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**SMART NANOSPONGE-BASED SYSTEMS
FOR ADVANCED APPLICATIONS**

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1 – General overview

1.1 Stimuli-responsive smart materials

Smart materials are a class of stimuli-responsive materials that constitute an interesting and expanding area of research, in view of both their possible applications and the conceptual issues implied. The ability of these materials to respond to different *stimuli* (such as mechanical stress, heat, light, gas, electricity, and pH) is related to their physical-chemical characteristics.¹⁻⁴ These systems can find applications in various fields, spanning from drug carrier/delivery devices⁵⁻⁷ to sensors,⁸ environment remediation devices,⁹⁻¹¹ optoelectronic devices, active packaging and so on.¹²⁻²² The *stimulus* can be internal or external, meaning that they can build up at the site of action or that they could be applied externally to achieve the desired effect. For example, redox conditions and pH vary in the different tissues and between intracellular and extracellular compartments. The properties of redox polymers (ionic, electrical, optical, mechanical or chemical) change depending on their oxidation state, offering potential uses for inclusion in actuators, biosensors and drug delivery systems.²³

The key for fabricating smart materials consists in introducing into their structures responsive groups or moieties able to respond to an external stimulus, and perform chemical and/or physical changes. Typical examples of responsive groups or moieties are: imine (C=N), able of protonation/deprotonation reaction; anthracene and vinyl (C=C), able to undergo the cycloaddition reaction with ¹O₂; polymer chains (i.e. poly-ethylene glycol (PEG), poly-vinyl alcohol (PVA) and poly-vinylidene fluoride (PVDF)) able of swelling/deswelling, that can be introduced to fabricate gas-responsive smart materials. Moreover, azo (N=N), spiropyran, dithienylethene and hydrazones are able to isomerization reaction and can be introduced to construct photoresponsive (i.e. light-responsive) smart materials.²⁴⁻²⁷ However, diverse smart materials (i.e., polymers, carbon materials, crystalline liquids, and hydrogels) have been constructed and reported in literature,²⁸ most of which present no or low structural order (i.e. responsive groups are disorderly distributed), inevitably affecting their performances (i.e. relatively low response rate and energy

transformation efficiency). Besides, it is challenging to analyze and unveil the response mechanism and the structure-property relationship for the amorphous materials, because of the lack of structural order.²¹ Thus, exploring new smart materials with clear response mechanisms and improved performance have great significance and urgent demand. For example, ferrocene, triphenylamine, catenanes, pseudo-rotaxane, azobenzene, spiropyran, pyridines, diarylethene and phthalocyanines are extensively investigated under light at various excitation wavelengths and applied into molecular machines, actuators, switches and electronic applications.²⁹⁻³⁴ Furthermore, electrochromic materials such as tetrathiafulvalenes (TTFs), thiophenes, naphthalenediimides (NDIs) and metalpolypyridyl complexes, due to the generation of mixed valence species, lead to artificial muscles, nanorobots and electronic noses. Likewise, extended diimides, porphyrins, tetrathiazolylthiophene, Schiff bases and borondipyrromethane moieties respond very well to sonication, stress/strain, heat and pH results the memory devices, drug-delivery and chemotherapeutic applications.³⁵⁻³⁹

In general, responsive polymers can be classified into two categories.⁴⁰ The first one is constituted by covalently cross-linked polymer networks that swell or shrink owing to a impulse. In this category, the volume of these polymer networks can be dramatically changed by changing the pH or the chemical compositions of the solvent. The second category is constituted by dynamic polymer networks with a variable topology, in which cross-links or chemical bonds can be broken and formed by external stimuli.⁴¹ The most important aspect of these materials is the ability of the network to reform broken bonds and rearrange its molecular structures in a completely different topology and shape. During this process, the association and dissociation of dynamic bonds, and the diffusion of polymer chains, play the most important roles.

Nature itself offers several examples of this type of behavior and an example is given by the ion exchange pumps present in neurons, whose transfer activity of the Na^+ and K^+ ions depends on the variation of the membrane potential.⁴² Another case of "biological material", able to respond to external stimuli, is represented by *spermaceti*, a waxy substance present in the head of sperm whales.⁴³ This mass, when cooled by the entry of water from the blowhole, crystallizes and increase its density, allowing the animal to go diving. Conversely, when heated, decreases its density and the *spermaceti* facilitates the ascent to the surface.

Moreover, one of the "tunability" factors taken into account is the pH. In fact, in the most varied application contexts, the variation of the acidity conditions of the environment in which a material can be found is a situation that is encountered very frequently. An example

is that of a pharmaceutical preparation taken orally, which passes from very acidic conditions of the stomach to neutral conditions of the following intestinal tract.

Recent literature reports many examples of "*pH-tunable*" materials, and it is appropriate to illustrate briefly some particularly significant examples.⁴⁴⁻⁴⁸ Microgels based on poly-acrylic acid and poly-N-isopropyl-acrylamide, able to stabilize Ag and Au nanoparticles, undergo variations in their size as a function of pH.⁴⁹ This causes the metallic nanoparticles included within them to move away or approach, and consequently a variation of their characteristic surface plasmon resonance band is observed in the surface-enhanced Raman scattering spectra (SERS). Given this particular property, these hybrid microgels could be used as a SERS nanosensor for measuring pH value.

Another mechanical effect can be found in the case of "*molecular machines*". This is due to the fact that hydrogen bonding, electrostatic and ion-dipole interactions are easily controlled by protonation and deprotonation processes.⁵⁰ An example of the use of this type is the induction of a translational motion in rotaxanes, with an acid/base mechanism, where the movement of the ring towards another favored position, is the results of the repulsion caused by the protonation of the amino groups of the site where it was previously "anchored" and vice versa.⁵¹ Furthermore, catalysts able to have the catalytic site protected or deprotected with a similar mechanism have been conceived.⁵² In addition, another category of pH-responsive materials, represented by modified porous nanoparticles, has raised particular interest. Systems of this type are generally designed for the inclusion and the delivery of a guest useful for a particular purpose (for example a drug). The guest to be transported is kept inside the pores thanks to an external chemical modification that closes them, to be subsequently "reopened" thanks to a reaction induced by a pH variation.⁵³⁻⁵⁵ These nanoparticles have been designed to act as carriers of anticancer drugs, using a mesoporous silica base covalently modified on the surface with a system consisting of the Indium-Salphen-Folic Acid complex.⁵⁶ This system has two fundamental characteristics: the folic acid, a natural targeting unit for tumor cells, gives the nanoparticles a better selectivity in the delivery of the drug; the complex acts as a "*nancontainer*" that can be open for the drug release once an acid environment is reaches. In fact, a pH value of 5-6 induced by tumor proliferation breaks the Indium-Salphen bond. In fact, systems of this type are quite versatile for this use due to their low toxicity and polyfunctional transport and selective release activity.

As it can be seen from the previous examples, the development of pH-responsive materials is closely linked to *Nanosciences* and to the vast field of *Nanomaterials* and

Nanotechnologies. Recently, the synthesis and the study of materials of the nanometric order have seen a strong growth of interest. As is well known, in fact, materials of this type have intermediate properties between those of systems with atomic/molecular dimensions and those of "bulk" systems.⁵⁷⁻⁵⁹ The most representative case is undoubtedly constituted by nanoparticles and their peculiar optical properties.⁴⁹ However, the concept of the "nano" system is also extended to other areas, including in particular nanotubes⁶⁰⁻⁶⁶ and especially nanosponges; the latter constitute the particular object of this work.

1.2 Nanosponge materials

Potential smart materials particularly studied in recent years are organic *nanosponges* (NSs). According to Trotta and Mele,⁶⁷ "Nanosponges" can be generally defined as "*porous materials having all the three external dimensions in the micro- or macrorange, and the internal cavities, pores or voids in the nanometer range*". This definition, clearly based on the physical and texture properties, allows to collect quite diverse materials from a chemical standpoint (even of inorganic nature). However, they are also frequently referred to as "*hyper-reticulated polymers*". In other words, the easiest way to conceptualize a nanosponge structure is the possibility to conceive it as an ensemble of constitutional moieties suitably joined by linker moieties. Of course, no limitations exist in the choice of both structural counterparts, provided that a chemically stable product is obtained. However, the "constitutional" moieties are frequently constituted by oligomeric or polymeric structures (an exemplificative though non exhaustive list is provided in Table 1.1). Moreover, no condition is required as long as the microscopic structure is concerned, in the sense that they are in general highly disordered amorphous solids. As "sponges", these materials are expected to be easily pervaded by, and interact, with solvent media, possibly undergoing more or less extensive swelling.

It is immediately apparent that the textural features of nanosponges critically depend on the choice of the constitutional and the linker moieties, and on their combination ratio. Therefore, the size of the nanoscopic pores or cavities within the overall structure can be largely modulated as a function of the synthesis conditions, the relative amount of the crosslinking agent and, if different types of constitutional moieties are used, also on their molar ratio.⁶⁸

Table 1.1. List of organic polymers and crosslinkers used for the production of NSs.

Polymers	Cross-linkers
Homopolymers	
Polystyrene	Diarylcarbonate
Native and modified Cyclodextrins	Diisocyanate
	Pyromellitic Anhydride
Copolymers	
Poly (valerolactone-allylvalerolactone)	Carbonyldiimidazole
Poly (valerolactone-allylvalerolactone-oxepandione)	Epichlorohydrin
Ethylcellulose	Glutaraldehyde
Polyvinyl alcohol	Dianhydride
	Dichloromethane

All the components contribute to the overall three-dimensional structure of the material and determine their chemical-physical characteristics, such as the hydrophilic/hydrophobic character,⁶⁹ or any acid-base properties. For example, if epichlorohydrin is used to crosslink the β -cyclodextrin units, highly hydrophilic nanosponges are obtained.⁷⁰ Conversely, linkers such as diphenylcarbonate, diisocyanates and others are used to synthesize more hydrophobic polymers.⁶⁹ According to the literature,^{5,69,71-72} for application purposes, the main characteristics of the nanosponges should be:

- Porous structures of the paracrystalline or crystalline type;
- Average pore size of nanometric order;
- Thermal stability up to $T \approx 300\text{ }^{\circ}\text{C}$;
- Stability in the pH range from 1 to 11;
- Insolubility in water and in organic solvents with formation of suspensions from which the polymer is mechanically recoverable;
- Non-toxicity;
- Recyclability of the polymer by thermal desorption or extraction by treatment with solvents of any included or adsorbed species.

As long as the constitutional moieties are concerned, one of the most frequent choices is constituted by macrocyclic oligomeric structures such as cyclodextrins, able to act as supramolecular hosts. Indeed, one of the most important features of nanosponges relies in their ability to interact with a solution in which a solute species is present, in such a way to

adsorbe or sequester it from the solution itself, or, contrariwise, to be loaded with a suitable solute to be more or less rapidly released in solution. Of course, the nanosponge-solute interaction must be an equilibrium process (an illustrative depiction is provided in Figure 1.1), and this implies the *recyclability* of the material, i.e. the possibility to elute away a possible adsorbed substance in order to get again the unloaded material with no loss of its adsorption abilities and (possibly) negligible mass losses. Therefore, thanks to their overall peculiar properties, nanosponges may find plenty of applications in diverse fields, particularly (but not only) in pharmaceutical, agricultural and environmental technologies.

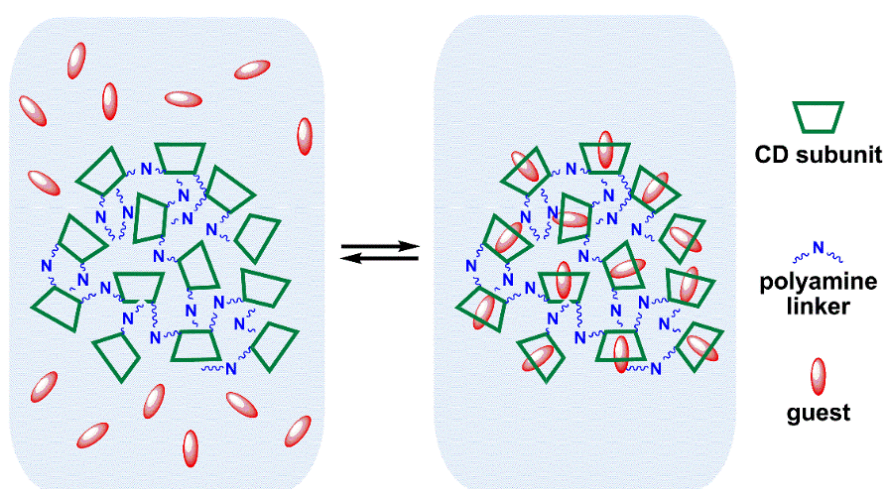


Figure 1.1. Pictorial representation of the host-guest equilibrium that is established in the case of a polyaminocyclodextrin nanosponge.

The formation of a complex between a nanosponge and a guest species is certainly influenced by the solvent medium. A hydrophilic medium pushes the organic molecule inside the nanocavities of the polymer, due to the hydrophobic effect; an organic solvent, on the other hand, favors the release of the molecule from the nanosponge.⁷³ Another factor that influences the inclusion is the temperature at which it occurs. In fact, increasing the temperature a decrease in the apparent stability constant of the drug-nanosponge complex occurs, due to the reduction of the interaction forces between the two parts, such as van der Waals forces and hydrophobic interactions.⁷² According to the type of interaction with the included molecule, three types of nanosponges can be distinguished.^{5,69,71-72} The first is represented by polyanion-based gels, such as alginate or pectin, where its pores are able to encapsulate molecules essentially through electrostatic interactions. The second type is made up of “*complexing*” systems that is able to bind the guests through the formation of

supramolecular complexes. Finally, the third type, defined as "*conjugant*", interacts with the included species by establishing covalent bonds.

The main application of nanosponges is probably their use in the pharmaceutical field such as drug-carrier/delivery systems, thanks to their ability to encapsulate both hydrophobic and hydrophilic molecules.⁶⁸ In particular, NSs allow to increase the water solubility of lipophilic drugs, to protect otherwise degradable substances, and to formulate alternative methods of drug administration to the oral way. For instance, an interesting case of vehiculation is represented by topical treatment.⁷⁴⁻⁷⁵ Hydrophilic nanosponges can increase the absorption of drugs at the level of their pharmaco-kinetics, in the step of drug distribution in the body, and improve the performance of rapid release formulations. On the other hand, hydrophobic nanosponges allow the controlled release of water-soluble drugs, improving their pharmacodynamics.⁷⁶ Furthermore, the functionalization degree of the supramolecular hosts employed in the nanosponges synthesis also influences the crosslinking.⁷⁷ The multiple interactions between nanosponges and bioactive molecules ensure that the latter are able to be solubilized inside the cavities. Therefore, the inclusion improves the bioavailability of poorly soluble molecules.⁶⁹ An example is a study by Swaminathan *et al.*, in which itraconazole, having a poor water solubility (10 ng mL^{-1}) appears 27 times more soluble when included into a nanosponge matrix.⁷⁸ At the same time, by adding the PVP (polyvinylpyrrolidone) to the formulation, the solubility further increases (55 times greater than itraconazole as such).

The nanosponges can also be used for environmental remediation purposes. For instance, cyclodextrins nanosponges can be used for the removal of organic pollutants from water, evidencing significant advantages with respect to other commonly used sorbents such as activated carbons (see Section 1.5 for more detailed discussion).⁷⁹ Furthermore, the three-dimensional microporous network of NSs also allows their use as separation media. For example, a possible application is presented in a work in which NSs made up of 70% of poly-(N-isopropylacrylamide) and of 30% of polymethacrylic acid, were used for the fractionation of peptides with the aim of obtaining a new separation system for analysis techniques such as MALDI-MS.⁸⁰ Given the high surface area, these NSs have proved to be a valid alternative to the use of two-dimensional separation media. The self-assembled planar monolayers of different columns, in fact, were less efficient for large molecules such as peptides.

Considering that most of the works reported in literature concern cyclodextrin-based nanosponges, it is appropriate to open a short parenthesis to illustrate the structure, the

properties and chemistry of this particular type of macrocycles. However, in literature, there are some examples of mixed cyclodextrin-calixarene nanosponges⁸¹⁻⁸⁷ and calixarene only-based nanosponges.⁸⁸⁻⁸⁹ For this reason, it is useful to provide herein a brief overview on the structure and properties of cyclodextrins and calixarenes.

1.3 Cyclodextrins

Cyclodextrins (CDs)⁹⁰⁻⁹¹ are natural oligosaccharides formed by α -(1,4)-linked glucopyranose units. The most common CDs are the α -, β -, or γ -cyclodextrin, which are formed by 6, 7, or 8 glucopyranose units, respectively (Figure 1.4). It is possible to produce them on industrial scale via enzymatic degradation of starch by means of bacteria such as *Bacillus amylobacter* or *B. macerans*.⁹²⁻⁹³ Since the glucopyranose units are in the chair conformation,⁹⁴ the macrocycle ring is usually described as a doughnut or a wreath-shaped truncated cone.⁹⁵ CDs have a hydrophobic cavity and hydrophilic rims, where the latter one is constituted by the hydroxyl groups in position 6 in the primary edge and in position 2-3 in the secondary edge. The inner cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges, respectively. The nonbonding electron pairs of the glycosidic oxygen bridges are directed toward the inside of the cavity producing a high electron density there and lending to it Lewis base characteristics. Of course, the size of the structure (volume and width of the cavity) vary according to the number of monomers that constitute it (Table 1.2).

The solubility in water is an important property for these molecules. Notably, the β -CDs have a limited solubility in aqueous medium both because of the unfavorable hydration energies compared to the other CDs, and because the latter ones are more flexible.⁹⁶ In fact, the β -CD molecule meets the best geometrical requirements to optimize the homodromic network of hydrogen bonds established at the secondary rim, which limits its interaction with water molecules and stiffens the overall structure. This occurs only to a minor extent with α -CD and γ -CD. In addition, it has been demonstrated that the cavity possesses an intrinsic dipole momentum, with the positive side directed towards the primary rim and the negative one towards the secondary edge, given by the vector sum of the dipoles of all the monomers making up the macrocycle (Figure 1.5).⁹⁷⁻⁹⁸

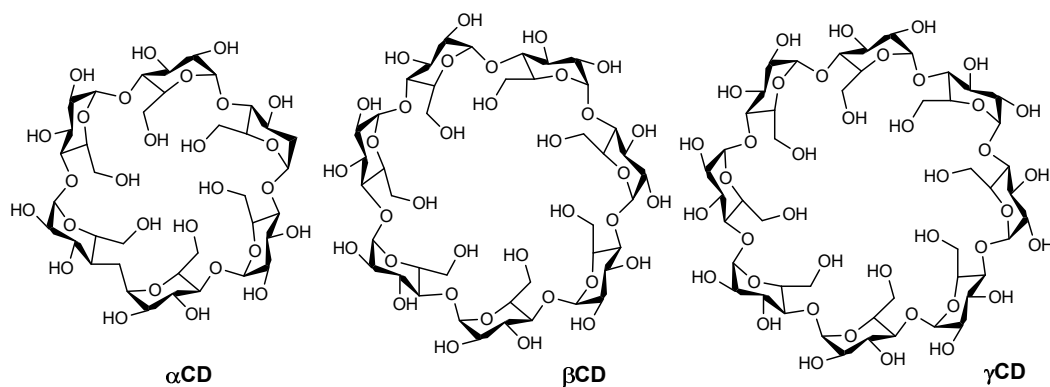


Figure 1.4. Structures of α -Cyclodextrin (α CD), β -Cyclodextrin (β CD) and γ -Cyclodextrin (γ CD).

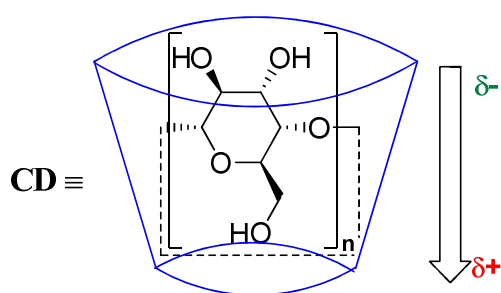


Figure 1.5. Dipole moment of cyclodextrins.

