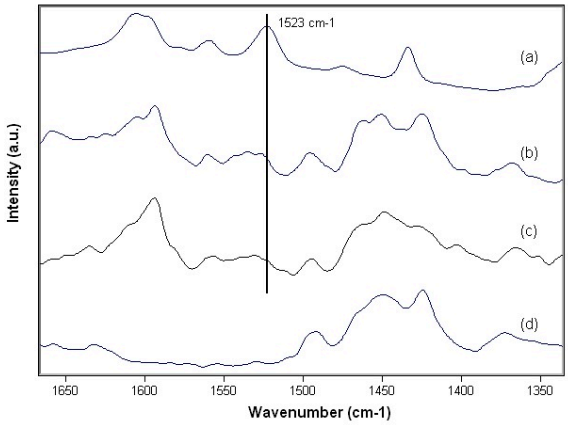

Figure 3.15. Raman spectra of pure Piroxicam (a); drug/poly(VP-co-AA) composites prepared in $scCO_2$ with 3% (w/w) of initial drug concentration at different monomer feed compositions: 40% (mol) (entry 3, Table 3.5) (b) and 60% (mol) (entry 4, Table 3.5) (c); poly(VP-co-AA) synthesised in $scCO_2$ in the absence of the drug (entry 1, Table 3.5) (d).

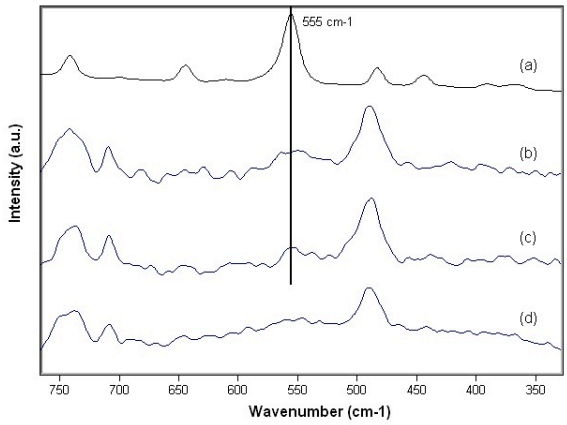

Figure 3.16. Raman spectra of pure Caffeine (a); drug/poly(VP-co-AA) composites prepared in $scCO_2$ with 3% (w/w) of initial drug concentration at different monomer feed compositions: 40% (mol) (entry 6, Table 3.5) (b) and 60% (mol) (entry 7, Table 3.5) (c); poly(VP-co-AA) synthesised in $scCO_2$ in the absence of the drug (entry 1, Table 3.5) (d).

To study the crystalline nature of the drug in the composites they were investigated by XRD. The pure drugs exhibit peaks typical of a crystalline material, while both copolymer and drug/copolymer composites gave diffused halo typical of amorphous matrixes. In the XRD pattern of drug-copolymer physical mixtures loaded with 15 % (w/w) of the bioactive compounds we observed characteristic diffraction peaks located at the same angle of the pure drugs, indicating no modification in the crystallinity of the pharmaceutical compounds. Anyway, when drug loadings in the physical mixtures were decreased at 3% (w/w), no clear diffraction peak could be detected in the spectra. We must conclude that drug concentration loaded in the composites are below the sensitivity threshold of the technique and then no clear conclusion can be drawn from the results of XRD characterization.

The ^{1}H-NMR spectra gave informations about the structure of Piroxicam or Caffeine inside the drug/poly(VP-co-AA) composites. The spectra of drug/poly(VP-co-AA) composites synthesised in the presence of Piroxicam or Caffeine showed typical signals of the two drugs: this is an indication of the fact that the drugs

maintain their initial chemical structure inside the polymer matrix, even if there are interactions.

3.7.3 Drug delivery experiments

The dissolution profiles of Piroxicam from the composites prepared in $scCO_2$ recorded at different pH of the buffer solution used to perform the tests are reported in Figure 3.17. The rate of dissolution of the drug was found to be pH-dependent and slower accumulation of the drug in the release medium were observed at lower pH. Moreover the sensitivity of the release rate on the pH value increased with the initial mole fraction of AA in the feed.

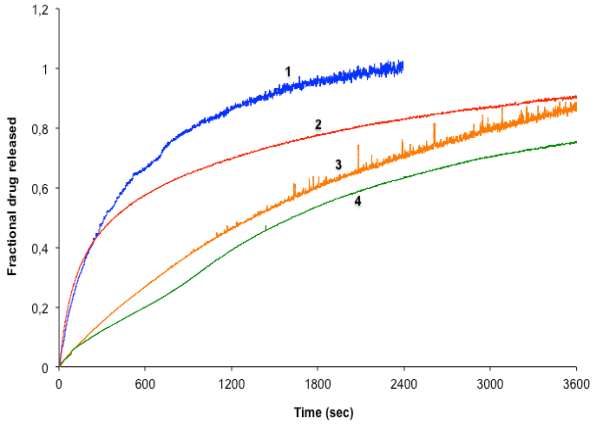

Figure 3.17. Fractional release of Piroxicam, at different values of pH, from drug/poly(VP-co-AA) composites prepared in experiments of Table 3.5 (entries 3-4): entry 3 at pH 6.8 (curve 1) and 2.5 (curve 2), entry 4 at pH 6.8 (curve 3) and 2.5 (curve 4).

The pH sensitivity of the studied copolymers can be attributed to the presence of AA repeat units in the macromolecular structure. When the pH is lower than 4.75, i.e. the pK_a of poly(AA) [27], the majority of the carboxyl groups in the macromolecular chains in the matrix remain non-ionized. In this condition, they are fully associated in forming intermolecular/intramolecular hydrogen bonding with VP repeat units,

thus providing a compact H-bonded structure to the copolymer. In this frozen conformation, the movements of polymeric segments are restricted and the rate of diffusion of the solvent in the matrix is depressed, thus leading to a slower dissolution rate of the drug from the polymer matrix. When the pH is higher than the pK_a of poly(AA), the majority of the carboxyl groups of AA units are ionized: this results in relaxation of polymeric chains due to electrostatic repulsion among negatively charged carboxylate groups along the macromolecular chains, with consequent disgregation of intermolecular/intramolecular hydrogen bondings, thus leading to a higher dissolution rate [19-21].

When we studied the release rate of Caffeine loaded in poly(VP-co-AA) matrixes (Figure 3.18), we found that the release rate was not affected by pH in the case of the composite prepared with 40% mol AA initial concentration in the feed. When the initial loading of AA in the feed was augmented to 60%, after a short initial period, the dissolution rate of Caffeine was slower in the release medium with lower pH level.

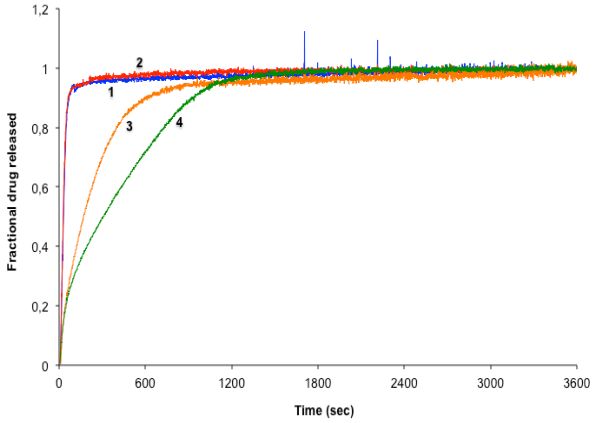

Figure 3.18. Fractional release of Caffeine, at different values of pH, from drug/poly(VP-co-AA) composites prepared in experiments of Table 3.5 (entries 6-7): entry 6 at pH 6.8 (curve 1) and 2.5 (curve 2), entry 7 at pH 6.8 (curve 3) and 2.5 (curve 4).

Also in the case of Caffeine loaded matrixes the release rate of drug from the composites decreased when the mole fraction of AA in the feed increased. This result can be explained by the difference in the AA mole fraction in the copolymer that determines the extent of interaction between VP and AA units.

Monomer reactivity ratios for the copolymerization of VP and AA were estimated in bulk to be r_{VP} =0.1 and r_{AA} =1.1 [28,29]. According to these values, the copolymer tends to be enriched in AA repeat units randomly alternated by VP repeat units.

It was observed that when PAA chains are synthesized by template polymerization using PVP as a template polymer, interpolymer complexes are formed by hydrogen bonds between the carboxyl groups of PAA and the carbonyl groups of PVP. The higher the mole fraction of incorporated AA the larger the number of hydrogen bond interactions with VP repeat units and the slower the observed dissolution rate [22].

As we have seen in the case of PVP/Piroxicam composites, an important parameter that can change the drug release from a polymer matrix is the interfacial area of the composite. We have studied the precipitation or dispersion copolymerization of VP and AA in the presence of Piroxicam (Table 3.5, entries 2-3).

Dispersion polymerizations rely on adsorbed or chemically grafted polymeric surfactants to prevent coagulation of growing polymer particles [23]. In principle, the surfactant leads to the formation of a dispersed phase with higher interfacial area, that should cause a higher dissolution rate of the drug. As showed in Figure 3.19, this is not true in our case: in fact, in the presence of the surfactant, the drug releases were slower. This experimental behaviour may be due to the hydrophobic nature of the surfactant that could hamper the water absorption and the swelling of the polymer, thus leading to slower release kinetics.

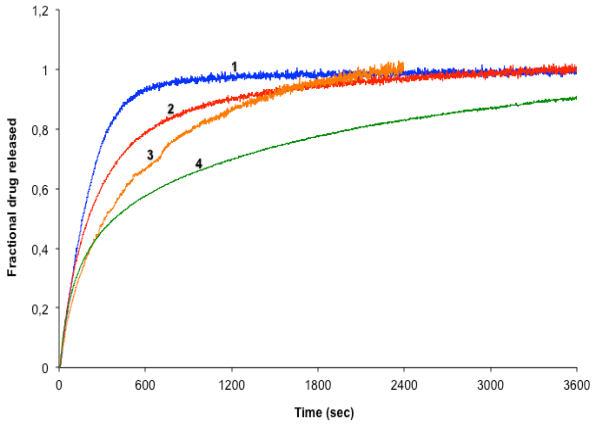

Figure 3.19. Fractional release of Piroxicam, at different values of pH, from drug/poly(VP-co-AA) composites prepared, through precipitation or dispersion polymerization, in experiments of Table 3.5 (entries 2-3): entry 2 at pH 6.8 (curve 1) and 2.5 (curve 2), entry 3 at pH 6.8 (curve 3) and 2.5 (curve 4).

3.7.4 Drug release from tablets

Dissolution experiments were performed also loading the drug/polymer composite in the buffer solution under the form of tablets, in order to study the effect of the different sample morphology, corresponding to two different pharmaceutical formulations, on the release rate. The idea was to create a better release system, able to control the drug dissolution as a function of the specific formulation.

The dissolution rates of Piroxicam from the tablets showed pH-dependency and were slower at lower pH (as in the case of powders); moreover, release rates of drug from tablets were slower than those from powders for all the investigated compositions (Figures 3.20-3.21).

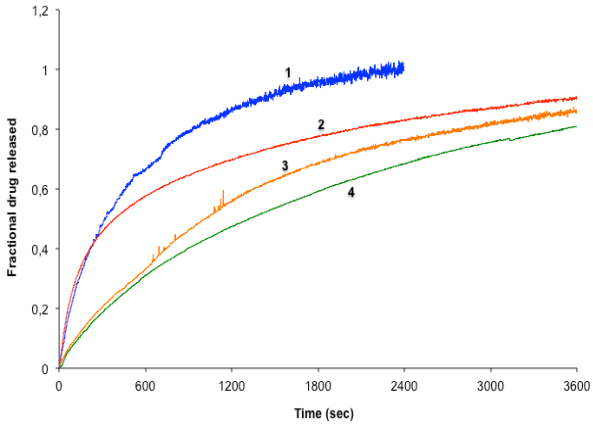

Figure 3.20. Fractional release of Piroxicam, at different values of pH, from drug/poly(VP-co-AA) composite in powder or tablet form, prepared in experiment of Table 3.5 (entry 3): powder at pH 6.8 (curve 1) and 2.5 (curve 2), tablet at pH 6.8 (curve 3) and 2.5 (curve 4).

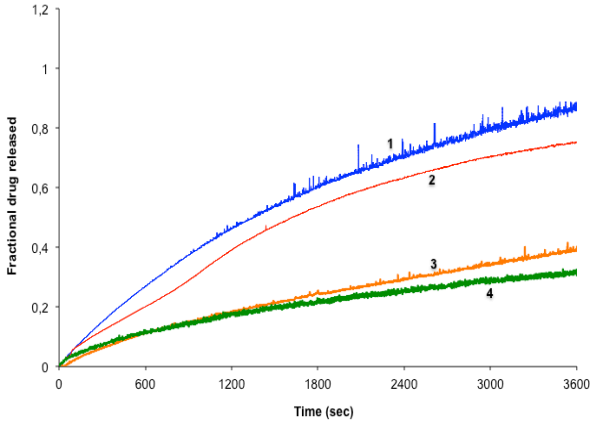

Figure 3.21. Fractional release of Piroxicam, at different values of pH, from drug/poly(VP-co-AA) composite in powder or tablet form, prepared in experiment of Table 3.5 (entry 4): powder at pH 6.8 (curve 1) and 2.5 (curve 2), tablet at pH 6.8 (curve 3) and 2.5 (curve 4).

3.8 Conclusions

Polymeric composites constituted by PVP and Piroxicam, selected as a model of drug with low water solubility, were prepared in a one-pot process performing the

dispersion polymerization of 1-vinyl-2-pyrrolidone in supercritical carbon dioxide in the presence of the bioactive compound. The composite was obtained with monomer yields higher than 80% under the form of spherical sub-micron particles characterized by high interfacial area. More than 70% of the pharmaceutical compound was charged inside the particles also when its initial concentration in the polymerization medium was significantly higher than its solubility. XRD and Raman spectroscopy suggest that Piroxicam is dispersed in the polymer matrix under non-crystalline morphology at all investigated operative conditions.

Dissolution rates of the anti-inflammatory agent from composites prepared in $scCO_2$ were significantly faster than those obtained both with the pure drug and from its physical mixtures with the polymer. The release kinetics were observed to increase with the drug loading of the polymer particles and with the initial concentration of the initiator, provided that high enough density of the polymerization mixture is adopted. More controversial was the effect of an increase of the surfactant concentration. Even if an increase of this parameter was always accompanied by an enhancement of the interfacial area of the composite, only with the lowest investigated AIBN concentration (0.33%, w/w) this effect was associated to an acceleration of the dissolution rate. The opposite happened when the same experiments were repeated at about 3% (w/w) initiator concentration. These results were tentatively attributed to the reactive nature of the adopted stabilizer.

Polymeric composites constituted by poly(VP-co-AA) and Caffeine or Piroxicam, selected as models of Class I and Class II drugs, were prepared in a one-pot process, as in the previous case. The composite was obtained with monomer yields higher than 95% under the form of spherical sub-micron particles. Raman spectroscopy suggested that the drugs were dispersed in the polymer matrix under non-crystalline morphology at all investigated operative conditions.

The release profiles showed pH-dependency and were slower at lower pH, even if this behaviour was not so marked for Caffeine/poly(VP-co-AA) composites; this may be due to the different interactions between pharmaceutical compound and polymeric matrix, that is a condition strictly dependent on the nature of the

considered drug. However, in both cases, the release rate of drugs from the composites increased as the mole fraction of AA in the copolymers decreased.

We compared release profiles from composites prepared through precipitation and dispersion copolymerization in $scCO_2$: even if the use of the surfactant leads to a higher interfacial area of the drug-polymer composite, this was not accompanied by an increase in the dissolution rates. This experimental behaviour may be due to the fact that using the surfactant, there is a significant amount of siloxane groups on the polymeric particles and thus a high hydrophobic polymer matrix that could hamper the water absorption and the swelling of the polymer, thus leading to slower release kinetics.

Finally, the dissolution rates of Piroxicam from the tablets showed pH-dependency and were slower at lower pH (as in the case of powders); moreover, release rates of drug from tablets were slower than those from powders for all the investigated compositions.

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CHAPTER 4

BIODEGRADABLE POLYESTERS: APPLICATIONS, PROPERTIES AND REACTION MECHANISMS

4.1 Introduction

In last years the studies about medical and pharmaceutical applications of biodegradable polymeric materials have greatly increased. Such materials have to satisfy several requirements: good mechanical and physical properties, compatibility with tissues and blood, possibility of degradation to non-toxic products [1]. The ideal scenario would be to use a material that achieves a specific therapeutic task and is subsequently, over time, degraded and removed harmlessly from the body.

Biodegradation has been defined by Albertsson and Karlsson [2] as an event which takes place through the action of enzymes and/or chemical decomposition associated with living organisms (bacteria, fungi, etc.) or their secretion products. However, it is necessary to consider abiotic reactions (e.g. photodegradation, oxidation and hydrolysis) that may also alter the polymer before, during or instead of biodegradation because of environmental factors.

Current research interest in biodegradable polymers is connected with well-defined areas of use. Biodegradable plastics offer one solution to managing packaging waste. There are also agricultural uses, e.g. for controlled release of fertilizers and pesticides, applications in the automotive industry and as surfactants. However, biomedical applications of biodegradable polymers generate an enormous amount of research interest.

Synthetic biodegradable polymers are attractive candidate materials for short-term medical applications, like sutures, drug delivery devices, orthopaedic fixation

devices, wound dressings, temporary vascular grafts, stents, different types of tissue engineered grafts, etc. Due to their transient nature, biodegradable polymers do not require surgical removal after their intended application and thus can circumvent some of the problems related to the long-term safety of non-degradable implanted devices.

A biomaterial can be defined as a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body. The essential prerequisite to qualify a material as a biomaterial is biocompatibility, which is the ability of a material to perform with an appropriate host response in a specific application. The tissue response to an implant depends on many factors ranging from the chemical, physical and biological properties of the materials to the shape and structure of the implant. In the case of biodegradable biomaterials, their active biocompatibility must be demonstrated over time. The chemical, physical, mechanical and biological properties of a biodegradable material will vary with time and degradation products may have different levels of tissue compatibility compared to the starting parent material [3].

Some of the important properties of a biodegradable biomaterial can be summarized as follows:

- it should not cause a sustained inflammatory or toxic response upon implantation in the body;
- it should have acceptable shelf life;
- the degradation time of the material should match the healing or regeneration process;
- the material should have appropriate mechanical properties for the indicated application and the variation in mechanical properties with degradation should be compatible with the healing or regeneration process;
- the degradation products should be non-toxic and able to get metabolized and cleared from the body;
- the material should have appropriate permeability and processability for the intended application.

For what concern polymeric biomaterials, given the complexity and the range of applications in which they can be used, there is not just one macromolecular system available that could be considered as an ideal biomaterial. This underlines the need to investigate the developing of biodegradable materials for implant fabrication that can appropriately match the specific and unique requirements of each individual medical application.

Both synthetic and biologically derived (or natural) polymers have been extensively investigated as biodegradable biomaterials. They degrade into products that are normal metabolites or that could be completely eliminated from the body with or without further metabolic transformations; the degradation products are either excreted by the kidneys or finally eliminated as carbon dioxide and water via well-known biochemical pathways [4].

4.2 Biodegradable polymers

Biodegradation involves two complementary processes: degradation and erosion. Degradation refers to the bond cleavage during which polymer chains are converted to low molecular weight fractions; erosion refers to physical phenomena such as dissolution and diffusion of these low molecular weight fractions from the polymer matrix [5].

Depending on the mode of degradation, polymeric biomaterials can be further classified into hydrolytically and enzymatically degradable polymers. The latter option is only effectively available for naturally occurring biopolymers like polysaccharides, proteins (gelatin and collagen) and poly(β-hydroxy acids), where appropriate enzymes are available. For most biodegradable materials, especially artificial polymers, passive hydrolysis is the most important mode of degradation. There are several factors that influence the velocity of this reaction: the type of chemical bond, the pH level, the copolymer composition and the water uptake are the most important.

Polymer erosion can be of two types, bulk erosion and surface erosion. In ideal bulk erosion, material is lost from the entire polymer volume at the same time, due to

water penetrating the matrix; in this case the erosion rate depends on the total amount of the material. In surface erosion, material is lost from the polymer surface only; the matrix gets smaller, but keeps its original geometric shape. This behaviour is generally observed with hydrophobic polymers or polymers containing a hydrophobic component wherein water cannot penetrate easily into the bulk. In ideal surface erosion, the erosion rate will be proportional to the surface area [6].

Biodegradable polymers are classified into three major categories: polyesters produced by microorganisms; natural polysaccharides and other biopolymers; synthetic polymers and particularly aliphatic polyesters.

Polyesters of microorganism origin are derived from renewable bioorganic resources, such as starch and fats, and are completely biodegraded in soil, rivers and sea. Furthermore, many different types of poly(hydroxyalkanoate)s can be produced by the proper choice of carbon sources. However, their production in an industrial scale is not so efficient, preventing cost reduction and hence their extensive use.

Natural macromolecules can be considered as the first biodegradable biomaterials used clinically. Biodegradable polymers based on natural polysaccharides, particularly starch, can be produced at low cost and in a large scale. As polysaccharides themselves do not have plasticity, they are often used after chemical modifications and/or as a blend with a biodegradable synthetic polymer. However, the effective combinations of such natural and synthetic polymers are rather limited. On the other hand, synthetic biodegradable polymers have interesting features, since recent advances in polymer science and technology have made possible to design and synthesize a great variety of polymers with desirable properties.

Synthetic biomaterials are generally biologically inert, they have more predictable properties and batch-to-batch uniformity and they have the unique advantage of having tailored property profiles for specific applications, overcoming many of the disadvantages of natural polymers. Among synthetic biodegradable polymers, aliphatic polyesters are the representatives. One of the main features of aliphatic polyesters is their compatibility with the natural environment and their ability to undergo hydrolytic and biological degradation. Synthetic routes to obtain polyesters are basically polycondensation and ring-opening polymerization (ROP). The former

method requires severe experimental conditions, such as high temperature and longer reaction times to obtain high molecular weight polymers, without controlled chain length and with the constraints arising from the separation of the condensate to reach high enough chain lengths. In contrast, ROP can be carried out under milder conditions and with a lower risk of side reactions, giving an easier control of the molecular weight. This synthetic route seems the best to synthesize this kind of polymers [7,8].

4.3 Biomedical applications

Utilization of polymers as biomaterials has greatly impacted the advancement of modern medicine. Polymeric biomaterials that are biodegradable provide the significant advantage of being able to be broken down and excreted or resorbed without removal or surgical revision, after they have served their function. To fit functional demand, materials with desired physical, chemical, biological, biomechanical and degradation properties must be selected [9].

4.3.1 Medical devices

Synthetic biodegradable macromolecules have attracted considerable attention for applications in medical devices. Important properties of polymer materials used for medical devices include mechanical strength and a degradation time appropriate to the considered medical purpose. In addition, the materials should not evoke toxic or immune responses and they should be metabolized in the body after fulfilling their tasks.

The most widely studied biodegradable polymers are the poly(α-ester)s and the microbial poly(hydroxyalkanoate)s; these have been shown to be non-toxic and biocompatible both as polymers and as their degradation products, which in most cases occur naturally in the body.

Research in the first half of the 20th century with polymers synthesized from glycolic acid and other α-hydroxy acids was abandoned for further development, because the resulting polymers were too unstable for long-term industrial uses. However, this high instability, leading to biodegradation, has proven to be immensely important in medical uses.

In the 1960s, poly(glycolide) was used to prepare completely biodegradable and bioresorbable sutures. Since then, poly(glycolide), poly(lactide) and other materials such as poly(dioxanone), poly(trimethylene carbonate) copolymers, poly(ε-caprolactone) homopolymers and copolymers and poly[d,l-(lactide-co-glycolide)] have been widely used for medical devices.

Orthopaedic devices made from biodegradable materials have a very important advantage over metal or non-degradable materials: they can be used as an implant and will not necessitate a second surgical event for removal. Moreover, the biodegradation may offer other therapeutic advantages. For example, a fractured bone, fixed with a rigid and non-biodegradable stainless steel implant, has a significant tendency for re-fracture upon removal of the implant, since the bone does not feel sufficient load during the healing process, because the load is carried by the rigid stainless steel, to reach high calcification level. An implant prepared from biodegradable polymer can be engineered to degrade at a rate that will slowly transfer load to the healing bone. Many commercial orthopaedic fixation devices such as pins and rods for bone fracture fixation, and screws and plates for maxillofacial repair are made of poly(l-lactide), poly(glycolide) and other biodegradable polymers [10].

Biodegradable polymers can be used as bioresorbable coatings on stents to control the release of drugs; moreover, they are also good candidates for fully biodegradable stents because of their good mechanical performance. A multitude of new stents incorporating fully biodegradable polymers, the most commonly used being PLA and PLGA, have been investigated. These devices are fully metabolized to water and carbon dioxide, leaving in situ a bare-metal stent after all the drug has been released.

Each drug-eluting stent (DES) comprises three components: the stent platform, the active pharmacologic compound, and a drug carrier vehicle (usually a polymer), which controls the elution rate of the bioactive compound. Research for the development of the next generations of DES has focused on each of these components. Since their arrival in 2002, DES have transformed the practice of interventional cardiology by drastically reducing restenosis and the need for repeat revascularization [11].

Many disposable medical devices, such as syringes, injection pipes, surgical gloves, pads, etc., are usually made of non-degradable plastics, resulting in serious environmental and economic issues. Therefore, biodegradable polymers are promising materials for use in disposable medical devices meeting environmental friendly requirements. They have been used to prepare some disposable medical devices and will have a widening commercial application.

4.3.2 Tissue engineering

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes used to restore, maintain or improve tissue functions.

Driven by the massive shortage of donor organs and increasing frequency of age-related diseases with unsatisfactory treatments, the closely allied fields of tissue engineering and regenerative medicine aim to repair, restore or replace damaged organs. In this context bone marrow transplantation demonstrates that cell based therapies can have outstanding success in treating previously fatal and untreatable diseases. However, quite frequently, the administration of cells alone does not result in integration and repair of the host tissue.

To overcome this, a central strategy of tissue engineering is to search for correct microenvironment for the cells using biomaterials as scaffold for tissue repair, regeneration or reconstitution in vivo or ex vivo. Typically, a therapeutic cell population is seeded onto a biodegradable scaffold, which has been modified to promote cell proliferation, differentiation and organization into the desired tissue.

Alternatively biomaterials loaded with the appropriate growth factors may be administered to the site of injury to encourage infiltration of the patients' own cells to promote healing.

A successful tissue engineering implant largely depends on the role played by three-dimensional porous scaffolds. The ideal scaffolds should be biodegradable and bioabsorbable to support the replacement of new tissues. In addition, the scaffolds must be biocompatible without inflammation or immune reactions and possess proper mechanical properties to support the growth of new tissues.

Also in the case of scaffold, biodegradable synthetic polymers offer a number of advantages in tissue engineering, mainly because they can be produced under controlled conditions and therefore exhibit in general predictable and reproducible mechanical and physical properties. So they have the ability to match mechanical properties and degradation kinetics with various applications. Synthetic polymers are also attractive because they can be fabricated into various shapes with desired pore morphologic features conducive to tissue in-growth; moreover, polymers can be designed with chemical functional groups that can induce tissue in-growth. A further advantage is the control of material impurities: possible risks such as toxicity, immunogenicity and favouring of infections are lower for pure synthetic polymers with constituent monomeric units having a well-known and simple structure.

Among synthetic polymers, polyesters have been attractive for these applications because of their ease of degradation by hydrolysis of ester linkage, degradation products being resorbed through the metabolic pathways in some cases and the potential to alter degradation rates by the control of the molecular structure. Poly(l-lactide), poly(ε-caprolactone), poly(glycolide) and poly[d,l-(lactide-co-glycolide)] have excellent biocompatibility and good mechanical properties and have been licensed by the Food and Drug Administration (FDA) for in vivo applications, so they have been the most widely used materials for tissue engineering scaffolds [12,13].