Table 1.2. Properties of Cyclodextrins (CDs).

	α CD	β CD	γ CD
Number of glucose unit	6	7	8
Molecular weight (g/mol)	972.85	1134.99	1297.14
Cavity diameter (Å)	4.7-5.2	6.0-6.4	7.5-8.3
External diameter (Å)	14.2-15.0	15.0-15.8	17.1-17.9
Depth (Å)	7.9-8.0	7.9-8.0	7.9-8.0
Internal cavity volume (Å³)	174	262	472
Solubility in water (25 °C, g/L)	145	18.5	232
Number of H₂O molecules in the cavity	6	11	17
Melting point (°C)	250-260	255-265	240-245
pK at 25 °C	12.332	12.202	12.081
[α_D] (deg)	+150.5	+162.0	+177.4

The main property of cyclodextrins is undoubtedly their ability to form supramolecular inclusion complexes (“*cavitates*”) with suitably sized and shaped organic molecules.⁹⁹⁻¹⁰³ Indeed, cyclodextrins are par excellence *the* typical and probably most known *hosts* of Supramolecular Chemistry. This ability relies on the simple fact that organic *guest* molecules

can be easily accommodated inside the cyclodextrin cavity, replacing the high free energy water molecules solvating the cavity itself, in a sort of ligand exchange process, as outlined in a seminal paper by Tabushi.¹⁰⁴ This replacement is one of the main “driving forces” of the complex formation process, which is conventionally considered as the sum of a “nominal” and an “environmental” counterpart.¹⁰⁵⁻¹⁰⁷ The former one refers to the strict host-guest interaction, which in turn involves either non-specific (van der Waals interactions, interactions dipolar, solvation effects) or of specific type (hydrogen bond, CH $\cdots$ π interactions).^{105,108-110} The environmental part of the process merely consists in the rearrangement of the solvation shells of the species. The conformational flexibility of cyclodextrins allows them to adapt their structure to the one of the included guest, in such a way to optimize the microscopic interactions involved. The latter fact, known as the “*induced fit effect*”,¹¹¹ implies that depending on the guest, different interactions can assume greater or lesser importance. Of course, all these factors reflect on both the enthalpic and the entropic contributions to the overall thermodynamics of the binding process. Enthalpy-entropy compensation (the so-called linear isoequilibrium correlation) can be observed, provided that structurally strictly homogeneous sets of guests are considered, and has been object of intense debate.^{105,107,112} As an extremely important consequence, the overall stability of the inclusion complex always results from a fine counterbalance of different and frequently contrasting factors. Hence, full and exhaustive rationalization of the outcome of the various types of interactions involved is in general very difficult, and subtle discussion of the experimental evidences is required, case by case, to individuate the actual driving force of the process.

The inclusion of the guest, in turn, involves a change in its chemical-physical properties, and in particular the solubility and, in specific cases, its reactivity. Consequently, the formation of complexes with CDs can be exploited for example to convey highly hydrophobic molecules into aqueous medium, which is widely used in pharmaceutical technologies.¹¹³⁻¹¹⁶ Furthermore, CDs can be used as confined reaction environments or even as enzyme-mimetics models.¹¹⁷⁻¹²³ Other applications can be found in industrial chemistry, in the textile, food and cosmetic sectors.^{120,124-129} To give some examples we can mention surfactants,¹³⁰ drugs,¹³¹⁻¹³² organic pollutants,^{122,133} dyes,^{119,127} and metal ions.¹³⁴

Cyclodextrins are chemically very versatile systems, thanks to the nucleophilic reactivity of the hydroxyl groups. Therefore, it is possible to obtain appropriately modified macrocycles in the most varied ways, as evidenced by the enormous literature on the subject.^{120,135-137} In principle, based on the particular reaction conditions used, it is possible to carry out the

reactions with a certain specificity. Under neutral conditions, the attack of electrophilic species on the less hindered primary hydroxyl groups is favored, whereas alkaline conditions favor the attack on the secondary hydroxyl groups (even if sometimes it is necessary to protect the primary groups). The possible functionalization depends both on the reaction chosen and on how many hydroxyl groups on the entire macrocycle are to be functionalized (Figure 1.6).

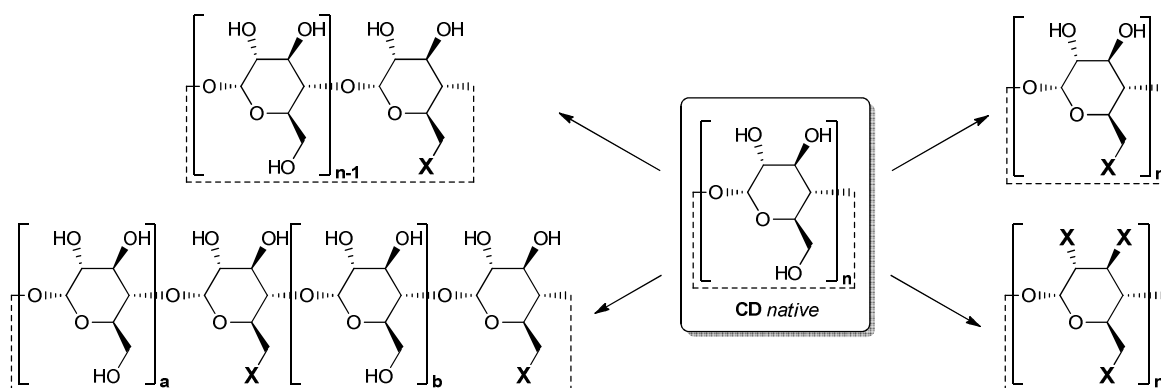


Figure 1.6 General strategies for the chemical modification of cyclodextrins.

In detail, if one acts using a defect of the reagent concerned, it is possible to obtain the mono- or bis-derivatives on specific sites. Alternatively, the exhaustive derivatization of a single rim is possible, by using an excess of the reactive, or even of both rims. Furthermore, functionalization can transform the hydroxyl groups (in particular the primary ones in position 6) into potential leaving groups, such as halogen or tosylate.¹³⁸⁻¹³⁹ This in turn opens the way to reactivity towards nucleophilic species. Therefore, through a SN2-type reaction it is possible to introduce even very complex preformed groups on the structure of the CD. The possibility of obtaining functionalized CDs enables broadening the amount of possible interactions that these may have with other molecules or ions, and consequently this allows the binding capacities of the macrocycle to be modulated within very wide limits.

The formation of cyclodextrin-based nanosponges exploits these general principles.^{5,69,71-72,140-141} In particular, the simplest way is to react a native CD (generally the β -CD, which is the cheapest and easiest to obtain) with a double electrophilic species, which reacts with the hydroxyl groups of two different CD units, acting as a “linker” between them. Of course, due to the presence of several -OH groups on each single unit, the reaction with the double electrophile will have the effect of linking the CD units in a random way, eventually forming a hyper-crosslinked structure. It is intuitively apparent that the physical and mechanical

characteristics of the hyper-crosslinked polymer formed (stability, porosity, fragility, etc.) will depend on both the chemical nature and structure of the linker, and the molar combination ratio between linker and monomer. In principle, this can also give a certain modulation of the adsorption properties of the polymer itself.

From a chemical point of view, the most common electrophilic linkers that are used (Figure 1.7) can be distinguished in: *i*) derivatives of carbonic acid¹⁴² and carbonyl-diimidazole,¹⁴³ through which a narrow carbonyl bridge between two CD units is created; *ii*) epichlorohydrin,¹⁴⁴ which creates a 2-hydroxy-propylene bridge; *iii*) bis-isocyanates¹⁴⁵⁻¹⁴⁶ and chlorides of dicarboxylic acids,¹⁴⁷ which instead create bis-urethane or bis-ester bridges respectively, with the advantage of enabling to vary the length of the bridge as desired, thus, modulating the porosity. A possible pictorial representation of the polymer structure is shown in Figure 1.8.

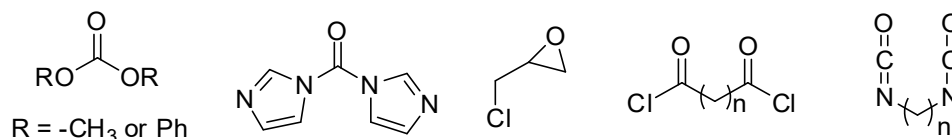


Figure 1.7. Structure of the most common linkers used for the synthesis of cyclodextrin NSs.

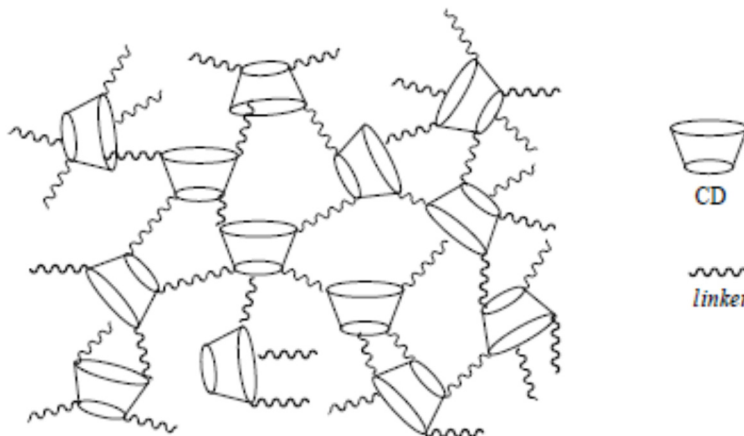


Figure 1.8. Structure of a generic cyclodextrin nanosponge.

In general, the syntheses of these β CD-based materials has been carried out using two different methods, namely a “*melt*” procedure¹⁴⁸ and a “*solvent*” method.¹⁴⁹⁻¹⁵⁰ In the first one, the mixture of finely homogenized crosslinker and the cyclodextrin is heated possibly under mechanical stirring to obtain a solid, which is then ground and washed with suitable solvents to remove secondary products and unreacted material. On the other hand, the “*solvent*” method provides the formation of the polymer in solution, and the reaction

conditions can be optimized by varying the ratio between β CD and crosslinker. Organic solvents such as dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) are frequently employed to solubilize the crosslinker and the β CD. The solution is heated at the suitable temperature for the proper time and then a solid product is obtained by adding water to the reaction crude. Finally, the solid product obtained is isolated by vacuum filtration, followed by purification by Soxhlet extraction.

1.4 Calixarenes

Calixarenes ($C[n]A$) are meta-cyclophanes, i.e. cyclic oligomers consisting of a certain number of phenolic units linked by methylene bridges in reciprocal *meta* position and look like vases (*calix crater*, Figure 1.9).¹⁵¹



Figure 1.9. The *calix crater* and the structure of calixarene.

They have a non-rigid calyx structure, with possible rotation of the arene units around the methylene bridges, giving rise to the possibility of forming different conformers.¹⁵²⁻¹⁵⁴ The mobility of the arene units can be limited by means of steric effects, alkylating the phenolic hydroxyls with sufficiently bulky groups.¹⁵⁵⁻¹⁵⁶ The distribution of the conformers depends on various factors such as the presence of hydroxyls, methoxyls or ethers on the lower edge. If the edge presents hydroxyl groups, since these are very close, they have the possibility to do intramolecular hydrogen bonds that will stabilize the cone conformation. If we pass to methoxyls or any other ether, however, there will be such a modification as to have a destabilization of this conformation as these hydrogen bonds are missing and this distribution depends on the solvent at which this evaluation is made. The cone conformation,

being the most polar, is the one favored in the presence of polar solvents. With other solvents, however, this conformation will not present this advantage. Clearly, the distribution of conformations between the title calixarene and functionalized calixarene can be different. The most common calix[n]arenes are formed by 4, 6 or 8 arenic units, and are obtained by condensation in a basic environment of para-substituted phenols with formaldehyde and subsequent thermolysis of the polymer,^{151-152,157-158} or through condensation in an acid environment of resorcinol with aliphatic or aromatic aldehydes.¹⁵⁹ Also in this case, the base structures can be subjected to chemical modification, both by exploiting the reactivity of the aromatic rings (SEA reactions)¹⁶⁰ and that of phenolic hydroxyls.¹⁶¹

In the past decades, considerable attention has been paid to the chemical modification of calixarenes, which has been used to deeply alter the chemical and supramolecular properties of the parent macrocycles.^{151,158} The transformations are usually performed by: *i*) the functionalization at the para positions of the aromatic rings; *ii*) the functionalization at the phenolic hydroxyls; *iii*) the substitution and replacement of methylene bridges by Si, S, SO₂, -CH₂-O-CH₂- and -CH₂-N(R)-CH₂- groups significantly changing the physical and chemical properties of the parent compound; *iv*) the less investigated modification of the aromatic "walls" of the calix cavity¹⁶² and *v*) the extension of the calix[n]arene cavity. The calixarene platform provides unique possibilities to organize several binding sites in an array complementary to a potential guest.

They too have a hydrophobic cavity, able to include both organic molecules and inorganic cations, mainly exploiting the possibility of establishing π - π or cation- π interactions.¹⁶³⁻¹⁶⁴ Very often, however, especially in the case of interaction with inorganic ions,¹⁶⁵⁻¹⁶⁶ an important role is played by suitable substituent groups linked to the structure. In general, however, it has been seen that C[n]A have quite different, and somewhat complementary, binding properties compared to CDs.

1.5 Cyclodextrin and Calixarene-based Nanosponges: synthesis, characteristics and applications

The possibility to modulate the cavity size, the low production cost, the stability and the complexing abilities make hosts like β -CDs and calixarenes ideal starting materials for the preparation of Nanosponges.¹⁵⁰ The first reported use of the term nanosponge was to label

insoluble polymers obtained by cross-linking β -cyclodextrins with organic diisocyanates linkers and these materials were used for the removal of organic pollutants as *p*-chlorophenol from wastewater.¹⁶⁷ As already mentioned in Section 1.3, different synthetic approaches are described in the literature.^{69,72,168-170} The most common one provides that all the ingredients are homogenized and heated to the proper temperature. The reaction takes place in a few hours under stirring; then, the crude is allowed to cool and the product is recovered and washed with different solvents to remove any unreacted residues and by-products.⁶⁹ An alternative method consists in solubilizing the crosslinker in an appropriate solvent and adding this solution to the monomeric host. The optimization of the process takes place by changing the combination ratio between linker and monomer, and the amount of solvent. The reaction temperature can be largely varied from 10 °C up to the evaporation temperature of the solvent, and the reaction time can be extended up to several days. The product is obtained by adding water to the reaction mixture and filtering the solid obtained.¹⁴⁹⁻¹⁵⁰ Further methods exploit sonochemistry; indeed, cyclodextrins can react with the linker in the absence of solvent (*solvent free*), by sonicating and heating (90 °C).⁶⁸ Moreover, the synthesis of cyclodextrin-based nanosponges carried out through the use of microwaves is also documented in the literature.¹⁷¹ In all cases, for the purposes of an application in pharmaceutical field, the purification step is very important, due to the possible toxicity of the by-products in the final reaction mixture. From an FTIR-ATR, solid state NMR and Raman analyses it can be seen that the hydroxyl groups are mainly involved in the cross-linking process where the reticulation process gives rise to channel-rich nanostructures that allow to describe these materials like sponges. The NSs mainly reported in the literature are spherical particles with an average diameter of 200-500 nm and a narrow polydispersity.

Cyclodextrin-based NSs can be conveniently distinguished according to the crosslinker used in their preparation. As already mentioned in Section 1.3, most used linkers include bis-electrophilic species, such as:

- Diisocyanates. Among these, the most common used are hexamethylene diisocyanate (HDI) and toluene-2,4-diisocyanate (TDI). The nanosponges obtained have been used in water purification; in particular, a polymer of the β -CD/TDI type has been reported, able to remove 84% of dissolved organic carbon (DOC).⁹
- Carbonates including carbonyldiimidazole (CDI) and triphosgene. Nanosponges of this type have been used as carriers of various bioactive molecules with anticancer properties, such as taxol and camptothecin, but also anti-inflammatory, such as dexamethasone, flurbiprofen, and others.⁶⁹

- Anhydrides. An example is certainly the pyromellitic dianhydride. The polymers obtained from the reaction between β -CD and this linker have been studied, for example, as carriers of the curcumin, an antioxidant.¹⁷²
- Polyamidoamines. The preparation of this type of nanosponges proceeds in water, making the cyclodextrin react with 2,2-bis-acrylamidoacetic acid (BAC). The product obtained is capable of absorbing and releasing albumin in a controlled manner and forms stable hydrogels.¹⁴⁷

The nanosponges listed above belong to the so-called first generation of cyclodextrin-based polymers, as reported in the literature.¹⁷³ In addition to these, we may recall:

- Second generation nanosponges. They are polymers decorated with fluorescent functionalities, electrically charged or hydrophobic groups, to modulate their polarity.
- Third generation nanosponges. They are polymers sensitive to external stimuli. These nanocarriers are known for their ability to release controlled starting from a stimulating signal, such as the variation of pH or the interaction with certain receptors.
- Fourth generation nanosponges. This is represented by polymers of the “MIPs” type (Molecularly Imprinted Polymers), for the synthesis of which a templating molecule is used during the crosslinking process. These materials are able to perform a highly specific and selective molecular recognition; have high stability and low production costs and are easily to prepare.

Chemical modification of cyclodextrins opens the way to virtually countless opportunities in supramolecular chemistry, as accounted for by an immense literature.^{5,69,120,135,142,168-170,173-175} An issue of particular interest is offered by modified CDs bearing amino or polyamino pendant groups. These derivatives can even dramatically change their binding abilities depending on the pH value of the solvent medium. For instance, a click chemistry approach has been exploited¹⁷⁶⁻¹⁷⁸ to graft suitable polyamine derivatives on the primary CD rim. More recently, a nucleophilic displacement reaction approach enabled to easily obtain in excellent yields polyaminocyclodextrin mixtures, by reacting a per-(6-deoxy)-(6-halo)-cyclodextrin with polyamines.¹⁷⁹⁻¹⁸⁰ These products can be used as capping agents for the stabilization of nanoparticles of noble metals such as Ag, Pd and Au.¹⁸¹⁻¹⁸² In particular, systems of this type can find interesting applications as micro-heterogeneous catalysts.¹⁸² These polyaminocyclodextrins have been used for obtaining pH-responsive nanosponges.¹⁸³

The synthesis simply provides their reaction with the same aforementioned per-(6-deoxy)-(6-halo)-cyclodextrins.

The use of host monomer units different from cyclodextrins to obtain nanosponges, has been by far less extensively explored. For instance, Calixarene-only polymers have been obtained exploiting a click-chemistry approach, by reacting via the well-known *CuAAC* copper-catalyzed cycloaddition reaction a propargyloxy-calix[4]arene with bis-azides or polyazide mixtures (Figure 1.10).⁸⁸⁻⁸⁹

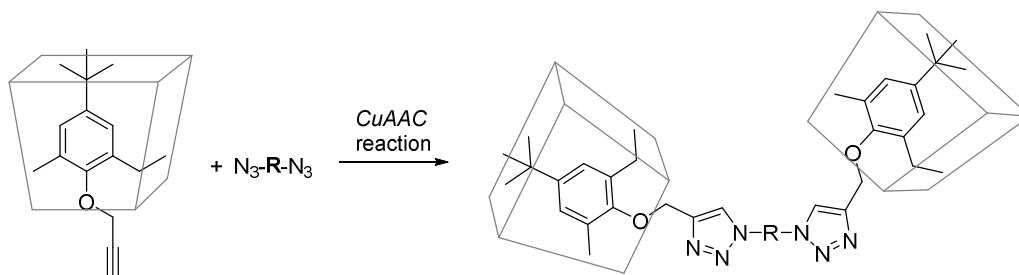


Figure 1.10. Synthesis of calixarene nanosponges.

The same synthetic approach has been used also to obtain mixed cyclodextrin-calixarene materials (CyCaNSs, Figures 1.11 and Figure 1.12).⁸¹⁻⁸² Using this approach it has been possible to obtain nanosponge materials that benefit from the presence of two different comonomers, having somehow complementary supramolecular host abilities, and also exploiting their viable chemical post-modification as well, which easily enables the introduction on the polymeric network of further functionalities such as amine or carboxyl groups.⁸²

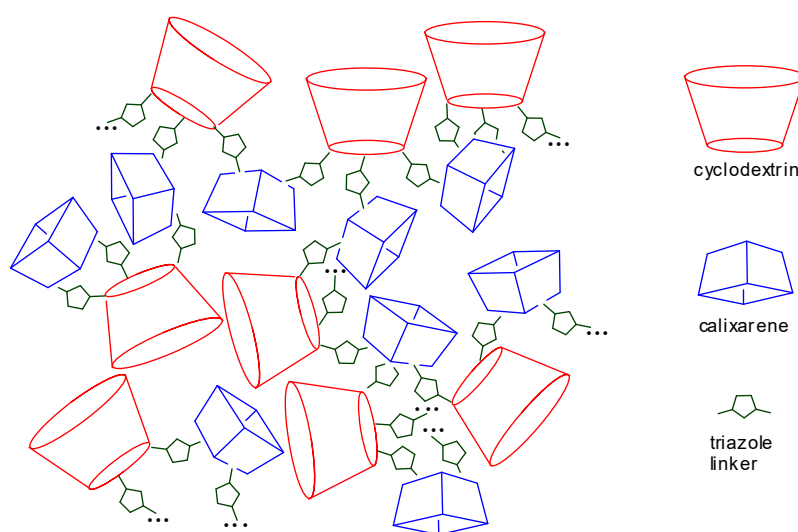


Figure 1.11. Structure of CyCaNSs.

In fact, it was observed that the formation of the polymer network occurs even though the co-monomers are reacted in non-stoichiometric amounts, in such a way that part of the functional involved in the cycloaddition process remain unreacted. Hence, they can be subjected to either Staudinger reduction of the excess azide groups, or further functionalization of the propargyl excess groups (Figure 1.12).

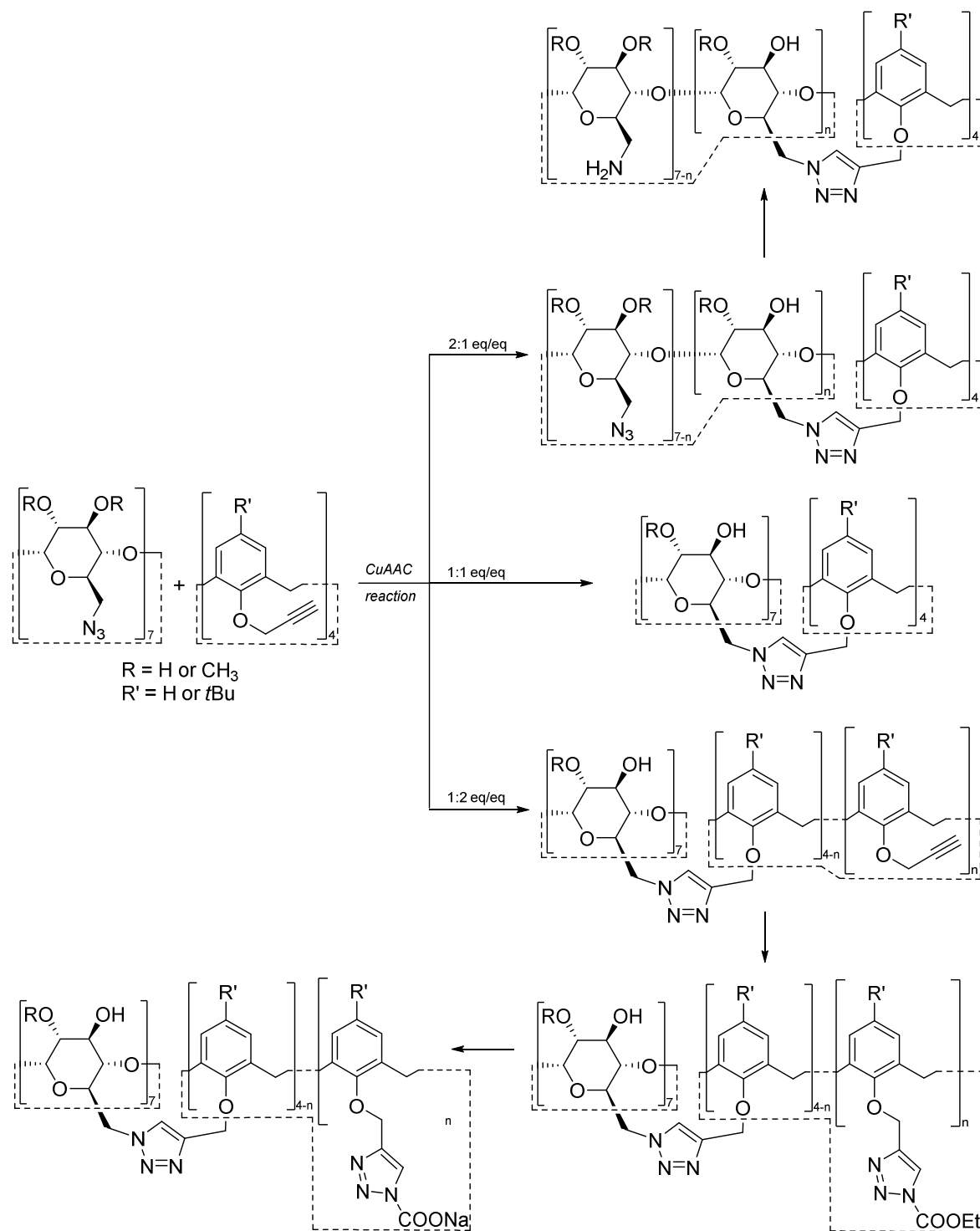


Figure 1.12. Synthesis of CyCaNSs

These co-polymers bearing 1,2,3-triazole linker units appeared interesting materials under several viewpoints. In fact, their adsorption and controlled release properties could be largely varied depending on the molar ratio between the co-monomers, and on the substituent groups present on each co-monomer scaffold as well. Furthermore, the introduction of ionizable groups by chemical post-modification enabled, once again, to obtain materials with remarkable pH-responsive properties.⁸³ On that occasion, it is noticed that fair responsivity to pH variations was showed even by non-modified CyCaNSs, and this behavior was tentatively attributed to the presence of the triazole linkers, which may act as weak bases. However, this hypothesis implies that the triazole units should largely increase their base strength (up to several orders of magnitude), due to their insertion in the polymeric network. Similar materials have been tested for the sequestration of both organic and inorganic species.

Considering the widespread use of cyclodextrins for the encapsulation and stabilization of drugs, a main application field of cyclodextrin based nanosponges is of course their use as drug carrier/delivery systems.¹⁸⁴⁻¹⁹⁰ Indeed, there is available such a massive amount of studies on the topic in recent literature, that an exhaustive overview would be simply impossible in the limited space of this presentation. Extremely diverse therapeutic agents have been associated to nanosponge architectures, such as itaconazole, acyclovir, dexamethasone, doxorubicin, resveratrol, and tamoxifen just to cite a few important examples.¹⁹¹⁻¹⁹² A lot of studies have been particularly focused on antitumor agents, for obvious reasons. In particular, good results were obtained using CD-NSs in the delivery of anticancer drugs such as paclitaxel and camptothecin.¹⁹³⁻¹⁹⁴

A particularly instructive example of stimuli sensitive cyclodextrin nanosponge for the targeted delivery of anticancer drugs has been obtained by inserting disulfide bridges in the polymeric structure. It is known that tumor cells show overexpression of glutathione (GSH, a principal cellular antioxidant tripeptide involved in defense against oxidative stress and in redox signaling) up to 1000 times than the concentration observed in the plasma. Moreover, chemoresistant tumor cells are reported to possess even higher levels of GSH.¹⁹⁵ Thus, GSH has been proposed to be an ideal stimulus for the rapid destabilization of nanocarriers inside cells to accomplish targeted and efficient intracellular drug release. Glutathione-sensitive NSs (Figure 1.13) were obtained in a single step by reacting the 2-hydroxyethyl disulfide in the presence of β -cyclodextrin and a suitable amount of the pyromellitic dianhydride as crosslinking agent; while the loading of the anticancer drug (i.e. the doxorubicin) was performed after the nanosponge synthesis.¹⁹⁶ The mechanism, which underlies the targeted

release, consists in the reduction of the disulphide bridges of the nanosponge to -SH groups, by the glutathione molecules. The breaking of these bridges causes an opening of the polymeric network of the nanosponge and a consequent increase in the release of the encapsulated drug. In fact, the release tests in the presence of variable amounts of glutathione, have shown that the kinetics are faster as the concentration of glutathione increases and the doxorubicin-loaded GSH-NSs displayed a higher antiproliferative effect than the free drug in cell lines having higher GSH concentrations. Studies performed on three different tumor cell lines (liver, colon, and ovary) showed greater inhibition of tumor cell proliferation both in cases treated with nanosponge and both in cases treated with doxorubicin alone.¹⁹⁴

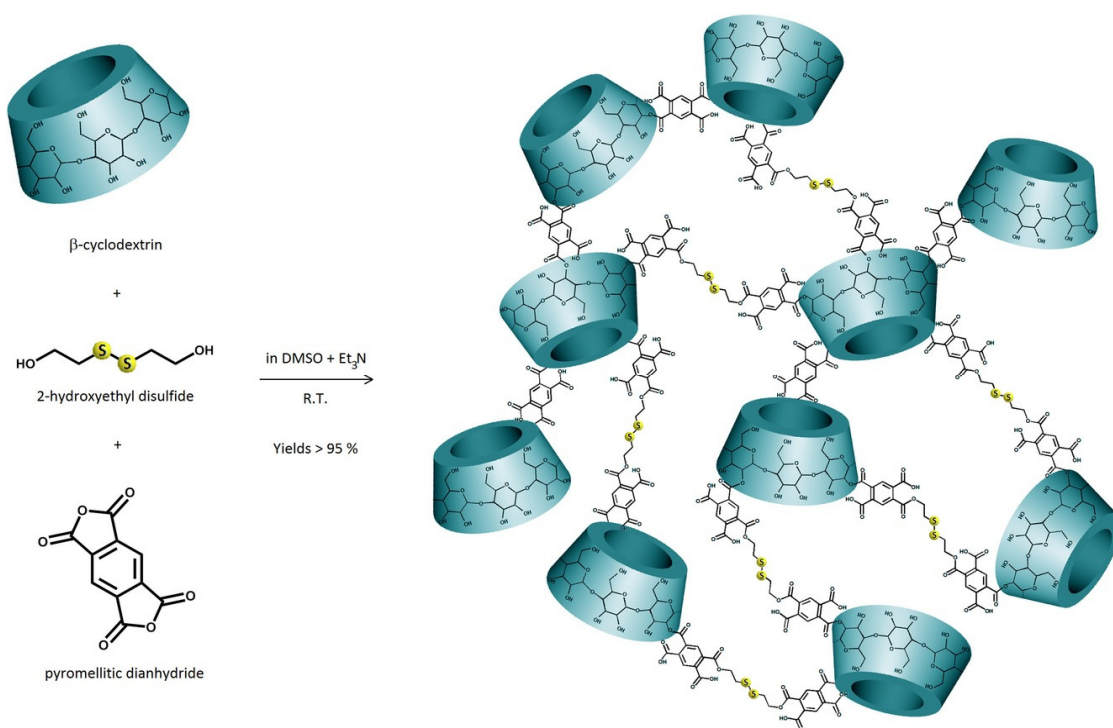


Figure 1.13. The one-step synthesis of GSH-responsive nanosponges.

The guest inside the nanosponge interacts with the monomers of the hosts in the nanocavities; moreover, its diffusion occurs within the nanochannels that formed during the cross-linking and establishes interactions even with the functional groups of the cross-linkers. This leads to an improvement in the complexing ability of the nanosponge compared to cyclodextrin alone, as occurs in the case of camptothecin¹⁹⁷ and other molecules.¹⁹⁸

In order to load a nanosponge with a drug, one can proceed by suspending the polymer in the solution of the molecule to be included (in excess); it is left to stir for 24 hours and then

the solid is filtered, which is subsequently lyophilized.¹⁴² The loading percentage can be determined with the appropriate analytical methods. Another procedure provides that drug is solubilized in a volatile organic solvent, the solution thus obtained is added to the solid nanosponge, and then the mixture is mechanically processed until the solvent evaporates.⁶⁹ The latter phenomenon occurs due to the absorption of mechanical energy by the mixture being processed (a characteristic feature of mechanochemistry).¹⁹⁹ It is an interesting alternative to classic operating methods, given the reduction in the amount of solvent used, the energy and time usually required.²⁰⁰ In order to monitor the release of the guest molecule from the polymer, the loaded nanosponge is dispersed in the appropriate amount of aqueous buffer in a donor compartment, such as a dialysis membrane. The acceptor compartment, placed in contact with the previous one, contains the aqueous buffer in which the molecules coming from the first diffuse; different aliquots of the solution from the acceptor compartment are withdrawn at set times. Through the proper analytical method, it is possible to evaluate the amount of guest released as a function of time and therefore the profile of the release kinetics. Finally, in addition to being excellent drug carriers, nanosponges are also able to carry enzymes, proteins, vaccines and antibodies.²⁰¹

On passing to other application fields, the general ability of nanosponges to absorb organic species from solution makes them particularly interesting materials for wastewater treatment and, more in general for environmental application purposes.^{119-120,202-204} These materials, if compared for example to more common sorbents such as activated carbons, show a low specific surface area (in the order of m^2/g per the first against $\sim 600 \text{ m}^2/\text{g}$ of activated carbon). This suggests that organic molecules are not simply adsorbed on the surface of the nanosponge, but are transported within the bulk of the nanoporous material. Moreover, nanosponges usually show higher affinity towards organic molecules than activated carbons. This implies that they can adsorb substrates at concentration ranges lower than the working concentrations of the activated carbons. Another important aspect of CD-nanosponges is that, if treated with suitable solvents such as methanol or ethanol, they release the previously sequestered guests and can therefore be regenerated and reused.²⁰⁵ Under the latter standpoint, the ability of the material to respond to pH variations can be extremely useful.¹⁸³ Interesting pH-dependent adsorption properties against a large variety of model organic pollutants have been also shown by mixed CyCaNS nanosponges.⁸¹ In addition, these materials benefit from complementary supramolecular host properties of the two co-monomers. Solid-state NMR studies have shown that for this purpose, they can exploit both the co-monomer host cavities and the nanochannels present in the structure. Controlled adsorption and release properties for CyCaNSs can be largely varied depending on the molar

ratio between the co-monomers, and on the substituent groups present on each co-monomer scaffold as well.^{81,83,85} Further introduction of ionizable groups (such as amine or carboxyl groups)⁸² by chemical post-modification enabled to obtain materials with remarkable pH-sensitive adsorption/release abilities towards various probe guests such as *p*-nitroaniline derivatives, dyes and bioactive molecules.⁸⁴ Their acid-base properties have been recently assessed and interestingly, it has been positively shown that the insertion in the polymeric network largely enhances (up to several order of magnitude) the basic strength of the triazole linker units.⁸² These materials have been tested to evaluate the possible sequestration abilities towards metal cations, such as the Pb²⁺ ion from aqueous solutions.⁸⁷

Other remarkable examples of applications of CD-based nanosponges are:

- Removal of organic matter, aromas and odors from water;⁷⁹
- Confined reaction medium for the enantioselective photoisomerization of 1,3-cyclooctadiene;²⁰⁶
- Delivery of gases, such as oxygen or carbon dioxide;¹⁴²
- Transport of fluorescent probes;⁸⁰
- Support of nanostructured catalysts.²⁰⁷

Regarding the latter point, it is worth mentioning here their use as support for metal nanoparticle catalysts able, in turn, to mediate various organic reactions. In particular, the borohydride reduction of nitroarenes, which is somehow a sort of “gold standard” reaction for assessing the catalytic efficiency of metal nanoparticle-based catalytic systems, has been performed using nanosponges loaded with silver²⁰⁷ or gold²⁰⁸ nanoparticles; Pd catalyzed C-C coupling reactions have been also reported,²⁰⁹⁻²¹⁰ as well as hydrogenation reactions mediated by ruthenium.²¹¹⁻²¹²

1.6 Aim of the work

On the grounds of the previous discussions, it is immediately apparent that nanosponges presently constitute a very active research field, and offer extremely interesting and exciting opportunities, challenges and perspectives. Despite so much work has been already carried out up-to-date, as testified by an impressive literature on the relevant topics, there still is at least as much to do, undoubtedly. Thus, the present PhD work aims at giving a contribution

to extend the knowledge on the synthesis and applications of nanosponges, having particular consideration of the aspects relevant to pH responsive smart systems for advanced applications. Under this perspective, it is important to underline that modern sciences are currently experiencing a somehow change of paradigm, moving towards complexity with a holistic approach (in agreement with the old Aristotelian idea that “*the whole is something besides the parts*”), and paying attention to sustainability issues.

There are at least four interesting aspects of Nanosponge chemistry, which have been particularly addressed in this work:

1) *Synthesis and characterization*. The properties of nanosponges can be even dramatically varied, depending on the host monomer and the linker units. The possibilities offered by the fact that both cyclodextrins and calixarenes can be chemically modified in many ways, that different linkers can be used and, most of all, that both the modification of the host and the choice of the linker allow to decorate the resulting nanosponge structure with virtually any functional group, offer unlimited opportunities. At the same time, some aspects of the synthetic procedures, in particular their optimization and the mechanistic issues involved, undoubtedly need detailed investigations (Chapter 2). Moreover, the textural characterization of these materials offers significant challenges (Chapter 3), and require an innovative experimental approach.

2) *Study of the tunable adsorption abilities*. The possible pH-dependent adsorption abilities of a cyclodextrin or calixarene-based nanosponge are positively related to the supramolecular host properties of the constituent hosts, but also depend on the possible functionalization introduced through both the host and the linker units. Moreover, even the synthetic conditions can have a significant outcome. Therefore, a rationale design of a sorbent material cannot neglect a detailed investigation on how all these factors affect the adsorption abilities (Chapter 4). Hence, the possibility to exploit nanosponges for obtaining composite sorbents with improved performances for environment remediation purposes such as wastewater treatment (Chapter 5), has been explored.

3) *Study of the controlled release abilities*. The ability of a nanosponge matrix to interact with a guest and the solvent medium as well, reflects on the rate at which the guest can be possibly released. Hence, it is necessary to clarify the microscopic aspect of the release phenomenon, relating them to the characteristics of the material (Chapter 6). This is essential piece of information in order to design effective drug carrier systems. Therefore, a study of the release abilities for some representative bioactive molecules was considered (Chapter 7).

4) *Functional supports for catalysts*. Last, but not least, the possibility to exploit nanosponges as functional support for nanostructured catalysts was studied (Chapter 8). In particular, the association of nanosponges with photocatalysts for the photodegradation of organic pollutant was considered, again in view of possible applications for environment remediation devices.

At the very dawn of the Nanoscience era, R. Feynman used to say that “*there is plenty of room at the bottom*”. There is undoubtedly plenty of room in the nanosponge world.