4.3.3 Controlled drug delivery

Controlled drug delivery is a very important and versatile application of biodegradable polymers. It has applications not only in medicine, but also in veterinary and agrochemical fields; active ingredients ranging from pesticides to contraceptives can be delivered by sustained release with the ultimate biodegradation of the carrier medium.

Plasmatic concentration profile of the drug against time can be used to compare traditional and controlled release methods of drug. Figure 4.1 illustrates typical drug concentration profiles, showing the advantages of controlled drug delivery.

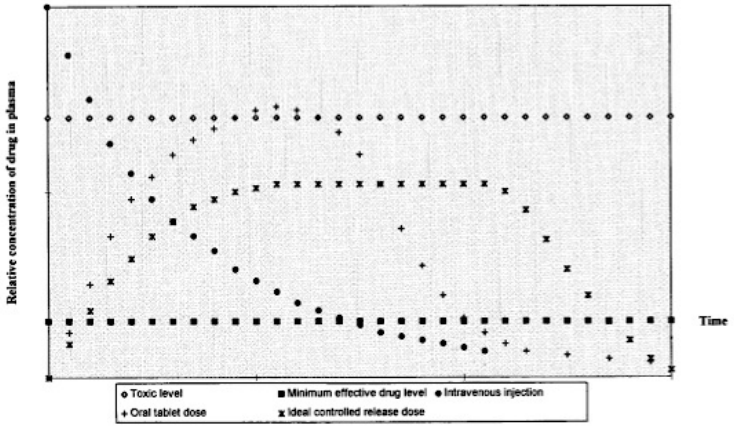


Figure 4.1. Plasma-drug concentration profiles for various methods of administration [2].

The controlled release of active ingredients aims at improving the therapeutic efficiency and increasing patient compliance. The encapsulation of a drug in a polymeric matrix allows the drug level to be maintained within a desired range, increase its therapeutic activity, decrease side effects, and reduce the number of administrations necessary (Figure 4.2). Moreover, such formulations can be used both to target the drug to the appropriate active site and to protect the active component from environmental and/or enzymatic degradation [14].

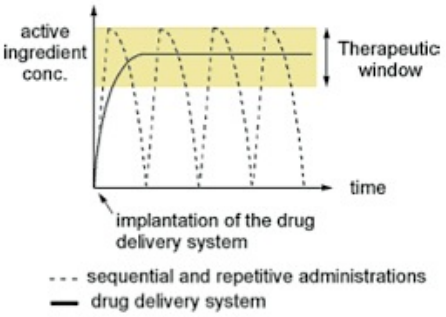


Figure 4.2. Schematic representation of the basic principle of drug delivery systems [14].

The initial drug release systems involved incorporation of the active substance into a polymer matrix, which was implanted into the patient in various ways, and a diffusion mechanism produced the maintained drug release followed by bulk hydrolysis of the polymer. Modern techniques allow the incorporation of drugs which vary in nature and molecular size (from low molecular weight steroids to polypeptides with M_w of 22000). A diffusion mechanism is too slow for the release of large molecules, so an erosion release mechanism may be required for some polymer matrices.

A wide variety of natural and synthetic biodegradable polymers have been investigated for drug targeting or prolonged drug release; however, only a few of them are actually biocompatible.

Natural biodegradable polymers like bovine serum albumin (BSA), human serum albumin (HSA), collagen, gelatin and haemoglobin have been studied for drug delivery; but the use of these natural polymers is limited due to their high costs and questionable purity. In last years, synthetic polymers have been increasingly used to deliver drugs, since, as previously mentioned, they are free from most of the problems associated with the natural polymers. Poly(amides), poly(amino acids), poly(alkyl-α-cyano acrylates), poly(esters), poly(urethanes) and poly(acrylamides) have been used to prepare various drug loaded devices; amongst them, the poly(esters) like poly(lactide) (PLA), poly(glycolide) (PGA), poly(lactide-co-

glycolide) (PLGA) and poly(caprolactone) (PCL) have generated an enormous interest due to their excellent biocompatibility and biodegradability [15].

4.3.4 Gene delivery

Gene delivery has great potential for treating various human diseases. Many carriers are non-degradable, so there is the risk of accumulation in the body, especially after repeated administration. Furthermore, most of cationic polymers show high cytotoxicity because of adverse interactions with the membranes crossed to enter the cells, causing loss of cytoplasmic proteins, permeabilization of cellular membranes and collapse of the membrane potential.

Despite the enormous potential of gene therapy, there are still numerous difficulties to be overcome before efficient clinical application is reached. Although remarkable advances have been made in identifying target structures for cancer gene therapy and synthesis or biotechnological production of nucleic acids has become feasible in larger quantities, progress is mainly hampered by the lack of a safe and efficient delivery system. A good gene carrier should be able to deliver the target gene to specific cells with high efficacy; it should also be degradable and be excreted from the body after a given time period. Consequently, there is a need for biodegradable gene delivery polymers [16].

4.3.5 Bioseparation and diagnostics applications

The development of biomedical polymers conjugated with peptide or protein has mostly focused on their use as bioactive materials in controlled drug delivery or tissue engineering. A new challenge arises in the development of materials for bioseparation and diagnostics applications. For these applications, biocompatible materials with reduced non-specific absorption and denaturation, that are able to amplify and transmit signals and that are beneficial for high-throughput screening with enhanced sensitivity and reduced sample size, are in great demand. To meet

these constraints, polymeric materials in various shapes, such as membranes, thin films, micro/nano-particles, hydrogels and micro/nano-fibers have been widely investigated [9].

4.4 Hydrolytically degradable polymers

Hydrolytically degradable polymers are characterised by a hydrolytically labile chemical bonds in their backbone that can be broken down, giving two species with one product gaining a hydrogen atom and the other gaining a hydroxyl group (Figure 4.3).

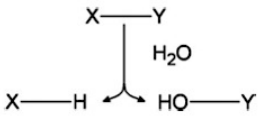


Figure 4.3. The hydrolytically sensitive bond X-Y is cleaved by a water molecule, yielding the products of X-H and HO-Y.

There are many degradable polymers that are susceptible to the presence of water, including esters, anhydrides, acetals, carbonates, amides, urethanes and phosphates. Depending on their degradation rates and erosion mechanisms, these polymeric families have the capacity to be used as biomaterials. An extensive investigation into a number of different degradable polymeric families (Table 4.1) showed that the degradation rates can vary considerably from very hydrolytically unstable (polyphosphazenes) to extremely hydrolytically stable (polyamides).

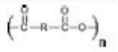
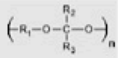
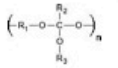
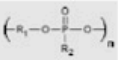
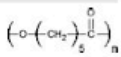
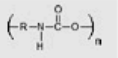
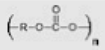
Polymer	Applications	Advantages	Disadvantages	λ, Degradation Rate Constant (s^{-1})	Structure
Polyphosphazenes	Tissue Engineering; Vaccine Adjuvant	Synthetic Flexibility; Controllable Mechanical Properties	Complex Synthesis	$4.5 \times 10^{-2} - 1.4 \times 10^{-7}$ (refs. 13 and 776)	
Polyanhydrides	Drug Delivery; Tissue Engineering	Significant Monomer Flexibility; Controllable Degradation Rates	Low-molecular Weights; Weak Mechanical Properties	$1.9 \times 10^{-3} - 9.4 \times 10^{-9}$ (refs. 17 and 777)	
Polyacetals	Drug Delivery	Mild pH Degradation Products; pH Sensitive Degradation	Low Molecular Weights; Complex Synthesis	6.4×10^{-5} (ref. 17)	
Poly(ortho esters)	Drug Delivery	Controllable Degradation Rates; pH Sensitive Degradation	Weak Mechanical Properties; Complex Synthesis	4.8×10^{-5} (ref. 17)	
Polyphosphoesters	Drug Delivery; Tissue Engineering	Biomolecule Compatibility; Highly Biocompatible Degradation Products	Complex Synthesis	1.4×10^{-6} (refs. 778 and 779)	
Polycaprolactone	Tissue Engineering	Highly Processable; Many Commercial Vendors Available	Limited Degradation	3.5×10^{-8} (ref. 27)	
Polyurethanes	Prostheses; Tissue Engineering	Mechanically Strong; Handle Physical Stresses Well	Limited Degradation; Require Copolymerization with Other Polymers	8.3×10^{-9} (ref. 780)	
Polylactide	Tissue Engineering; Drug Delivery	Highly Processable; Many Commercial Vendors Available	Limited Degradation; Highly Acidic Degradation Products	6.6×10^{-9} (ref. 17)	
Polycarbonates	Drug Delivery; Tissue Engineering; Fixators	Chemistry-Dependent Mechanical Properties; Surface Eroding	Limited Degradation; Require Copolymerization with Other Polymers	4.1×10^{-10} (ref. 285)	
Polyamides	Drug Delivery	Conjugatable Side Group; Highly Biocompatible Degradation Products	Very Limited Degradation; Charge Induced Toxicity	2.6×10^{-13} (ref. 17)	

Table 4.1. Summary of different polymeric families' applications, advantages, disadvantages, degradation rate and structure [17].

Degradation rates are correlated with other factors determining how a degradable polymeric biomaterial will erode, such as water diffusion in the macromolecular matrix, monomer solubility and diffusion, and device geometry and size.

Erosion can occur as surface erosion, bulk erosion or a combination of the two. In surface erosion, the rate of polymer degradation and mass relief at the water-device interface is much greater than the rate at which water diffuses into the bulk of the material; this leads to a device that degrades almost entirely at its surface. Bulk erosion occurs when water diffusion is much faster than degradation, leading to

mass loss distributed throughout the bulk of the material. These classifications are extremely important in determining which material is best for a desired application. For example, in controlled drug delivery, a material that can undergo surface erosion may be desired, because a stable release can be maintained and kinetics can be more easily tailored. Instead in tissue engineering, for which there is the requiring of a permeable membrane, bulk eroding materials would allow for necessary hydrolytic diffusion.

Poly(α-esters) contain a hydrolytically labile aliphatic ester bond in their backbone. Many polyesters are commercially available and they are theoretically degradable, but only polyesters with reasonably short aliphatic chains can be utilized for biomedical applications. Although these polymers are often mildly hydrophobic, ester bond stability causes them to undergo bulk erosion [3,17].

Poly(α-esters) comprise the earliest and most extensively investigated class of biodegradable polymers. The uniqueness of this class of polymers lies in its immense diversity and synthetic versatility. Among this class, the most extensively investigated polymers are poly(glycolide), poly(lactide), poly(ε-caprolactone) and their copolymers.

4.4.1 Polyglycolide

Polyglycolide or poly(glycolic acid) (PGA) was one of the first biodegradable synthetic polymers investigated for biomedical applications (Figure 4.4).

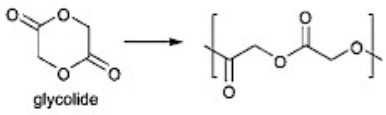


Figure 4.4. Chemical structure of polyglycolide.

It is characterised by a high crystallinity (45-55%) and therefore exhibits a high tensile modulus with very low solubility in organic solvents; its glass transition

temperature ranges from 35 to 40°C, while its melting point is greater than 200°C. Even if it has low solubility, this polymer has been produced into a variety of forms and structures.

Polyglycolide was initially investigated for developing resorbable sutures, thanks to its excellent mechanical properties and fiber forming ability. The first biodegradable synthetic suture called DEXON®, approved by the United States Food and Drug Administration (FDA), has been actively used since 1970. Due to its good mechanical properties, from 1984 to 1996, PGA was marketed as an internal bone pin under the name of Biofix®; but since 1996 Biofix® has been converted to a poly(l-lactide) base for better long-term stability.

Because of PGA's rapid degradation and insolubility in many common solvents, limited research has been conducted with PGA-based drug delivery devices. Instead, most recent research has focused on short-term tissue engineering scaffolds and the utilization of PGA as a filler material coupled with other degradable polymer networks.

Polyglycolide is a bulk degrading polymer: it loses its strength in 1-2 months when hydrolysed and its mass within 6-12 months. In the body, PGA is broken down into glycine, which can be excreted in the urine or converted into carbon dioxide and water via the citric acid cycle [3].

However, the biomedical applications of polyglycolide are limited, due to the high rate of degradation, acidic degradation products and low solubility. Therefore, several copolymers containing glycolide units are being developed to overcome the inherent disadvantages of the homopolymer [17,18].

4.4.2 Polylactide

Lactide is a chiral molecule and exists in two optically active forms, l-lactide and d-lactide. The polymerization of these monomers leads to the formation of semi-cristalline polymers; the polymerization of racemic (d,l)-lactide and meso-lactide, however, results in the formation of amorphous polymers.

Polylactides come in four forms: poly(l-lactide) (PLLA), poly(d-lactide) (PDLA), poly(d,l-lactide) (PDLLA) and meso-polylactide (Figures 4.5).

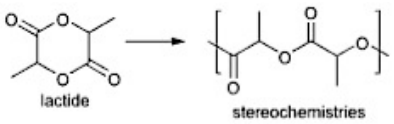


Figure 4.5 (a). Chemical structure of polylactide.

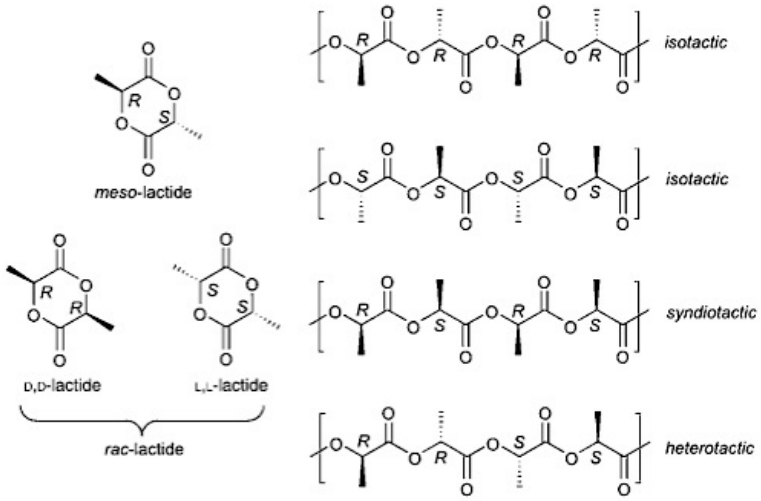


Figure 4.5 (b). Stereochemical possibilities observed in polylactide synthesis.

PLLA and PDLLA are used in biomedical applications and thus they have been extensively studied. The additional methyl group in PLA causes the polymer to be much more hydrophobic and stable against hydrolysis than PGA.

Polylactide undergoes hydrolytic degradation via the bulk erosion mechanism; it degrades into lactic acid, a normal human metabolic by-product, which is broken down into water and carbon dioxide via the citric acid cycle.

Poly(l-lactide) has a crystallinity of about 37%, depending on the molecular weight and polymer processing parameters; it is characterised by a glass transition temperature of 60-65°C and a melting temperature of approximately 175°C. Poly(l-

lactide) is a slowly-degrading polymer compared to polyglycolide, has good tensile strength, low extension and high modulus. Because of the slow degradation time, limited research has been conducted into drug delivery by PLLA systems alone; instead, it has been considered an ideal biomaterial for orthopaedic fixation devices. Some of the approved PLLA-based orthopaedic products are: the Phantom Soft Thread Soft Tissue Fixation Screw®, Phantom Suture Anchor® (DePuy), Full Thread Bio Interference Screw® (Arthrex), BioScrew®, Bio-Anchor®, Meniscal Stinger® (Linvatec) and the Clearfix Meniscal Dart® (Innovasive Devices). PLLA was also used as fibre, when FDA approved in 1971 its use for an improved suture over DEXON®. Due to the high strength of PLLA fibres, it has been investigated as material for the production of scaffolds [19,20]. Some PLLA fibre-based devices are currently under investigation as long-term blood vessel conduits. Recently, an injectable form of PLLA (Sculptra®) has been approved by the FDA for the restoration or correction of facial fat loss or lipoatrophy in people with the human immunodeficiency virus [3].

The degradation rate of PLLA is very low: in fact, it is reported that high molecular weight PLLA takes greater than 5 years to be completely resorbed in vivo [10,21]. The rate of degradation, however, depends on the polymer crystallinity and on the porosity of the matrix. The polymer loses its strength in approximately 6 months when hydrolysed, but no significant changes in mass will occur for a very long time. To reduce degradation time, investigators have either developed modification techniques or have blended or copolymerized PLLA with other degradable polymers. Several co-polymers of l-lactides with glycolides or d,l-lactides are currently under investigation for the development of polymers with better property modulation.

Poly(d,l-lactide) is an amorphous polymer and has a glass transition temperature of 55-60°C. Due to its amorphous nature, the polymer shows much lower strength compared to poly(l-lactide); this polymer loses its strength within 1–2 months when hydrolysed and undergoes a loss in mass within 12–16 months [22]. Being a low strength polymer with faster degradation rate compared to PLLA, it is a

preferred candidate for developing drug delivery vehicles and as low strength scaffolding material for tissue regeneration.

4.4.3 Poly(lactide-co-glycolide)

The most investigated degradable polymer for biomedical applications is the random copolymer of PLA (both l- and d,l-lactide forms) and PGA, known as poly(lactide-co-glycolide) (PLGA): it has been used in sutures, drug delivery devices and tissue engineering scaffolds. One particular advantage is that, because PLA and PGA have significantly different properties, careful choice of copolymer composition allows for the optimization of PLGA properties for the specific application: with 25–75% lactide composition PLGA forms amorphous polymers, due to the disruption of the regularity of the polymer chain by the other monomer. These copolymers are very hydrolytically unstable compared with the more stable homopolymers; this is evident in the degradation times of 50:50 PLGA, 75:25 PLGA, and 85:15 PLGA being 1–2 months, 4–5 months, and 5–6 months, respectively [10].

PLGA copolymers at different ratios of the two monomers have been commercially developed and are being investigated for a wide range of biomedical applications. PuraSorb®PLG is a semicrystalline bioresorbable copolymer of l-lactide and glycolide, with a monomer ratio of 80L:20G. The multifilament suture Vicryl® was used since 1974 and was constituted by a copolymer containing 90% glycolic acid and 10% l-lactic acid; a modified version of the suture, Vicryl Rapid®, is currently on the market, which is an irradiated version of the suture, in order to increase the rate of degradation. Panacryl® is another commercially developed suture from the copolymer, with a higher LA/GA ratio in order to decrease the rate of degradation. Several copolymers have subsequently been developed for the fabrication of monofilament sutures. Other applications of PLGA are in the form of meshes (Vicryl Mesh®), suture reinforcements and skin replacement materials [3].

PLGA has been shown to undergo bulk erosion; the rate of degradation depends on the LA/GA ratio, the molecular weight, the shape and the structure of the matrix.

The major popularity of these biocompatible copolymers can be attributed in part to their approval by the Food and Drug Administration (FDA) for use in humans, its good processability which enables fabrication of a variety of structures and forms, controllable degradation rates and their success as biodegradable sutures compared to the earlier suture materials.

Several drug delivery vehicles composed of PLGA have been developed for the controlled release of drugs or proteins. Depending on the composition of the PLGA used, it is reported that the drug or protein have different interactions with the base polymer, resulting in rapid or prolonged release profiles. Moreover, if PLGA is used as a protein delivery vehicle, there is the possibility of protein denaturation, due to the bulk degradation mechanism of the polymer that leads to the formation of acidic products. This has led to the search for surface eroding polymers as ideal candidates for developing drug delivery vehicles [3,17].

PLGA demonstrates good cell adhesion and proliferation properties, making it a potential candidate for tissue engineering applications. Various studies have been performed, using micro- and nano-fabrication techniques to form three-dimensional scaffolds based on PLGA [17].

4.4.4 Polycaprolactone

Polycaprolactone (PCL) is a semicrystalline polyester (Figure 4.6) with great organic solvent solubility, a melting temperature of 55-60°C and glass transition temperature of about 60°C; it has the ability to form miscible blends with wide range of polymers.

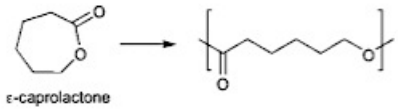


Figure 4.6. Chemical structure of polycaprolactone.

The polymer undergoes hydrolytic degradation, with a rate rather slow (2–3 years). Due to the slow degradation, high permeability to many drugs and non-toxicity, PCL was initially investigated as a long-term drug/vaccine delivery vehicle. For example, Capronor® is a commercial contraceptive PCL product that is able to deliver levonorgestrel in vivo for over a year and has been on the market for over 25 years [17].

Due to its excellent biocompatibility, PCL has been extensively investigated as scaffolds for tissue engineering. It has low tensile strength, but very high elongation at breakage, making it a very good elastic biomaterial.

Extensive research is concentrated to develop various micro- and nano-sized drug delivery vehicles based on PCL; but the degradation rate (2-3 years) is a significant issue for pure PCL products to be FDA approved for this use. So several copolymeric systems containing PCL have been investigated to improve the properties of the native polymer. Copolymers of ε-caprolactone with d,l-lactide have yielded materials with more rapid degradation rates. Similarly, a copolymer of ε-caprolactone and glycolide resulted in fibers that were less stiff compared to those made of polyglycolide and are currently on the market as a monofilament suture (MONACRYL®). Another bioresorbable multiblock copolymer composed of ε-caprolactone, glycolide, lactide and poly(ethylene glycol) units has been developed as a drug delivery vehicle for small and medium sized biologically active molecules (SynBiosys®) [3].

4.5 Synthesis of polyesters by polycondensation

Polyesters can be synthesised via the direct polycondensation of alcohols and acids. The pioneering studies of Carothers on polycondensation in the 1930s established a firm base for systematic studies of mechanisms of aliphatic polyester formation. Various catalysts and coupling reagents may be used, but tipically the polyesters formed in this manner have low and uncontrolled molecular weight and are not suitable for biomedical applications. Thus, the high melting points inherent to aliphatic polyesters, together with the difficulty in obtaining high molecular

weight polymers, had prevented a wide usage of aliphatic polyesters as polymeric materials for a long time.

The step-growth polymerization technique relies on the condensation of hydroxyl-acids or of mixtures of diacids and diols (Figure 4.7). The major drawbacks of this polycondensation mechanism are the high temperatures and long reaction times generally required that favour the side reactions, together with the deleterious effect on the molecular weight of any short deviation from the reaction stoichiometry. Such reaction is also limited to equilibrium: water must thus be removed from the polymerization medium to increase the conversion and the molecular weight [23].

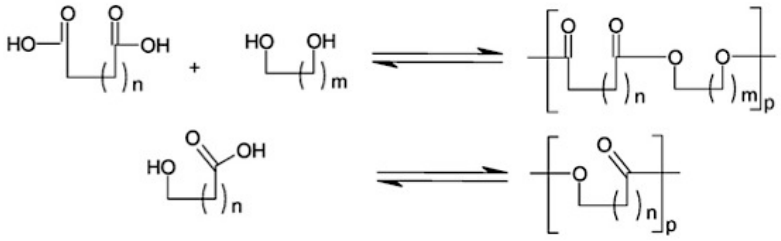


Figure 4.7. Synthesis of aliphatic polyesters by step-growth polycondensation [23].

4.5.1 Thermodynamics and kinetics

Polyesterification may be based on homo-polycondensation of hydroxycarboxylic acid or hetero-polycondensation of a diol with dicarboxylic acid. This is a reversible process and, in order to prepare a high molar mass polymer, at least two important prerequisites must be fulfilled:

- the equilibrium constant of condensation has to be high enough;
- in the case of hetero-polycondensation, 1:1 stoichiometry must be strictly obeyed.

The degree of polymerization depends on the equilibrium constant of condensation and on the degree of conversion of the reactive groups. If the equilibrium constant is not sufficiently high (as is generally in the case for polycondensation of alcohols

with carboxylic acids), the condensation side products (usually water) must be removed from the reaction mixture in order to obtain a reasonably high degree of polymerization. Furthermore, for majority of polyesters a high degree of polymerization is needed in order to obtain the required physical properties; this corresponds to degree of conversion of the reactive groups not less than 0,99 and in turn would require an high equilibrium constant of condensation.

Such a situation creates one of the practical limitations in the synthesis of polyesters directly by polycondensation. In addition, high viscosity of the system at higher degrees of conversion hinders removal of the low molar mass byproducts, such as water.

Another important factor is related to the stoichiometry of the substrates. Even if in the feed the 1:1 stoichiometry is secured, one of the components may be partially lost during the polycondensation process; this may be due to the volatilization, since high reaction temperatures are often used, or to reactant losses by side reactions.

The rate of polycondensation agrees with simple kinetic expressions throughout the entire process. Changes in the reaction mixture properties, such as viscosity or dielectric constant, influence the course of the reaction, even if the assumption of equal reactivity of functional groups is obeyed (independently on the material chain length). However, polyesterification is a relatively slow process: this means that long reaction times are required to achieve high conversion and high molecular weight. Furthermore, this process may be accompanied by the appearance of a certain fraction of macrocyclic products; but usually cyclization is considered as a side reaction of a minor importance, because critical concentrations of macrocycles are low [7].

4.6 Polyesters by ring-opening polymerization (ROP)

Ring-opening polymerization of lactones is an effective method for synthesizing biodegradable polyesters. It has some advantages over polycondensation of diacids (or their derivatives) with diols: the former does not require equimolar balance of functional groups, that is essential for the synthesis of

high molecular weight polymer by the latter; in addition, ring-opening polymerization occurs under milder reaction conditions. Thanks to ring-opening polymerizations, high molecular weight polyesters can be easily prepared under mild conditions from lactones of different ring-size, substituted or not by functional groups. Due to the kinetic control of the entire process, it's possible to obtain a more narrow molar mass distribution respect to that obtained by polycondensation, with a polydispersity index not much higher than 1.

A polyester is formed when cyclic esters are reacted with a catalyst or initiator; Figure 4.8 presents the reaction pathway for the ring-opening polymerization of cyclic esters.

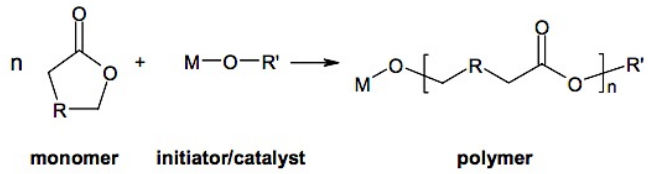


Figure 4.8. Schematic representation of the ring-opening polymerization of a cyclic ester. R=$(CH_2)_{0-3}$ and/or (CHR") [24].

Each macromolecule formed will generally contain one chain end terminated with a functional group originating from the termination reaction and one terminus end-capped with a functional group originating from the initiator. By altering the catalyst or initiator and the termination reaction, the nature of the functional groups can be varied to fit the application of the polymer. The types of initiator and end-group play an important role in both the thermal stability and hydrolytic stability of the resulting polyester [24].

Under rather mild conditions, high-molecular weight aliphatic polyesters of low polydispersity can be prepared in short time. Problems associated with condensation polymerization, such as the need for exact stoichiometry, high reaction temperatures and the removal of low molecular weight by-products (for example, water) are excluded in ROP.

4.6.1 Thermodynamics and kinetics

In ROP conducted at constant pressure, the change of enthalpy is mostly due to the monomer ring strain energy, when the ring has been opened, if specific interactions monomer-polymer-solvent can be neglected. The major contributions to the ring strain come from: deviation from the nondistorted bond angle values, bond stretching and/or compression, repulsion between eclipsed hydrogen atoms and nonbonding interactions between substituents (angular, conformational and transannular strain, respectively). Moreover, polymerization of the majority of monomers is accompanied by an entropy decrease. Polymerization is permitted thermodynamically when the enthalpic contribution into free energy prevails; therefore, the higher the ring strain, the lower the resulting monomer concentration at equilibrium. But thermodynamic polymerizability cannot be taken as a direct measure of monomer reactivity, because it can be related to the structure of active species. Comparison of the lactone ring sizes with the relative polymerization rates shows that the larger the lactone ring, the lower is its reactivity. It can be expected that in the transition state of propagation, the ring strain is partially released and the resulting enthalpy of activation is lower for strained monomers in comparison with the nonstrained ones. This is the main reason why the reactivity of lactones decreases as their sizes are increased, with a constant value eventually being reached for larger rings. Other factors, such as electrophilicity of the monomer acyl atom or steric hindrance, hampering approach of the active species to the lactone ester group, probably play a minor role [7].

The ROP proceeds mainly via two major polymerization mechanisms depending on the used organometallics. Some of them acts as catalysts, and activate the monomer by complexation with the carbonyl group (Figure 4.9); polymerization is then initiated by any nucleophile (water or alcohol) present in the polymerization medium as impurities or as compound added on purpose.

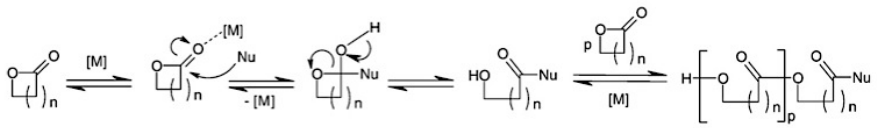


Figure 4.9. Ring-opening polymerization of lactones catalysed by organometallic species [M] in presence of nucleophiles (Nu) [23].

In the second mechanism, the organometallic plays the role of initiator and the polymerization proceeds through a "coordination-insertion" mechanism (Figure 4.10). Metal alkoxides are typical initiators, which first coordinates the carbonyl of the monomer, followed by the cleavage of the acyl–oxygen bond of the monomer and simultaneous insertion into the metal alkoxide bond.

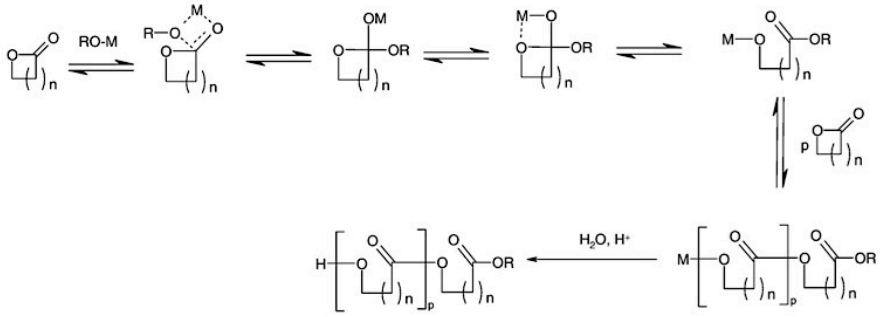


Figure 4.10. Ring-opening polymerization of lactones by the "coordination-insertion" mechanism [23].

Whenever the polymer repeating units contain the same heteroatoms as those present in monomers and reacting in the propagation step, then chain transfer to polymer with chain scission occurs.

Intermolecular transesterification is the process by which the metal alkoxide of one chain attacks the carbonyl of another growing chain instead of inserting a monomer. This kind of reactions modifies the sequences of copolylactones and prevent the formation of block copolymers.

Intramolecular transesterification reactions, back-biting, cause degradation of the polymer chain and the formation of cyclic oligomers of various lengths. Both types of transesterification reactions broaden the molecular weight distribution. As displayed in Figure 4.11, each intramolecular transesterification randomly breaks the polymer chain; an attack on the polymer chain leads to a free residual polymer and a new randomized, modified polymer.

Chain transfer can occur also by having water or alcohol present: in this case, the polymer chain is eliminated from the metal center and transferred onto the alcohol or water, where it remains dormant at a constant molecular weight while the metal center continues to propagate a new polymer chain. Subsequent chain transfer leads to reactivation of the dormant chain toward propagation [14].

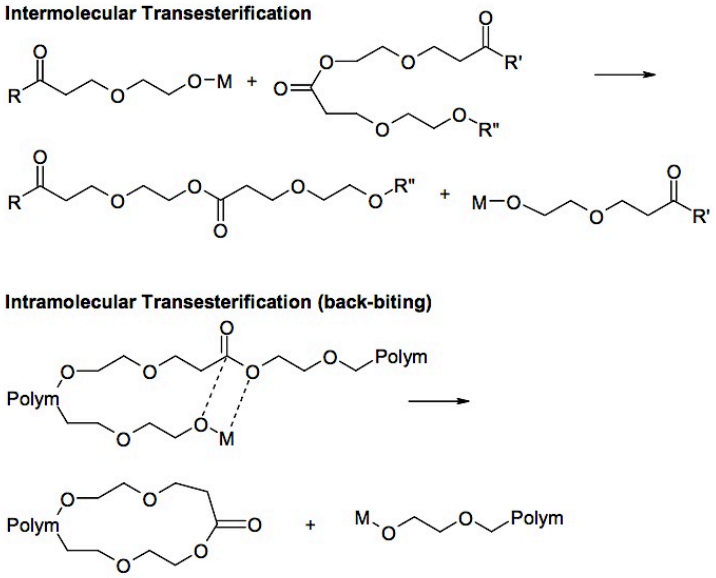


Figure 4.11. Reaction scheme for intermolecular and intramolecular transesterifications reactions [24].

Parameters that influence the number of transesterifications are temperature, reaction time, type and concentration of catalyst or initiator. High temperatures and high reaction times lead to much more transesterification reactions.

Chain transfer is often a beneficial side reaction and used to control the molecular weight of the polymer. According to IUPAC, a living polymerization is characterized by the absence of any irreversible termination event. In these processes, the polymer grows as long as there is monomer present and, with the addition of a transfer agent such as an alcohol, the lengths of the chains can be controlled. Each alcohol grows a polymer chain, and if the rate of exchange between transfer agents is more rapid than the rate of ring enchainment, then a narrow polydispersity is maintained.

Depending on the initiator or catalyst, the polymerization proceeds according to three different major reaction mechanisms: cationic, anionic or "coordination-insertion" mechanism.

4.6.2 Cationic ring-opening polymerization

The cationic ring-opening polymerization involves the formation of a positively charged species, which is subsequently attacked by a monomer; the attack results in a ring-opening of the positively charged species (Figure 4.12).

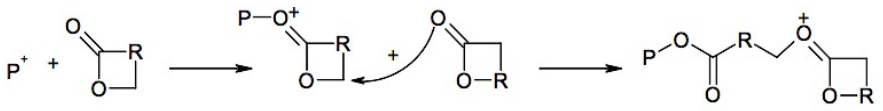


Figure 4.12. The reaction pathway for the ring-opening polymerization of cyclic ester by cationic initiation [24].

The cationic polymerization is difficult to control and often only low-molecular weight polymers are formed.

Among the cyclic esters, 4-, 6- and 7-membered rings form polyesters when reacted with cationic catalysts [24].

4.6.3 Anionic ring-opening polymerization

Anionic ring-opening polymerization of cyclic ester monomers takes place by the nucleophilic attack of a negatively charged initiator on the carbonyl carbon or on the carbon atom adjacent to the acyl-oxygen, resulting in a linear polyester (Figure 4.13). The propagating species is negatively charged and counter-balanced with a positive ion. Depending on the nature of the ionic propagating chain end and the solvent, the reacting complex varies from completely ionic to almost covalent [24].

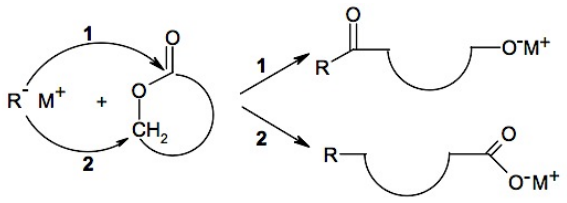


Figure 4.13. The reaction pathway for the ring-opening polymerization of a cyclic ester by anionic initiation. Ring-opening of monomer by 1) acyl-oxygen bond cleavage and 2) alkyl-oxygen bond cleavage [24].

A problem associated with the anionic ROP is the extensive back-biting, and in some cases only polyesters of low molecular weight are achieved. Thus, although higher activities might be anticipated for anionic promoters that typically display strong nucleophilic and/or basic character, the deleterious contribution of transesterification and racemization reactions might be expected to be significantly more important when naked or loosely bonded anionic species are involved [14].

4.6.4 "Coordination-insertion" ring-opening polymerization

The pseudo-anionic ring-opening polymerization is often referred to as "coordination-insertion" ROP, since the propagation is thought to proceed by coordination of the monomer to the active species and then insertion of the monomer into the metal-oxygen bond by rearrangement of the electrons (Figure 4.14).

The first step consists of the coordination of the monomer to the Lewis-acidic metal centre. The monomer subsequently inserts into one of the aluminum-alkoxide bonds via nucleophilic addition of the alkoxy group on the carbonyl carbon, followed by ring opening via acyl-oxygen cleavage. Hydrolysis of the active metal-alkoxide bond leads to the formation of a hydroxyl end-group, while the second chain end is capped with an ester [14].

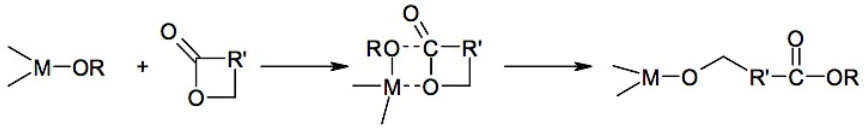


Figure 4.14. The proposed reaction pathway for the ring-opening polymerization of a cyclic ester by the coordination-insertion mechanism [24].

The coordination-insertion type of polymerization has been thoroughly investigated since it may yield well-defined polyesters through living polymerization. When two monomers of similar reactivity are used, block copolymers can be formed by sequential addition to the "living" system.

In coordination-insertion polymerizations, the efficiency of the molecular-weight control depends from the initiation and propagation reactions, but also from the extent of transesterification side reactions. These transesterification reactions can occur both intramolecularly (backbiting leading to macrocyclic structures and shorter chains) and intermolecularly (chain redistributions). The polymerization/depolymerization equilibrium should also be taken into account as a particular case of intramolecular transesterification reaction. All of these side

reactions result in broader molecular-weight distributions, sometimes making the molecular weights of the resulting polymers irreproducible. The extent of these undesirable transesterification reactions strongly depends on the metallic initiator [14].

4.7 Metal-based initiators or catalysts for ROP

Ring-opening polymerization of cyclic esters has been studied using various transition metal, lanthanide and main group metal initiators. The polymerization is achieved most often using a catalyst in the form of LM-X, where L is an inert ligand set, M is the metal center, and X is the initiating group. The initiating group must be sufficiently nucleophilic in order to attack the carbonyl bond, with the most commonly studied X groups being the alkoxides, carbonates and oxides. Many reactions catalysed by metal complexes are highly specific and, by careful selection of metal and ligands, reactions can be generated to form a desired polymer structure.

4.7.1 Aluminum alkoxides

The high selectivity of aluminum alkoxides is the major reason for their choice to produce well-defined polyesters. The polymerization rate is high and the molecular weight is very well controlled; both chain end-groups are well-defined and predictable. Indeed, the polyester chains are α-end-capped by an ester group RO–C(=O), where RO is the alkoxy group of the initiator. Because a huge variety of aluminum alkoxides can be prepared by reaction of triethylaluminum with an alcohol ROH, the choice of the α-end-group is very flexible. An excess of triethylaluminum is usually used to form the monoalkoxide initiator since the di- and tri-alkoxides strongly aggregate and are thus not useful in the aliphatic polyester synthesis.

A two-step “coordination-insertion” mechanism has been proposed, which consists of lactone complexation to the initiator, followed by monomer insertion into the

metal-oxygen bond. Hydrolysis of the active metal-alkoxide bond leads to the formation of a hydroxyl end-group; the second chain end is capped with an ester, carrying the alkoxy radical of the initiator. The propagation is characterized by the almost total absence of side-reactions such as transesterification reactions, at least until complete monomer conversion has occurred.

The controlled synthesis of high molecular weight polyesters requires the complete absence, or at least severe limitation, of side inter- and intramolecular transesterification reactions, which perturb chain propagation and broaden the molecular weight distribution. The very good control imparted to ring-opening polymerization by aluminum alkoxides is a unique platform for the macromolecular engineering of aliphatic polyesters, making comb-like, star-shaped, graft and hyperbranched (co)polyesters available [25].

For safety reasons, residual aluminum must be removed from the material before use in food or biomedical applications.

Most metal alkoxides are aggregated in solution and, as a result, an induction period during which the initiator is rearranged to form the active species often characterizes the polymerization. The type and size of the aggregates depend on the solvent polarity, the nature of the substituents, and the presence of coordinative ligands such as amines and alcohols. The groups involved in coordinative aggregation are not active in propagation.

Moreover, systems have been developed where the aluminum alkoxide is covalently bonded to solid porous silica. This system takes advantage of the exchange reaction between the alkoxide and the hydroxyl-terminated free molecules to produce a catalytic process, to produce a larger number of polymer chains than aluminum complexes present. The initiator/catalyst used can easily be recovered by filtration and recycled. In addition, the polymers obtained are free from metal residues [26].

Tin (IV) alkoxides can be used as substitutes for aluminum alkoxides, although the polydispersity of the chains is higher (~1.5). The cyclic tin-alkoxides were originally studied because of their resistance towards hydrolysis.

4.7.2 Tin octoate

Tin compounds have proven to be an extremely versatile and highly efficient group of catalysts or initiators. The most popular initiator for ring-opening polymerization of aliphatic polyesters is tin(II) bis-(2-ethylhexanoate), also referred as tin octoate ($Sn(Otc)_2$) (Figure 4.15). It is accepted as a food additive by the US Food and Drug Agency (FDA) and thus no purification of the polymers is needed for applications such as packaging. However, tin compounds have the disadvantage of a significant cytotoxicity and thus it would be desirable to find a suitable substitute for pharmaceutical or biomedical applications. As a result, several research groups are exploring the usefulness of catalysts/initiators based on less or non toxic metals, such as magnesium, calcium, iron, zinc, or bismuth [27,28].

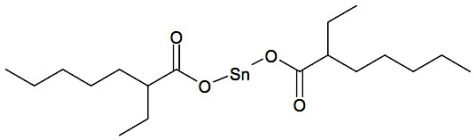


Figure 4.15. Chemical structure of tin octoate.

Tin octoate is the most often-used initiating compound in the polymerization of cyclic esters, but also the least understood and several mechanisms of initiation have been proposed. In the literature, two major ROP mechanisms are proposed: the activated monomer mechanism and the coordination-insertion mechanism. Both mechanisms are thought to be alcohol-initiated, since the degree of polymerization is clearly dependent on the monomer-to-alcohol ratio, and the end groups of the polymer have hydroxyl functionalities. The coordination-insertion mechanism provides an explanation of the highly stereoregular polymers obtained with $Sn(Oct)_2$.

In the activated monomer mechanism, $Sn(Oct)_2$ forms a donor-acceptor complex with a monomer; this activates the monomer toward alcohol attack. A hydroxyl-ended macromolecule attacks the carbonyl carbon and ring-opening proceeds. Thus, if alcohol or water (ROH) are present, then initiation and propagation involve

reaction of three simultaneously interacting compounds: for example, in propagation, a macromolecule fitted with a terminal -OH group, monomer, and $Sn(Oct)_2$. In every propagation step, a macromolecule with an -OH at its end is reformed as a longer one by one monomer unit and one intact $Sn(Oct)_2$, either "free" or complexed, emerges from this step. It is enough to stress that, according to this mechanism, $Sn(Oct)_2$ survives during polymerization as such and is not converted into any other species chemically different from $Sn(Oct)_2$. Thus, in this case, tin octoate is merely a catalyst.

In the coordination-insertion mechanism, a compound containing a hydroxide group (purposely added or adventitiously present in the reacting mixture) is believed to react with $Sn(Oct)_2$ to form the actual initiator, that is an alkoxide covalently bound to tin. Then the elementary reaction of the polyester chain growth was assumed to proceed via monomer insertion as for the other metal alkoxide active centres [29-31].

It must be stressed that, in spite of the existing differences, all of these mechanisms assume necessity of some contribution of hydroxyl group containing compound (ROH), namely H_2O, alcohol or hydroxycarboxylic acid. The most promising mechanism is a coordination-insertion mechanism where a hydroxyl functional group is thought to coordinate to $Sn(Oct)_2$, forming the initiating tin-complex. $Sn(Oct)_2$ is neither a catalyst nor initiator exclusively by itself, it reacts with an alcohol added in order to produce the true initiator. The rate of polymerization increases with added alcohol as long as there is sufficient $Sn(Oct)_2$ in the system to provide the initiator. The deliberate addition of a predetermined amount of alcohol to the polymerization medium is an effective way to control the molecular weight by the monomer-to-alcohol molar ratio [32].

Tin octoate is also efficient in copolymerization of various lactones. It is a strong transesterification agent and resulting copolymers normally have a randomized microstructure. Consequently, the reaction mechanism is very difficult to determine by kinetics studies or from analysis of end groups and reaction products. It is also difficult to elucidate the structure of the actual initiating and propagating species of ROP by spectroscopic or chromatographic methods. An increase in reaction

temperature or reaction time increases the amount of transesterification reactions. Playing on the composition of such copolymers allows tailoring their properties, that are very important for their applications. These systems are of particular importance because $Sn(Oct)_2$ is the most often used initiator in preparing various grafts, block copolymers and multibranched products starting from polymers with hydroxyl groups and using cyclic esters [30].

However, as mentioned before, even if tolerated in food regulations, due to tin toxicity, it has to be avoided in materials used for biomedical application; when added in stoichiometric amounts for polymerization, careful extraction from the obtained polymer is required before exploitation in the medical field.

4.8 Supported-metal catalysed ROP

Whenever toxic metallic catalyst is a must, the heterogeneous polymerization by using metal alkoxides grafted onto a solid porous supports is a valuable strategy to produce polyesters free from metallic residues. It was found that the solid support not only has a mechanical function, but also is a chemical ligand of the active centre, which modifies the intrinsic reactivity of the active centres. Therefore, a synergy appears, brought about by the combination of the support and the active centre itself, which allows a more rapid polymerization of monomers.

In addition, these heterogeneous initiators/catalysts can be used in batch production, but their easy recycling/reactivation is promising for continuous processes, using a fixed-bed reactor. In that case, the transfer reaction on the solid support is the key step, since it allows the polymer chains to move along the reactor. This concept of a continuous polymerization process is different from that using a continuous feed of both monomer and initiator, without or with the presence of additional alcohol molecules, since the active centres are grafted onto a solid support. One of the main advantages of this process is to obtain polymers directly free from any solid residue. In addition, the use of another alcohol allows to change easily the functionality of the polymer chains.

Al, Nd, Y, Sm, Zr metal alkoxides have been grafted onto silica or alumina and successfully used for the synthesis of poly-ε-caprolactone. Nevertheless, the alcohol that is released in the reaction medium can react with the silanol groups of silica and form water. Therefore, part of the active species can be lost by hydrolysis [33].

4.9 Enzymatic polymerization

As stressed before, the availability of aliphatic polyesters uncontaminated with possible toxic metallic residues is essential for biomedical applications. Enzyme-catalysed ring-opening polymerization is one of the most promising tools, because it avoids the use of organometallic catalysts.

A vast number of enzymes catalyse metabolic reactions via biosynthetic pathways in living cells; natural polymers such as polysaccharides, proteins, polyesters, etc. are synthesized in nature by enzymes. The need to develop environment-friendly processes and products has culminated in alternative routes for the generation of synthetic polymers and in-vitro enzyme catalysis is one of the most promising options. The use of enzymes for in-vitro polymer synthesis has been actively pursued in the last decade. Enzymatic polymerization is an in-vitro polymerization via non-biosynthetic pathways catalysed by an isolated enzyme. This approach was found to be effective and useful in cellulose synthesis and was then expanded to the synthesis of other polysaccharides as well as polyesters, polyaromatics, etc. [34].

The key for enzymatic polymerization to proceed lays in the combination of the substrate monomer and the enzyme.

Enzyme catalysis has provided a new synthetic strategy for useful polymers, most of which are otherwise difficult to produce by conventional chemical catalysts. It may greatly contribute to global sustainability by using non-petrochemical renewable resources as starting substrates for functional polymeric materials. On the other hand enzymes are natural catalysts and therefore better candidates for ROP. The enzymatic polymerization can thus be regarded as an environment-friendly synthetic process for polymeric materials, providing a good example of “green polymer chemistry”.

Thus, enzyme catalysed synthesis of polyesters is a very important tool, with the aim to get high molecular mass polymers/copolymers with desired molecular architecture to meet the specific and stringent requirements of biomedical field.

4.9.1 Enzyme catalysed polymerization vs chemical polymerizations

Enzyme catalysis offers several advantages over chemical catalysis. Biodegradable polyesters synthesized by chemical means generally use organometallic catalysts which have to be completely removed for biomedical applications. The organometallic catalysts used for the ROP of lactones or lactides are based on derivatives of metals such as Zn, Al, Sn or Ge, which may be toxic in nature. This is of concern in biomedical applications of these polymers as it is difficult to remove the metallic impurities and residues from these polyesters, which may become concentrated within matrix remnants after degradation of the polymer. In addition, extremely pure monomers and anhydrous conditions are required for carrying out polymerizations by chemical means.

Enzyme catalysed polymerization, on the other hand, is a benign process and can be carried out at low temperature.

The benefits of enzyme-catalysed polymerization are:

- Enzyme-catalysed reactions proceed under mild reaction conditions (temperature, pressure, pH, etc.) with high enantio- and regio-selectivity.
- Enzymes are derived from renewable resources; they are recyclable, eco-friendly, non-toxic materials and can be easily separated from the synthesized polymers. However, the need for their complete removal from the polymers is not so stringent.
- Enzymes can be used in bulk, organic media and at various interfaces.
- Polymers with well-defined structures can be formed by enzyme-catalysed processes.

- Lipases do not require the exclusion of water and air when used as catalyst for polyester synthesis. This is in contrast to traditional chemical initiators, where strict precautions are to be taken to exclude air and water from the system.
- Small (4-7 member) cyclic lactones have ring strains and are easily polymerized by organometallic initiztors, but the polymerization of large ring lactones (macrolides) is slow and only low molecular weight products are obtained. Enzymes have shown the capability to polymerize macrolides under normal polymerization conditions [35-38].

4.9.2 Polymerization mechanism

In polymerizations of cyclic esters promoted by enzymes, the best results have been obtained for the lipase-catalysed processes.

Lipases are ubiquitous enzymes and have been found in most organisms from microbial, plant and animal kingdom. They are esterases, which can hydrolyse triglycerides (or esters) at water–oil interface. The hydrolytic capability of these enzymes has been utilized by nature in degradation of food and fats. They also find application as valuable drugs against digestive disorders and diseases of the pancreas, as detergent additive for removal of fat stains and as catalysts for the manufacture of specialty chemicals and for organic synthesis. Lipases are the most versatile classes of biocatalysts in synthesis of organic compounds: this is primarily because lipases can accommodate a wide variety of synthetic substrates, while still showing regio-selectivity or chiral recognition. Lipases have evolved unusually stable structures that may survive effect of the organic solvents. The lipase-catalysed hydrolysis in water can be easily reversed in non-aqueous media into ester synthesis or transesterification.

Regardless of the organism from which they have been isolated and the variation in molecular masses, all lipases show a remarkable structural and functional similarity. A unique structural feature common to most lipases is a lid or flap composed of amphiphilic α-helix peptide sequence, which in its closed conformation prevents

access of the substrate to the catalytic site. After the lid is opened, a large hydrophobic surface is created to which the hydrophobic supersubstrate (oil drop) binds. The active site is composed of a nucleophilic serine (Ser) residue activated by a hydrogen bond in relay with histidine (His) and aspartate (Asp) or glutamate [35].

The polyester chain growth process most probably involves ring-opening of the monomer by nucleophilic attack of the catalytic site of lipase (the serine residue), followed by hydrolysis of the acyl-enzyme intermediate or its esterification with low molar mass alcohol or hydroxyl-terminated polyester chain. Thus, the polymerization can be explained as a "monomer-activated" mechanism (Figure 4.16). The rate-determining step in enzymatic polymerization is the formation of the lactone–lipase complex, which is more favourable for more hydrophobic large-size lactones. The key step is the reaction of lipase with the lactone, involving the ring-opening of the monomer, with formation of an acyl–enzyme intermediate ("enzyme-activated monomer", EM). The initiation is a nucleophilic attack of water, which is contained partly in the enzyme, onto the acyl carbon of the intermediate to produce ω-hydroxycarboxylic acid (n=1), the shortest propagating species. In the propagation stage, the intermediate is nucleophilically attacked by the terminal hydroxyl group of a propagating polymer to produce a one-unit-more elongated polymer chain [39,40].

In cyclic compounds, reactivity is generally dependent upon their ring size; the ring strain of small and moderate ring size compounds is larger than that of macrocyclic ones, and hence they show higher ring-opening reactivity. On the other hand, the macrolides show unusual reactivity toward enzymatic catalysis. The enzymatic polymerizability increases as a function of the ring size, and the large enzymatic polymerizability is governed mainly by the reaction rate and not by the binding abilities, i. e., the reaction process of the lipase-lactone complex to the acyl-enzyme intermediate is the key step of the polymerization [40].

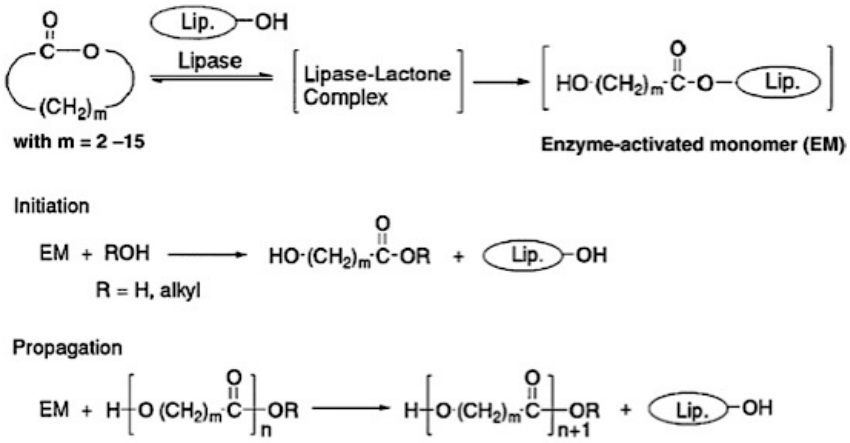


Figure 4.16. Mechanism of enzymatic ring-opening polymerization [40].

Unfortunately, the control imparted to ROP is not as good as the one obtained by chemical ROP, with Mw/Mn higher than 2 [40]. One advantage of the enzymatic route is to be found in the optical activity of lipases, which opens up a new route to stereoselective ROP.

The rapidly increasing number of publications evidences the potential of the enzyme catalysis, providing a wide range of polymer structure. In the near future, the development of production systems and feasibility studies for industrial production should provide a novel interesting source of metal free polyesters.

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CHAPTER 5

RING-OPENING POLYMERIZATION OF CYCLIC ESTERS USING STANNOUS OCTOATE AS CATALYST

5.1 Introduction

The most popular metal-based initiator for the ring opening polymerization of aliphatic polyesters is tin(II) bis-(2-ethylexanoate), also referred as tin octoate ($Sn(Oct)_2$). It is accepted as a food additive by the US Food and Drug Agency (FDA) and thus no purification of the polymers is needed for applications such as packaging. Different is the situation if the synthesised polymer has to be used in the pharmaceutical or biomedical field: in this case, indeed, due to tin toxicity, it has to be removed from the obtained product. The purity of the polymer product is of fundamental importance, because residual monomers, catalysts and solvents pose risks when such materials are in contact with biological fluids or tissues [1,2].