2 – Synthesis of the Nanosponge materials

2.1 Introduction

The development of the experimental activities performed in the course of the present PhD work provided to design and perform the syntheses of a significant number of new diversely structured nanosponges, and of composite materials embedding catalysts or co-sorbents into a nanosponge matrix as well. Indeed, a main point of this work was focused on was the investigation of how the constitution of the materials, i.e. the nature of host monomers, the structure of the linkers, the possible presence of ionizable functional groups in the constituent synthons, and so on, would possibly affect their performances. For this purpose, a study of the optimization of the synthetic conditions was required. This, in turn, led to critically reconsider with a systematic approach the characteristics of the polymer network formation process from a microscopic viewpoint. As discussed in the Introduction Chapter, there is already available a large amount of literature on the synthesis of cyclodextrin-based nanosponges.^{5,9,68,71-72,142,150,168,175,197,201,213-214} In most cases the synthetic paradigm basically provides the reaction of the cyclodextrin with a double electrophile. Different strategies have been explored only occasionally. In particular, the use of calix[4]arene as a (co)-monomer has been reported by Lo Meo and co-workers, who exploited a click-chemistry approach based on the well-known copper catalyzed azide-alkyne cycloaddition reaction.⁸¹⁻⁸² A further alternative is the reaction of an electrophilic cyclodextrin derivative (usually a per-(6-halo)-(6-deoxy)-cyclodextrin) with a poly-nucleophile such as a polyamine.^{81,179-180} Indeed, the synthesis of CD-NSs, with cross-linker units able of providing further binding sites to the included guests allows to obtain materials with improved adsorption abilities, which can be largely modulated by changing the kind of cyclodextrin or cross-linker used, and by varying the combination ratio between cross-linker and host. It is possible to obtain materials with a different degree of cross-linking, a different size of the nanochannels and different microscopic interaction abilities. For instance, the CD-NSs obtained with pyromellitic dianhydride as the linker bear free carboxylic groups and, consequently show a

high tendency to swell, a negative ζ -potential, and the ability to include cationic organic species.^{140,172,196} In general, the properties of NSs can be widely tuned by using reticulating agents bearing ionizable (polyamines, triazoles, pyromellitic anhydride) or other stimuli-sensitive groups;^{69-70,142-143,145-146} hence, functional tailored materials can be obtained.

On the grounds of the state-of-the-art, the present work particularly aimed at further exploring the tunability of the adsorption and release abilities of nanosponge materials, and at clarifying the relationships between tunability and material structure in the perspective of the rationalization of the synthetic design. Therefore, it seemed interesting to focus onto the simple reaction of cyclodextrins and/or calixarenes with bis-isocyanates, paying attention on the possibility to modulate the properties of the product by modulating the structure of the reticulating agent. More in detail, the idea was pursued to combine a simple commercial product such as hexamethylene diisocyanate (H) with two different chain extenders, namely 1,6-hexanediol (D) and 3,3'-diamino-N-methyldipropylamine (A) (Figure 2.1).

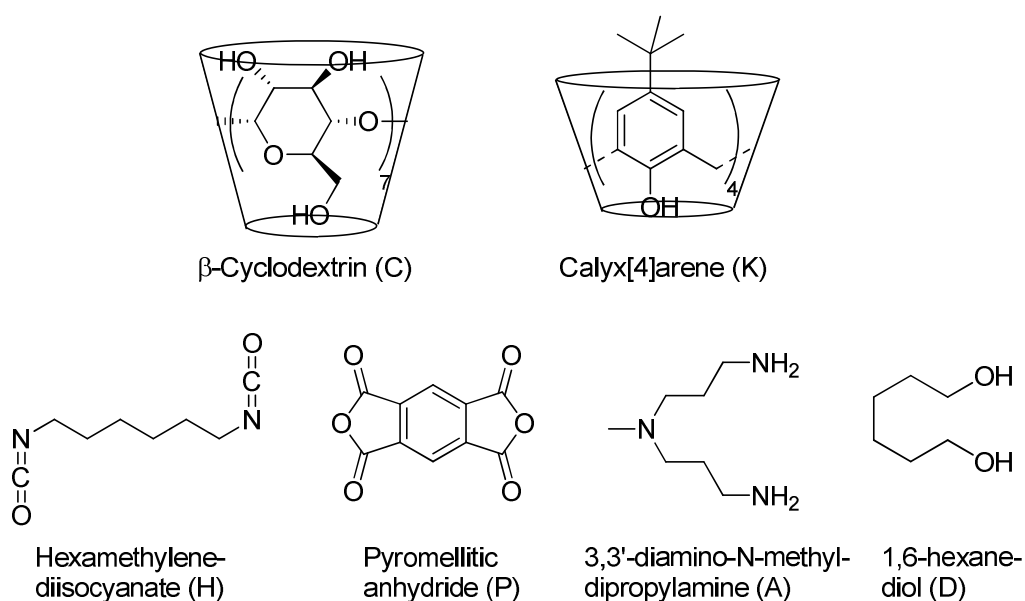


Figure 2.1. Host, linkers and chain extenders structures.

It is worth noting that combining H and D, long polyurethane chains can be obtained, bearing no ionizable group; conversely, combining H and A polyurea chains are obtained containing basic tertiary amine groups. Moreover, the use of pyromellitic dianhydride (P) as (co)reticulating agent was also considered, in order to gain the possibility of decorating the nanosponge structure with carboxyl acid functional groups (Figure 2.2). Hence, various

combinations of the co-linkers and the chain extenders with β CD, or calix[4]arene, or both hosts as the monomers, led to design and study the synthesis of a panel of materials according to more than 30 different reactants combinations that are summarized in Table 2.1.

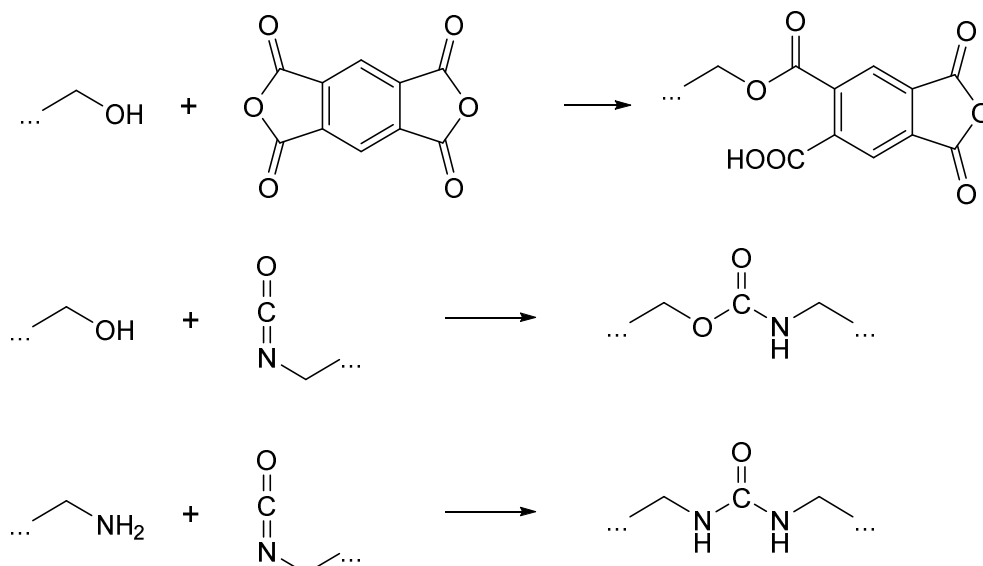


Figure 2.2. Functional groups formed during the cross-linking reactions.

It must be stressed here that the present chapter deals only with “*plain*” materials, i.e. those not charged with possible co-sorbents or catalysts: the latter ones, will be addressed in Chapters 5 and 8 respectively. To each combination a code is attributed, composed by a capital letter identifying the host monomer (namely, C for β CD, K for calixarene, CK if both are present), and a progressive number that identifies a particular molar combination ratio between the reactants. Moreover, it is important to outline that, as it will be clear in the proceeding of the present discussion, these codes do not necessarily identify the actual materials obtained. In fact, in several cases different reactions conditions were studied for the same combination of reactants, often leading to products having so much different physical properties, to be actually considered as different materials. In these particular cases, the real materials obtained will be specifically identified with additional sub-codes.

Table 2.1. Complete panel of Nanosponges (NS).

Code	Host		linker/chain extender				mole ratio	Notes	Amount of host units (mmoles/g)	
									C	K
C1	C	-	H	-	-	-	1 : 2	a, b, c	0.680	-
C2	C	-	H	-	-	-	1 : 3	a, b, c, e	0.611	-
C3	C	-	H	-	-	-	1 : 4	a, b, c	0.554	-
C4	C	-	H	-	-	-	1 : 6	b, c	0.467	-
C5	C	-	H	-	-	-	1 : 8	a,b,c	0.404	-
C6	C	-	-	P	-	-	1 : 2	a	0.637	-
C7	C	-	-	P	-	-	1 : 4	a, b	0.612	-
C8	C	-	-	P	-	-	1 : 7	a, b	0.375	-
C9	C	-	H	P	-	-	1 : 2 : 2	a, b	0.522	-
C10	C	-	H	P	-	-	1 : 2 : 4	a, b, c	0.427	-
C11	C	-	H	P	-	-	1 : 4 : 4	a, b, c	0.373	-
C12	C	-	H	P	-	-	1 : 8 : 4	c	0.299	-
C13	C	-	H	-	-	D	0.1 : 6 : 5	c	0.059	-
C14	C	-	H	-	-	D	0.5 : 1 : 2	c, d, e	0.490	-
C15	C	-	H	-	-	D	0.5 : 2 : 3	c, d, e	0.383	-
C16	C	-	H	-	-	D	1 : 1 : 3	c	0.570	-
C17	C	-	H	-	-	D	0.5 : 6 : 5	c	0.231	-
C18	C	-	H	-	-	D	1 : 6 : 5	c	0.366	-
C19	C	-	H	-	A	-	1 : 8 : 4	c	0.328	-
K1	-	K	H	-	-	-	1 : 2		-	0.623
K2	-	K	H	-	A	-	1 : 8 : 6		-	0.350
K3	-	K	H	-	-	D	0.1 : 6 : 5		-	0.060
K4	-	K	H	-	-	D	0.5 : 6 : 5		-	0.261
K5	-	K	H	-	-	D	1 : 6 : 5		-	0.446
CK1	C	K	H	-	-	-	1 : 1 : 8		0.320	0.320
CK2	C	K	H	-	-	-	1 : 1 : 4		0.408	0.407
CK3	C	K	H	-	-	-	0.5 : 1 : 8		0.196	0.391
CK4	C	K	H	-	-	-	2 : 1 : 8		0.469	0.235
CK5 _a	C	K	H	-	A	-	0.5 : 1 : 8 : 3		0.167	0.335
CK5 _b	C	K	H	-	A	-	0.5 : 1 : 8 : 3		0.426	0.335
CK5 _c	C	K	H	-	A	-	0.5 : 1 : 8 : 3		0.172	0.335
CK6	C	K	H	-	A	-	2 : 1 : 8 : 3		0.680	0.213
CK7	C	K	H	-	-	D	0.5 : 1 : 8 : 2		0.611	0.344

a: DMSO “*thick paste*” method; b: DMF “*solution*” method; c: DMF “*smart dilution*”; d: variable temperature; e: variable concentration.

2.2 Optimizing the synthetic procedure: the problem of the solvent choice

Two general problems to cope with in the synthesis of nanosponges are the choice of the most suitable type of solvent and the actual amount of solvent per a given reactant mass. Reports in recent literature regarding the preparation of cyclodextrin nanosponges provide the use of a relatively little amount of DMSO (300~500 μL per g of reactants),^{82,215} in such a way that the reactants can be first mechanically mixed up to form a *thick paste* that is then allowed to react still at 60 °C overnight. The reaction crude is subsequently crunched and subjected to a multiple washing protocol (see Experimental section). This procedure usually leads to the desired product in satisfactory yields, but has some drawbacks. In particular, when applied to the reaction of βCD with plain di-isocyanide H (samples C1-C5) it affords very hard materials, difficult to subject to subsequent mechanical grinding. Further problems arose when the same procedure was applied to the samples prepared with the addition of pyromellitic anhydride P (samples C6-C8, containing only P as the linker, and samples C9-C12, containing both H and P). In fact, in these cases, the mass of the recovered products was frequently much lower than the mass of the mixed reagents, even taking into account the unavoidable mechanical losses that occur on processing the crude and recovering the material obtained. This might be justified considering that a significant amount of the crosslinking agent is not incorporated in the polymeric material and is then lost. Indeed, it can be also observed that gaseous by products are formed during the reaction, as accounted for by macroscopic observation of the reaction crude. In the particular case of samples C9-C11, when a liquid mixture of H and DMSO was added to a solid mixture of βCD and P and the system was rapidly stirred at r.t., the reactant mass rapidly overheated and turned brown, underwent an enormous increase in volume, and unambiguously presented the strong unpleasant smell typical of dimethylsulfide. At the end of the reaction, the crude product appeared as a rubbery mass that became fragile after the usual washing work-up procedure (the product did not lose its dimethylsulfide smell even after repeated Soxhlet extraction with dichloromethane). These observations can be easily explained assuming the occurrence of a Swern-like reaction between the solvent and the dianhydride. Indeed, such a process occurs up to a minor extent even in the case in which H alone is used as the linker (C1-C5), although good overall weight yields are anyway found. Conversely, the materials having only P as the linker (C6-C8) gave significantly low yields. The latter products showed a high hydrophilic character and large swellability in water. These findings are perfectly consistent with the presence in their structure of the ionizable carboxyl groups carried by the

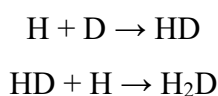
pyromellitic linker units discussed above. In aqueous medium, these materials gave rise to diaphanous suspensions, which became almost clear under mild alkaline conditions. In detail, material C6 (prepared with a 1:2 β CD/P mole-to-mole combination ratio) showed a significant solubility in water, and could never be recovered in satisfactory amount; therefore, it was not subject to further investigation. The material C7 (prepared with a 1:4 β CD/P mole-to-mole combination ratio), after suspension in water and subsequent filtration, could be recovered with some difficulty, because it too formed a jelly precipitate on the filter; also in this case recover was not satisfactory. Conversely, product C8 (prepared with a 1:7 β CD/P mole-to-mole combination ratio) resulted much easier to collect. As a final remark, samples C9-C12 prepared with the both H and P could be recovered in fair yields, showing significant swellability in water only under strong alkaline conditions.

Based on these observations, the possibility to change the nature and the amount of the solvent was explored. In particular, DMF was considered as a good alternative to DMSO, because both solvents share some interesting characteristics: they are both dipolar high-boiling aprotic solvents, with similar dielectric constant values, and good solvent abilities towards the reactants, in particular the otherwise scarcely soluble β CD. Regarding the amount of solvent, the “*thick paste*” procedure (0.3 mL of solvent per g of reactants) was compared to a “*solution*” procedure using a ten-fold volume (i.e. 3 mL per g of reactants). In detail, the second procedure provides to completely dissolve the reactants in the proper amount of solvent, keep the solution under magnetic stirring for 3 h at 60 °C, and then allow the reaction to complete at the same temperature without stirring. In this way, the formation of the reticulated host network occurs under almost homogeneous conditions (moreover, the dispersion of possible further co-sorbents or catalysts becomes much more satisfactory, see Chapters 5 and 8). The replied syntheses in DMF were performed in particular for the samples C1-C3, C8 and C11. In the case of the “*thick paste*” procedure the products obtained were nearly indistinguishable from those obtained in DMSO (but for the lack of dimethylsulfide smell!); in fact, they presented similar stiffness, the same spectroscopic and relaxometric properties (see Chapter 3) and roughly the same adsorption abilities towards some model guests (see Chapter 4). The “*solution*” procedure, in turn, gave very interesting results, because it could be observed that the reaction systems showed a peculiar behavior. In fact, the initially liquid system suddenly underwent a large increase in viscosity within the first three hours of reaction. Then, after the reaction crude had been kept still at 60 °C overnight, it appeared as a glassy or gummy mass, which could be easily crunched and subjected to the usual work-up procedure, to afford a solid that could be easily grinded in an agate mortar. Once again, the spectroscopic features and the adsorption abilities of the

materials obtained resulted almost indistinguishable from those of the ones obtained with the “*thick paste*” procedure. However, it is immediately apparent that, from a practical standpoint, the “*solution*” procedure in DMF appears much more viable and manageable, and is by far preferable to the old “*thick paste*” procedure in DMSO. Hence, the new procedure was chosen to perform the syntheses of the cyclodextrin materials C13-C19 with the 1,6-hexanediol (D) and the polyamine (A) chain extenders, the calixarene-only materials K1-K5, and the mixed cyclodextrin-calixarene materials CK1-CK7. As long as the synthesis of mixed materials is concerned, it is interesting to notice that the order of addition of the various synthons can have a significant outcome. An interesting example is given by the CK5 combination (i.e. β -cyclodextrin/calix[4]arene/H/A in a 0.5:1:8:6 mole-to-mole ratio), in which the speed and the order of mixing was found to affect the degree of reticulation and the properties of the product. In details, three different products were prepared by: *i*) quickly adding the amine A to the mixture of all other reagents; *ii*) slowly adding dropwise the amine A to the mixture of all other reagents; *iii*) first adding dropwise A to the solution of H in DMF, and then adding the solid mixture of cyclodextrin and calixarene. The three different products (referred to as CK_{5a}, CK_{5b} and CK_{5c} respectively), though apparently similar in their macroscopic appearance, showed different adsorption and release properties (see Chapter 4 and Chapter 6).

2.3 Optimizing the synthetic procedure: the problem of the chain extender

As we mentioned previously, the use of a chain extender is interesting because it offers the opportunity to modify up to a significant extent the channel network of the nanosponge materials. For this purpose, the use of the diol D was examined in particular detail. In principle, by reacting D with H in the appropriate stoichiometric amounts (Figure 2.3), a new bis-isocyanide of given length should be obtained. Unfortunately, this reaction cannot actually afford a single product, but rather leads to a mixture of statistically distributed products. For instance, in the simplest case of a reaction with a 2:1 stoichiometry the H₂D adduct should be obtained in two steps:



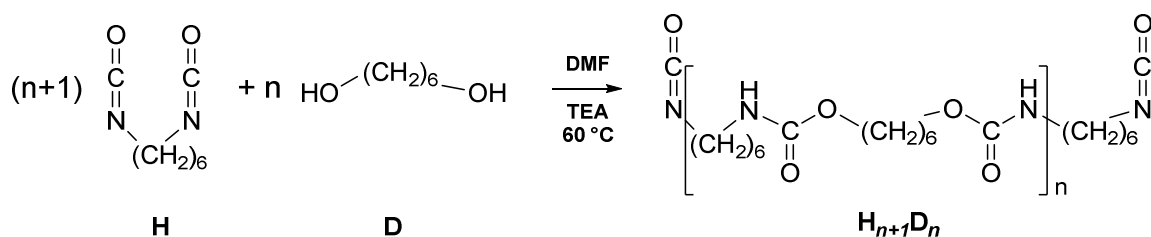
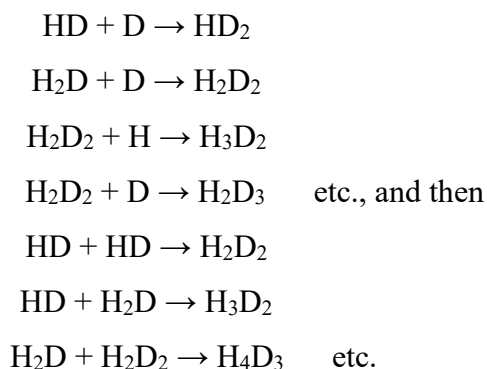
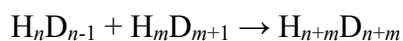


Figure 2.3. Synthesis of the linker units.