In a first stage of the research on biodegradable polymers, we have decided to study the ring opening polymerization of cyclic esters in the presence of tin octoate as catalyst, using supercritical carbon dioxide, at a pressure low enough to operate in a liquid phase constituted by the molten monomer expanded by CO_2. Working in the presence of CO_2, problems associated with the use of a solvent in polymerization of polyesters (i.e. the presence of organic solvents, such as methylene chloride, chlorobenzene, benzene or toluene, and thus the necessity of a purification step to eliminate them from the obtained product) can be avoided [3]. In this way, it is possible to use a nontoxic solvent which can be easily separated from the polymer by reducing the pressure of the solvent-polymer phase, leading to a clean and dry product [4-8]. This is a very important point, because with this operative procedure

it could be possible to reduce a large amount of cost for the product recovery at industrial scale.

In this part of the research activity we have studied the ring opening polymerization of lactide and glycolide in the presence of tin octoate as catalyst and using supercritical carbon dioxide as swelling agent. The objective of this research was to study a process for the production of bioabsorbable polymeric matrixes to evaluate if the polymerization could enjoy advantages from the swelling of the reaction phase induced by dissolution of dense CO_2.

In the literature there are some works on the ring opening polymerization of cyclic esters using metal-based initiators in the presence of supercritical carbon dioxide. The first studies on the ROP of lactide and glycolide in $scCO_2$ have been reported by Bratton [4] and Hile [5,6]. Homopolymers and copolymers were synthesized in reaction systems pressurized with $scCO_2$, using stannous octoate as catalyst.

5.2 Materials

The monomers l-lactide, d,l-lactide and glycolide were purchased from Sigma-Aldrich; they were purified by re-crystallization in ethyl acetate and stored in a dry place at 4°C (l-lactide and d,l-lactide) or at -20°C (glycolide) until use. The ring-opening reaction was catalysed by tin octoate ($Sn(Oct)_2$), also purchased from Sigma-Aldrich; CO_2 (99.998% pure) was supplied by Rivoira.

5.3 Polymerization apparatus and reaction procedure

The experiments were carried out in a stainless steel fixed volume view cell with a volume of 54 mL from Thar Technologies (designed to operate up to 50.0 MPa and 423 K), stirred by a magnetic stir bar (Figure 5.1). The temperature control of the system was ensured by a heating tape, controlled by a PID controller with an accuracy of ± 0.3 K. The pressure vessel was equipped with a pressure transducer Barksdale UPA3 (estimated accuracy by calibration with an high precision

manometer ± 0.05 MPa) and a thermocouple (estimated accuracy ± 0.3 K). Reactions were visually monitored through two sapphire optical windows assembled at opposite ends of the reactor.

The proper amounts of monomers and catalyst were loaded in the vessel weighting their masses using an electronic scale. The cell was put into a vacuum oven at 50°C for at least 2 hours to decrease the water content of the reactants. Then liquid CO_2 was delivered by means of a Maximator air-driven pump until the final selected pressure was reached. The reaction temperature was set at 120°C; after 24 hr polymerization, the reaction was stopped by the immersion of the reactor in an ice-water bath to cool it to room temperature before slowly depressurization of the gaseous phase. Finally, the amount of formed polymer was determined gravimetrically.

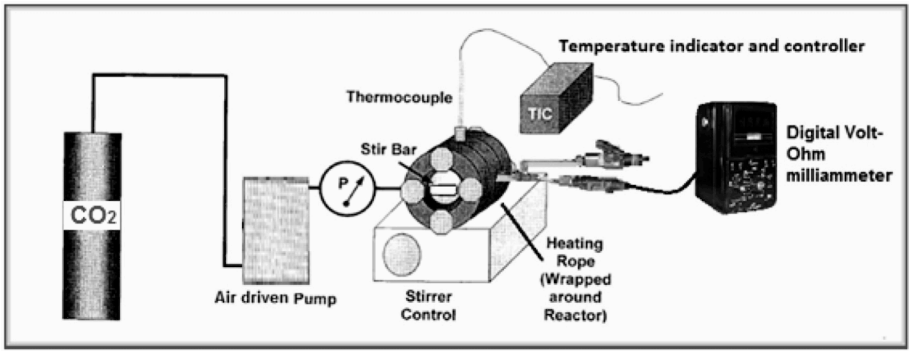


Figure 5.1. Schematic representation of the equipment used in the polymerization reactions.

5.4 Polymer characterization

Polymer yields were determined gravimetrically. Polymer morphologies were analysed with a Philips scanning electron microscope (SEM). Samples were sputter-coated with gold to a thickness of 200 Å.

Calorimetric analyses of polymers were performed with a Perkin Elmer Jade-DSC calorimeter. A pre-weighed amount of the material was loaded into an aluminium pan and placed in the differential scanning calorimeter (DSC), together

with an empty pan used as a reference. The samples were heated twice under nitrogen atmosphere; thermograms covering a range from 30°C to 190°C were recorded at heating and cooling rates of 10°C/min.

The thermal stability of the polymers was studied by thermogravimetric analysis (TGA) using a Perkin Elmer STA 6000 simultaneous thermal analyser. Samples were inserted in a small crucible made of refractory ceramic material, which is chemically unreactive towards most materials. The analysis were performed under a stream of nitrogen having a flow rate sufficient to sweep volatiles from the furnace, to avoid the build-up of decomposition products in the close proximity of the sample, but not so fast as to cool the sample. The samples were heated from 30°C to 700°C at a rate of 10°C/min and the diminution of their weight was recorded by the instrument as a function of temperature.

The microstructure of the obtained polymers was studied by means of proton nuclear magnetic resonance (^{1}H-NMR) with a Bruker AN 400 spectrometer, using chloroform as the solvent, at 25°C. Chemical shifts were given in parts per million with tetramethylsilane as an internal reference.

Molecular weight distribution of selected samples was characterized by size exclusion chromatography (SEC) (Agilent, 1100 series), equipped with two detectors, ultraviolet (UV) and differential refractive index (RI), and a pre-column with two PLgel 5 μm MIXED-C columns (Polymer Laboratories, length of 300 mm and diameter of 7.5 mm, measuring range: 2000-2000000 Da). Chloroform was used as eluent at flow rate of 1 mL/min and temperature of 30°C; the calibration based on polystyrene standards was used. These analyses were kindly carried out by the research group of Prof. Giuseppe Storti and Prof. Massimo Morbidelli of the ETH of Zurich (Switzerland).

5.5 ROP of l-lactide assisted by supercritical carbon dioxide

The ROP homopolymerization of l-lactide was studied using scCO_2 instead of organic solvents. The sorption of CO_2 into polymers, indeed, results in their swelling and changes their mechanical and physical properties. The plasticization is

accompanied by a reduction of the number of entanglements between polymer chains and decreases the viscosity of the system, thus enhancing the rate of diffusion of the monomer to the site of reaction [6,9,10]. Indeed, it is reported in the literature [11] that, at a CO_2 pressure higher than 70 bar, the plasticized polymer is at a relatively low viscosity, but as the pressure is lowered the viscosity of the mixture increases significantly. The ability to decrease the viscosity of the polymer phase could be an important feature in the process under consideration because the viscosity of PLLA significantly increases during polymerization [4,12].

It was possible to prepare PLLA homopolymers (nominal monomer feed concentration=0.9 M, $Sn(Oct)_2$=1/1000 mol/mol monomer and CO_2 pressure of about 23.0 MPa) with yields higher than 98% (as determined from ^{1}H-NMR spectra), working in a relatively low density system (0.47 g/mL). In the presence of CO_2, the system resulted divided into two phases: the heaviest was constituted by the expanded liquid monomer; above this, there was a light phase rich in CO_2.

The ^{1}H-NMR spectra of PLLA (Figure 5.2) show signals at 5.1-5.3 ppm and 1.6 ppm, that are characteristic of the chemical structure of PLLA: the peaks around 5.1-5.3 ppm are due to the methine protons in LA repeat units, while the peaks at 1.6 ppm are related to methyl protons; signals due to methine protons in the end groups of the polymers appear at about 4.4 ppm [13].

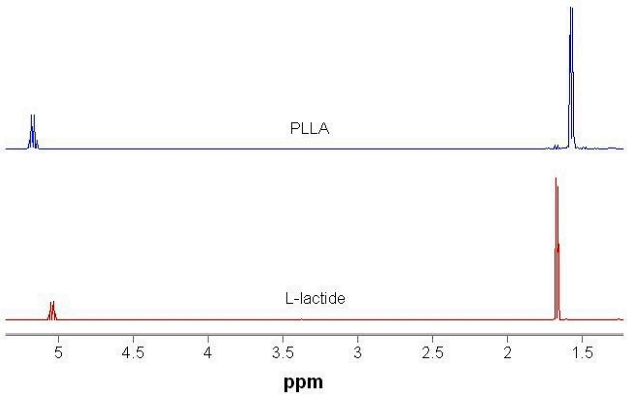


Figure 5.2. ^{1}H-NMR spectrum of PLLA compared with that of l-lactide monomer.

The samples were also analysed by DSC and TGA (Figures 5.3-5.4). The melting temperature and the thermal degradation behaviour of the synthesised polymers confirmed that, working in the CO_2 expanded liquid monomer, it was possible to prepare PLLA homopolymers.

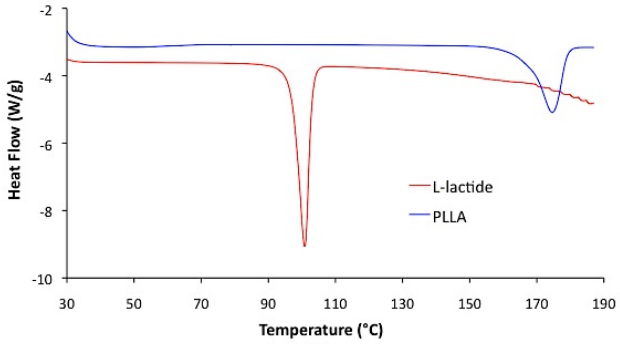


Figure 5.3. DSC thermogram of PLLA compared with that of l-lactide monomer (1st heating).

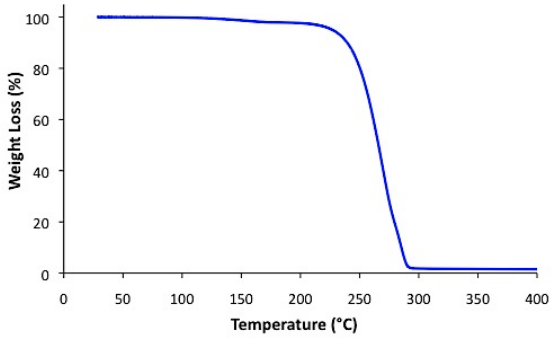


Figure 5.4. TGA thermogram of PLLA.

The number-average molecular weights (M_n) of PLLA homopolymers were determined by ^{1}H-NMR spectra by comparing the integrated area of peaks at 5.1-5.3 ppm with that of the peak at 4.4 ppm [13,14]. From this analysis, we obtained M_n of about 11500 g/mol for all the PLLA homopolymers synthesised in the presence of

CO_2. Thus, it seems that, working in a CO_2 expanded polymerization system, it was possible to synthesise PLLA homopolymers in a simple way.

5.6 ROP of lactide and glycolide assisted by supercritical carbon dioxide

Another investigated process was the copolymerization of l-lactide or d,l-lactide with glycolide assisted by $scCO_2$, with initial LA/GA molar ratios in the feed of 80/20. It was possible to prepare PLGA copolymers with yields higher than 98% and with LA/GA molar ratio in the obtained polymer close to that in the feed (Table 5.1).

Entry	Monomers	P, MPa	Conversion LA/GA, %	[LA]/[GA] (polymer)
1	l-LA, GA	bulk	99.5/99.8	78.4/21.6
2	l-LA, GA	9.3	99.5/99.6	78.7/21.3
3	l-LA, GA	15.6	99.0/99.9	78.8/21.2
4	l-LA, GA	21.5	99.1/99.7	76.8/23.2
5	l-LA, GA	29.3	99.1/99.9	76.7/23.3
6	d,l-LA, GA	23.0	98.7/99.7	78.1/21.9

Operating conditions: T=120°C, t=24 hr, monomers feed concentration: [LA]+[GA]=0.9 M; [LA]/[GA] (feed)=80/20; $Sn(Oct)_2$=1/1000 mol/mol monomers; magnetic stirrer rate=500 rpm.

Table 5.1. Copolymerization of lactide and glycolide at different values of CO_2 pressure.

Bulk polymerizations were conducted in the melted monomers and have been used as reference to determine the CO_2 effect on the process and on the characteristics of the obtained product.

In bulk reactions, at the beginning the system was liquid, because the reaction temperature was higher than the melt temperature of the monomers; as the reaction proceeded, there was a progressive increase in the viscosity of the melted phase, until the mass became solid.

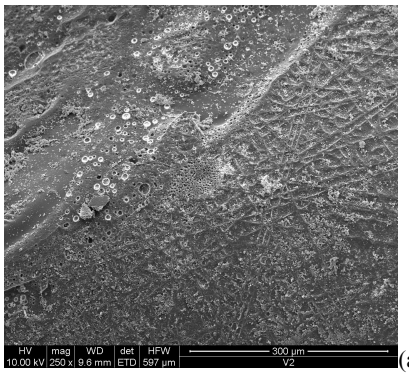
The use of CO_2 allowed a sensible decrease in the melting point of the monomers (under the adopted conditions, the system resulted completely liquid at about 50°C instead of 95°C) and in the viscosity of the molten phase. In the presence of CO_2, at the beginning of the polymerization, the system was divided into two phases: the

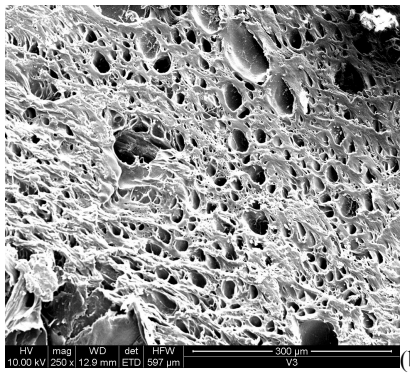
heaviest constituted by the molten monomers expanded by CO_2; the second one was above and was rich in CO_2, even if there was a little amount of monomers dissolved in it. As a result, there were two different reaction loci.

The solubility of the monomers and the polymer in $scCO_2$ depends mainly on pressure and temperature. It can be expected that the monomers are only slightly soluble in $scCO_2$ and their solubility increases with pressure. A change of pressure can modify the relative importance of each polymerization locus, as higher pressure increases the solubility of the monomers in supercritical medium and faster polymerization rate can be expected in the liquid monomers with respect to that in the CO_2 rich phase [7,15]. As the polymerization advances, unsoluble polymer chains precipitate.

All these considerations lead to the hypothesis of the existence of an optimal CO_2 pressure, that allows to obtain high plasticization and diffusion rates, but, at the same time, minimizes the monomers dissolution in $scCO_2$.

It is interesting to note that, in bulk polymerization, we obtained a very hard and compacted polymer that was difficult to extract from the reactor; while in polymerization assisted by $scCO_2$, after the depressurization of the system, we obtained polymers more porous that were easier recovered from the reactor (Figure 5.5). These considerations are coherent with the above-mentioned properties of CO_2, whose dissolution in the cyclic monomers induced plasticization of the forming polymer phase and, after depressurization, allowed the generation of a porous expanded polymer matrix.



(a)


(b)

Figure 5.5. SEM micrographs of PLGA synthesised without CO_2 (entry 1, Table 5.1) (a) and with CO_2 (entry 3, Table 5.1) (b).

The thermal behaviour and stability of PLGA copolymers were studied by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The DSC analysis confirmed that the synthesised polymers were amorphous. Thermograms of copolymers synthesized in the absence of CO_2 or at different values of CO_2 pressure are shown in Figure 5.6: the polymer prepared without CO_2 showed a weight loss in the range of 75-115°C, whereas, in the presence of CO_2, the thermal stability of the material depended on the value of the pressure. It was observed the presence of an optimal range of CO_2 pressure between 15.0 and 21.0 MPa, that allowed to control side reactions and molecular weight. We have seen that monomers are only slightly soluble in $scCO_2$; anyway their solubility increases with pressure, while the polymer is substantially insoluble in $scCO_2$. This change could be responsible for the changes in the molecular weight, because the polymerization rate is slower in CO_2 than in the expanded monomers, and for the presence of transesterification reactions, that lead to the formation of shorter polymer chains.

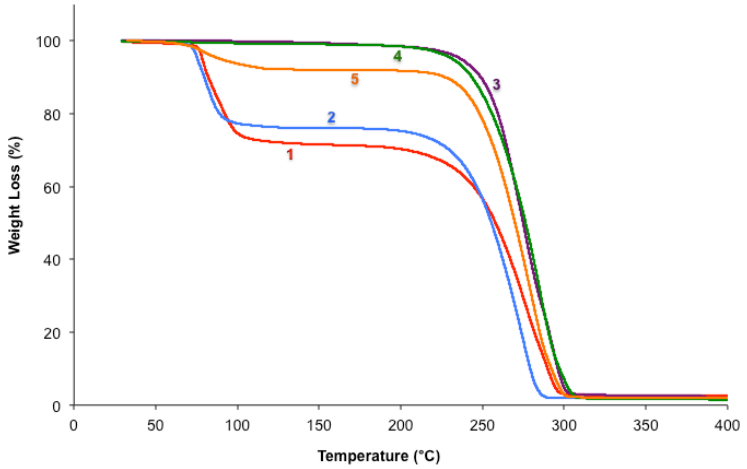


Figure 5.6. TGA thermograms of PLGA synthesised in experiments in Table 5.1. Bulk polymerization (entry 1, curve 1) and at different values of CO_2 pressure: 9.3 MPa (entry 2, curve 2), 15.6 MPa (entry 3, curve 3), 21.5 MPa (entry 4, curve 4) and 29.3 MPa (entry 5, curve 5).

Besides, comparing the molecular weight, calculated from GPC analysis, of two samples prepared in the absence or in the presence of CO_2 (entries 1-3, Table 5.1), it is important to underline that, even if the molecular weight M_n was about the same (14000 g/mol), with CO_2 it was possible to prepare a polymer with a narrower molecular weight distribution.

The analysis of the copolymer microstructure was performed by means of ^{1}H-NMR spectroscopy. The ^{1}H-NMR spectrum of PLGA exhibited peaks at δ=5.20 ppm (CH, PLA), δ=4.60-4.90 ppm (CH_2, PGA) and δ=1.60 ppm (CH_3, PLA). The LA/GA molar ratio in all PLLGA copolymers was about 80/20, which was estimated from the ratio of the integrals of the peaks at δ=5.20 ppm and at δ=4.60-4.90 [16,17].

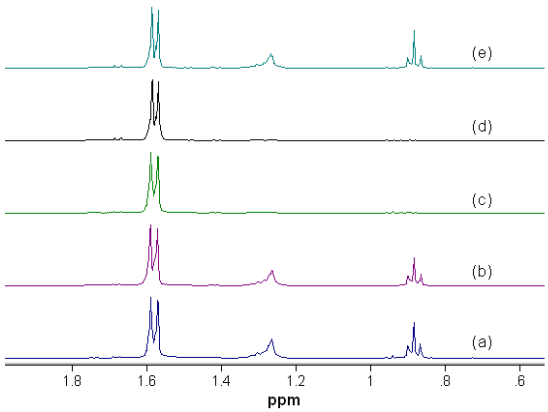


Figure 5.7. ^{1}H-NMR spectra of PLGA synthesised in experiments of Table 5.1. Bulk polymerization (entry 1, a) and at different values of CO_2 pressure: 9.3 MPa (entry 2, b), 15.6 MPa (entry 3, c), 21.5 MPa (entry 4, d) and 29.3 MPa (entry 5, e).

It is interesting to note that also from the ^{1}H-NMR analysis it seems that there is an optimal value of CO_2 pressure.

The explanation of this trend may be due to the equilibrium between propagating species and dormant species. When a tin catalyst is used, the chain end of the growing chain is carbonated; the reversible insertion of CO_2 into the Sn-O bond leads to a carbonated tin compound. It is reported in the literature that tin-carbonate species are not active species in ROP of ε-CL [18] (Figure 5.8) and, thus, presumably the same behaviour could be assumed for others cyclic esters.

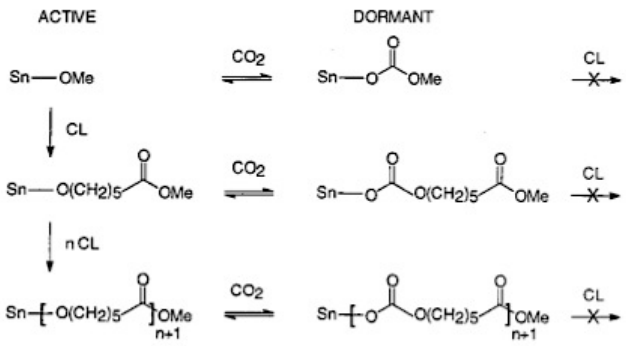


Figure 5.8. Main steps of the reaction mechanism of the ring opening polymerization of ε-CL [18].

At higher CO_2 pressure values, the monomers solubility in $scCO_2$ increases and the carbonatation reaction is presumably more relevant, leading to the formation of more dormant species. At lower CO_2 pressure values, the carbonatation reaction has less influence on the reaction kinetics, but there is however the formation of by-products, owing to the transesterification reactions. Thus, it is important to work at such experimental conditions in order to minimize the two effects.

5.7 Conclusions

PLLA homopolymers and PLGA copolymers were prepared using tin octoate as catalyst in the presence of supercritical carbon dioxide. The polymers were obtained with yields higher than 98% and with LA/GA molar ratio in the obtained polymer close to that in the feed (for copolymers), working in the liquid monomers expanded by CO_2.

Working in the presence of CO_2, it was possible to obtain polymers more porous and characterised by a narrower molecular weight distribution.

From results collected until now, it seems that there is the presence of an optimal CO_2 pressure, that allows to obtain high plasticization and diffusion rates, but, at the same time, restricts the loss of monomers by dissolution in the CO_2 rich phase.

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CHAPTER 6

ENZYME CATALYSED RING-OPENING POLYMERIZATION OF CYCLIC ESTERS

6.1 Introduction

As mentioned before, metal-based initiators, in particular tin octoate, are characterised by cytotoxicity and thus they have to be removed from the synthesised polymer, if they are targeted to the biomedical field. In fact, the availability of aliphatic polyesters uncontaminated with toxic metallic residues is essential for biomedical applications. In Chapter 5 we have seen that it was possible to synthesize polyesters working in a molten phase constituted by the monomers expanded by supercritical carbon dioxide. In this chapter the idea is to work in a completely "green" system, using an enzyme as catalyst for the ring opening polymerization and avoiding the use of organic solvents by using CO_2, which thanks to its plasticizing effect is able to decrease the viscosity of the polymerization system. By this approach the costly removal of the toxic metals from the produced polymers is removed [1-5].

Enzymes do not require the exclusion of water or air and catalyse the degradation as well as the synthesis of polyesters. The activity and selectivity of the process is highly dependent on the method of enzyme preparation, the type of solvent and the temperature of the reaction. Moreover, the presence of water is required to trigger enzyme activity by providing a partial hydration layer around the enzyme, thus providing flexibility to its structure [4].

Lipases are the most versatile classes of biocatalysts in synthesis of organic compounds. In fact, there has been an extremely rapid increase in research in the

area of in-vitro enzyme-catalysed polymerizations during last decade. Lipases catalyse the ring opening polymerization of lactones and lactides to produce aliphatic polyesters. The broad synthetic potential of lipases is largely due to the fact that they accept a wide range of substrates, they are quite stable in organic solvents and thus, depending on the solvent system used, can be applied to hydrolysis reactions or ester synthesis [6].

6.2 Materials

The monomers l-lactide, d,l-lactide, glycolide, ε-caprolactone and the macroinitiator poly(ethyleneglycol)methylether, average M_w 750 or 2000, were purchased from Sigma-Aldrich. The monomers l-lactide, d,l-lactide and glycolide were purified by re-crystallization in ethyl acetate and stored in a dry place at 4°C (lactide) or at -20°C (glycolide) until use, while ε-caprolactone was used as it was. The ring-opening reactions were catalysed by Lipase Candida Antarctica immobilized on acrylic resin (Novozyme 435, activity ≥10000 U/g) or by Amano Lipase PS, derived from Burkholderia Cepacia (activity ≥30000 U/g), both purchased from Sigma-Aldrich; CO_2 (99.998% pure) was supplied by Rivoira. Chloroform HPLC grade (Sigma-Aldrich) was used as received.

6.3 Polymerization apparatus and reaction procedure

The experiments were carried out in a stainless steel fixed volume view cell; the description of the polymerization apparatus has been discussed in the previous chapter, the schematic representation of the equipment is shown in Figure 5.1.

The proper amounts of monomers (and eventually the macroinitiator) with the enzyme were loaded in the vessel weighting their masses using an electronic scale. Then the cell was put into a vacuum oven at 50°C for at least 2 hours to decrease the water content of the reactants. Liquid CO_2 was delivered by means of a Maximator air-driven pump until the final selected pressure was reached. The reaction

temperature was set at 65°C or 100°C, depending on the catalytic system and monomers used; after polymerization, the reaction was stopped by the immersion of the reactor in an ice-water bath to cool it to room temperature before slowly depressurization of the gaseous phase. The reaction mixture was dissolved in chloroform and the insoluble biocatalyst was removed by filtration [7]. Finally, the amount of the obtained polymer was determined gravimetrically.

6.4 Polymer characterization

Calorimetric analyses of polymers were performed with a Perkin Elmer Jade-DSC calorimeter. A pre-weighed amount of the material was loaded into an aluminium pan and placed in the differential scanning calorimeter (DSC), together with an empty pan used as a reference. The samples were heated twice under nitrogen atmosphere; thermograms covering a range from 30°C to 190°C or 250°C were recorded at heating and cooling rates of 10°C/min.

The thermal stability of the polymers was studied by thermogravimetric analysis (TGA) using a Perkin Elmer STA 6000 simultaneous thermal analyser. Samples were inserted in a small crucible made of refractory ceramic material, which is chemically unreactive towards most materials. The analysis were performed under a stream of nitrogen. The samples were heated from 30°C to 700°C at a rate of 10°C/min and the diminution of their weight was recorded by the instrument as a function of temperature.

The microstructure of the obtained polymers was studied by means of proton nuclear magnetic resonance (^{1}H-NMR) with a Bruker AN 400 spectrometer, using chloroform as the solvent, at 25°C. Chemical shifts were given in parts per million with tetramethylsilane as an internal reference.

Infrared spectra were recorded on a Perkin-Elmer Spectrum 2000 Explorer FTIR with an averaging of 30 scans at a resolution of 1 cm^{-1} using a near-IR fast recovery deuterated tryglicine sulphate detector. Analyses were performed on the polymer powder mixed with anhydrous KBr and then compressed to produce solid pellet.

6.5 Enzyme catalysed ROP of l-lactide and glycolide

The ring opening polymerization of lactide and glycolide catalysed by enzymes has been widely studied in bulk or in organic solvents; moreover, the enzyme catalysed homopolymerization of l-lactide has been also studied in supercritical carbon dioxide [7]. There are some works in the literature concerning this kind of reaction: generally it is necessary to conduct the reaction for long reaction times in order to obtain high monomer conversions and high polymer molecular weights. Matsumura [8] was the first to report the ROP of PLLA using Lipase PS at 100 °C in bulk for 7 days; he also reported unsuccessful polymerization of either l-lactide or d-lactide in bulk by immobilized Novozyme 435. Subsequently, Fujioka [9] claimed the first ROP of l-lactide reaching a M_w of 2440 g/mol using immobilized Novozyme 435 in bulk, but polymer yields were not reported. The author also reported significant polymerization (1440 g/mol) at identical conditions but without enzyme; he indicated that the polymerization observed in bulk ROP of l-lactide without the enzyme might be attributed to cationic polymerization mechanism induced by traces of hydroxyacid from the monomer, which might be formed at such operational temperatures. Huijser [10] reported the successful bulk copolymerization of lactide and glycolide using Lipase PS at 130 °C, employing a molar ratio of 80/20 between L and G monomers, even if the obtained molecular weights were not very high and the corresponding polydispersity index higher than 2.

It is interesting to note that, in reactions conducted in bulk, it is necessary to operate at high temperatures in order to reach the melting point of the monomers; this may cause a partially deactivation of the enzyme and thus it would be preferable to operate at lower temperatures, since the 55-70 °C temperature range is reported as the most suitable for the synthesis of polymers mediated by lipases [6,11,12]. As we have seen in the previous chapter, this could be possible working in the presence of CO_2, which leads to the decrease of the monomer melting temperature and of the viscosity of the polymeric system [7].

First we have decided to study the ROP of l-lactide and glycolide catalysed by two different enzymes, Novozyme 435 and Lipase PS. The operative conditions

were chosen on the basis of the data reported in the literature [7-9,13].

By studying the phase behaviour of the l-lactide/glycolide system in the presence of the two different enzymes, we have seen that the system resulted liquid at 35°C with a CO_2 pressure of about 15.0 MPa. Differently from the reactions with stannous octoate, in this case, at the reaction temperature the system was liquid; as the reaction proceeded, it seemed that the viscosity increased, but not so much as we have seen in the case of polymerization catalysed by the metal compound. This behaviour prompts to think that the enzymatic catalysts should be less active than the metallic one.

6.5.1 ROP in the presence of Novozyme 435

PLLA homopolymers and PLGA copolymers were synthesised using Novozyme 435 as catalyst. In this case, it was possible to prepare the polymers with low yield, as determined from ^{1}H-NMR spectra (Table 6.1). For the homopolymerization reactions, yield of about 14% was obtained for lactide after 72 hours; for the copolymerization reactions, yields were about 33% and higher than 90% for lactide and glycolide respectively after a reaction time of 16 hours. It was possible to see that, as the reaction time increased, the lactide conversion also increased and this was accompanied by a corresponding increase on the amount of lactide in the copolymer, reaching the feed value.

These results suggested that, owing to the higher reactivity of glycolide, at low reaction times there were long blocks of glycolide in the polymeric chain; as the reaction time was increased, the lactide conversion increased.

Entry	Monomers	Novozyme 435, %(w/w)	T, °C	Time, hr	P, MPa	Conversion LA/GA, %	[LA]/[GA] (polymer)
1	l-LA	5	100	24	bulk	7.5/0	100/0
2	l-LA	5	100	24	16.5	6.3/0	100/0
3	l-LA	5	100	24	27.3	6.3/0	100/0
4	l-LA	10	100	24	8.0	5.3/0	100/0
5	l-LA	10	100	72	7.9	14.3/0	100/0
6	l-LA, GA	5	65	4	26.8	3.8/94.6	38.3/61.7
7	l-LA, GA	5	65	8	29.1	4.2/91.8	58.3/41.7
8	l-LA, GA	5	65	16	26.1	33.3/96.8	80.1/19.9

Monomers feed concentration: for homopolymerization reactions [LA]=0.9 M, for copolymerization reactions [LA]+[GA]=0.9 M, [LA]/[GA] (feed)=80/20. Magnetic stirrer rate=500 rpm.

Table 6.1. Polymerization of lactide and glycolide using Novozyme 435 as catalyst.

The low efficiency of Novozyme 435 in the homopolymerization and copolymerization of lactide may be due to the high enantioselectivity of the enzyme towards R-configured secondary alcohols [14]. For the lactide monomers, i.e. with water as initiator, a secondary alcohol end group is formed upon ring opening (Figure 6.1). It can be hypothesized that l-lactide and d,l-lactide are not polymerizable due to the S-configuration of the secondary alcohol, which is formed upon the ring opening of the monomer. The secondary alcohol obtained from d-lactide has R-configuration and is able to react with another enzyme activated monomer and thus to propagate the chain.

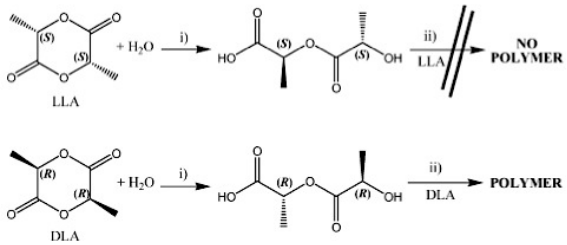

Figure 6.1. Hypothesis concerning the enzymatic ring opening polymerization of l-lactide (LLA) and d-lactide (DLA) by Novozyme 435: initiation (i) and chain propagation (ii) step [14].

It seemed that the use of CO_2 did not induce differences on the monomer conversion. This result may lead to think that the growing process of polymer chains was not controlled by the monomer diffusion to the active catalytic site.

Due to the low amount of polymer and to the presence of low molecular weight products, the thermal properties of the obtained products reflected those of the monomers rather than those of polymers, as shown in typical DSC and TGA thermograms reported in Figures 6.2 and 6.3.

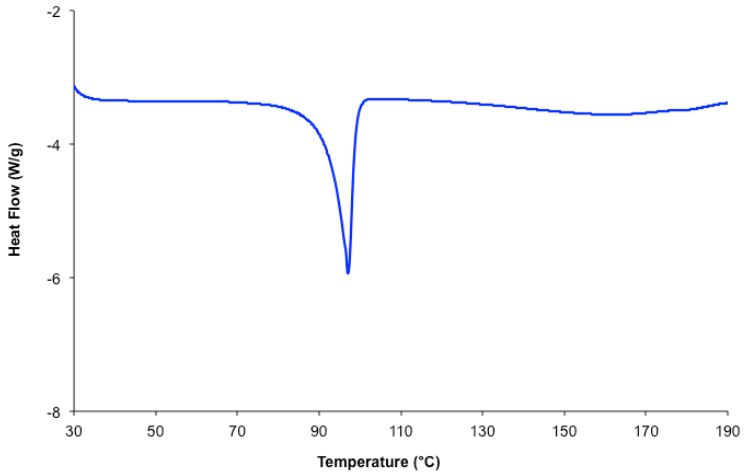

Figure 6.2 (a). DSC thermogram of PLLA (1st heating) synthesised in the presence of Novozyme 435 as catalyst (entry 5, Table 6.1).

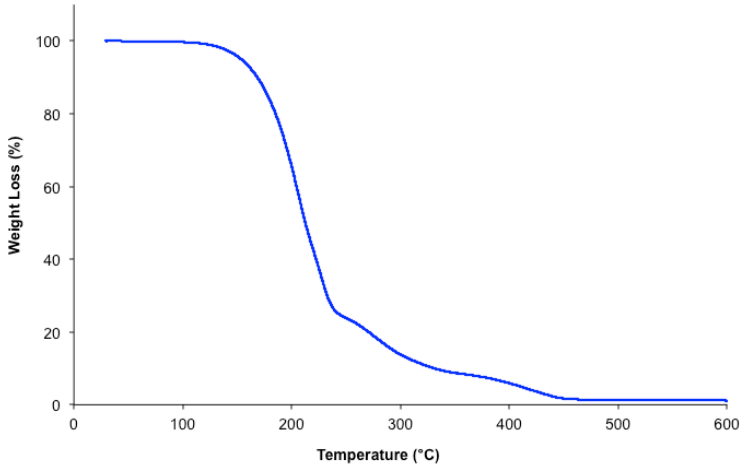

Figure 6.2 (b). TGA thermogram of PLLA synthesised in the presence of Novozyme 435 as catalyst (entry 5, Table 6.1).

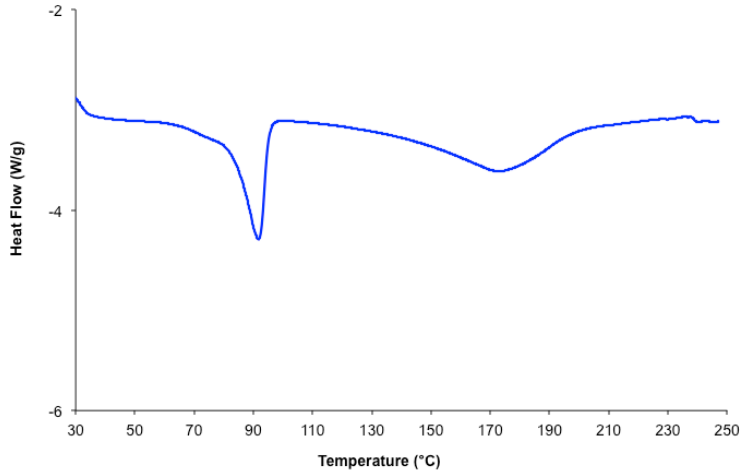

Figure 6.3 (a). DSC thermogram of PLGA (1st heating) synthesised in the presence of Novozyme 435 as catalyst (entry 8, Table 6.1).

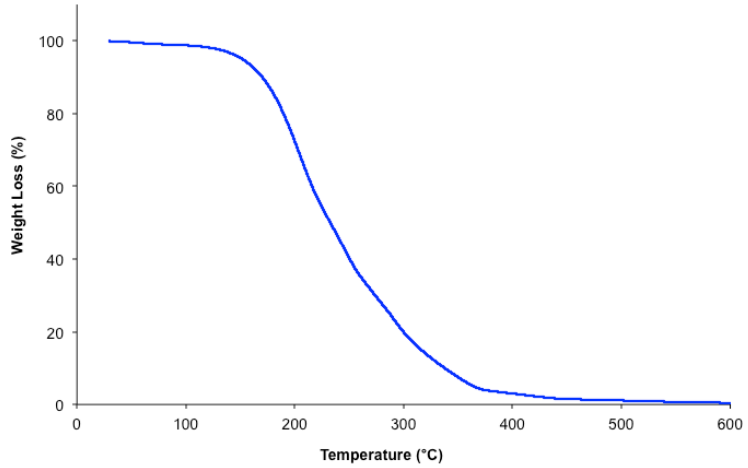

Figure 6.3 (b). TGA thermogram of PLGA synthesised in the presence of Novozyme 435 as catalyst (entry 8, Table 6.1).

Thus, the thermal analysis confirmed that, using Novozyme 435 as catalyst for the ring opening polymerization, it was not possible to obtain polymers with high molecular weight, due to the low conversion of lactide under the adopted conditions. In fact, if we compare the TGA curves of PLLA and PLGA prepared in the presence of the enzyme (Figures 6.2 (b), 6.3 (b)) with those of PLLA and PLGA prepared in the presence of stannous octoate (Figures 5.4, 5.6 (curves 3-4)), we can clearly see the differences in the polymer weight loss and, consequently, in the polymer molecular weight. For homopolymers or copolymers prepared using the metallic catalyst, the materials showed thermal stability up to about 220°C. For homopolymers or copolymers prepared using the enzyme, instead, the materials showed thermal stability up to about 120°C; moreover, at about 230°C, they lost 50% of the initial mass. This can be considered a further indication of the lower activity of Novozyme 435 towards l-lactide.

6.5.2 ROP in the presence of Lipase PS

PLLA homopolymers and PLGA copolymers were also synthesised using Lipase PS as catalyst. It was possible to prepare the polymers with low yields, as determined from ^{1}H-NMR spectra (Table 6.2). In the homopolymerization reactions, we obtained yields up to about 14% for lactide after 72 hours; in the copolymerization reactions, after 48 hours, yields reached a value of about 25% for lactide, while were always greater than 92% for glycolide. In this case, the composition of the obtained copolymer was different from that of the feed.

Entry	Monomers	Lipase PS, %(w/w)	T, °C	Time, hr	P, MPa	Conversion LA/GA, %	[LA]/[GA] (polymer)
1	l-LA	10	100	48	bulk	13.0/0	100/0
2	l-LA	10	100	48	8.2	8.6/0	100/0
3	l-LA	10	100	72	8.4	13.7/0	100/0
4	l-LA, GA	10	100	48	bulk	26.2/96.9	58.0/42.0
5	l-LA, GA	10	100	48	8.2	24.3/92.0	60.5/39.5
6	d,l-LA, GA	10	100	48	bulk	25.9/96.3	57.2/42.8
7	d,l-LA, GA	10	100	48	8.4	25.1/98.1	56.6/43.4

Monomers feed concentration: for homopolymerization reactions [LA]=0.9 M, for copolymerization reactions [LA]+[GA]=0.9 M, [LA]/[GA] (feed)=80/20. Magnetic stirrer rate=500 rpm.

Table 6.2. Polymerization of lactide and glycolide using Lipase PS as catalyst.

As previously reported for polymers prepared using Novozyme 435, also polymers synthesized in the presence of Lipase PS exhibited thermal properties typical of the monomers (Figures 6.4 and 6.5).

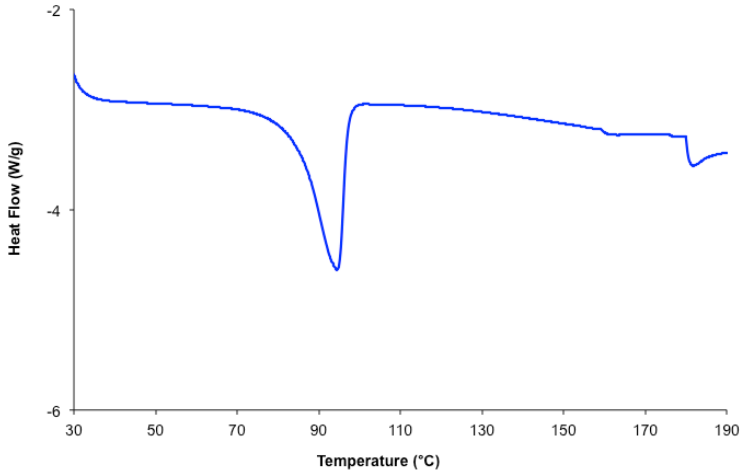

Figure 6.4 (a). DSC thermogram of PLLA (1st heating) synthesised in the presence of Lipase PS as catalyst (entry 3, Table 6.2).

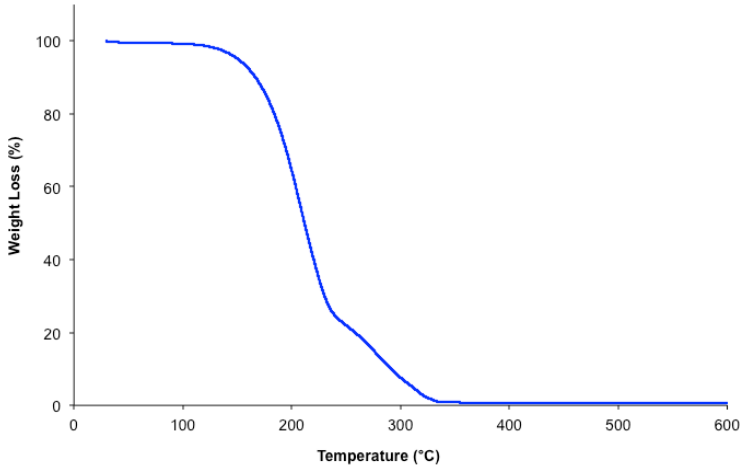

Figure 6.4 (b). TGA thermogram of PLLA synthesised in the presence of Lipase PS as catalyst (entry 3, Table 6.2).

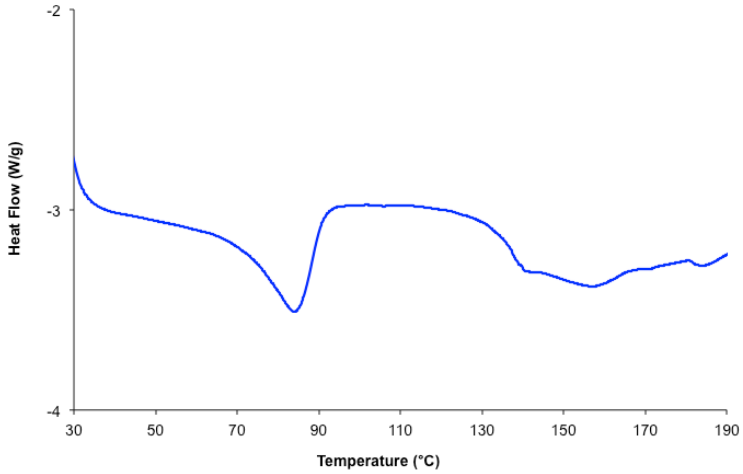

Figure 6.5 (a). DSC thermogram of PLGA (1st heating) synthesised in the presence of Lipase PS as catalyst (entry 5, Table 6.2).

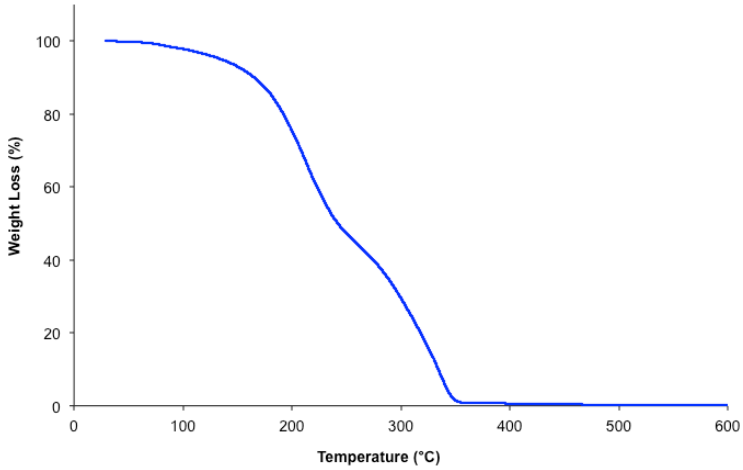

Figure 6.5 (b). TGA thermogram of PLGA synthesised in the presence of Lipase PS as catalyst (entry 5, Table 6.2).

For homopolymers and copolymers prepared using Lipase PS as catalyst, all the considerations above discussed in the case of Novozyme 435 are valid. The thermal

instability of the synthesised materials at low temperatures means that they were characterised by low molecular weights.

Thus, in conclusion, we have seen that the two different enzymes used for the ring opening polymerization of lactide and glycolide were not very efficient as catalysts under the adopted experimental conditions.

6.5.3 Polymer microstructure: effect of the catalytic system

A very important feature of the polyesters obtained through ring opening polymerization of the cyclic monomers is the microstructure.

We have performed the FT-IR analysis of some selected samples prepared with Novozyme 435 (Figure 6.6).

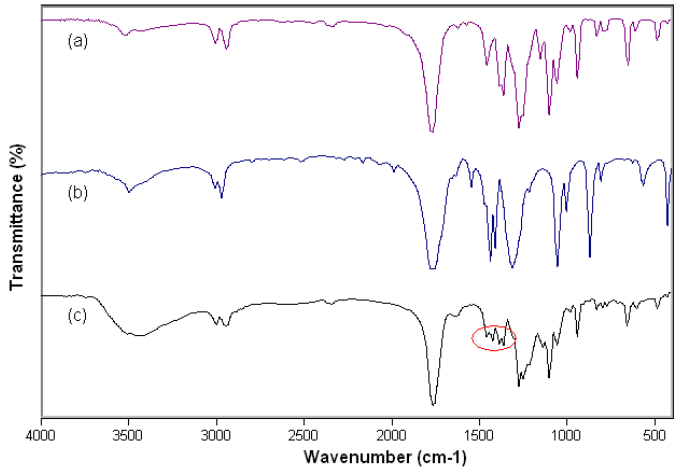

Figure 6.6. FT-IR spectra of l-lactide (a) and glycolide (b) monomers compared with a PLGA copolymer synthesised using Novozyme 435 as catalyst (entry 8, Table 6.1) (c).

From the analysis of the FT-IR spectra, we can clearly see that the synthesised samples were polymers, because there is the presence of a large band at about 3500 cm^{-1}, which corresponds to the hydroxyl group present at the end of the polymer chains. In the spectra of copolymer samples synthesised with Novozyme 435, there

is the presence of a peak between 1420 and 1423 cm^{-1} and a peak at 1385 cm^{-1}, which are not present in the monomer spectra: the first may be attributed to glycolide-glycolide bonds in the polymer chain, while the second to lactide-glycolide bonds. Moreover, in the case of polymers prepared in polymerization characterised by longer reaction times, there is a peak at 1453 cm^{-1}, which may be attributed to lactide-lactide bonds [15].

From ^{1}H-NMR spectra it is interesting to note that the polymer microstructure of the samples synthesised through enzymatic copolymerization was different from that of samples synthesised using the metallic catalyst (Figure 6.7).

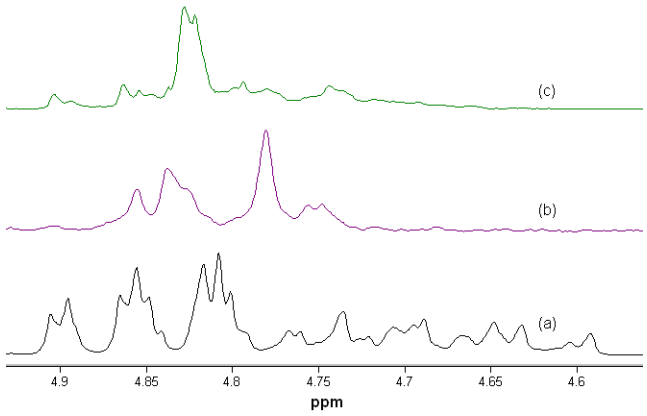

Figure 6.7. ^{1}H-NMR zone relative to polyglycolide in the copolymer chain for PLGA samples synthesised through ROP of cyclic monomers using stannous octoate (a), Novozyme 435 (b) or Lipase PS (c) as catalyst.

6.6 Enzyme catalysed ROP of ε-caprolactone

The first studies of enzyme catalysed ROP of ε-caprolactone appeared in 1993 [16,17], when Uyama and Kobayashi reported that performing the ROP of ε-caprolactone with Pseudomonas fluorescens lipase at 60°C in bulk after 10 days high monomer conversion were obtained, that resulted in the formation of a polyester with average molecular weight of about 7000 g/mol [17]. While a range of

lipases have been investigated for ring opening polymerization, probably the most commonly applied in the non-aqueous ROP of cyclic ester monomers is Candida antarctica lipase B, which is often immobilised on a macroporous support available as Novozyme 435.

Despite significant advancements in recent years, a number of problems still exist in transferring the protocols of in vitro enzyme-catalysed polyester synthesis from the laboratory to the industrial scale. Of particular concern is the relatively low catalytic activities displayed by enzymes when used in non-aqueous media. In fact, the water content is a very important variable in the enzymatic polymerization. Kobayashi [18] and Dong [19] described the ring opening polymerization in bulk or in organic solvents, putting in evidence the effect of the water content. It is reported that both low (0.1%) and high (16%) water content negatively affected the initiation. At low water contents, the conformation of the enzyme is relatively rigid and this leads to the decrease of its activity and, consequently, to the reduction of the initial polymerization rate. Nevertheless, the presence of water leads to the hydrolysis of the formed polymer and thus to the formation of oligomers. It was shown that the water content decreased sharply at the beginning and increased slowly at later stages, suggesting that linear condensation polymerization became dominant during the later stages of reaction [19].

A similar work was conducted by Gross and Kumar [20], which performed investigations about the role of the enzyme concentration, the effect of temperature, the choice of the organic solvent and the presence of water in the ROP of ε-caprolactone. It was shown that the highest activities and polymer molecular weights were obtained in toluene or iso-octane solution; more polar solvents were found to deactivate the enzyme probably as a consequence of conformational changes. Higher temperatures led to increased reaction rates and polymerisations carried out at 90°C reached 90% monomer conversion within 2 hours. The water content of the solvent was shown to be critical to control the molecular weight of the polymer.

Loeker [12] investigated the enzyme catalysed ROP of ε-caprolactone in supercritical carbon dioxide, demonstrating that this compressible medium can be a highly effective solvent for the process. This study revealed that molecular weights

comparable to those obtained in organic solvents could be obtained, but with both narrower polydispersities and higher yields (up to 98%).

Thurecht [21] showed that there is an optimal water content with respect to the monomer conversion and that the molecular weight of polycaprolactone decreased with increasing water concentration. However, unlike traditional metal catalysts, precise control over the absolute molecular weight and polydispersity could not be obtained using the enzyme as catalyst, because there was the presence of transesterification reactions. The main reason for this lack of control, especially at higher conversion, is due to the high instance of side reactions that are catalysed by the enzyme (Figure 6.8). Inter-transesterification (Figure 6.8-III) occurs when an ester group within a polymer chain is activated by the enzyme, which then reacts with the hydroxyl moiety of another chain, leading to linear polymer with variable molecular weight. On the other hand, intra-transesterification (Figure 6.8-IV) leads to cyclic moieties via activation of an in-chain ester group, followed by reaction with the terminal hydroxyl group of the same chain. In addition to transesterification, degradation of the polymer by hydrolysis can be catalysed by the enzyme if a suitable nucleophile is present (for example, water).

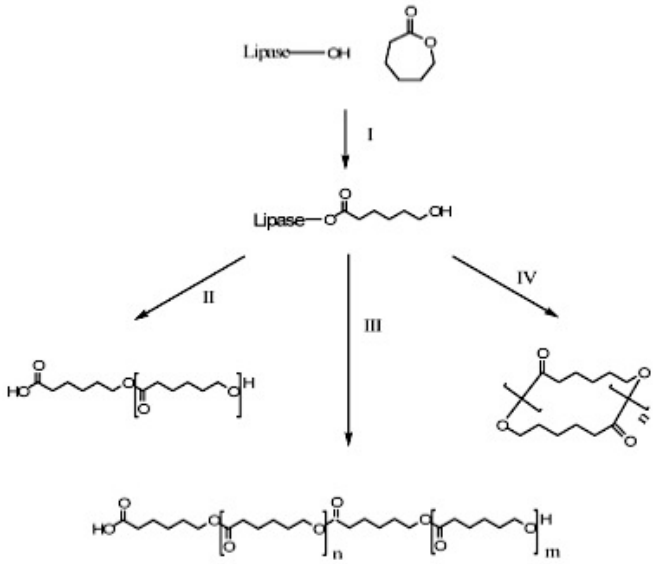

Figure 6.8. Mechanism outlining the enzymatic ring opening polymerization of ε-caprolactone and relevant side reactions (I, enzyme-activated monomer; II, polymer formation; III, inter-transesterification; IV, intra-transesterification) [21].

In last years great interest has been focused to the design of polymeric micelles composed by self-assembly of hydrophobic-hydrophilic segment, potentially useful as drug carriers due to their unique characteristics such as core-shell structure and prolonged blood circulation. Drug delivery systems based on polymeric micelles composed by copolymers of poly(ethylenglycole) (PEG) and polycaprolactone showed excellent biocompatibility, nontoxicity, high drug-loading capacity, long circulation time in the bloodstream, and high biodegradability [22,23].

The copolymerization of ε-caprolactone with PEG catalysed by tin octoate has been widely studied, obtaining amphiphilic copolymers characterised by high molecular weights and low polydispersity indexes [24-27].

For what concerns the copolymerization of ε-caprolactone with PEG catalysed by enzymes, in the literature there are few works; but it is reported that the enzymatic

ROP in bulk or in toluene is efficient and fast, giving copolymers characterised by high enough molecular weights [28].

Copolymers constituted of PCL and PEG are very important in the pharmaceutical field, because they may be used to prepare supramolecular aggregates which allow the conveying of hydrophobic drugs into the core constituted by the PCL chains, while the shell constituted by the PEG chains ensures the stability of the aggregate in aqueous environment. In the copolymerization of ε-CL with PEG, the latter acts as macroinitiator, leading the attack to the lactone ring activated by the enzyme, according to the reaction scheme reported in Figure 6.9 [29].

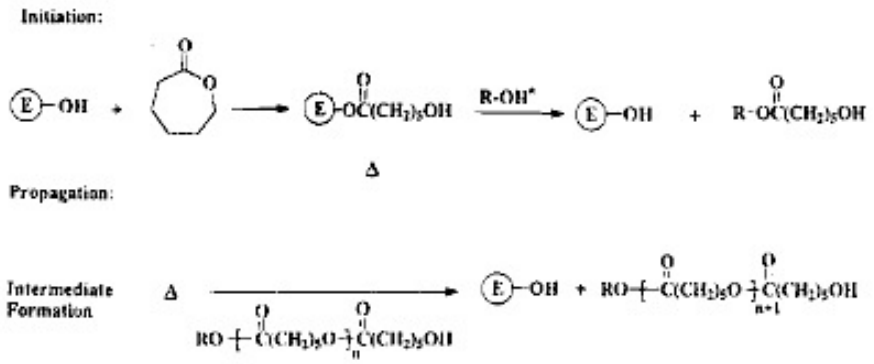

Figure 6.9. Reaction scheme of the copolymerization of ε-CL with PEG (in the scheme represented by R-OH) [29].