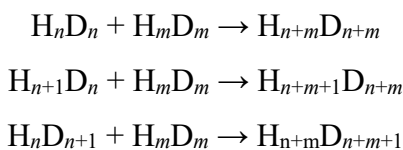
However, as the two adducts HD and H₂D are formed, they can react with the starting precursors H and D, forming further adducts, which can also react together:



Of course, the possible combinations are virtually countless. Only in the case in which H is reacted in very large excess with respect to D, the desired adduct H₂D could be obtained as the only product, but in this case a lengthy and expansive separation would be necessary. Considering that nanosponges are highly disordered materials at a microscopic level, in their synthesis the fact that the crosslinking agent is a pure species or a mixture is barely important, provided that the composition of the mixture can be reasonably evaluated. Indeed, once the H/D combination ratio has been fixed, it is sufficient to evaluate the relative amount of the various adducts present and then define an "*average length*" of the linker system as the weighted average of the lengths of the various adducts. This distribution can be indeed calculated applying a combinatorial analysis method. In fact, starting from the assumption that the reactivity of the functional groups is independent of the length of the (linear) chain to which they are linked, the rate of the generic isocyanate-alcohol addition reaction ($\text{R}-\text{N}=\text{C}=\text{O} + \text{R}'-\text{OH} \rightarrow \text{R}-\text{NH}-\text{CO}-\text{O}-\text{R}'$) should only depend on the equivalent concentration of functional groups present in solution. Hence, indicating with k the kinetic constant for the addition reaction reported above, simple statistical considerations lead to conclude that for all generic reactions between adducts of the type:



the kinetic constant must be as large as $4k$, whereas for the generic reactions between adducts of the type:



the kinetic constant must be $2k$ (noticeably $n, m \geq 0$). On the grounds of these considerations, it is possible to write down a kinetic scheme to exhaustively describe the formation of all possible adducts of type $H_{n-1}D_n$, H_nD_n e $H_{n+1}D_n$, with $n \leq 7$, starting from the reactants H and D. From this scheme (79 reactions), the kinetic expressions in differential form for all the possible species present in the system (22 overall) can be obtained. These expressions constitute a system of differential equations that cannot be exactly solved, but can be worked out by setting up a numerical integration procedure with the aid of an MS Excel worksheet. Hence, it was possible to implement an algorithm able to calculate the evolution in time of the concentrations of all the possible species and their final distribution, as a function of the initial concentrations of the reactants H_0 and D_0 . This calculation was carried out systematically for a whole series of possible combinations, many of which were then effectively used for setting up the synthesis of the systems studied. In particular, three different H/D mole-to-mole combination ratios were taken into consideration, namely 3:2, 2:1 and 3:1, fixing the difference between the initial concentrations of H and D ($H_0 - D_0$) at the values of 0.250 M, 0.200 M, 0.167 M and 0.125 M. The calculation results are collected in Table 2.2. The application of the developed algorithm shows:

- i) in all the cases analyzed, once the reaction is complete, there is always a significant amount (not less than 31%) of unreacted H;
- ii) the sum of the final concentrations (C_{eq}) of all the species of the $H_{n+1}D_n$ type ending with two isocyanate groups (including the precursor H), i.e. those which can effectively contribute to the formation of the hyper-crosslinked polymer network with the β CD, is always very close (within 0.5%) to the difference between the initial concentrations of the reactants ($H_0 - D_0$);
- iii) the final concentrations of all species of the H_nD_{n+1} type (i.e. all those adducts ending with two hydroxyl functions) are always negligible, as well as the residual concentration of the initial diol;

Table 2.2. Simulated percentages of $H_{n+1}D_n$ adducts.

H_0 (M)	D_0 (M)	$\frac{H_0}{D_0}$	H%	H ₂ D %	H ₃ D ₂ %	H ₄ D ₃ %	H ₅ D ₄ %	H ₆ D ₅ %	H ₇ D ₆ %	H ₈ D ₇ %	<i>b</i>	C_{eq} (M)
0.750	0.500	3:2	31.0	20.3	16.2	11.0	8.6	6.6	4.9	1.4	44.6	0.251
0.600	0.400	3:2	31.8	20.5	16.2	10.9	8.4	6.3	4.6	1.2	43.7	0.201
0.500	0.333	3:2	32.8	20.8	16.1	10.8	8.1	6.0	4.3	1.0	42.7	0.168
0.375	0.250	3:2	34.8	21.4	16.0	10.4	7.6	5.4	3.7	0.7	40.5	0.126
0.500	0.250	2:1	50.4	23.6	13.1	6.5	3.5	1.9	0.9	0.1	27.8	0.250
0.400	0.200	2:1	50.8	23.6	13.0	6.4	3.4	1.8	0.9	0.1	27.6	0.200
0.333	0.167	2:1	51.3	23.6	12.9	6.3	3.3	1.7	0.8	0.1	27.2	0.167
0.250	0.125	2:1	52.8	23.6	12.4	5.9	3.0	1.5	0.7	0.1	26.3	0.125
0.375	0.125	3:1	67.4	21.0	7.7	2.5	0.9	0.3	0.1	0.0	19.0	0.250
0.300	0.100	3:1	67.6	21.0	7.6	2.5	0.9	0.3	0.1	0.0	18.9	0.200
0.250	0.083	3:1	68.0	20.8	7.5	2.4	0.8	0.3	0.1	0.0	18.7	0.167
0.187	0.062	3:1	69.2	20.5	7.1	2.2	0.7	0.2	0.1	0.0	19.3	0.125

vi) the sum of the final concentrations of all the H_nD_n -type adducts (ending with one alcohol function and one isocyanate function) is also quite low, generally between 0.4% and 4.5% with respect to the H_0 - D_0 difference. Hence, also the presence of these species can be considered negligible, at a first approximation;

v) the concentration of the longer adduct H_8D_7 is always equal to or less than 1.4% of the sum of the various bis-isocyanates, and therefore is negligible at a first approximation. The latter finding points out that the kinetic scheme used in the simulation of the system reasonably models the composition of the linker species mixture, and that it is not necessary to consider a more complex scheme involving the formation of higher adducts.

As mentioned above, from the percentages of the adducts $H_{n+1}D_n$, it is possible to calculate an average length (***b***) of the linker units as a weighted average, i.e.:

$$\mathbf{b} = \Sigma [c_n \cdot \%_{H_{n+1}D_n}] / 100$$

where the coefficients c_n represent the total number of C, N and O atoms present in the single urethane linker unit bridged between two cyclodextrin units (i.e. $c_0 = 10$; $c_1 = 28$; $c_2 = 46$; c_3

= 64; $c_4 = 82$; $c_5 = 100$; $c_6 = 118$; $c_7 = 136$). From the calculations carried out it is found that the average length of the linker depends mainly on the ratio H_0/D_0 between the initial concentrations of the reactant and to a lesser extent on this absolute concentration. In particular, it results between 40 and 45 for $H_0/D_0 = 3/2$, and between 26 and 28 for $H_0/D_0 = 2$. It should be noted that these values are reasonably similar to those of 46 and 28, relevant to the ideal products H_3D_2 and H_2D respectively. For the systems in which $H_0/D_0 = 3$, it must be said that this combination ratio would be the one ideally corresponding to an equimolecular mixture of the species H and H_2D , for which it would have been $\langle b \rangle = (10 + 28)/2 = 19$. It is noteworthy that in the systems simulated with this ratio, the calculated value of b is actually very close to 19. As a final remark, it is immediately evident that the same formalisms can be applied to any chain extender, and therefore can be used for considering the cases where the polyamine A is used in place of the diol D.

2.4 Optimizing the synthetic procedure: the problem of the reaction conditions and the mechanistic course

The calculations discussed in the previous section served as a guide for designing the subsequent experimental work. Indeed, the influence of the reaction conditions (temperature, concentration of the reactants, combination ratios, etc.) on both the macroscopic/mechanical and microscopic/textural features of the nanosponge product obtained, is a problem that has seldom been addressed with a systematic approach. However, it must be considered that the ability to design materials with tailored characteristics is essential to foresee a possible use in any applicative field. Under this standpoint, the observation of the sudden viscosity variation of the system during the reaction course appeared particularly intriguing. In fact, such a phenomenon has actually been well documented in the literature.²¹⁶⁻²¹⁷ It is usually referred to as the “*gel point*”, and can be suitably related (see later) to the kinetics of the polymer network formations. Its occurrence has been reported, in particular, for the copolymerization of the di-isocyanate H with dihydroxylated polybutadienes²¹⁸ and for the cationic polymerization of epoxides.²¹⁹

On the grounds of the previous discussion, the determination of the *gel point* was investigated for systems C1-C4, prepared with the pure H as the linker, and C14-C16

prepared with the chain extender D, in such a way to continuously increase the value of the average length of the linker units $\langle b \rangle$ from 10 to 45. It is worth mentioning here that, from a practical standpoint, the mixed linkers are pre-formed *in situ*, by reacting the appropriate amount of H and D in anhydrous DMF at 60 °C for 1 h under magnetic stirring. Then, the proper amount of β CD is added, and stirring is continued for 3 h, during which the system was kept under strict observation in order to individuate the time (t_g) needed for the *gel point* to occur. The system is then kept overnight still at 60 °C to ensure completion of the crosslinking process. In the case of the reaction between β CD and pure H, the procedure is followed obviously omits the initial preparation step of the linker system. Noticeably, a further parameter to be fixed in designing of the synthesis of a nanosponge material is the combination ratio between linker and the β CD (i.e. the ratio C_{eq}/CD mole-to-mole ratio). For systems C14-C16 this ratio is 2:1, whereas for systems C1-C4 it varies from 2:1 to 6:1. In a first set of experiments, the reaction temperature was fixed to 60 °C, and the amount of DMF solvent was set in such a way to have an initial concentration of cyclodextrin (CD_0) as large as 0.125 M. In a second set of experiments, systems C14 and C15 were examined more in detail by varying the reaction temperature between 50 °C and 80 °C. Finally, for systems C2, C14 and C15 the dependence of the *gel point* on the reactant concentration was also studied, by varying CD_0 in the range 0.050-0.125 M. The results are collected in Table 2.3. Noticeably, for clarity the various samples obtained and reported in the Table 2.3 are identified with an additional sub-code (individuating the initial β CD concentration).

Table 2.3. t_g values of nanosponge-like materials (average on three replicates).

Code	T (°C)	C_{eq} (M)	β CD (M)	t_g (min)	Code	T (°C)	C_{eq} (M)	β CD (M)	t_g (min)
C1 _[125]	60	0.250	0.125	-	C14 _[125]	50	0.250	0.125	-
C2 _[125]	60	0.375	0.125	85 ± 8	C14 _[62]	60	0.200	0.062	-
C2 _[83]	60	0.250	0.083	-	C15 _[125]	80	0.251	0.125	7 ± 1
C2 _[62]	60	0.187	0.062	-	C15 _[125]	75	0.251	0.125	9 ± 1
C2 _[50]	60	0.150	0.050	-	C15 _[125]	70	0.251	0.125	16 ± 1
C3 _[125]	60	0.500	0.125	35 ± 5	C15 _[125]	65	0.251	0.125	23 ± 1
C4 _[125]	60	0.750	0.125	22 ± 3	C15 _[125]	60	0.251	0.125	30 ± 2
C14 _[125]	80	0.250	0.125	13 ± 1	C15 _[125]	55	0.251	0.125	46 ± 3
C14 _[125]	75	0.250	0.125	18 ± 1	C15 _[125]	50	0.251	0.125	-
C14 _[125]	70	0.250	0.125	30 ± 1	C15 _[100]	60	0.201	0.100	46 ± 1
C14 _[125]	65	0.250	0.125	45 ± 1	C15 _[83]	60	0.168	0.083	-
C14 _[125]	60	0.250	0.125	60 ± 1	C15 _[62]	60	0.126	0.062	-
C14 _[125]	55	0.250	0.125	92 ± 4	C16 _[125]	60	0.250	0.125	90 ± 10

Interestingly, the *gel point* was observed in 15 cases out of 26; after 24 h, the reaction system appeared as a gummy residue, from which the desired product could be easily isolated by the usual washing procedure. Surprisingly, in the other 9 cases the *gel point* was not observed, and the reaction mixture was found still liquid after 24 hours; however, the subsequent processing of the reaction crude carried out in the usual way, led to products apparently identical from the previous ones in terms of spectroscopic characterization (FT-IR, CP-MAS NMR). Analysis of the t_g trends provided further interesting details. Considering the systems C14 and C15, a regular decrease in t_g values is observed on increasing the reaction temperature (Figure 2.4). Indeed, plotting the values of $\ln(t_g)$ against the inverse of the absolute temperatures, $1/T$, in an Arrhenius-like graph, the resulting trends are two almost parallel lines (Figure 2.5).

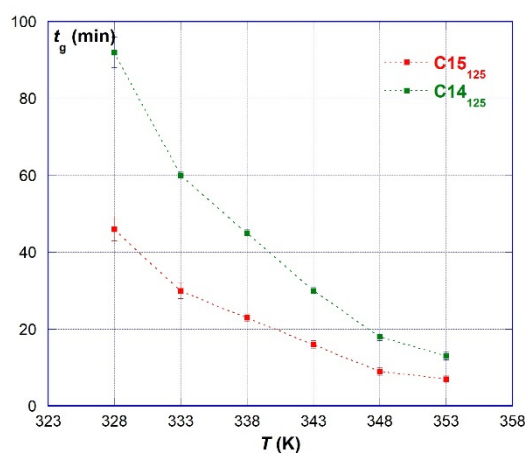


Figure 2.4. Trends of t_g vs. temperature.

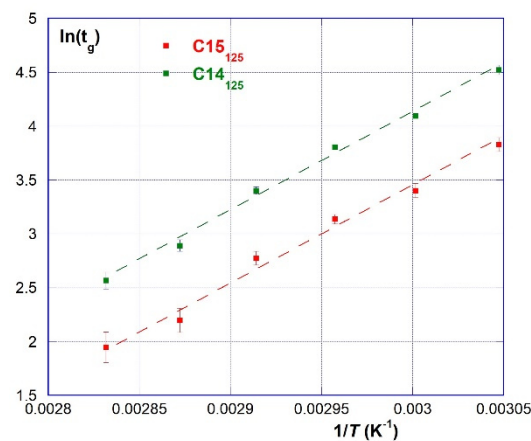


Figure 2.5. Arrhenius plot.

Therefore, data were subjected to simultaneous regression analysis using a biparametric equation, affording the following results:

for **C14**: $\ln(t_g) = (9.0 \pm 0.3) \cdot 10^3/T - (22.9 \pm 0.8)$; $r = 0.9963$, $f = 9$

for **C15**: $\ln(t_g) = (9.0 \pm 0.3) \cdot 10^3/T - (23.5 \pm 0.9)$

(for completeness of information, the analysis returned a difference between the intercepts of the two parallels equal to 0.67 ± 0.04).

The linearity of $-\ln(t_g)$ vs $1/T$ plots suggested to critically reconsider the meaning that can be attributed to the parameter t_g . On the grounds of literature,²¹⁹ it is reasonable to assume that the *gel point* occurs when the system has achieved a sufficiently extensive crosslinking, i.e.

when the progress of the reaction has reached a suitable limit value. The number of equivalents of the -OH groups in reaction is more than 10 times larger than the isocyanate groups. Therefore, for the urethane groups formation reaction, the isocyanate is the limiting reactant, and from a kinetic point of view, the reaction indeed occurs under pseudo-first order conditions. Under such circumstances, it is possible to define an extent of reaction (α) for the formation process of polymeric network via the residual equivalent concentration of isocyanate groups,²²⁰ as:

$$\alpha(t) = \frac{|NCO_{t=0}| - |NCO|(t)}{|NCO_{t=0}|}$$

It is immediately apparent that the value of α must vary over time according to a first order exponential law of the type: $\alpha(t) = 1 - \exp(-kt)$, where k is the apparent kinetic constant of the reaction between the individual isocyanate and hydroxyl groups. Hence, it can be assumed that the *gel point* occurs when t_g reaches a certain threshold value α_g . Because it can be assumed that α_g does not vary with the temperature,²¹⁹ then the value of t_g can replace the true kinetic constant for the construction of the Arrhenius plot.²²¹ In fact, it can be obtained:

$$\alpha_g = 1 - \exp(-k \cdot t_g) \quad \text{and with a few passages:}$$

$$k \cdot t_g = -\ln(1 - \alpha_g) = \text{const} \quad \text{from which}$$

$$k = \text{const}/t_g \quad \text{then:}$$

$$k = \text{const}/t_g = A \cdot \exp(-E_a/RT) \quad \text{and finally, switching to logarithms and rearranging:}$$

$$\ln(t_g) = (\ln \text{const} - \ln A) + (E_a/R) \cdot (1/T)$$

from which the linear dependence of $\ln(t_g)$ on $1/T$ is evident. It is remarkable that the activation energy of the process can be calculated by having carried out no real kinetic experiment, which in our case would be difficult to perform. For the same very reason, t_g can also be used to calculate the $\Delta H^\ddagger$ using the Eyring equation (Figure 2.6). In fact:

$$\ln(k/T) = \ln(\text{const}/(t_g \cdot T)) = \ln \text{const} - \ln(t_g \cdot T) \quad \text{from which it can be easily obtained:}$$

$$\ln(t_g \cdot T) = (\Delta H^\ddagger/R) \cdot (1/T) - [(\Delta S^\ddagger/R) + \ln(k_B/h) - \ln \text{const}]$$

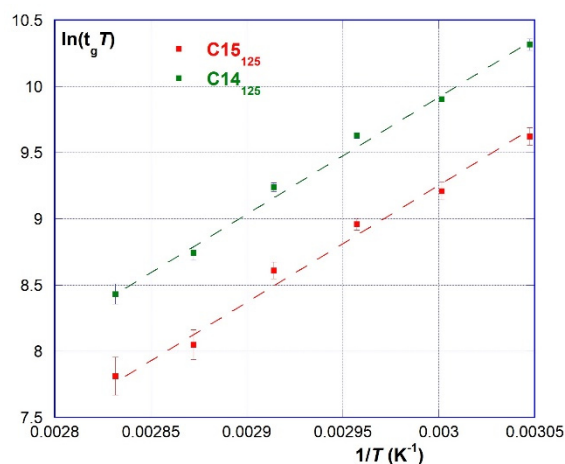


Figure 2.6. Eyring-type graph for t_g values.

From the data reported in Table 2.2 one can obtain: $E_a = 75 \pm 2 \text{ kJ}\cdot\text{mol}^{-1}$ and $\Delta H^\# = 72 \pm 2 \text{ kJ}\cdot\text{mol}^{-1}$. These results are in agreement with those reported in the literature for the synthesis of urethane polymers.^{218,220,222} This confirms the actual descriptive value of the parameter t_g , the use of which can be therefore generalized for the analysis of other systems.

At this point, it is interesting to discuss the difference in behavior of the systems C14 and C15, which mathematically is accounted for by the differences in the intercepts of the Arrhenius and Eyring linear plots. In fact, at the same temperature, the shortest linker system shows the longest t_g . This trend is confirmed by the analysis of systems C16 and C1_[125]. In fact, the first one shows a t_g value of $90 \pm 10 \text{ min}$ at 60°C . Noticeably, the latter datum has a large uncertainty, because this system showed a much worse reproducibility. Furthermore, at the *gel point* the reaction system had also a different macroscopic appearance (a rubbery and opaque rather than a jelly transparent mass). By contrast, the C1_[125] system showed no *gel point* after three hours. These observations can be justified from a mathematical standpoint assuming that the value of α_g is different in the various systems. From the previously reported relation $k \cdot t_g = -\ln(1 - \alpha_g)$, being k the intrinsic rate constant for the reaction between the functional groups -NCO and -OH , it derives that a higher value of t_g must correspond to a higher value of α_g . This leads to the conclusion that at the *gel point* the crosslinking process must be more advanced as the length of the linker decreases.

The effect of the concentration of the reagents can be deduced considering the samples C15_[100], C15_[83] and C15_[62]. In fact, only the first showed a t_g value of 46 min (to be compared with the 30 min of C15), whereas the other two showed no *gel point*. These results appear anomalous because the apparent value of the apparent (pseudo-first order) kinetic

constant k in the equation $k \cdot t_g = -\ln(1 - \alpha_g)$ should linearly depend on CD_0 . Consequently, one would expect that the values of t_g should be inversely proportional to the latter. The experimental datum indicates that in the case of C15_[100] the value of t_g is greater than the expected one. This fact, together with the lack of *gel point* for C15_[83] and C15_[62], can be explained by assuming that the formation of the polymer network is actually the result of two distinct processes. One is the actual chemical cross-linking due to the formation reaction of urethane bridges. The other one can be identified in a physical process, i.e. in the possible tangling of linear portions of different polymeric pre-aggregates, which leads to the limit of a concatenation of links, in a somehow similar way as in the formation of catenanes (Figure 2.7).

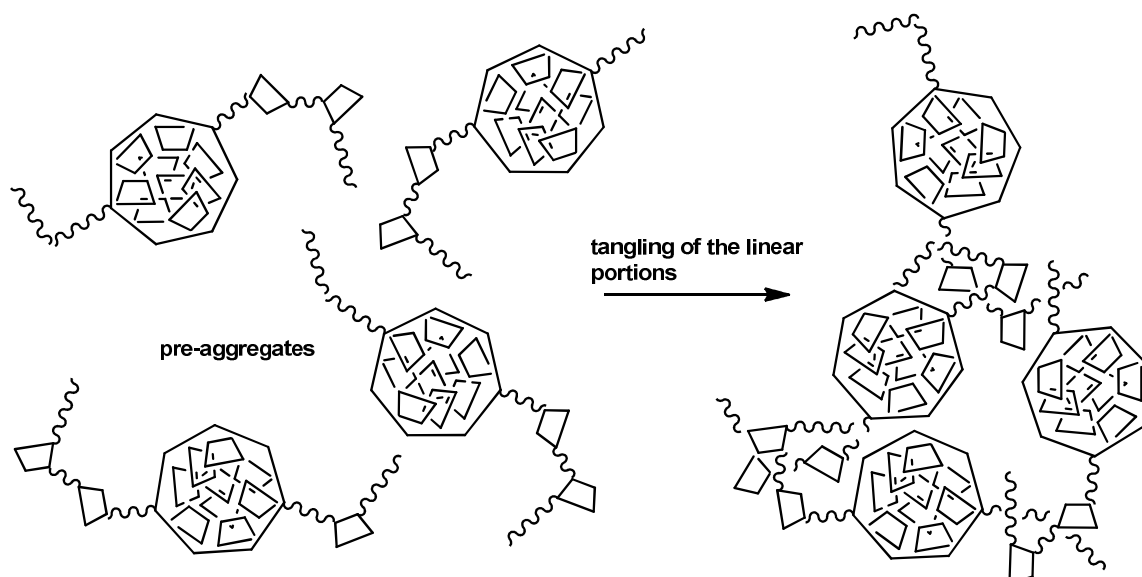


Figure 2.7. Physical interconnection between pre-aggregates.

This interpretation is confirmed by the comparison between the C14_[125] and C14_[62] systems; in fact, the second one does not present any *gel point*. Considering the effect of the temperature, the C15_[125] and C14_[125] systems also do not show a *gel point* within three hours below 55 °C. This fact could not be explained if the network formation process relied on a purely chemical process, because in this case the *gel point* should have been observed anyway (after about 75 min and 150 min respectively in the two different cases, on the basis of the calculated E_a values).

The systems C1-C4 and C16 showed a somehow different behavior than the systems C14-C15, because the *gel point* is less sharp, the corresponding t_g values are less reproducible

and the macroscopic appearance of the material is more “*rubbery*”, rather than gelatinous, and appears opaque. Something similar is actually observed also for the systems C14 and C15 at 80 °C. To better investigate the point, the C1-C4 systems were considered by varying the combination ratio between the reactants. As shown in Table 2.2, the minimum H/C ratio at which it is possible to verify the occurrence of the *gel point* is 3:1. Again, the t_g values are not inversely proportional to the crosslinking agent concentrations, going from 85 ± 8 min for C2_[125] at 35 ± 5 min for C2_[83] up to 22 ± 3 min for C4_[125]. Furthermore, the effect of dilution is confirmed by the comparison between the systems C2_[83], C2_[62], and C2_[50], where only the first one shows the viscous transition.

The fact of finding so many systems that do not present a *gel point*, and remain liquid after the 24 hours, suggested the opportunity for further investigations. The relevant reaction crudes were subjected to the usual work-up procedure (precipitation in water, washing and drying), from which products were obtained as subtle powders or very friable solids. These products resulted indistinguishable on spectroscopic analysis (FT-IR and NMR in solid state) from the corresponding nanosponges obtained in the “*bulk*” syntheses. A simple observation at the optical microscope (10x) under polarized light (Figure 2.8) showed that the products are actually formed by tiny granules of irregular shape and variable dimensions, but rarely larger than 50 μ . From the photographs taken with the optical microscope, it can be seen that part of the sample is actually made up of particles of sub-micrometer size. Hence, the images could not be subjected to statistical analysis to obtain the size distribution (since the evaluation of diameters smaller than 2 μ is not reliable). However, even only at a qualitative level, the images clearly show a progressive decrease in the average size of the particles passing from C2_[83] to C2_[62] and to C2_[50], i.e. as the dilution of the system increases. For the sake of information completeness, the polarized light microscope observation of the corresponding liquid reaction systems clearly shows the presence of globular aggregates, which can easily be identified as nanosponge granules strongly swelled up by the solvent.

These observations allow us to make some general considerations on the mechanism of formation of the polymeric network of the synthesized materials. Starting from the initial situation of a homogeneous solution of β CD and bis-isocyanate species, as the urethane bridges formation reaction starts, initially linker unit covalently connect different cyclodextrin units. As the process goes on, the forming aggregates progressively become larger and larger, but also more and more rare.

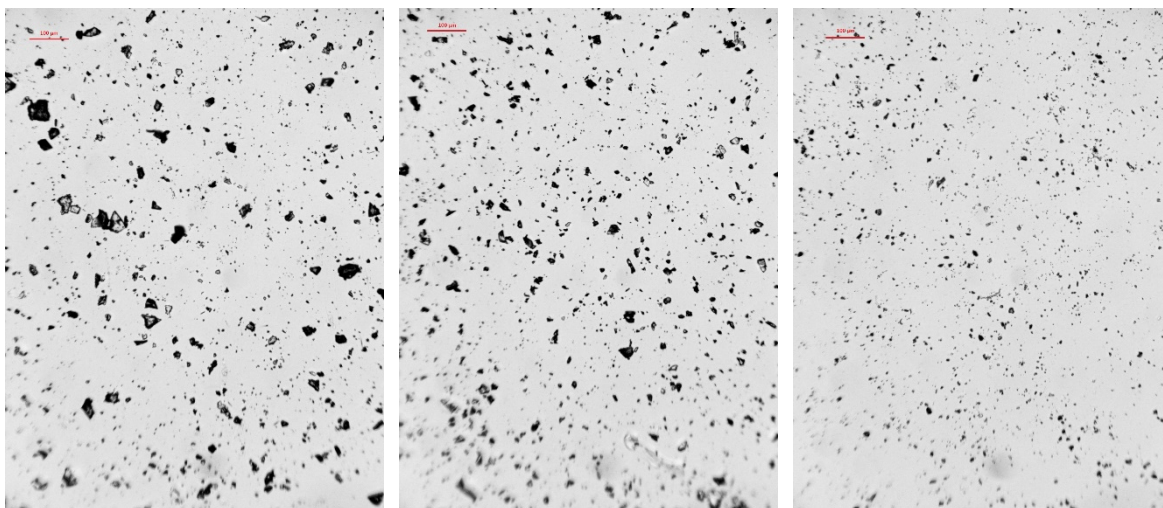


Figure 2.8. 10x optical microscope images of the materials C2_[83], C2_[62] and to C2_[50] (from right). The red reference bars represent 100 μ .

At this point, the reaction system has different possibilities. *i)* If at the overall concentration is large enough, and at the same time the linker units are long enough, and the temperature falls within the right range, then the physical tangling between linker chains (or in any case between linear polymer portions) belonging to different pre-aggregates has a significant probability to occur. Physical interconnection significantly contributes to the growth of the network (which at this point will be connected both chemically and physically), thus leading to the sudden increase in viscosity resulting in the *gel point*. Subsequently, the reaction of the residual linker units only consolidates the three-dimensional network, leading to the massive material and the ultimate formation of a glassy or rubbery-like system. *ii)* If the linker units are shorter, or if the system concentration is lower than a given threshold value, or if the temperature is so low to make the connection between linear subunits statistically unlikely, the pre-aggregates fail to physically interconnect. Subsequently, as they grow, at some point the last remaining reacting linker units will close in a ring by binding on different -OH groups of the same aggregate, thus preventing further growth. In the absence of an indefinitely extended network, the reaction system remains liquid. In this context, it should be noted that a too high reaction temperature ($> 75\text{ }^{\circ}\text{C}$) negatively affects the physical binding of the chains, favoring chemical cross-linking (i.e. the formation reaction of urethane bridges). This can explain the particular behavior of the C14 and C15 systems at $80\text{ }^{\circ}\text{C}$. The binding process evidently involves a significant contribution to the activation entropy of the network formation process, but not to its activation enthalpy, which is in fact the same for the two materials.

2.5 Optimizing the synthetic procedure: the “*smart dilution*” method

The hypothesis that the *gel point* substantially corresponds to the formation of the widely extended polymer network, suggested the possibility to stop the cross-linking process at this specific level, i.e. before the last chemical-type “*completion*” phase. This could be smartly obtained by diluting the reaction system immediately after the *gel point* with a suitable amount of solvent. In particular, starting from the samples C14, C15, C16 and C2_[125] prepared at 60 °C, an amount of DMF as large as 1.5 times the initial volume was added at the *gel point*, together with a little amount of methanol (0.02 mL per 1 mL of overall DMF), in order to quench the remaining unreacted isocyanate groups. Interestingly, the systems obtained appeared as gelatinous masses, which by subsequent work-up afforded solid products that showed some peculiar differences in comparison with the corresponding nanosponges obtained with the usual *bulk* (“thick paste” or “solution”) procedures. Indeed, these products resulted indistinguishable from the *bulk* ones upon spectroscopic (FT-IR, NMR) characterization; however, they were much more friable and showed an outstandingly large swellability in DMF (up to 1200% in weight!) and water (up to 66% in weight, Table 2.4). It was easily verified that the absorption of DMF is perfectly reversible; the gelatinous system obtained is stable for months, and can be extruded with little effort from a syringe through a 22G needle (0.7 mm lumen), keeping its characteristics unaltered. Noticeably, similar swelling abilities are also shown by the finely divided materials obtained from systems C14_[62], C15_[62] and C2_[62] (i.e. those showing no *gel point*).

Table 2.4. Swelling rates in DMF and water (average of four replicates).

	% DMF (w/w)	% H ₂ O (w/w)
C15_[125]	1100 ± 100	49 ± 10
C14_[125]	1180 ± 60	57 ± 6
C16	480 ± 90	38 ± 5
C2_[125]	1250 ± 50	66 ± 5

2.6 Concluding highlights

A large panel of diverse tailored materials has been obtained varying the type and amount of the host monomers, the cross-linkers and the possible chain extenders. The optimization

of the synthetic procedure is an important issue; indeed, even the speed and order of mixing of the reactant can have a significant outcome on the final product (in terms of its microscopic disorder). Hence, three different methodologies, namely a “thick paste”, a “*solution*” and a “*smart dilution*” procedures were developed, by which products can be obtained having either a hard, or a gelatinous or a micro-powder appearance.

Then, the mechanism of formation of the polymeric network has been more thoroughly understood, by addressing the study of the effect of the linker chains length on the gel transition point. The data indicate that the mechanism involved both a strictly chemical process (namely, the isocyanate-alcohol addition reaction to afford the urethane bridge) and a physical interconnection process. This second process is favored by increasing the length of the linker chains, but is prevented if the temperature is not within the right range (55~75 °C) or if the reaction system is excessively diluted.

2.7 Experimental section

All commercial reagents and solvents needed were as purchased (Aldrich, Fluka, Cyclolab) with no further purifications. The β CD was dried in vacuum over P_2O_5 at 90 °C overnight before use. The anhydrous DMF was prepared by equilibrating the commercial product for at least two days with a quantity equal to 20 % by weight of A4 molecular sieves, in turn activated by keeping them overnight in a vacuum drying gun on P_2O_5 at 160 °C. The solvent thus obtained was preserved with the same sieves under an argon atmosphere.

For the synthesis of composite materials different approaches were used which differ in the type and in the amount of solvent used (DMF and DMSO). The necessary amount of reagents and solvent must be set ad hoc according to the particular material, and are summarized in Table 2.5.

2.7.1 “*Thick paste*” method

A proper amount of C and of cross-linker P (if required by the synthesis), calculated according to the various molar or weight ratios required for the particular material to be prepared, is carefully mixed in an agate mortar. The mixture of powders obtained is introduced into a sufficiently large glass vial and then mixed with 300 μ L of anhydrous

solvent, in which the appropriate amount of H had been dissolved. The reaction system is then vigorously mixed with a thin metal rod for a few minutes, up to obtain a homogeneous mass. The system is then left to react in an oven at 60 °C for 18 hours.

2.7.2 “Solution” method

A proper amount of anhydrous solvent is placed in a sufficiently large glass vial with a proper amount of triethylamine (TEA). A proper amount of host (C or K) is dissolved, then it is brought under magnetic stirring at 60 °C (heating plate) and the cross-linking reagents (H, P) are added in the appropriate calculated amount. If the synthesis required the presence of the chain extender D, the 1,6-hexandiol is dissolved in a proper amount of DMF and the triethylamine and the bis-isocyanate H are added. Once the addition is complete, the system is heated at 60 °C and left to react under magnetic stirring for 1 h. At this point, the C is added, under vigorous stirring (360 rpm) to ensure its rapid dissolution. If the synthesis required the use of the chain extender A, the 3-3'-diamino-N-methyldipropylamine are dissolved in 1.0 ml of anhydrous DMF and then, is added drop by drop in the solution that contained the H. After 10 minutes, a proper amount of C and TEA are added and the system is left to react at 60 °C under magnetic stirring. For the synthesis of the materials CK, a given amount of *p-tert*-butyl-calix[4]arene are dissolved in a solution of H and anhydrous DMF in presence of TEA. The system is left to react for 1 hour in a bath oil at 60 °C, after which a given amount of C are added and, then, the reaction system is allowed to react until it polymerizes at 60 °C under magnetic stirring. For the synthesis of materials C14, C15 and C16, the appropriate amount of diol D (solid) is dissolved in DMF and then the triethylamine and the bis-isocyanate H are added using a Hamilton-type microsyringe. Once the addition is complete, the system is heated at 60 °C and left to react under magnetic stirring for 1 h. At this point, the C is added, under vigorous stirring (360 rpm) to ensure its rapid dissolution, and the system is recorded and kept under observation for the time necessary for the occurrence of any *gel point*.

Subsequently, all the reaction system is kept in the oven at 60 °C for 18 hours. The isolation of the final product, for both methods, is carried out as follows. The reaction mass is recovered, mechanically crushed, and suspended in 40 mL of ethyl acetate. The suspension is sonicated for 15 min and the solid is isolated by decantation. The same washing with ethyl acetate is carried out two more times, then the recovered solid is resuspended in 40 mL of methanol, sonicated and left to stand overnight. The final product is filtered, dried for 24 hours in a vacuum gun on P₂O₅ at 65 °C, and finally ground by ball milling and passed through a mesh sieve of 150μ.

2.7.3 Rubbery-like or glassy systems

The reaction mixture is maintained at 60 °C without stirring for one night. Subsequently, it was disintegrated mechanically, poured into 40 mL of distilled water and sonicated for 10 minutes. The precipitated solid was decanted and treated again in the same way by suspending it first in water, then in methanol (2 times) and finally in diethyl ether. The residue, which now appears as a very hard solid, was filtered, dried in a rotavapor at 60 °C until it reaches constant weight, and then subjected to effective mechanical grinding (by ball milling). The obtained powder was resuspended in ethanol and the suspension was passed through a 5 μ mesh sieve to separate the more finely divided part (the part remaining on the sieve is ground again). The ethanolic suspension is finally evaporated to dryness and the residue kept overnight in a vacuum gun over P₂O₅ at 60 °C.

2.7.4 Gel-like systems: “smart dilution” method

A few minutes after observing the *gel point*, given amount of commercial (non-anhydrous) DMF equal to one and a half times the initial volume of the system and 150 μ L of methanol are added to the system. The raw reaction product is always kept at 60 °C under magnetic stirring (60 rpm) until total absorption of the added liquid. At this point, the magnetic stirring is stopped and the mixture is brought to room temperature. The system looks like a stable jelly for indefinite times. To isolate the dry material, the system is suspended, sonicated (10 min) and decanted first twice into a quadruple volume of water, then twice into methanol and finally into diethyl ether. The mass obtained is then dried in a rotavapor, ground and passed through a 150 μ mesh sieve. The product is finally kept for at least one night in a vacuum gun on P₂O₅ at 60 °C.

2.7.5 Liquid systems

In cases in which the *gel point* is not observed within 3 hours of mixing the reactants, the obtained pseudo-solution is left to stand for one night at r.t.; then, it is poured dropwise into a tenfold volume of methanol under vigorous magnetic stirring and the resulting suspension is then centrifuged (3800 rpm) for 10 min. The residue is re-suspended and re-centrifuged two more times with acetone (same volume) to isolate a first fraction of solid product. The supernatant liquids are dried in a rotavapor, and the residue is re-suspended again in acetone and centrifuged to obtain a second small fraction of solid which is combined with the previous one. The combined solids are then dried for at least one night in a vacuum gun over P₂O₅ at 60 °C.

Table 2.5. Synthesis of materials.

NS	Host		Linker/chain extender				solvent		TEA (μ L)	Yield (mg)
	CD (mg)	CA (mg)	H (mg)	P (mg)	D (mg)	A (mg)	DMSO (mL)	DMF (mL)		
C1	568	-	168	-	-	-	0.3	-	-	721
C1 _[125]	284	-	84	-	-	-	-	2.0	35	365
C2 _[125]	284	-	126	-	-	-	-	2.0	35	401
C2 _[83]	284	-	84	-	-	-	-	3.0	35	365
C2 _[62]	284	-	84	-	-	-	-	4.0	35	363
C2 _[50]	284	-	84	-	-	-	-	5.0	35	364
C3	568	-	336	-	-	-	0.3	-	-	885
C3	568	-	336	-	-	-	-	0.3 or 3.0	-	807
C3 _[125]	284	-	168	-	-	-	-	2.0	35	448
C4 _[125]	284	-	251	-	-	-	-	2.0	35	520
C5	568	-	670	-	-	-	-	0.3 or 3.0	-	810
C6	1135	-	-	436	-	-	1.5	-	100	1422
C7	1220	-	-	536	-	-	1.5	-	100	1682
C8	1135	-	-	1530	-	-	1.5	-	100	2166
C8	324	-	-	436	-	-	-	0.3 or 3.0	-	693
C9	568	-	173	218	-	-	0.8	-	-	557
C10	1135	-	336	872	-	-	1.2	-	-	1758
C11	1135	-	670	873	-	-	0.85	-	-	2196
C11	568	-	336	436	-	-	-	3.0	-	938
C12	568	-	670	436	-	-	-	0.3 or 3.0	-	1139
C13	114	-	1010	-	591	-	-	3.0	140	1348
C14 _[125]	284	-	168	-	59	-	-	2.0	35	490
C14 _[62]	284	-	168	-	59	-	-	4.0	35	492
C15 _[125]	284	-	251	-	118	-	-	2.0	35	550
C15 _[100]	284	-	251	-	118	-	-	2.5	35	552
C15 _[83]	284	-	251	-	118	-	-	3.0	35	548
C15 _[62]	284	-	251	-	118	-	-	4.0	35	549
C16	284	-	126	-	29.5	-	-	2.0	35	460
C17	284	-	503	-	295	-	-	2.5	70	687
C18	567	-	503	-	295	-	-	2.5	70	541
C19	284	-	336	-	-	144	-	2.5	70	531
K1	-	324	336	-	-	144	-	2.5	70	492
K2	-	162	336	-	-	216	-	2.5	70	475
K3	-	65	1010	-	591	-	-	3.0	140	1614
K4	-	162	503	-	295	-	-	2.5	70	747
K5	-	324	503	-	295	-	-	2.5	70	961
CK1	284	162	336	-	-	-	-	2.5	70	750
CK2	284	162	168	-	-	-	-	2.5	70	294
CK3	142	162	336	-	-	-	-	2.5	70	687
CK4	567	162	336	-	-	-	-	2.5	70	586
CK5	142	162	336	-	-	108	-	2.5	70	625
CK6	567	162	336	-	-	108	-	2.5	70	857
CK7	284	324	670	-	177	-	-	2.5	140	985

3 – Characterization of the Nanosponge materials

3.1 Introduction

Once the synthesis and isolation had been carried out, the materials were subjected to suitable characterization, in order to: *i*) verify the actual occurrence of the reticulation process and to confirm the presence of the various constituent moieties; *ii*) quantify and assess the dissociation constant of the ionizable functional groups (if present); *iii*) assess the texture properties and functional mobility of a possible solvent medium within the nanosponge pore network.