On the basis of these considerations, we have decided to use the Novozyme 435 to catalyse first the homopolymerization of ε-caprolactone and after its copolymerisation with the PEG as macroinitiator.

6.6.1 ROP of ε-caprolactone in the presence of Novozyme 435

We have studied the possibility of using Novozyme 435 as catalyst to prepare polycaprolactone (PCL), varying the reaction time and the nature of the

polymerization medium (pure bulk monomer as such or expanded with a low CO_2 pressure) (Table 6.3).

Entry	Novozyme 435, %(w/w)	T, °C	Time, hr	P, MPa	Conversion ε-CL, %	M_n, g/mol
1	10	65	2	5.0	88.7	1310
2	10	65	4	5.1	89.1	1940
3	10	65	6	5.3	97.8	2350
4	10	65	8	4.9	96.2	2600
5	10	65	24	5.0	98.6	3300
6	10	65	24	bulk	96.1	1660

Monomer feed concentration: [ε-CL]=0.84 M; magnetic stirrer rate=500 rpm.

Table 6.3. Polymerization of ε-caprolactone using Novozyme 435 as catalyst.

The operative pressure was chosen lower than 16.0 MPa on the basis of the data reported in the literature [30] to work at a reaction temperature of 65°C in a biphasic system.

The enzyme was dried under vacuum at 50°C for at least 2 hours, decreasing its water content in order to optimize the monomer conversion and to obtain high molecular weights polymer.

As the reaction time increased, the viscosity of the system progressively increased too as determined by visual observation of the polymerization mixture. This feature was an indication of the good catalytic activity of Novozyme 435 for the ROP of ε-caprolactone monomer, differently from what we have seen in the case of lactide and glycolide: the rate-determining step in enzymatic polymerization is the formation of a lactone-lipase complex, which is more favourable for more hydrophobic large-size lactones [1,8,19,21].

Besides, in the synthesis assisted by CO_2, the obtained polymers were visually porous, while those prepared in bulk resulted compact. However, it was possible to prepare PCL with high conversions (≥ 88%), determined by ^{1}H-NMR spectra [29], both in CO_2 and in bulk.

It is interesting to note that, as the reaction time increased, the molecular weights M_n of the obtained polymer increased (estimated by comparison of the intensities, using ^{1}H-NMR spectrometer integration values, of the methylene protons adjacent to the oxygen of intra-chain repeat units at 4.06 ppm with respect to the methylene protons

adjacent to the hydroxyl end-group at 3.65 ppm [31]), while the monomer conversion was found close to 90% also in shortest polymerization run (Figure 6.10).

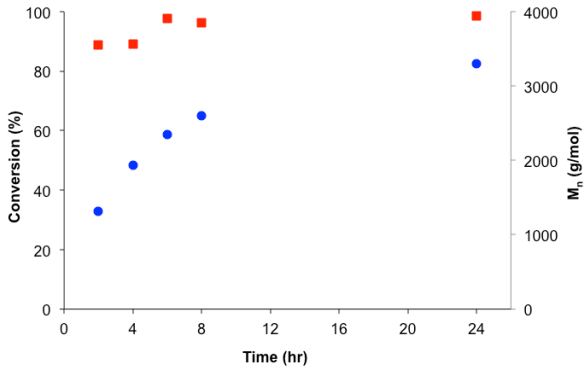

Figure 6.10. Effect of reaction time on the monomer conversion (■) and on the molecular weight M_n of the obtained polymer calculated from ^{1}H-NMR spectra (●).

If we compare polymerization reactions conducted for 24 hours in the presence of CO_2 and in pure bulk monomer (entries 5-6, Table 6.3), we can see that, even if the conversion was high in both cases, using CO_2 it was possible to obtain a polymer with higher molecular weight. This was confirmed by DSC and TGA analysis (Figure 6.11), since in the presence of CO_2 we obtained a polymer with a higher melting temperature [32,33] and more thermally stable with respect to that synthesised in bulk.

This is an important information, because, starting from these results, it could be possible to study a reaction procedure in order to better exploit the particular properties of CO_2 towards aliphatic polyesters.

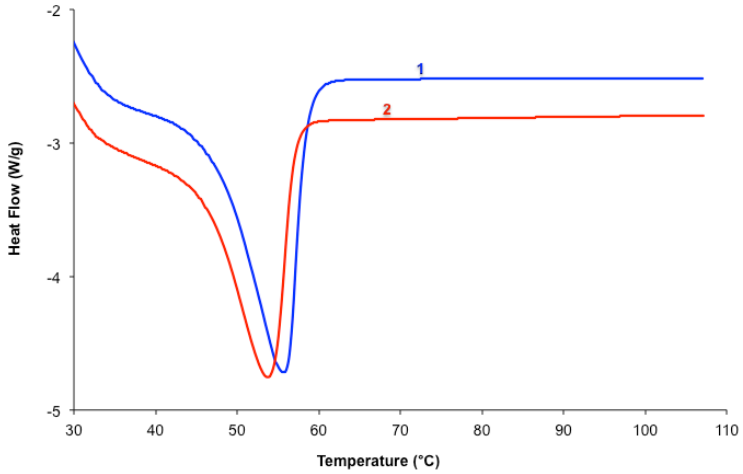

Figure 6.11 (a). DSC thermograms of PCL (1st heating) synthesised with Novozyme 435 in the presence of CO_2 (entry 5, Table 6.3, curve 1) or in bulk (entry 6, Table 6.3, curve 2).

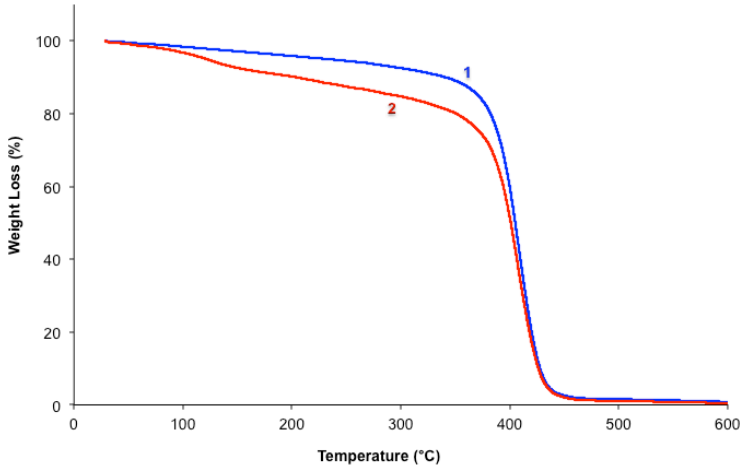

Figure 6.11 (b). TGA thermograms of PCL synthesised with Novozyme 435 in the presence of CO_2 (entry 5, Table 6.3, curve 1) or in bulk (entry 6, Table 6.3, curve 2).

6.6.2 ROP of ε-caprolactone with poly(ethylenglycole) in the presence of Novozyme 435

On the basis of the results obtained studying the homopolymerization of ε-CL, we have tried to synthesize block copolymers of poly(ethylenglycole)-polycaprolactone (PEG-b-PCL).

The solubility of PEG in CO_2 is significantly lower than that of ε-CL and decreases as its molecular weight increases [34]. Thus, under the adopted conditions used for the homopolymerization reactions, that led to the presence of a liquid monomeric phase, even PEG was insoluble in CO_2. In particular, the visual observation of the system at the beginning of the polymerization reaction, allowed to verify that, working at pressures lower than 6.0 MPa, the system was constituted by two phases: one rich in ε-caprolactone, in which PEG was soluble, and another one rich in CO_2. At low enough operative pressure (about 5.0 MPa) the amount of ε-CL solubilized in CO_2 should be minimum.

^{1}H-NMR analyses of polymer products allowed us to verify that it was possible to prepare PCL-PEG copolymers with different length of the hydrophobic block as a function of reaction time, with ε-CL conversions higher than 87% (Table 6.4). The degree of polymerization of each component is indicated as subscript at the repeating polymeric units: for example, the block copolymer PEG_{17}-b-PCL_{22} has 17 repeating units of EG and 22 of ε-CL.

Entry	Monomers	[ε-CL]/[PEG] (feed)	Novozyme 435, %(w/w)	Time, hr	P, MPa	Conversion ε-CL/PEG, %	(M/I)[a]	DP_n[b]	M_n, g/mol
1	ε-CL, PEG 750	80/20	10	4	5.1	90.1/10.6	22.0	PEG_{17}-b-PCL_{22}	3250
2	ε-CL, PEG 750	80/20	10	24	4.9	93.2/6.9	46.2	PEG_{17}-b-PCL_{46}	6010
3	ε-CL, PEG 750	80/20	10	24	11.1	90.1/9.5	38.8	PEG_{17}-b-PCL_{39}	5170
4	ε-CL, PEG 750	80/20	10	24	bulk	91.5/12.5	25.4	PEG_{17}-b-PCL_{25}	3650
5	ε-CL, PEG 750	95/5	10	24	4.9	98.8/47.1	205.5	PEG_{17}-b-PCL_{205}	24180
6	ε-CL, PEG 2000	80/20	10	4	5.0	87.2/7.8	32.6	PEG_{45}-b-PCL_{33}	4460
7	ε-CL, PEG 2000	80/20	10	24	5.0	94.0/4.1	84.8	PEG_{45}-b-PCL_{85}	10410
8	ε-CL, PEG 2000	80/20	10	24	10.1	91.6/3.0	119.8	PEG_{45}-b-PCL_{120}	14400
9	ε-CL, PEG 2000	80/20	10	24	bulk	94.6/10.8	25.0	PEG_{45}-b-PCL_{25}	3600
10	ε-CL, PEG 2000	95/5	10	24	5.0	98.7/25.0	298.2	PEG_{45}-b-PCL_{298}	34740

The amount of reactants have been calculated with a fixed amount of ε-CL of 1 g. Magnetic stirrer rate=500 rpm, T=65°C.

[a] Number of caprolactone units for PEG chain in the copolymer, obtained from ^{1}H-NMR.

[b] Average degree of polymerization of PEG units (determined from nominal molecular weight) and of caprolactone units (determined from ^{1}H-NMR).

Table 6.4. Polymerization of ε-caprolactone with PEG using Novozyme 435 as catalyst.

The ^{1}H-NMR spectra of the PEG-b-PCL copolymers are complicated (Figure 6.12), due to the overlap of some peaks characteristic of the two different constituent units and to the possibility that there is PEG non linked to the PCL chains.

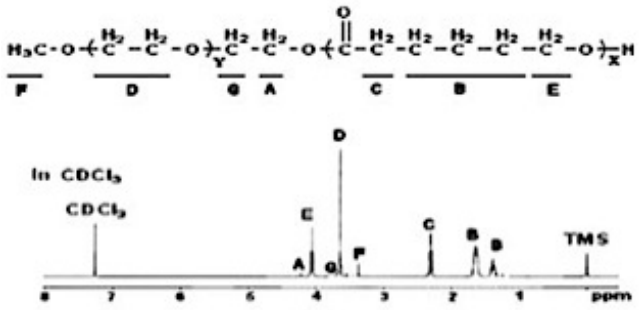

Figure 6.12. Typical ^{1}H-NMR spectrum of PEG-PCL copolymer in chloroform ($CDCl_3$) [35].

It was possible to determine the ε-CL conversion (with the same approach used for homopolymers), but also the amount of PEG linked to a CL repeat units, by comparing the peak at 4.20 ppm, attributed to the methylene protons of the -OCH_2- group of PEG end blocks linked with the PCL blocks, and the peak at 3.38 ppm, due to the protons of the -OCH_3- group corresponding to the end groups in PEG blocks:

in fact, the first one refers only to the PEG chains in the copolymer, while the second one is present as end-group in all the PEG chains [35].

The structure of the block copolymer has been characterised by the calculation of the average degree of polymerization of the ε-CL from the ratio of the peak of the methylene protons of the group -CH_2O- in PCL blocks at 4.06 ppm and the peak at 4.20 ppm of the aforementioned -OCH_2- group in PEG [35]; for the PEG, we have used as average degree of polymerization that calculated from its nominal molecular weight (17 for PEG 750, 45 for PEG 2000). Known the average degrees of polymerization, it was possible to determine the M_n of the block copolymer by using the equation 6.1:

$$M_n = M_{nPEG} + 114 \times DP_{PCL} \qquad 6.1$$

where M_{nPEG} is the nominal molecular weight of the specific used PEG, DP_{PCL} is the average degree of polymerization of PCL (determined from ^{1}H-NMR spectra) and 114 is the molar mass of ε-CL repeat units.

A typical ^{1}H-NMR spectrum of synthesised PEG-b-PCL copolymers is reported in Figure 6.13.

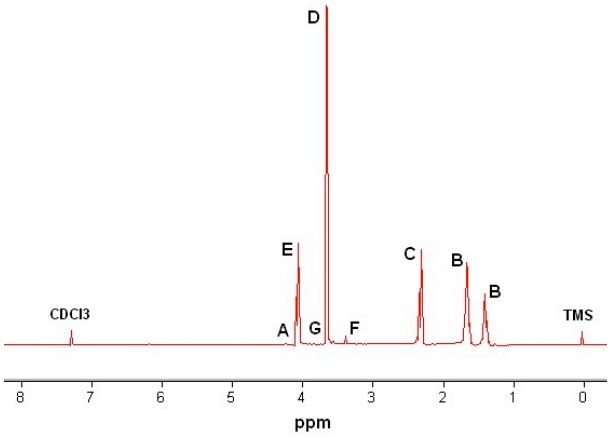

Figure 6.13. Typical ^{1}H-NMR spectrum of synthesised PEG-b-PCL copolymers.

The ε-CL conversions were always high, but the fraction of PEG linked to caprolactone units was quite low; this confirmed the high activity of Novozyme with respect to ε-CL monomer and the role of macroinitiator of the PEG.

The average degree of polymerization of the PCL block increased with the reaction time (entries 1-2 and 6-7, Table 6.4) and in the presence of CO_2 (entries 2-3-4 and 7-8-9, Table 6.4). According to their definition, M_n values reached their highest values for copolymers synthesised at 24 hours and in the presence of CO_2.

It is interesting to note that the effect of CO_2 pressure (entries 3 and 8, Table 6.4) was different depending on the chain size of used PEG. In fact, when the pressure was increased from 5 to about 10 MPa, the length of the PCL block decreased when PEG 750 was used, while the same length became larger with the higher molecular weight polyether macroinitiator. The increase of pressure enhances the density of the CO_2 rich phase, thus increasing its solvent power, and decreases local viscosity of the PEG rich phase owing to the higher amount of CO_2 that dissolves in it. These two phenomena differently affect the length of the CL block. The increase of solvent power influences the partitioning of the monomer towards the catalyst free CO_2 rich phase, thus decreasing the local concentration of monomer in the polymerization phase. The lower viscosity increases the mass transfer rate of monomer to the active catalyst, thus promoting the chain growth.

Using PEG 750, it is possible that the dissolution of monomers in the light phase prevails, leading to the decrease of the molecular weight of the obtained copolymer. Differently, using PEG 2000, the solubility of the CL in the PEG could be enhanced so that the viscosity decreasing effect can be prevalent.

The effect of the concentration of the feed mixture was also investigated (entries 2-5 and 7-10, Table 6.4). When the concentration of PEG decreased, there was an increase both in the ε-CL and PEG conversions and in the PCL average degree of polymerization, leading to a copolymer with higher molecular weight. Thus, decreasing the amount of PEG, it was possible to better optimize the process, incorporating a grater amount of PEG in the copolymer chain and obtaining a longer PCL block.

6.7 Conclusions

It was possible to prepare PLLA homopolymers and PLGA copolymers using two different enzymes (Novozyme 435 and Lipase PS) in the presence of CO_2, but the results collected until now showed that they were not very efficient as catalysts under the adopted experimental conditions. In fact, while the glycolide conversion was always quite high (>91%), those of l-lactide and d,l-lactide were low (<34%), independently on the used enzyme. The thermal analysis confirmed that it was not possible to obtain polymers with high molecular weight. This means that the enzymes have different activity towards the different monomers.

From FT-IR and ^{1}H-NMR analysis it was possible to have informations about the microstructure of the synthesised PLGA copolymers: not only the copolymer was formed using the enzymes as catalysts, but its microstructure was different from that of samples synthesised using tin octoate. Thus, the copolymer microstructure appears to depend strongly by the specific catalytic system used.

It was possible to prepare PCL homopolymers using Novozyme 435 as catalyst with high conversions ($\geq$ 88%), both in the presence of CO_2 and in bulk monomer. In the case of the synthesis assisted by CO_2, the obtained polymers were porous and characterised by higher molecular weights.

It was possible to prepare PCL-PEG copolymers using the same reaction system, with different length of the hydrophobic block as a function of reaction time. The average degree of polymerization of PCL increased with reaction time and in the presence of CO_2; the M_n values followed this trend, reaching higher values for copolymers synthesised in reactions carried out for 24 hours and with CO_2. The effect of CO_2 pressure changed depending on the different type of PEG used (750 or 2000), presumably because of the different interactions between CO_2 and PEG and of the different partition equilibrium of the cyclic monomer between light and heavy phases.

It was possible to change copolymer composition and average molecular weight decreasing the PEG fraction in the feed, obtaining the incorporation of a greater amount of PEG in the copolymer chain and longer PCL blocks.

In the last considered case, the use of the enzyme as catalyst for the ROP of ε-CL with PEG gave good results and its high cost could be justified by the specific application that these copolymers may have in the pharmaceutical field.

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CHAPTER 7

RING OPENING POLYMERIZATION OF CYCLIC ESTERS BY CYCLODEXTRINS

7.1 Introduction

As discussed in the previous chapter, enzymes are characterised by high costs, thus their practical use could be justified only for specific applications of the synthesised polyesters. Due to this drawback, we have also tested the possibility of using cyclodextrins as initiators for the ring opening polymerization of cyclic esters. They are cyclic oligosaccharides consisting of six (α-CD), seven (β-CD) or eight (γ-CD) α-(1,4)-linked glycosyl units (Figure 7.1); they have cone like structures with a hydrophobic internal cavity and a hydrophilic outside. Cyclodextrins show an enzyme-like behaviour, but they are characterised by lower costs: they are semi-natural products, produced in thousands of tons per year amounts from starch by a relatively simple enzymatic conversion, and thus their prices are acceptable for most industrial purposes. Moreover, thanks to their ability of forming inclusion complexes, important properties of the complexed substances can be significantly modified. Due to their favourable safety profile, they can be used as ingredients of drugs, foods or cosmetics [1,2].

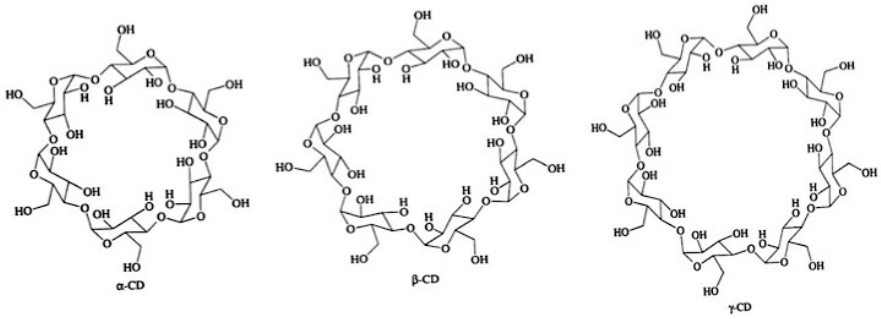

Figure 7.1. Chemical structures of α-CD, β-CD and γ-CD.

The main properties of native cyclodextrins are given in Table 7.1. β-cyclodextrin is the most accessible, the lowest-priced and generally the most useful. Apart from these native cyclodextrins, many CD derivatives have been synthesised, produced by aminations, esterifications or etherifications of primary and secondary hydroxyl groups. Depending on the substituent, the solubility of the CD derivatives is usually different from that of their parent compounds.

Property	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin
Number of glucopyranose units	6	7	8
Molecular weight (g/mol)	972	1135	1297
Solubility in water at 25 °C (%, w/v)	14.5	1.85	23.2
Outer diameter (Å)	14.6	15.4	17.5
Cavity diameter (Å)	4.7–5.3	6.0–6.5	7.5–8.3
Height of torus (Å)	7.9	7.9	7.9
Cavity volume ($Å^3$)	174	262	427

Table 7.1. Cyclodextrins properties [3].

An important feature of cyclodextrins is their ability to form inclusion complexes with guest molecules. The formation of inclusion complexes is a function of two factors. The first is steric and depends on the relative size of the cyclodextrin to the size of the guest molecule or certain functional groups within the guest. The second critical factor is the thermodynamic interactions between the different components of the system: there must be a favourable net energetic driving force that pulls the guest into the cyclodextrin, in order to form the complex [3,4].

7.2 State of the art

The choice of using cyclodextrins as initiators for the ROP of cyclic esters was based not only on considerations about the price of the CD with respect of that of enzymes, but also on the data reported in the literature for their utilization as catalysts in these polymerization processes. Harada et al. [5-7] reported the ring-opening polymerization of cyclic esters initiated by cyclodextrins operating in bulk at 100°C (Figure 7.2). These works were based on the idea that, given that CDs easily hydrolyse polyesters and lactones in water, if lactones are heated with CDs without water, they might form polymers because hydrolysis can not occur. They studied the polymerization of β-butyrolactone, δ-valerolactone and ε-caprolactone employing α-, β- and γ-cyclodextrins as catalysts, obtaining different results as a function of the specific monomer and of the different CD.

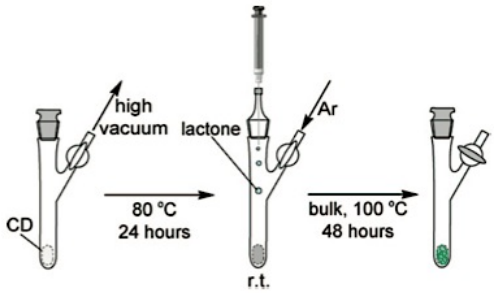

Figure 7.2. Polymerization of lactones with CDs [5].

In particular, for what concerns ε-caprolactone (Figure 7.3), good results were reported working with [ε-CL]/[CDs]=5 in the presence of β-cyclodextrin; but they obtained yields lower than 15% for reactions conducted for 72 hours (Figure 7.4). This means that, in order to obtain higher yields, they should continue the reactions for several days. The authors associated this behaviour to the lower reactivity of ε-CL with respect to the other monomers; additionally they commented that another important drawback may be the fact that, increasing the monomer conversion, the viscosity of the system increased and thus the mass transfer of the cyclic ester to the

growing chains was slowed down, limiting the reaction rates and the molecular weights of the growing polymer chains.

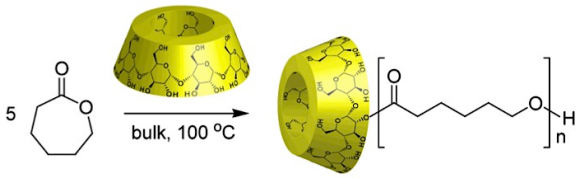

Figure 7.3. Reaction scheme of the polymerization of ε-CL in the presence of CD [6].

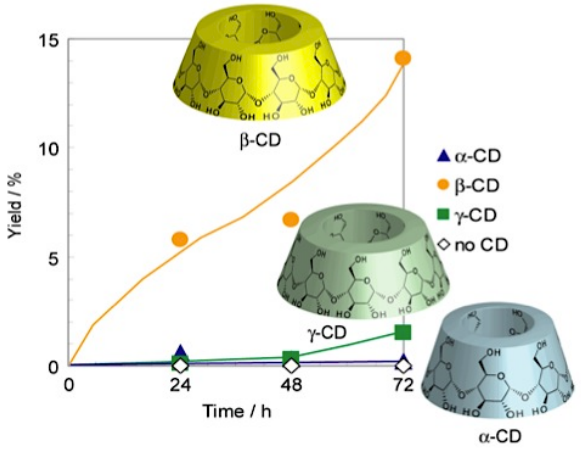

Figure 7.4. Dependence of yield on time for the polymerization of ε-CL initiated by various cyclodextrins in bulk at 100°C ([ε-CL]/[CDs]=5) [6].

On the basis of these considerations, given the results obtained with the other catalytic systems employed (Chapters 5 and 6), we decided to exploit the particular properties of CO_2 (in particular its plasticizing effect) in order to reduce the viscosity of the system, to study how the performances of the CD are affected by the improved mobility of the polymer in the polymer melt.

Most of this experimental part was carried out at the "Centre for Education and Research on Macromolecules" (CERM) of the University of Liège (Belgium).

7.3 Materials

The monomer ε-caprolactone (99%) was purchased from Sigma-Aldrich and used as it was or dried overnight over CaH_2, distilled under vacuum and deoxygenated by nitrogen bubbling. The ring-opening reactions were conducted in the presence of β-cyclodextrin (≥97%), water (HPLC grade) or benzyl alcohol (99%), all purchased from Sigma-Aldrich; CO_2 (99.998% pure) was supplied by Rivoira.

7.4 Polymerization apparatus and reaction procedure

For bulk polymerizations we used glass tubes: first the β-cyclodextrin (or water) was inserted and, in some cases, dried at 80°C under vacuum for two hours; then the monomer was added under a N_2 atmosphere. Once charged all the reagents, the mixture was stirred and heated up to the reaction temperature. At the end of the reaction, the glass tubes were inserted in a dry ice bath and cooled down to room temperature.

The majority of polymerization reactions under pressure were performed in an 80 mL AISI 316 high-pressure reactor, equipped with a mechanical stirring system and an electric heating mantle; pressure, temperature, and stirring speed were monitored on a digital display control rack. In some cases, the experiments were carried out in a 20 mL AISI 316 high-pressure reactor, equipped with a magnetically coupled stirring system and inserted in an oil bath; pressure and temperature were controlled, respectively, by a manometer and a thermocouple. Before each test, the reactor was first carefully cleaned with analytical grade THF alone, then loaded with 20-30 mL of THF, closed and pressurized with liquid CO_2 and heated to 65°C, reaching a pressure that was always higher than 20.0 MPa and always corresponded to single phase condition in the reactor. This last step was performed to clean ducts and channels difficult to be rinsed with liquid solvents. After 2-3 hours the reactor was vented and carefully purged with compressed CO_2 for about 15 min. The proper amounts of ε-caprolactone (distilled or not) with β-cyclodextrin, water or benzyl

alcohol were loaded in the vessel; for some experiments, β-cyclodextrin was dried before starting the reaction under vacuum at 80°C for two hours, in order to remove its water content. Once reached the reaction temperature, the reactor was rapidly filled with liquid CO_2 using a high-pressure syringe pump (ISCO) or with compressed liquid monomer or N_2, up to reach the desired pressure value. Before venting, the reactor was cooled to room temperature with a dry ice mixture.

7.5 Polymer characterization

The thermal stability of the samples was studied by thermogravimetric analysis (TGA) using a Perkin Elmer STA 6000 simultaneous thermal analyser. Samples were inserted in a small crucible made of refractory ceramic material chemically unreactive towards most materials. The analysis were performed heating the samples under a stream of nitrogen from 30°C to 700°C at a rate of 10°C/min and the diminution of their weight was recorded by the instrument as a function of the temperature.

The molecular weight and the molecular weight distribution of the synthesised polymers were determined by gel permeation chromatography measurements (GPC) with a SFD S5200 auto sampler liquid chromatograph equipped with a SFD refractometer index detector 2000. Three PL gel 5μm (10^5 Å, 10^4 Å, 10^3 Å and 100 Å) columns were eluted with THF (flow rate of 1 mL/min at 45°C) and calibrated with polystyrene standards. For the determination of PCL molecular weights, the following Mark-Houwink relation was used: $M_n(PCL)=0.259\ M_n(PS)^{1.073}$.

The microstructure of the obtained polymers was studied by means of proton nuclear magnetic resonance (^{1}H-NMR) with a Bruker AN 400 spectrometer, using chloroform as the solvent, at 25°C. Chemical shifts were given in parts per million with tetramethylsilane as an internal reference. The molecular weight M_n of the obtained polymer was estimated by comparison of the intensities of the methylene protons adjacent to the oxygen of intra-chain repeat units (CH_2O) at 4.06 ppm with respect to the methylene protons adjacent to the hydroxyl end-group (CH_2OH) at

3.65 ppm. The conversion of ε-CL to PCL was determined by comparing the spectral integration intensities of the aforementioned peak at 4.06 ppm and the corresponding peak due to the cyclic monomer at 4.20 ppm [10,15].

Solubility tests of selected samples were done in a Soxhlet extractor using water or THF, close to their boiling point, as solvents.

Infrared spectra were recorded on a Perkin-Elmer Spectrum 2000 Explorer FTIR with an averaging of 30 scans at a resolution of 1 cm^{-1} using a near-IR fast recovery deuterated tryglicine sulphate detector. Analyses were performed on the polymer powder mixed with anhydrous KBr and then compressed to produce solid pellet.

The MALDI-TOF mass spectra were recorded on a MALDI-TOF-TOF Bruker Daltonics Ultraflex II spectrometer and were kindly carried out by the research group of Prof. Eric Monflier of the University of Artois (France).

Calorimetric analyses of polymers were performed with a Perkin Elmer Jade-DSC calorimeter. A pre-weighed amount of the material was loaded into an aluminium pan and placed in the differential scanning calorimeter (DSC), together with an empty pan used as a reference. The samples were heated twice under nitrogen atmosphere; thermograms covering a range from 18°C to 350°C were recorded at heating and cooling rates of 10°C/min.

7.6 Preliminary results

On the basis of the results reported in the literature, at the beginning we have decided to perform the polymerization of ε-caprolactone both in the bulk liquid monomer and in the presence of CO_2 using β-cyclodextrin as catalyst.

7.6.1 Reactions carried out at 100°C

On the basis of the data reported in the literature, we have studied the polymerization reactions at 100°C in bulk or in the presence of CO_2 (Table 7.2). The

operative CO_2 pressure (about 15.0 MPa) was chosen at such a value as to limit the dissolution of the monomer in the light phase [8], since the β-cyclodextrin is not soluble in CO_2 [9] and thus the reaction occurred in the melted phase constituted by the monomer. The monomer ε-caprolactone was used both distilled that not treated and β-cyclodextrin dried or not. In fact, the cyclic oligosaccharide contains about 13% (w/w) concentration of water in its native structure as determined from TGA analyses reported after. The [ε-CL]/[β-CD] ratio was fixed at 100 (differently from the data reported in the literature), because we would like to evaluate if it was possible to synthesise the polymer using low molar concentration of CD.

Entry	ε-CL	β-CD	[ε-CL]/[β-CD]	T, °C	Time, hr	P, MPa	Conversion ε-CL, %
1			100	100	24	bulk	0.5
2		dried	100	100	24	bulk	0.5
3	distilled	dried	100	100	24	bulk	0.5
4	distilled		100	100	24	bulk	0.5
5			100	100	24	15.2[a]	2.0
6	distilled		100	100	24	15.0[a]	0.2

Monomer feed concentration: [ε-CL]=0.84 M, magnetic stirrer rate=500 rpm. When the CD was dried, it was maintained for 2 hours at 80°C under vacuum, in order to remove its water content.

[a] Pressure of CO_2.

Table 7.2. Polymerization of ε-CL in the presence of β-cyclodextrin.

The monomer conversion was always very low and this was confirmed by GPC analysis, where we could clearly see the signal caused by the monomer at about 34 minutes of elution time (Figure 7.5).

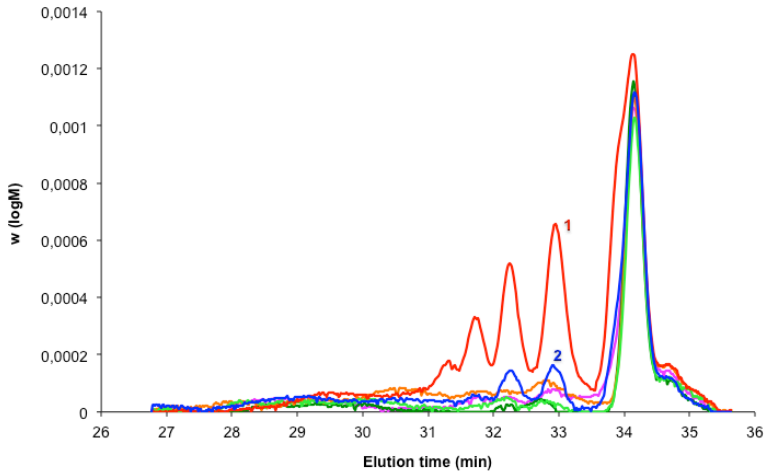

Figure 7.5. GPC elution traces for the samples prepared in experiments of Table 7.2: PCL synthesised using CO_2, with ε-CL and β-CD as they were (entry 5, curve 1); PCL synthesised using CO_2, with distilled ε-CL and β-CD as it was (entry 6, curve 2); the other curves are relative to samples prepared in bulk (entries 1-2-3-4).

The collected data confirmed the results reported in the literature by Harada et al. [5-7], in fact the conversions were very low for reactions conducted for 24 hours. CO_2 promoted expansion of the polymerization mixture did not change significantly the results. Thus, the process conducted under these experimental conditions seemed very slow at all adopted conditions.

7.6.2 Reactions carried out at 120°C in bulk

Due to the results obtained in experiments conducted at 100°C, probably caused by the low activity of the monomer under the adopted conditions, we decided to increase the reaction temperature at 120°C to enhance the polymerization rate. It was reported in the literature that the cyclodextrin decomposes at temperatures higher than 100°C [5], but we performed TGA analysis to verify the thermal stability of the cyclic oligosaccharide (Figure 7.6).

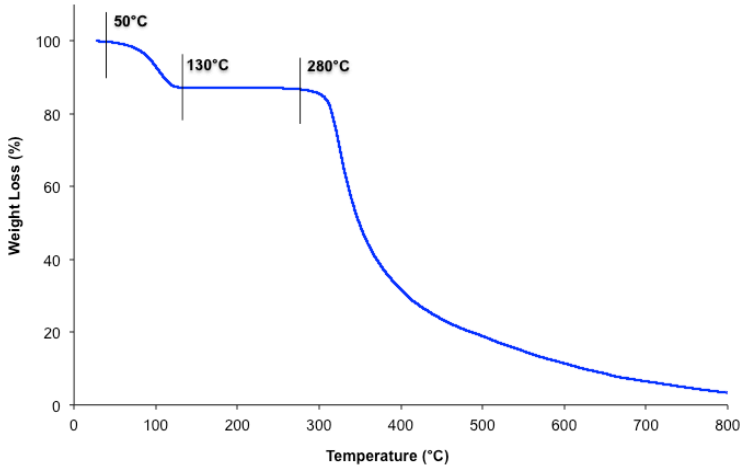

Figure 7.6. TGA thermogram of β-CD.

The CD lost about the 13% of its initial mass in a temperature range comprised between 50°C and 130°C, reasonably as a consequence of water evaporation; the degradation occurred at temperatures higher than about 280°C. This thermogravimetric behaviour was confirmed by data reported in the literature [11]. Thus, from the degradation point of view, working at 100°C or at 120°C seemed the same, because the CD lost only the water molecules contained in its structure, but did not degrade. Besides, working at 120°C, the CD did not change its colour (it remained white) and this was an additional indication that it maintained unchanged its chemical structure.

We performed several polymerization reactions of ε-CL in bulk (Table 7.3), using CD or water as initiator, because also water could initiate the polymerization of ε-CL, according to the scheme reported in Figure 7.7. The obtained products were always liquid, with monomer conversions lower than 2%, even for reactions prolonged for 48 hours. Moreover, the corresponding molecular weights (determined from ^{1}H-NMR spectra) were characteristic of oligomers.

Entry	ε-CL	β-CD	[ε-CL]/ [β-CD]	H_2O[a], μL/g	T, °C	Time, hr	Conversion ε-CL, %	$M_{n\ (H\text{-}NMR)}$[b], g/mol	$M_{n\ (GPC)}$[c], g/mol
1			100	13.1	120	24	0.5	320	-
2			100	13.1	120	48	0.8	470	870
3	distilled		100	13.1	120	24	0.7	300	-
4	distilled		100	13.1	120	48	1.2	330	920
5		dried	100	-	120	24	0.5	420	830
6		dried	100	-	120	48	0.6	160	830
7	distilled	dried	100	-	120	24	0.6	360	790
8	distilled	dried	100	-	120	48	1.0	250	950
9		-	-	2.6	120	24	0.8	770	-
10		-	-	26.1	120	24	0.5	510	-
11		-	-	52.2	120	24	0.7	280	-
12		-	-	104.4	120	24	1.9	200	-

Monomer feed concentration: [ε-CL]=0.84 M, magnetic stirrer rate=500 rpm. When the CD was dried, it was maintained for 2 hours at 80°C under vacuum, in order to remove its water content.

[a] Water content in the reaction system, due to the CD (13% (w/w) of its initial mass) and to water opportunely added inside the reactor.

[b] Molecular weight calculated from ^{1}H-NMR spectra.

[c] Molecular weight obtained from GPC analysis.

Table 7.3. Bulk polymerization of ε-CL in the presence of β-cyclodextrin or water.

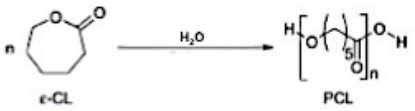

Figure 7.7. Reaction scheme of the polymerization of ε-CL in the presence of water.

Also in this case, the process seemed very slow; however, the ε-CL conversion increased with time. It seems reasonable to think that, in order to obtain high enough conversions and also high molecular weights, it should be necessary to perform the reactions for several days.

It is interesting to note that, when we used water as initiator instead of cyclodextrin, even if the conversion was low, the molecular weight decreased as the water content increased: this probably may be due to the fact that, when there were more molecules of water, there were more species that initiated the polymerization, leading to macromolecules with lower molecular weight.

However, the results obtained under these experimental conditions were not satisfactory and confirmed the low activity of β-CD in the ROP of ε-CL in bulk polymerization system.

7.6.3 ROP of ε-CL at 120°C in the presence of CO_2

We decided to study the polymerization at 120°C in the presence of CO_2, performing the experiments at a CO_2 pressure of about 15.0 MPa (Table 7.4).

Entry	ε-CL	β-CD	[ε-CL]/ [β-CD]	H_2O^a, μL/g	T, °C	P, MPa	Time, hr	Conversion ε-CL, %
1			100	12.9	120	15.2	24	96.0
2		dried	100	-	120	14.7	24	0.5
3	distilled	dried	100	-	120	15.0	24	0.6
4	distilled		100	12.9	120	15.2	24	84.8

Monomer feed concentration: [ε-CL]=0.84 M, magnetic stirrer rate=500 rpm. When the CD was dried, it was maintained for 2 hours at 80°C under vacuum, in order to remove its water content.

[a] Water content in the reaction system, due to the CD (13% (w/w) of its initial mass).

Table 7.4. Polymerization of ε-CL using β-cyclodextrin, in the presence of CO_2.

The first consideration which arises from the analysis of the collected data is that, working under CO_2 pressure and with not dried CD (thus with 13% (w/w) amount of water in its structure), there was a significant increase in the monomer conversion. Anyway, it is interesting to note that high monomer conversion coupled with the formation of a solid product were obtained only when the CD was not dried (with a water content of about 13 μL/g, due to its presence in the native CD). Differently very low conversions were obtained when the CD was dried (the product was liquid). These results are evident in ^{1}H-NMR spectra: in fact, in spectra of samples 2 and 3 the peaks due to the monomer are present, while in those of samples 1 and 4 there are also the peaks due to the polymer; these considerations are confirmed by GPC analysis (Figures 7.8, 7.9).

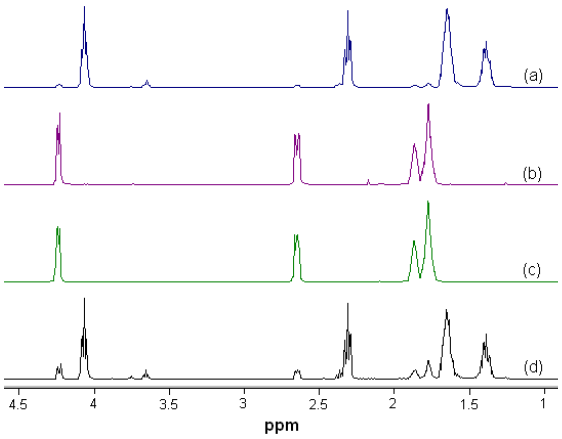

Figure 7.8. ^{1}H-NMR spectra of samples synthesised in experiments of Table 7.4: entry 1 (curve a), entry 2 (curve b), entry 3 (curve c), entry 4 (curve d).

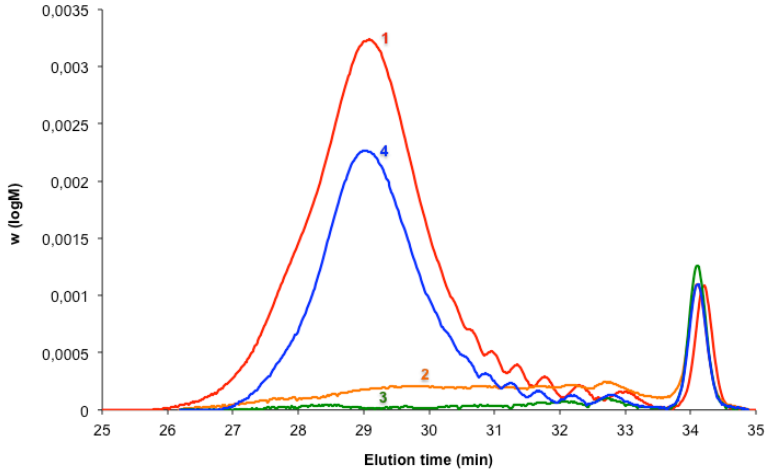

Figure 7.9. GPC elution traces for the samples prepared in experiments of Table 7.4 (the curve number corresponds to the experiment number).

From these preliminary results it seemed that CO_2 pressure could play a significant acceleration effect on the kinetics of the ROP when water is present in the reaction mixture.

7.7 Synthesis of PCL in the presence of CO_2

First we decided to investigate the role of different parameters on the reaction kinetics and on the synthesised products, making the polymerization reactions in the presence of CO_2.

7.7.1 Effect of water content

To highlight the effect of water, we decided to perform a set of experiments adding to the reaction mixture suitable amounts of water, in the presence or not of CD (dried or as it was) (Table 7.5).

Entry	ε-CL	β-CD	[ε-CL]/[β-CD]	H_2O[a], μL/g	T, °C	P, MPa	Time, hr	Conversion ε-CL, %	$M_{n\ (H\text{-}NMR)}$[b], g/mol	$M_{n\ (GPC)}$[c], g/mol
1		-	-	2.6	120	17.0	24	86.4	1820	1770
2		-	-	26.1	120	15.0	24	99.3	1390	1400
3		-	-	52.2	120	15.0	24	99.2	680	800
4		-	-	104.4	120	17.0	24	99.1	670	790
5			100	12.9	120	15.2	24	96.0	1550	1320
6	distilled		100	12.9	120	15.2	24	84.8	1400	1280
7	distilled		100	19.3	120	15.0	24	98.9	1010	1000
8	distilled	dried	100	-	120	15.0	24	0.6	-	-
9	distilled	dried	100	0.6	120	15.2	24	0.6	-	-
10	distilled	dried	100	6.5	120	15.1	24	1.2	250	390
11	distilled	dried	100	25.9	120	15.2	24	6.0	440	540

Monomer feed concentration: [ε-CL]=0.84 M, magnetic stirrer rate=500 rpm. When the CD was dried, it was maintained for 2 hours at 80°C under vacuum, in order to remove its water content.

[a] Water content in the reaction system, due to the CD (13% (w/w) of its initial mass) and to water opportunely added inside the reactor.

[b] Molecular weight calculated from ^{1}H-NMR spectra.

[c] Molecular weight obtained from GPC analysis.

Table 7.5. Polymerization of ε-CL in the presence of water or β-cyclodextrin, adding different amount of water, as initiator.

We performed several experiments at a CO_2 pressure of about 15.0 MPa, using the monomer distilled or not and water or cyclodextrin (dried or not) as initiator for the ring opening polymerization.

Differently from what observed in glass vials at room pressure, when we used water alone (entries 1-4), we obtained high monomer conversion and the formation of a solid product already at the lowest investigated concentration, that led to a monomer

conversion of about 86%. Moreover, the polymer molecular weight decreased as the water content increased (Figure 7.10). All this data are in agreement with the fact that water can initiate the ROP and more water molecules can generate more polymer chains that grow during the process reaching a low molecular weight.

We performed also experiments in the presence of cyclodextrin as it was or dried to remove the amount of water loaded in the system and using the monomer distilled or not. When not dried cyclodextrin was used (entries 5-7), it seems that the process was activated by the presence of water inserted into the system together with the CD; in fact, the conversion is high and the polymer molecular weight similar to that obtained in the presence of water only. When dried cyclodextrin was used (entries 8-11), quite surprisingly even adding controlled amounts of water to the reaction mixture very low monomer conversions were reached. Moreover, as the water content increased, the monomer conversion also increased (Figure 7.10).

It is very difficult to separate the effect of water from that of cyclodextrin. Moreover, it seems that there is an interaction between the hydrophilic shell of the CD and the water molecules.

In fact, it is interesting to underline that when dried CD is present, some sort of interaction with water molecules must be postulated. If we compare two experiments conducted under the same operative conditions and with the same water content, but one with dried CD and one without (entries 2 and 11), we can clearly see that both conversions and molecular weights are very different.

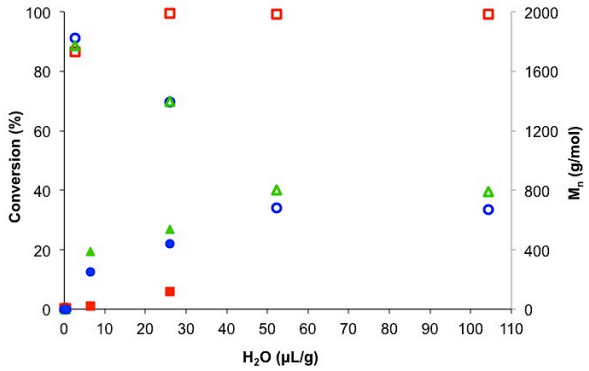

Figure 7.10. Effect of water content opportunely added to the system on the monomer conversion and molecular weight of experiments of Table 7.5. Samples synthesised in the absence of β-CD (entries 1-4): conversion (□), M_n calculated from ^{1}H-NMR spectra (○) and obtained from GPC analysis (△); samples synthesised in the presence of β-CD (entries 7-10): conversion (■), M_n calculated from ^{1}H-NMR spectra (●) and obtained from GPC analysis (▲).

7.7.2 Effect of [ε-CL]/[β-CD] ratio

The effect of the [ε-CL]/[β-CD] ratio was studied in order to investigate if the amount of CD could influence the polymerization process (Table 7.6).

Entry	ε-CL	β-CD	[ε-CL]/[β-CD]	H_2O^a, µL/g	T, °C	P, MPa	Time, hr	Conversion ε-CL, %	$M_{n\,(H\text{-}NMR)}{}^b$, g/mol	$M_{n\,(GPC)}{}^c$, g/mol
1			50	25.9	120	15.0	24	99.2	1090	1080
2			100	12.9	120	15.2	24	96.0	1550	1330
3			500	2.6	120	14.8	24	19.1	740	810
4	distilled		50	25.7	120	15.2	24	99.3	1120	1060
5	distilled		100	12.9	120	15.2	24	84.8	1400	1280
6	distilled		500	2.6	120	15.2	24	1.1	330	420

Monomer feed concentration: [ε-CL]=0.84 M, magnetic stirrer rate=500 rpm. When the CD was dried, it was maintained for 2 hours at 80°C under vacuum, in order to remove its water content.

[a] Water content in the reaction system, due to the CD (13% (w/w) of its initial mass).

[b] Molecular weight calculated from ^{1}H-NMR spectra.

[c] Molecular weight obtained from GPC analysis.

Table 7.6. Polymerization of ε-CL using β-cyclodextrin, in the presence of CO_2: effect of [ε-CL]/[β-CD] ratio.

We performed several experiments at a CO_2 pressure of about 15.0 MPa, using the monomer distilled or not and changing the [ε-CL]/[β-CD] ratio.

As the ratio increased, the amount of CD decreased (and consequently decreased the amount of water inserted with it), with a corresponding decrease in the monomer conversion. This trend was observed both in the case of distilled monomer and when it was not. Besides, in the case of the highest ratio used for the polymerization reactions, the obtained product was liquid, while in the other cases it was solid. The molecular weight slightly increased when the [ε-CL]/[β-CD] ratio was 100 and decreased with low amount of CD. From the analysis of these data, it seemed that the better results in term of conversion and molecular weight were obtained with a [ε-CL]/[β-CD] ratio of 100 (Figure 7.11).

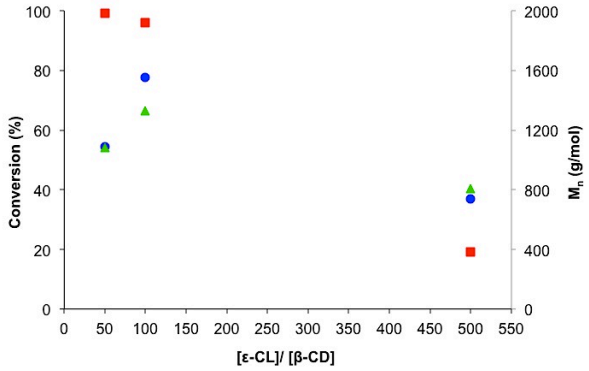

Figure 7.11. Effect of [ε-CL]/[β-CD] ratio on the monomer conversion and molecular weight (entries 1-3, Table 7.6): conversion (■), M_n calculated from ^{1}H-NMR spectra (●) and obtained from GPC analysis (▲).

On the other hand if we filter the collected experimental data pointing the attention to the amount of water loaded in the system together with the CD, it is high the suspect that it could be the water to initiate the reaction. On this base part the research activity was aimed to better understand the role of water in the ring opening polymerization of ε-CL.

7.7.3 Effect of CO_2 pressure

To study the effect of CO_2 presence we performed several experiments at different CO_2 pressure values between about 9.0 MPa and 25.0 MPa, maintaining unchanged the other parameters (Table 7.7).

Entry	ε-CL	β-CD	[ε-CL]/ [β-CD]	H_2O[a], µL/g	T, °C	P, MPa	Time, hr	Conversion ε-CL, %	$M_{n\ (H\text{-}NMR)}$[b], g/mol	$M_{n\ (GPC)}$[c], g/mol
1			100	12.9	120	9.5	24	93.5	1860	1730
2			100	12.9	120	15.2	24	96.0	1550	1320
3			100	12.9	120	25.3	24	28.4	650	710
4	distilled		100	12.9	120	9.2	24	87.7	1760	1550
5	distilled		100	12.9	120	15.2	24	84.8	1400	1280
6	distilled		100	12.8	120	25.1	24	6.5	410	430

Monomer feed concentration: [ε-CL]=0.84 M, magnetic stirrer rate=500 rpm. When the CD was dried, it was maintained for 2 hours at 80°C under vacuum, in order to remove its water content.

[a] Water content in the reaction system, due to the CD (13% (w/w) of its initial mass).

[b] Molecular weight calculated from ^{1}H-NMR spectra.

[c] Molecular weight obtained from GPC analysis.

Table 7.7. Polymerization of ε-CL using β-cyclodextrin, in the presence of CO_2: effect of the pressure.

It is interesting to note that, when we performed the reaction at the highest investigated pressure values (entries 3 and 6), we obtained the lowest conversions and, in fact, the obtained products were liquid. With lowest adopted CO_2 pressure values (entries 1-2 and 4-5), instead, the products were solid and the corresponding conversions higher than 84%. The molecular weights reflected this trend, leading to polymers with higher M_n at pressure values of about 9.0 MPa and 15.0 MPa. This was probably due to the fact that, at higher pressure, the amount of ε-CL solubilised in CO_2 was so high to significantly deplete the concentration of the monomer in the melted phase.

7.8 Effect of the compressing agent

As previously reported, the presence of CO_2 induced a faster polymerization rate with respect to that observed at room pressure in glass vials. At this stage of the research, we wanted to test if this enhancement effect can be associated with

chemico-physical properties of CO_2 or if it is dependent on the higher operative pressure in itself. Due to these considerations, we decided to investigate the ring opening polymerization of ε-CL using also N_2 or the compressed liquid monomer (CLM).

7.8.1 Experiments using water as initiator

The pressure effect was studied first by comparing experiments carried out in the presence of water as initiator (Table 7.8).

Entry	ε-CL	H_2O[a], μL/g	T, °C	P, MPa	Time, hr	Conversion ε-CL, %	$M_{n\ (H\text{-}NMR)}$[b], g/mol	$M_{n\ (GPC)}$[c], g/mol
1		2.6	120	bulk	24	0.8	770	-
2		26.1	120	bulk	24	0.5	510	-
3		52.2	120	bulk	24	0.7	280	-
4		104.4	120	bulk	24	1.9	200	-
5[d]		2.6	120	17.0	24	86.4	1820	1770
6[d]		26.1	120	15.0	24	99.3	1390	1400
7[d]		52.2	120	15.0	24	99.2	680	800
8[d]		104.4	120	17.0	24	99.1	670	790
9[e]		25.0	120	17.9	24	96.3	1500	1530
10[f]		0.2	120	15.0	24	20.0	4230	2370
11[f]		4.8	120	15.0	24	89.4	3120	1930
12[f]		9.7	120	15.0	24	96.3	1720	1110

Monomer feed concentration: [ε-CL]=0.84 M, except for experiments conducted in compressed liquid monomer for which [ε-CL]=9.0 M. Magnetic stirrer rate=500 rpm.

[a] Water content in the reaction system, opportunely added inside the reactor.

[b] Molecular weight calculated from ^{1}H-NMR spectra.

[c] Molecular weight obtained from GPC analysis.

[d] Pressure of CO_2.

[e] Pressure of N_2.

[f] Pressure of compressed liquid monomer.

Table 7.8. Polymerization of ε-CL using water as initiator: effect of the compressing agent.

In the experiments conducted in bulk, the monomer conversions and the corresponding molecular weights were low (entries 1-4). Completely different were these values when the reaction was conducted under pressure (entries 5-12); in fact, with amount of water higher than about 2 μL/g, almost quantitative conversions

were obtained independently on the medium adopted to generate (i.e. CO_2, N_2 or compressed liquid monomer).

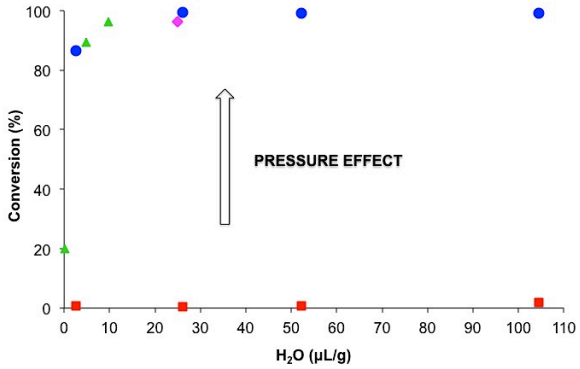

Figure 7.12. Pressure effect on the monomer conversion in experiments of Table 7.8: bulk (entries 1-4) (■), with CO_2 (entries 5-8) (●), with N_2 (entry 9) (♦) and with compressed liquid monomer (entries 10-12) (▲).