The first task was pursued by ordinary spectroscopic techniques, namely FT-IR and ^{13}C CP-MAS solid-state NMR. For the second task, the materials prepared with reticulating agents possessing ionizable groups were subjected to simple acid-base titrimetric determinations. In details these determinations were carried out for the materials C7-C12 prepared with the pyromellitic anhydride P, which bear two carboxyl groups per linker unit, and for the materials C19, CK5-CK6 and K2 prepared with the polyamine A, which bear one tertiary amine group per linker unit. The third task was accomplished by exploiting the FFC-NMR relaxometry, which provides the evaluation of the longitudinal relaxation rates (R_1) of a solvent saturated material in a magnetic field varying between $2.35 \cdot 10^{-4}$ T and $2.35 \cdot 10^{-1}$ T (corresponding to the proton Larmor frequency range 0.01~10 MHz). The theoretical bases of the latter technique and of its application to the characterization of porous materials will be discussed in details in Section 3.4, before suitably addressing the results obtained in section 3.5.

3.2 FT-IR and $^{13}\text{C}[^1\text{H}]$ CP-MAS NMR characterization of the materials

In general, FT-IR spectroscopy allowed to unambiguously identify the signals expected for the various functional groups originated in the reticulation reactions, and the typical fingerprints of the moieties from the constituting synthons (cyclodextrins, calixarenes, linkers). In particular, as long as the functional groups are concerned, FT-IR allows to immediately assess the absence of possible unreacted isocyanide groups from H and the formation of the urethane and urea groups, as well as the formation of the aryl ester groups from P. The relevant attributions are summarized in Table 3.1. In general, features to be particularly noted in the FT-IR spectra are:

i) the modification of the intense -OH str. band in the $3200\text{-}3500\text{ cm}^{-1}$ region. This is mainly due to the reaction of these groups with the electrophilic reticulating agent. In particular, in the case of materials K1-K5, the -OH phenolic groups are quantitatively converted into carbamates, which results in a neat shift of the signal from 3240 to 3330 cm^{-1} . Moreover, in the case of materials prepared with cyclodextrin, it should be also kept into account that unreacted -OH groups experience significant environmental changes with respect to the pure reactant, which affect their abilities in hydrogen bonding formation.

ii) the presence of new signals in the carbonyl region $1750\text{-}1500\text{ cm}^{-1}$. This feature positively accounts for the actual presence of the expected carbamate and urea functional groups occurring from the reticulation reaction. Under this standpoint, the materials CK1-CK9 represent an interesting case of study. The spectrum of CK5_c is reported in Figure 3.1 as an illustrative example. In fact, the aforementioned region features a complex set of signals, the deconvolution of which enables to recognize the bands relevant to the groups originated in the reactions depicted previously in Figure 3.1. Noticeably, it is possible to discriminate between the alkyl urethane deriving by the reaction of H with the alcoholic -OH groups of the cyclodextrin and diol D (Amide-I-type at ca. 1665 cm^{-1} and Amide-II-type at ca. 1540 cm^{-1}), and the aryl urethane group deriving from the reaction between H and the calixarene phenolic -OH groups of the cyclodextrin and diol D (Amide-I at ca. 1725 cm^{-1} and Amide-II at ca. 1530 cm^{-1}).

iii) The FTIR spectra of materials C7 and C8 show a wide band centered at ca. 2620 cm^{-1} , attributable to the secondary band of the -OH stretching of the -COOH group. There is also a very intense band centered at 1721 cm^{-1} of C=O stretching, accompanied by another intense band at 1251 cm^{-1} of CO-O stretching, both characteristics of the esters of aryl-carboxylic

acids. Therefore, these three signals fully confirm the presence of the 1,2,4,5-phenyl-tetracarboxylic-1,4-diester linker which is generated by the reaction of P with the hydroxyl groups of β CD.

iv) Finally the spectra allow also to recognize the peculiar fingerprint signals due to the cyclodextrin and calixarene scaffolds.

Table 3.1. Diagnostic FT-IR signals expected for the various functional groups and moieties.

Band		$\tilde{\nu}$ (cm ⁻¹)
str. -OH	Cyclodextrin	3350-3450
	Calixarene	3240-3250
	carboxyl.acid	2500-2700
str. -NH-	urethane, urea	3320-3340
str. C=O	aroate ester	1710-1715
str. C=O (Amide I-type)	aryl carbamate	1720-1730
str. C=O (Amide I-type)	alkyl carbamate	1640-1690
str. C=O (Amide I-type)	alkyl urea	1620-1630
bend. -NH- (Amide II-type)	aryl carbamate	1525-1535
bend. -NH- (Amide II-type)	alkyl carbamate	1535-1545
bend. -NH- (Amide II-type)	alkyl urea	1575-1585
fingerprints:		
poly-urethane/urea		1257, 1222, 1144, 1076, 1055, 1005
cyclodextrin		1156, 1079, 1030, 947
calixarene		1487, 1466, 1363, 1303, 1290, 1244, 1205, 1118

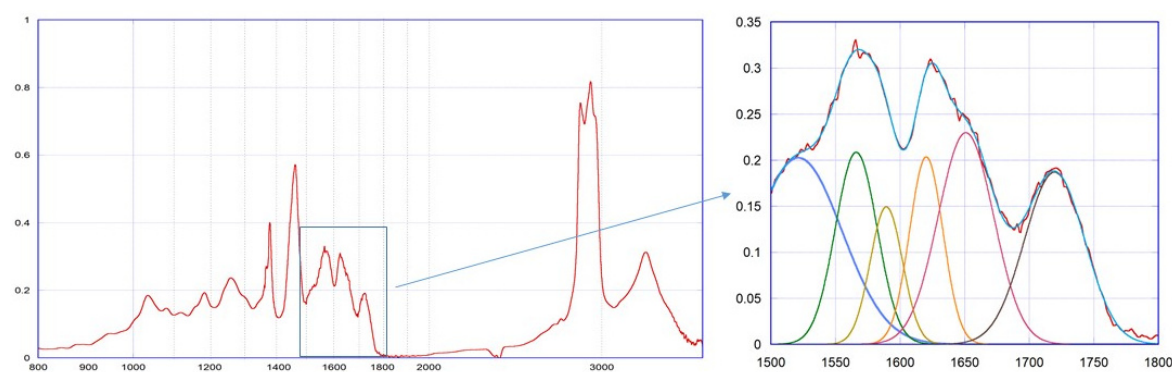


Figure 3.1. FT-IR spectra (absorbance mode) and deconvolution of CK5_c.

On passing to the ¹³C CP-MAS solid-state NMR characterization, spectra fully confirmed the presence of the constituents and the expected functional groups in the materials obtained.

The complete attribution of NMR signals is summarized in Table 3.2. The spectrum of material CK2 is reported in Figure 3.2 as an illustrative example.

Table 3.2. Diagnostic signals in the ^{13}C CP-MAS solid-state NMR spectra		
Signal		ppm range
Cyclodextrin	C(1)	100~105
	C(2,3,5)	70~75
	C(4)	81~84
	C(6)	60~65
Calixarene	<i>t</i> -butyl	31~33 (methyl); 34~35 (quaternary)
	-CH ₂ -	32~33
	C aromatics	125~128; 131~136; 146~147; 155~157
Aliphatic linker/chain extender	-CH ₂ - (not bound to O,N)	26~31
	-CH ₂ - (bound to O,N)	40~60
	C aromatics (from P)	128; 133
	urethane, urea	158~160
	aroate ester/carboxyl	160~170

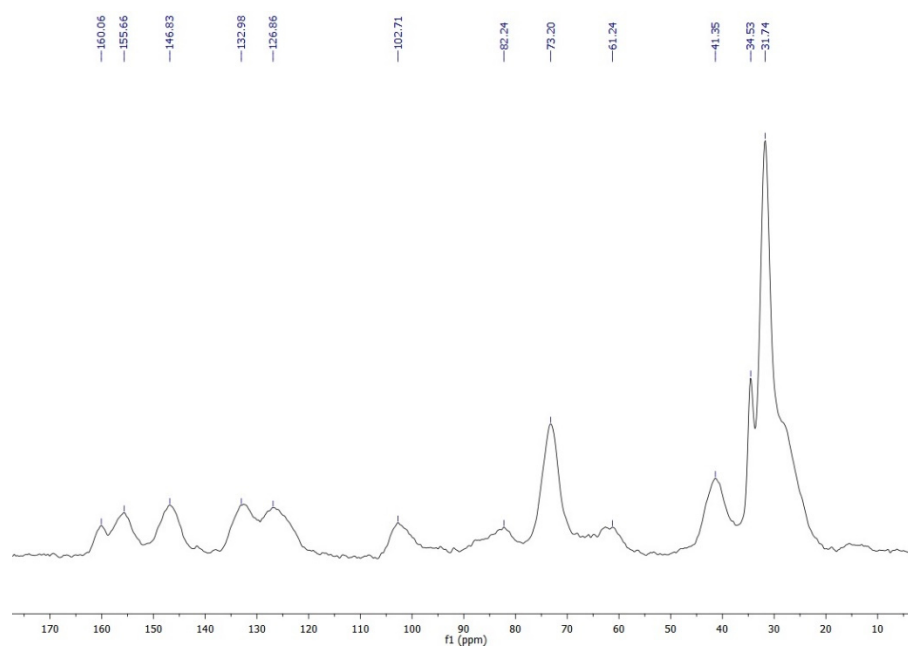


Figure 3.2. ^{13}C CP-MAS solid-state NMR spectrum of material CK2.

All the spectra present the three characteristic signals of cyclodextrin, at ca. 102.9 (C1), 82.1 (C4) and 73.0 ppm (cumulative for C2, C3 and C5). The spectra of the materials C7 and C8 show a system of three overlap signals in the aromatic carbon zone (131.2, 134.0 and 136.9

ppm), plus two further overlap signals at 167 and 169 ppm, that was attributable to the carboxylic C. Passing from material C7 to material C8, all these signals significantly increase in intensity if they are compared to those related to cyclodextrin carbons. Finally, it should be noted that the signals expected for C6 of the free cyclodextrin (approx. 61 ppm) and esterified (approx. 64 ppm), are not very visible in the spectra, due to the fact that they are partially or totally covered by the nearby very intense signal at 73 ppm. Furthermore, it is also interesting to note the presence of a small signal at 53.8 ppm in the C8 material, in a position consistent with that of the methyl ester of benzoic acid. The latter finding could be justified by admitting that, during the processing of the material, in the first washing with methanol, small amounts of unreacted P undergo a nucleophilic acyl substitution reaction by the washing solvent. As a last observation, the $^{13}\text{C}[^1\text{H}]$ CP-MAS solid-state NMR spectra of the other materials show, in addition to the cyclodextrin and aromatic ligand signals, two signals at 28.0 and 40.8 ppm. These signals were attributable to the $-\text{CH}_2-$ groups not bound or bound to an N atom respectively. Furthermore, the expected signal at 60.5 ppm for C6 of the cyclodextrin units is clearly present.

3.3 Titrimetric characterization of the materials

The chemical characterization of the materials required to assess the acid-base properties of those ones containing ionizable groups. In particular, was necessary to establish the amount of cross-linking agent present in materials C7-C11, C19, CK5-CK6 and K2, in order to explain the lower than theoretical yields for the materials containing P (C7-C8) and HP (C9-C11) as cross-linkers. In these cases, considering that the resulting materials contain two carboxyl groups for each pyromellitic linker residue, it is immediately apparent that the actual amount of P subunits per g of materials can be immediately obtained from the quantification of the $-\text{COOH}$ groups. In fact, taking into account the number of equivalents of carboxylic groups per gram of material, the percentage of linker in the material and the degree of incorporation can be easily obtained through stoichiometric calculations. The data for the materials synthesized with DMSO are summarized in Table 3.3. In a similar way, the amount of polyamine units in materials C19, CK5-CK6 and K2 can be obtained.

It is worth recalling here that the titrimetric method provides considering the material as a mixture of virtual monoprotic weak acids in solution (whose number must be set case by

case, based on the fit goodness). This method has been already used in previous works^{84,89,183} for the characterization of polyamino-cyclodextrins NSs and cyclodextrin-calixarene NSs (the latter were native and post-functionalized NSs with ionizable groups). However, it should be pointed out that the reliability of the results strictly depends on the nature of the material. In fact, in the case of polyamino-cyclodextrin NSs, which possess a very hydrophilic nature, the determination actually leads to an exhaustive quantification of the ionizable groups present. Conversely, for the much more hydrophobic mixed cyclodextrin-calixarene materials, this method leads to the determination of an amount of ionizable groups generally not larger than 50% of the theoretical. This fact was explained by admitting the presence of highly hydrophobic micro-domains within the texture of the material, which cannot be permeated by the aqueous medium,⁸⁴ in such a way that the potentially ionizable groups cannot be subjected to titration. Data reported in Table 3.3 are in agreement with these trends.

Table 3.3. Potentiometric titration data of materials.									
NS	Notes	apparent ioniz. groups	$pK_{a,app}$	β CD (mmole/g) K (mmole/g)		P or A (mmole/g)		host:ioniz.linker mole-to-mole ratio	
		(meq/g)		Theor.	Exp.	Theor.	Exp.	Theor.	Exp.
C7	a)	1.98 ± 0.04	4.5 ± 0.2	0.50	0.63	1.99	1.32	1:4	1:2.1
		0.66 ± 0.04	6.2 ± 0.4						
C8	a)	2.28 ± 0.05	4.2 ± 0.06	0.37	0.46	2.62	2.18	1:7	1:4.7
		1.49 ± 0.09	5.58 ± 0.10						
		0.60 ± 0.11	7.6 ± 0.2						
C8	b)	3.12 ± 0.11	4.5 ± 0.2	0.37	0.58	2.62	1.56	1:7	1:2.7
			6.3 ± 0.3						
C8	c)	3.00 ± 0.10	4.2 ± 0.2	0.37	0.59	2.62	1.51	1:7	1:2.6
			5.5 ± 0.5						
C9	a)	1.55 ± 0.08	4.8 ± 0.1	0.52	0.56	1.05	0.78	1:2	1:1.4
C10	a)	1.74 ± 0.04	4.7 ± 0.1	0.43	0.55	1.71	0.87	1:4	1:1.6
C11	a)	0.97 ± 0.08	5.9 ± 0.3	0.37	0.46	1.49	0.79	1:4	1:1.7
		0.60 ± 0.15	9.3 ± 0.3						
C11	c)	0	n.d.	0.37	n.d.	1.49	0	1:4	n.d.
C19	c)	0.78 ± 0.04	7.7 ± 0.3	0.33	0.33	1.31	1.25	1:4	1:3.8
		0.47 ± 0.04	9.1 ± 0.5						
CK5_a	c)	0.56 ± 0.03	9.0 ± 0.1	0.17	0.18	1.00	0.56	1:2:6	1:2:3.1
				0.33	0.36				
CK5_c	c)	0.48 ± 0.04	8.6 ± 0.2	0.17	0.18	1.00	0.48	1:2:6	1:2:2.6
				0.33	0.36				
CK6	c)	0.60 ± 0.03	8.2 ± 0.1	0.43	0.43	0.64	0.60	2:1:3	2:1:2.8
				0.21	0.21				
K2	c)	0.80 ± 0.04	8.2 ± 0.1	0.35	0.44	2.10	0.80	1:6	1:1.8
K2	c), g)	1.05 ± 0.03	8.0 ± 0.2	0.35	0.42	2.10	1.05	1:6	1:2.5

a): DMSO “thick paste” method; b): DMF “solution” method; c): DMF “smart dilution”; g): after one night equilibration with HCl (see experimental).

In detail, as long as polyamine-containing materials are concerned, in the case of the more hydrophilic derivatives C19 (made up with β CD only) and CK6 (having β CD and K in a 2:1 mole-to-mole ratio) the quantification of polyamine chains affords results reasonably similar to the expected theoretical. Conversely, for the more hydrophobic materials CK5_a and CK5_c

(having β CD and K in a 2:1 mole-to-mole ratio) and K2 (containing only K), the amount of ionizable groups found is largely lower than the theoretical. Noticeably, in the case of K2 it was also attempted to equilibrate the material with an excess of hydrochloric acid overnight before titration. This led to a ca. 30% increase of the titrated groups found; however, this result still underestimates the amount of groups actually present. Furthermore, also in the materials C7-C11 the P component seems incorporated in a much lower amount than the theoretical. The observed apparent pK_a values, ranging from 4.2 to 7.6, are quite interesting. In fact, the former ones are perfectly consistent with the characteristic values of carboxylic acids. On the other hand, the progressive deprotonation makes the dissociation of the residual groups more and more difficult, which justifies the second apparent pK_a values.

Materials C8 and C11 offer an interesting case of study, because the apparent amount of ionizable groups seems to depend also on the synthetic procedure (in particular on the solvent chosen to perform the synthesis). In order to clarify the point, the materials were subjected to alkaline hydrolysis, in order to disrupt all the ester groups present and make free the pyromellitic tetra-carboxylic acid units. The alkaline hydrolysates were then subjected to titration analysis, in such a way to obtain an independent determination of the P units actually bound by the synthetic procedure. The results are collected in Table 3.4. The apparent amount of P units for the materials obtained in DMSO does not seem to change in the hydrolysate determination. Conversely, for the material obtained in DMF the new datum is significantly larger, and perfectly agrees with the one for the material obtained in DMSO. This indicates that in the syntheses (both in DMSO and DMF) part of the P component is actually lost. However, the use of different solvents leads to a different degree of microscopic order. In fact, the apparent increase in the amount of ionizable groups on passing from the pristine material to its hydrolysate implies the existence of highly hydrophobic microdomains that do not seem present in the material obtained in DMSO. The same interpretation can be suitably applied also to the data relevant to materials C8 and C11.

Table 3.4. Potentiometric titration data for C8 and C11 materials subjected to alkaline hydrolysis.								
NS	notes	apparent ioniz. groups	β CD (mmole/g) K (mmole/g)		P (mmole/g)		host:ioniz.linker mole-to-mole ratio	
		(meq/g)	Teor.	Exp.	Teor.	Exp.	Teor.	Exp.
C8	a)	7.6 ± 0.1	0.37	0.46	2.62	2.17	1:7	1:4.7
C8	b)	7.7 ± 0.1	0.37	0.46	2.62	2.18	1:7	1:4.7
C11	c)	3.5 ± 0.2	0.37	0.43	1.49	1.03	1:4	1:2.4

a): DMSO “thick paste” method; b): DMF “solution” method; c): DMF “smart dilution” method.