The polymer with the highest molecular weight was obtained using compressed liquid monomer at the lowest water content used (entry 10) (Figure 7.13); as the water content increased, the molecular weight decreased.

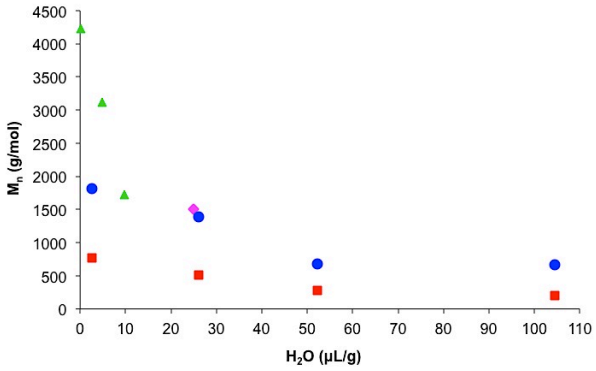

Figure 7.13. Pressure effect on the molecular weight (calculated from ^{1}H-NMR spectra) of samples synthesised in experiments of Table 7.8: bulk (entries 1-4) (■), with CO_2 (entries 5-8) (●), with N_2 (entry 9) (♦) and with compressed liquid monomer (entries 10-12) (▲).

However, in this discussion we have compared experiments in bulk, performed in glass tubes, with experiments carried out under pressure with the polymerization mixture confined in AISI 316 reactors. To verify any catalytic effect of the reactor material in the ROP, we performed bulk experiments in the absence of water or adding about 17 μL/g of water to the monomer also in the AISI 316 reactors. In water free experiments, a free flowing liquid was obtained, while a viscous liquid was collected in the other case. This result was very important because this means that there was also a wall effect due to the reactor material on the polymerization reaction of ε-CL, in addition to the pressure effect.

In any case, it seems that monomer conversions reached are lower with respect to that obtained in the presence of CD.

7.8.2 Experiments in the presence of β-cyclodextrin

The effect of the compressing agent was also studied in the presence of β-cyclodextrin (Table 7.9). We performed several experiments using CO_2, N_2 or compressed liquid monomer, in order to put in evidence differences in the obtained polymeric product.

Entry	ε-CL	[ε-CL], mol/l	[ε-CL]/ [β-CD]	T, °C	P, MPa	Time, hr	Conversion ε-CL, %	$M_{n\ (H\text{-}NMR)}$[a], g/mol	$M_{n\ (GPC)}$[b], g/mol
1		-	100	120	bulk	24	0.5	320	-
2	distilled	-	100	120	bulk	24	0.7	300	-
3[c]		0.84	100	120	15.2	24	96.0	1550	1320
4[c]	distilled	0.84	100	120	15.2	24	84.8	1400	1280
5[c]		7.4	100	120	8.6	24	99.3	1460	1460
6[d]		0.84	100	120	23.1	24	99.3	1560	1560
7[d]		7.4	100	120	17.3	24	99.4	1460	1470
8[e]		9.0	100	120	15.0	24	90.9	880	560
9[e]		9.0	100	120	11.5	24	99.3	1600	1660

Magnetic stirrer rate=500 rpm. Water content in the reaction system, due to the CD (13% (w/w) of its initial mass)=13 μL/g.

[a] Molecular weight calculated from ^{1}H-NMR spectra.

[b] Molecular weight obtained from GPC analysis.

[c] Pressure of CO_2.

[d] Pressure of N_2.

[e] Pressure of compressed liquid monomer.

Table 7.9. Polymerization of ε-CL in the presence of β-cyclodextrin: effect of the compressing agent.

Also in this case, there was a pressure effect on the monomer conversion and on the molecular weight of the obtained polymer.

It is interesting to consider that Harada et al. [5-7] have observed a very low kinetics of ROP of ε-CL in the presence of β-cyclodextrin working in bulk at room pressure (in glass tubes). Instead, working under pressure in stainless steel reactors allowed to synthesise PCL with high monomer conversions in 24 hours.

7.9 Effect of the co-initiator

As we have discussed in the previous section, the performances of the process were enhanced working under pressure and, in particular, using compressed liquid monomer. The effect of water content was studied to better investigate the effect of this parameter and to better correlate the pressure effect with the water content inside the reaction system.

The effect of water in the absence of CD was studied in CLM (Table 7.10).

Entry	ε-CL	H_2O[a], μL/g	T, °C	P, MPa	Time, hr	Conversion ε-CL, %	$M_{n\ (H\text{-}NMR)}$[b], g/mol	$M_{n\ (GPC)}$[c], g/mol
1		-	120	13.2	24	1.5	260	-
2		0.2	120	15.0	24	20.0	4230	2370
3		4.8	120	15.0	24	89.4	3120	1930
4		9.7	120	15.0	24	96.3	1720	1110

Monomer feed concentration: [ε-CL]=9.0 M, magnetic stirrer rate=500 rpm.

[a] Water content in the reaction system, opportunely added inside the reactor.

[b] Molecular weight calculated from ^{1}H-NMR spectra.

[c] Molecular weight obtained from GPC analysis.

Table 7.10. Polymerization of compressed liquid ε-CL using water as initiator.

As the water content increased, the monomer conversion increased, whereas the molecular weight decreased (Figure 7.14).

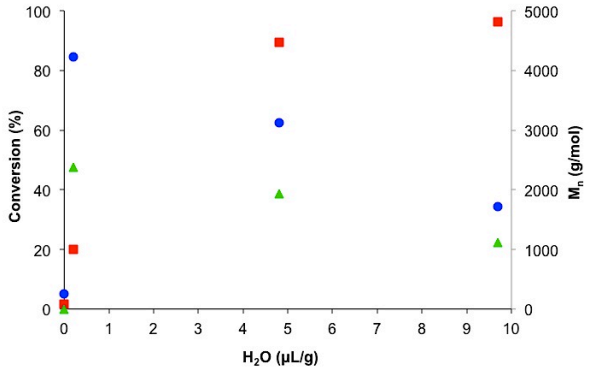

Figure 7.14. Effect of water content on the monomer conversion and molecular weight: conversion (■), M_n calculated from ^{1}H-NMR spectra (●) and obtained from GPC analysis (▲).

This behaviour is in agreement with the presence of more water molecules that initiate the reaction, leading to shorter polymer chains; these trends of conversion and molecular weight as a function of the water content are analogous to those observed in the presence of CO_2 (Figure 7.15).

These considerations led to think that working under pressure the ring opening polymerization of ε-CL could be initiate just by water.

In spite of the low molecular weight obtained, the fact that PCL can be produced in the absence of metallic catalyst makes these polyesters interesting for the preparation of polyurethanes foams for pharmaceutical, biomedical or even domestic applications [12-14].

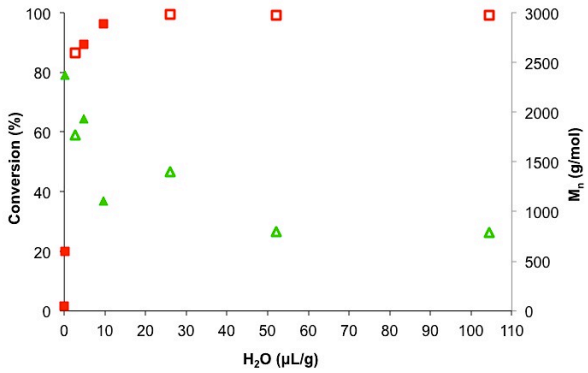

Figure 7.15. Effect of water content opportunely added to the system on the monomer conversion and molecular weight (determined from GPC analysis). Experiments carried out in the presence of CO_2 (entries 1-4, Table 7.5): conversion (□) and M_n (△); experiments performed using compressed liquid monomer (entries 1-4, Table 7.10): conversion (■) and M_n (▲).

The effect of water content was also studied as a function of [ε-CL]/[β-CD] ratio in CLM (Table 7.11).

Entry	[ε-CL]/[β-CD]	H_2O[a], μL/g	T, °C	P, MPa	Time, hr	Conversion ε-CL, %	$M_{n\ (H\text{-}NMR)}$[b], g/mol	$M_{n\ (GPC)}$[c], g/mol
1	100	13.2	120	15.0	24	88.6	1450	810
2	100	15.4	120	15.0	24	96.5	1280	730
3	100	17.8	120	15.0	24	96.0	1250	660
4	500	2.8	120	15.0	24	91.8	3840	2680
5	500	5.0	120	15.0	24	96.6	2600	1660
6	500	7.4	120	15.0	24	99.3	2200	1400

Monomer feed concentration: [ε-CL]=9 M, ε-CL and CD were used as they were; magnetic stirrer rate=500 rpm.

[a] Water content in the reaction system, due to the CD (13% (w/w) of its initial mass) and to water opportunely added inside the reactor.

[b] Molecular weight calculated from ^{1}H-NMR spectra.

[c] Molecular weight obtained from GPC analysis.

Table 7.11. Polymerization of compressed liquid ε-CL in the presence of β-cyclodextrin, adding different amount of water.

We performed several experiments with the AISI 316 reactor completely filled with the polymerization mixture so that a total pressure of about 15.0 MPa was reached at the polymerization temperature, using ε-CL and β-CD as they were, changing the [ε-CL]/[β-CD] ratio and adding different amount of water.

In all cases, the monomer conversion was high, confirming that the polymerization process in the presence of CD (and consequently water inserted with it) occurred in a good manner, even if, at this research stage, we did not know yet if it acted as initiator or not. In both two [ε-CL]/[β-CD] ratios considered, as the water content increased, the molecular weight decreased. It is interesting to note that we obtained polymers characterised by higher molecular weights when we used a ratio of 500. Moreover, in the reactions with the highest [ε-CL]/[β-CD] ratio (entries 4-6), the total amount of water was less with respect to that of the other reactions (entries 1-3): this confirmed that, in the presence of less amount of water, it was possible to obtain polymers characterised by higher molecular weights. This behaviour is opposite of that observed for reactions carried out in the presence of CO_2, for which conversions were very low (section 7.7.2).

Thus, based on these considerations, it seemed that it was only water which initiated the polymerization reaction; it was very difficult to separate the specific effect of water from that of CD, since it contained water also in its original structure. In order

to overcome this trouble, we performed experiments adding benzyl alcohol inside the reaction system, instead of water, in the absence or in the presence of dried β-cyclodextrin (Table 7.12).

Entry	ε-CL	β-CD	[ε-CL]/ [β-CD]	Benzyl alcohol, μL/g	T, °C	P, MPa	Time, hr	Conversion ε-CL, %	$M_{n\,(GPC)}$[a], g/mol
1		-	-	6.9	120	14.9	24	6.4	410
2		dried	100	6.9	120	15.4	24	99.3	720

Monomer feed concentration: [ε-CL]=9.0 M, magnetic stirrer rate=500 rpm.

[a] Molecular weight obtained from GPC analysis.

Table 7.12. Polymerization of compressed liquid ε-CL using benzyl alcohol as initiator, in the absence or in the presence of β-CD.

It is interesting to note that, under the same experimental conditions, when we operated in the presence of dried CD the conversion had a significant increase. This was the confirmation that the CD had a specific role in the process, because it activated the benzyl alcohol. Therefore, in the case of benzyl alcohol it seems that the cyclodextrin behaved as an enzyme-like catalyst.

7.10 Microstructure of synthesised PCL

Harada et al. [5-7], working under anhydrous conditions, from MALDI-TOF analysis determined that CD is incorporated in the polyester chain and thus acts as initiator. In their sample, in fact, in the spectrum there is the presence of a peak due to the CD and all the others are multiple of this at intervals corresponding to the monomer unit (Figure 7.16).

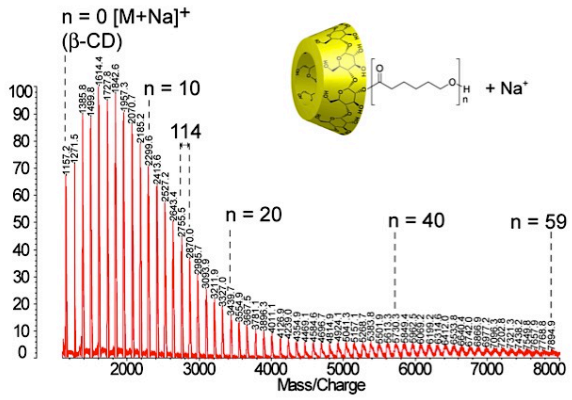

Figure 7.16. MALDI-TOF mass spectrum of β-cyclodextrin attached to poly(ε-caprolactone) synthesised by Harada et al. [6].

MALDI-TOF analysis of selected samples synthesised in compressed liquid monomer using water alone (Figure 7.17) or in the presence of CD, at different [ε-CL]/[β-CD] ratios (Figures 7.18), were performed to investigate if the CD was linked to the polymer chain. Each signal appeared at 114 amu intervals (corresponding to a ε-CL monomer unit). Signals of chains having water fragments OH- and H- as end-groups were detected; only a clear signal at 1157.4 g/mol relative to the free CD was detected, thus suggesting that the cyclic oligosaccharides was not incorporated in polymer chains.

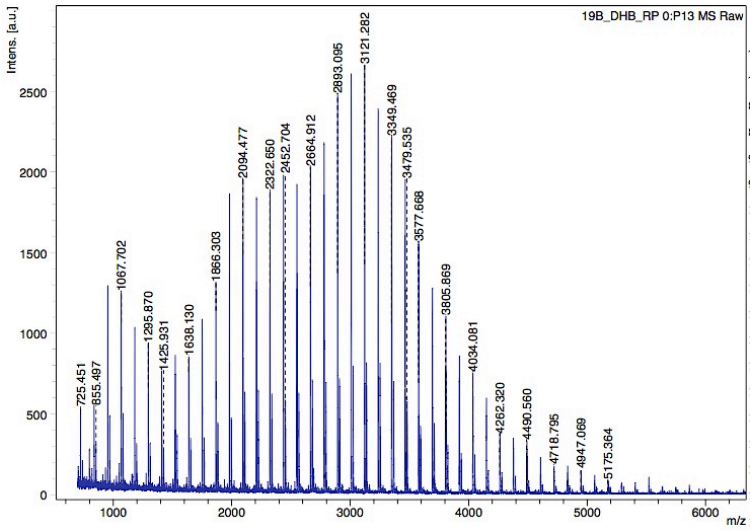

Figure 7.17. MALDI-TOF mass spectrum of a sample synthesised in the absence of β-CD, using water as initiator of the polymerization reaction.

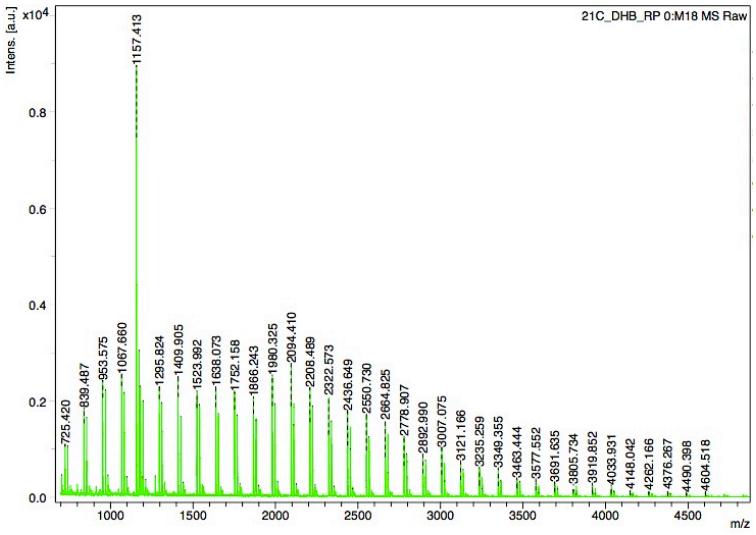

Figure 7.18 (a). MALDI-TOF mass spectrum of a sample synthesised in the presence of β-CD ([ε-CL]/[β-CD]=100).

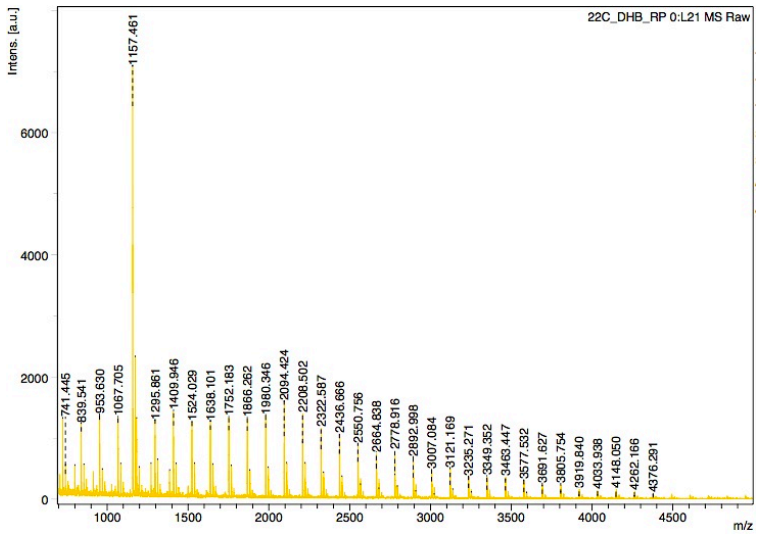

Figure 7.18 (b). MALDI-TOF mass spectrum of a sample synthesised in the presence of β-CD ([ε-CL]/[β-CD]=500).

These considerations led to think that it was water to initiate the polymerization reaction, but cyclodextrin had a specific function in the process.

In fact, it is reported in the literature that cyclodextrins can form inclusion complexes with aliphatic polyesters to give crystalline compounds: the CD encircles the polymer chain so that the chain assumes a polyrotaxane structure [16-20], as is shown as example in Figure 7.19 in the case of α-CD.

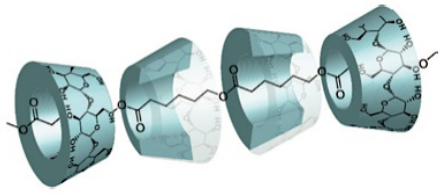

Figure 7.19. Inclusion complex of polyesters with α-CD [5].

Some experimental evidences suggest that, under the operative conditions adopted to perform the polymerization, part of the CD formed an inclusion complex with the synthesized polymer chains.

In fact, when we solubilised in THF or $CHCl_3$ (solvents used for GPC and ^{1}H-NMR analysis) polymer samples synthesised in the presence of CD, we always found an insoluble portion. Free CD is well known to be insoluble in aforementioned solvents and then it must be certainly a component of this fraction. To have information on the composition of this fraction we performed on it FTIR analysis.

In Figure 7.20 the FTIR spectra of free β-CD (curve a) and of a sample synthesised in the presence of β-CD, divided in portion insoluble (curve b) and soluble (curve c) in THF are shown. The soluble portion was characterised by a peak at 1730 cm^{-1}, which can be attributed to PCL [21]. It is interesting to note that the insoluble portion was not constituted by free β-CD only: there was, in fact, the peak of the carbonyl at 1730 cm^{-1} and a not very pronounced peak at 1644 cm^{-1}, probably due to the CD. The presence of the peak due to the carbonyl can be considered an indication that part of PCL chains are made insoluble in THF by complexation with CD. As additional element to support this consideration, it must be underlined that polymer synthesised in the presence of CD was very hard and quite difficult to recover from the stainless steel reactor despite the relatively low molecular weight obtained from GPC analysis.

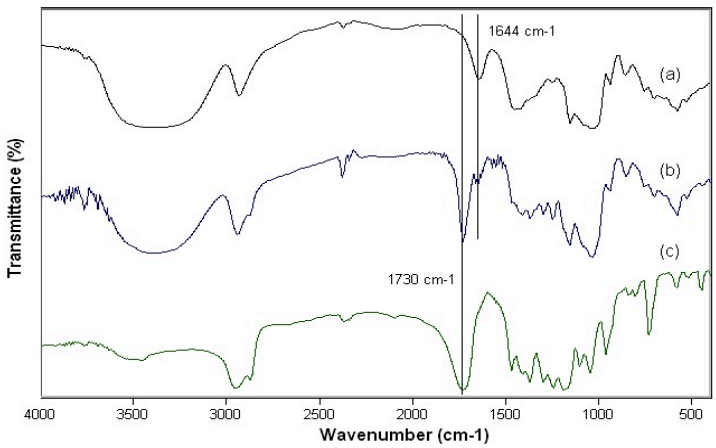

Figure 7.20. FTIR spectra of β-CD (a) and of a sample synthesised in the presence of CO_2: portion insoluble (b) and soluble (c) in THF.

To have additional informations on the structure of the synthesised polymer, we analysed two different samples obtained after Soxhlet extractions with boiling THF or water respectively (Figure 7.21): the first is a good solvent for PCL, the second for β-CD. The residue obtained after the extraction with THF (curve b) presented the carbonyl peak at 1730 cm^{-1} and a little peak at 1644 cm^{-1} due to the CD. The insoluble fraction obtained after the extraction with water, instead, was characterised only by the carbonyl peak at 1730 cm^{-1}, showing that the CD was removed by the treatment.

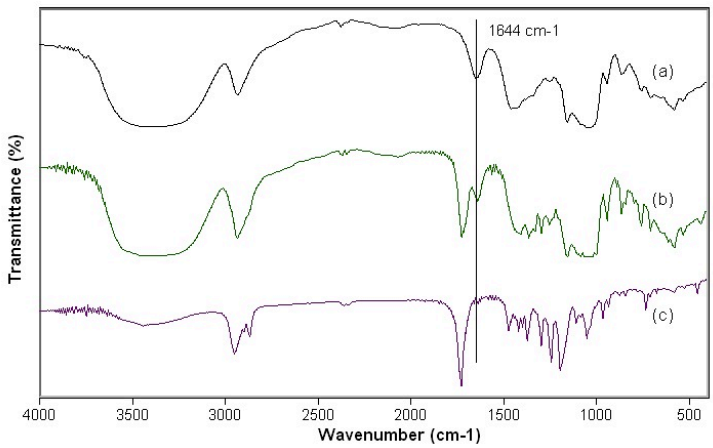

Figure 7.21. FTIR spectra of β-CD (a) and of a sample synthesised in the presence of CO_2 after Soxhlet extraction with THF (b) or water (c).

In Figure 7.22 it is reported the FTIR spectrum for some PCL samples synthesised under pressure (CO_2, N_2 or compressed liquid monomer) in the presence of β-CD. Regardless of the different type of reaction system adopted, the FTIR spectra were very similar, showing the characteristic peak of the carbonyl of PCL at 1730 cm^{-1}.

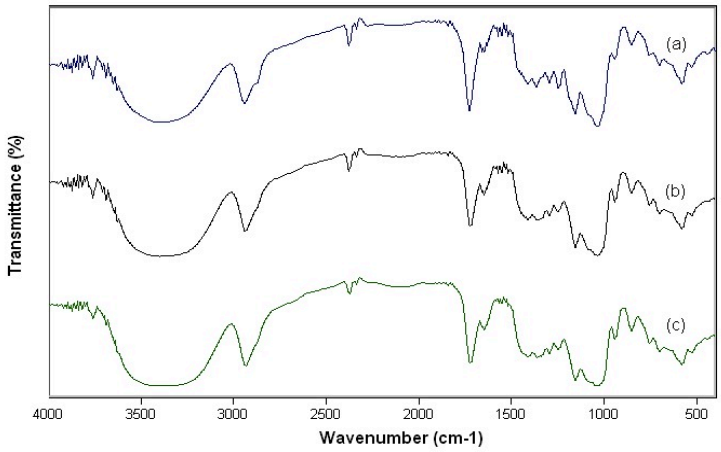

Figure 7.22. FTIR spectra of samples synthesised under pressure of CO_2 (a), N_2 (b) and with compressed liquid monomer (c).

A further indication of the complexation of PCL with CD was given by DSC analysis (Figure 7.23) of selected samples synthesised using compressed liquid monomer in the presence of β-CD (curve 1) or water (curve 2) as initiator. The polymer synthesised in the presence of CD, in addition to the melting peak relative to the PCL at about 57°C, has a little peak at about 140°C, which is not present in the polymer synthesised with water and could be due to the melting of polyrotaxanes domains.

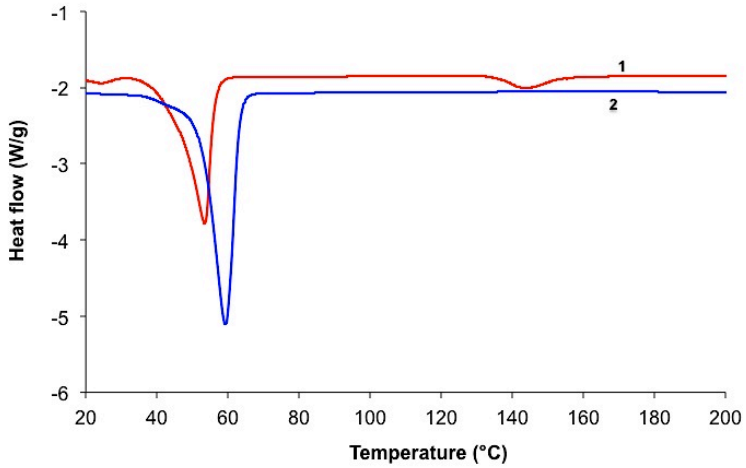

Figure 7.23. DSC thermograms of PCL (1st heating) synthesised in the presence of β-CD (curve 1) or water (curve 2) as initiator.

CD-based polyrotaxanes particular structures which may be widely applied as drug delivery systems by conjugating drug or functional proteins on the CD rings. Polyrotaxanes are very promising for this kind of drug delivery, thanks to several unique structural characteristics. One of the structural features seen in polyrotaxanes is the absence of any covalent binding between the CD and the linear polymeric chain. Moreover, cyclodextrins are formed by many hydroxyl groups that could be easily modified by chemical reactions, allowing the conjugation of bioactive agent on the CD rings. Self-assembled polyrotaxanes display high mobility of cyclodextrins as a result of the CD rotating around the polymer chain; this flexibility

is promising for applications such as targeting drugs, drug-mediated drug delivery and tissue engineering. Cyclodextrins can unthread through the polymer chain when the end is capped through biodegradable linkage, which insures the release of drug into the cell [22,23].

7.11 Conclusions

It was possible to polymerize ε-caprolactone with high monomer conversions in the presence of β-cyclodextrin.

Some experimental results obtained adding co-initiators like water and benzyl alcohol suggested the possibility that the CD can mime the behaviour of an enzymatic catalyst. If confirmed, this results would be highly interesting given the much cheaper cost of the cyclic oligosaccharide in comparison with that of enzymes traditionally adopted for these polymerization processes. A pressure effect on the reaction kinetics was observed: working in bulk, the conversions and the corresponding molecular weights were very low, while working under CO_2 or N_2 pressure or using compressed liquid monomer, the performances of the process were increased, even taking into account the wall effect played by AISI 316 on water co-initiated ROP. Interesting results were obtained working with compressed liquid monomer. It must be underlined that, due to the presence of many interactions between the components of the polymerization system, the interpretation of the experimental data is quite complicated and some carefully designed experiments should be performed to clearly decouple the different effects.

To this regard, since the polymerization of ε-CL in AISI 316 vessels occurred both in the absence or in the presence of CD, provided that water was present inside the reaction system, we found that water can initiate the polymerization as clearly confirmed by MALDI-TOF analyses.

It was quite difficult to separate the specific effect of water from that of CD, since it contained significant amount of water also in its native form. To overcome this trouble, experiments adding benzyl alcohol, instead of water, were performed in the absence or in the presence of dried β-cyclodextrin. The monomer conversion

significantly increased working in the presence of CD and this can be considered a confirmation that CD had a specific role in the activation of the process.

FTIR and DSC analyses suggested that inclusion complexes of CD with PCL are formed under adopted polymerization condition.

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PUBLICATIONS AND CONFERENCES

Galia, O. Scialdone, G. Filardo, **T. Spanò**, "A one-pot method to enhance dissolution rate of low solubility drug molecules using dispersion polymerization in supercritical carbon dioxide", International Journal of Pharmaceutics, 377 (1), p.60-69, 2009.

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