3.4 FFC-NMR relaxometric characterization of the materials: theory

In order to complete the structural characterization of the materials, and also to aid the rationalization of the experimental results achieved in the course of this PhD work, it was necessary to acquire information on the organization of the pore network present in the materials, i.e. to determine their textural features, and in general to assess the functional mobility of a possible solvent medium within the pore network. However, it has been repeatedly reported in literature that ordinary investigations based on the adsorption/desorption of gases such as BET/BJH analyses do not work well with nanosponge materials.²¹⁵ In fact, unless the linker units are constituted by relatively long and rigid structural moieties, unreliable very low apparent values can be found for the specific surface area and the specific pore volume of nanosponges. The reasons of this failure of the BET method are not clear, and are currently object of debate. Other alternative methods based on the adsorption of probe guests, such as *p*-nitrophenol, provide much more “reasonable” results.²²³⁻²²⁵ However, questionability arises from the fact that the values obtained strictly depend on the choice of the probe molecule. Indeed, one could argue that the notion itself of textural properties for nanosponge materials somehow fades away, because their “surface” might even be viewed as a sort of fractal object, and because the void volume within the pore network (and the pores diameter as well) changes as a consequence of possible swelling phenomena, which in turn depend on the solvent medium the material is equilibrated with. Under this perspective, the FFC-NMR relaxometry provides an interesting alternative investigation tool. This very informative technique is not new, indeed, but it has been exploited only occasionally by chemists, being in fact as a niche technique. Therefore, it is appropriate to present in this section its basic principles and applications, in such a way to subsequently discuss the results obtained by its application to the nanosponge materials studied in this work.

The ordinary NMR spectroscopy is the main tool for acquiring structural and conformational information on molecular systems. Moreover, by studying the kinetics of nuclear relaxation further information can be obtained on molecular dynamics. The application of pulses (generally of 90° or 180°) to a sample consisting of nuclei with a non-zero spin momentum placed into a magnetic field, causes the spin states of the system to be doubly perturbed, because the absorption of energy quanta and the establishment of a phase coherence

simultaneously occur. These perturbations are canceled over time by two independent and distinct *relaxation* processes, characterized in the simplest cases by a first order kinetics; the relevant kinetic constants R are referred to as the “relaxation rates”, whereas their inverses $T = 1/R$ are referred to as the “relaxation times”. The *longitudinal* relaxation (or spin-lattice) is a direct consequence of the loss of quantum of energy while the *transversal* relaxation (or spin-spin) is given by the loss of phase coherence previously induced by the impulse. The term “*relaxometry*” refers to the study of both these processes and the determination of their speed; however, if not specified otherwise, the study of longitudinal relaxation only is implicitly intended.

Longitudinal relaxation, in the case of a condensed phase molecular system, is physically related to the distribution of magnetic field fluctuations due to random molecular motions occurring in the solvent lattice.²²⁶ Nuclei in spin excited states can relax when they meet a local fluctuation occurring at a frequency equal to their Larmor frequency. The rate of relaxation of a system is closely related to the microscopic dynamics. Therefore, relaxometry can be an effective tool to obtain information on this phenomenon. Indeed, relaxometry finds several applications in various fields, including polymer chemistry and materials science in general. Of particular interest are systems or materials containing water since the ^1H nuclei in water molecules and the related relaxation processes are observed.²²⁷⁻²²⁸ The motion of the water can be correlated, in turn, with the texture properties of porous systems.^{229 230}

According to the theory by Bloch, Wangsness and Redfield,^{226,231-232} the rates of both the longitudinal and transversal relaxation processes can be expressed as:

$$R_{1\text{or}2} = K[J_1(\omega_L) + 2J_2(2\omega_L)] \quad (1)$$

where K is a parameter whose value depends on Plank's constant, the gyromagnetic constant and the spin quantum number, while J_1 and J_2 are referred to as the so-called “*spectral density functions*” which, through the autocorrelation functions for the nucleus subject to relaxation, relate to the related spin Hamiltonians; finally, $\omega = \nu_L/2\pi$. The subsequent theory elaborated by Bloembergen, Purcell and Pond²²⁶ provides that in the case of a “*simple*” molecular system subjected to a single dynamic regime, the longitudinal and transversal relaxation rate depends on the strength of the magnetic field according respectively to the following relationships (the so-called BPP functions):

$$R_1 = \frac{1}{T_1} = \frac{3}{10} \frac{\mu_0^2 \hbar^2 \gamma^4}{r^6} \left(\frac{\tau_c}{1 + (\omega_L \tau_c)^2} + \frac{4\tau_c}{1 + 4(\omega_L \tau_c)^2} \right) \quad (2)$$

$$R_2 = \frac{1}{T_2} = \frac{3}{20} \frac{\mu_0^2 \hbar^2 \gamma^4}{r^6} \left(3 \tau_c + \frac{5 \tau_c}{1 + (\omega_L \tau_c)^2} + \frac{2 \tau_c}{1 + 4(\omega_L \tau_c)^2} \right) \quad (3)$$

where r is the average internuclear interaction distance and τ_c is the “*correlation time*”, i.e. the average time taken by the molecule to rotate by one radian or to move a distance equal to its diameter of rotation. Therefore, τ_c quantifies the speed of molecular motions. The typical trends of the relaxation times T_1 and T_2 expressed by equations (2) and (3) as a function of τ_c are shown in Figure 3.3. Analyzing equations (2) and (3) it can be seen that the rate of transverse relaxation monotonically increases as the rate of the molecular motions increases, regardless of the value of the Larmor frequency and, consequently, of the intensity of the generated magnetic field. Conversely, T_1 passes through a minimum, whose position depends on the intensity of the magnetic field. As a consequence, under “high” magnetic field conditions (> 2.5 T, or $\nu_L > 100$ MHz) the relaxation rate increases on decreasing τ_c , i.e. on increasing the speed of molecular motions, whereas the opposite occurs under “low” magnetic field conditions (< 1 T, or $\nu_L < 40$ MHz). The curve that expresses the dependence of $\text{Log}(R_1)$ vs $\text{Log}(\nu_L)$ is referred to as the “NMR dispersion curve” (NMRD); its regression analysis leads back to the correlation time τ_c . The τ_c value cannot be obtained using ordinary high-field NMR instrumentation, which operates under fixed magnetic field values since the relaxation rates would be obtained at that single magnetic field value. Rather, the low-field Fast-Field Cycling (FFC) NMR technique can be very profitably exploited for the job.

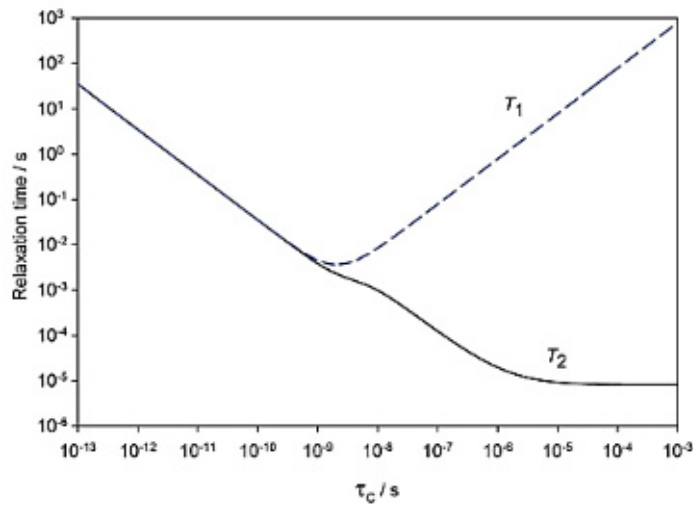


Figure 3.3. Dependence of relaxation times on correlation time.

The FFC-NMR technique relies on the estimation of the longitudinal relaxation rates (R_1) of samples placed in a “low” magnetic field that can be varied within a significantly large range

(ideally between 0.001 and 100 MHz in terms of Larmor frequencies ν_L). Figure 3.4 shows the typical FFC-NMR experimental phases. Two types of sequences can be employed, namely a pre-polarized sequence (PP) and a non-polarized one (NP). The PP sequence consists of a first phase in which the nuclei are immersed in a magnetic field (B_{pol}) for a fixed time interval (T_{pol}) in order to generate a longitudinal magnetization. After this phase, the nuclei are subjected to a different magnetic field defined as the relaxation field (B_{rlx}) for a period of time τ during which the magnetization of the nuclei varies as a function of the molecular dynamics of the system. Finally, the magnetic field is again varied on another fixed value defined acquisition field (B_{Acq}) and, at this point, an impulse of 90° is applied (with a suitable phase cycle) necessary to generate the observable and to acquire the Free Induction Decay (FID). The other sequence, i.e. the non-polarized one, differs from the previous one for the sole lack of the B_{pol} which is, in fact, zero.

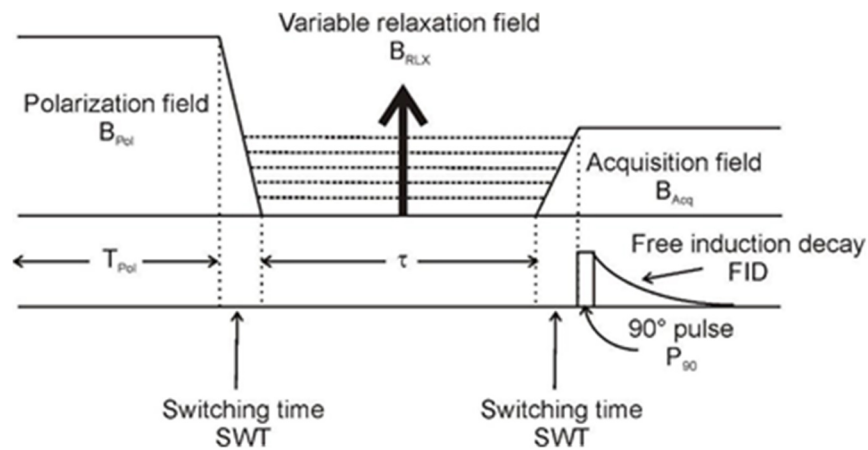


Figure 3.4. Scheme of the FFC-NMR sequence.

The observed values of the longitudinal relaxation times (T_1) of the nuclei are obtained for each value of B_{rlx} through a regression analysis of the signal intensity as a function of the value of τ , according to equations (4) and (5), the which hold respectively for the sequence PP and NP:

$$M(\tau) = a + \sum_{i=1}^N b_i \exp\left\{-\frac{\tau}{T_{1i}}\right\} \quad (4)$$

$$M(\tau) = a + \left(\sum_{i=1}^N b_i \exp\left\{-\frac{\tau}{T_{1i}}\right\}\right) = \left(a + \sum_{i=1}^N b_i\right) - \sum_{i=1}^N b_i \exp\left\{-\frac{\tau}{T_{1i}}\right\} \quad (5)$$

However, the equations just described cannot be used to adequately describe the distributions of T_1 when multiphase systems are studied because the best model to describe different

components of molecular dynamics, described by T_1 very close to each other, is that of the inverse Laplace transform, whose equations for the experiments conducted with PP and NP sequences are respectively:

$$M(\tau) = \int_{T_{1min}}^{T_{1max}} D(T_1) \exp\left[-\frac{\tau}{T_1}\right] d(T_1) + \sigma \quad (6)$$

$$M(\tau) = \int_{T_{1min}}^{T_{1max}} D(T_1) \left\{1 - \exp\left[-\left(\frac{\tau}{T_1}\right)\right]\right\} d(T_1) + \sigma \quad (7)$$

where T_{1min} and T_{1max} are the longitudinal relaxation time limits within which all T_1 values fall; $D(T_1)$ is the distribution function to be determined by solving equations (6) or (7), depending on the type of sequence used. The inverse Laplace transform can be obtained through the use of various methods, one of which is the UPEN algorithm developed by Fantazzini *et al.*²³³⁻²³⁵ It is worth stressing that under the low magnetic field conditions exploited by the technique, any spectral resolution is lost. Consequently, the actual physical state (liquid, solid, gel, etc.) and the possible complexity of the examined system has no impact on the possibility to perform the relaxometric study. Thus, the FFC-NMR can be used for any sample irrespective of its status or complexity. However, it is immediately evident that a real complex system cannot be simply considered in terms of the basic BPP treatment. Therefore, in order to describe a real systems, where the dynamics of molecular motions and reorientation are complex, it would be necessary to derive theoretically the corresponding autocorrelation functions and obtain from these the NMRD profiles, through the appropriate mathematical treatment.²³⁶ Approaches of this type are present in the literature;^{237-238 232,239} however, they require the ability to handle very complex mathematical tools, and result into equations the validity of which is strictly limited to a particular class of systems. Thus, as an alternative, the so-called "*model-free*" approaches have been developed and have progressively acquired popularity. The bases of *model-free* methods have been set up in a series of seminal papers by Halle *et al.*²⁴⁰⁻²⁴² In brief, starting from the hypothesis that even in a complex system the molecular motions can be assimilated to Brownian motions, the NMRD curve of a generic system can be approximated to a summation of an arbitrary number of Lorentzian terms:

$$\frac{1}{T_1} = \sum_i \frac{c_i \tau_i}{1 + (\omega \tau_i)^2} \quad (8)$$

where the coefficients c_i are empirical weight coefficients, and from which a weighted average correlation time can be obtained through the relation:

$$\tau_c = \frac{\sum_i c_i \tau_i}{\sum_i c_i} \quad (9)$$

Kruk's research group has recently addressed the problem of the NMRD curves of solid-phase proteins,²⁴³⁻²⁴⁴ by applying a different model-free approach. In detail, the NMRD curve is modelled by a relation of the type:

$$R_1 = R^{HH} + R^{NH} + R_0 \quad (10)$$

where the R^{HH} term keeps into account the homonuclear H-H relaxation as a linear combination of three independent BPP-like components; R^{NH} takes into account the presence of the “*quadrupolar tips*”,²⁴⁵⁻²⁴⁹ i.e. the enhancement of the relaxation rate due to the quadrupolar interaction with the ^{14}N atoms present in the peptide bonds (modeled by means of a complex combination of trigonometric functions); finally, R_0 is an offset term that keeps into account the contribution to relaxation deriving from very fast motions (such as the rotation of small alkyl groups, or hydrogen-bond exchange). This type of approach can be suitably extended to the analysis of dairy products, in order to obtain useful information for the traceability of the products themselves.

The use of *model free* approaches in general has the considerable problem of introducing a certain degree of arbitrariness, since the number of Lorentian or BPP components to be used cannot fixed a priori, but should rather be chosen as a sensible compromise based on fitting reliability. To overcome this drawback, Lo Meo et al. recently proposed a heuristic model-free approach based on a smart development of Kruk's equation.²⁵⁰⁻²⁵¹ The starting assumption is made that a complex system can be considered as the superposition of a continuum of “*simple*” systems, whose relaxation can be described by a BPP function. Thus, the existence of a distribution function $f^*(\tau_c)$ can be considered, in such a way that the homonuclear R^{HH} term in Kruk's equation can be reduced to an integral form, i.e.:

$$R^{HH} = \int_0^\infty \left[\frac{\tau_c}{1 + (\omega\tau_c)^2} + \frac{4\tau_c}{1 + 4(\omega\tau_c)^2} \right] f^*(\tau_c) d\tau_c \quad (11)$$

Therefore, the function $f^*(\tau_c)$, which constitutes the inverse integral transform of the NMRD curve with the BPP function as the kernel, would describe the overall microscopic dynamics of the system. This function can be retrieved by means of a suitable numerical integration procedure such an approach has been successfully applied to diverse types of systems, including wet biochars and soils, dairy products and carbohydrate-based materials. By applying this approach, it has been surprisingly found that the integral transform function $f^*(\tau_c)$ reduces to a set of delta-like peaks. Thus, the NMRD curve is actually transformed into a correlation time spectrum and individuates a limited number of dynamic regimes in

the materials, the physical identification of which can be reasonably attempted. The contribution (C^H) of each component of the correlation time spectrum to the overall relaxometric response (i.e. the weight of the component in the NMRD curve) can be then calculated as:

$$C^H = \lim_{\omega \rightarrow 0} \int_0^{\infty} \left[\frac{\tau_c}{1 + (\omega\tau_c)^2} + \frac{4\tau_c}{1 + 4(\omega\tau_c)^2} \right] f^*(\tau_c) d\tau = 5 \cdot \tau_c \cdot f^*(\tau_c) \quad (12)$$

By comparing the original BPP function with the latter expression, it is immediately apparent that, after suitable rearrangement, it is possible also to calculate the average internuclear interaction distance (r) for each dynamic domain regime. In particular, after having attributed the correct values to the various fundamental constants from equation (6), the following relation can be obtained:

$$r = 12.28 \cdot (\tau_c / C^H)^{1/6} \text{ \AA} \quad (13)$$

where τ_c and C^H must be expressed in μs and ms^{-1} respectively.

A further development of Lo Meo's approach consisted in implementing a MonteCarlo procedure on the calculation of the limit function C^H , in order to account for the experimental uncertainties of the relaxation rates data.²⁵¹ In this way, the NMRD curve is transformed into a real correlation times distribution function (referred to as the inverse integral transform IIT function), which enables to reliably identify the real dynamic regimes of the physical system (i.e. ruling out possible artifacts that can occasionally be present in the original $f^*(\tau_c)$ or C^H spectrum functions) and to estimate the confidence ranges for the relevant correlation time values. A pictorial representation of the data analysis process is illustrated in Figure 3.5.

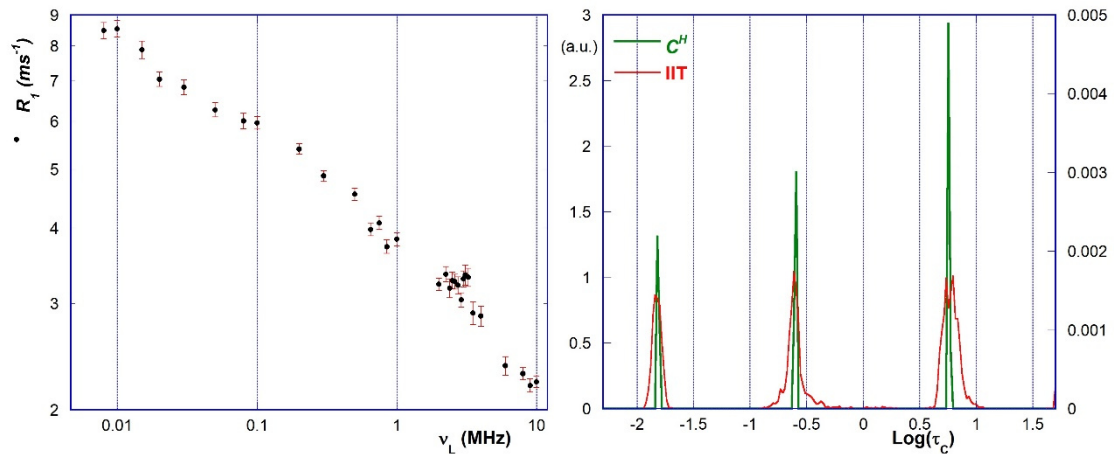


Figure 3.5. Typical NMRD curve (left) and relevant C^H and IIT transforms (right; water saturated material C19, “slow” component)

From an applicative standpoint, the FFC-NMR has proven to be an excellent tool for studying the behavior of water (or any other liquid) on the surface of a porous material, which enables to obtain valuable information on the physical and textural properties of the material itself.^{229,252-255} In fact, the water molecules saturating a porous material partly interact with the surface of the pores and partly flow inside the pore network. Therefore, they experience different types of motion regimes: the ones closest to the surface mainly diffuse horizontally, whilst those inside the channels are freer to undergo rotational and translational movements. The “*surface*” molecules constitute indeed the solvation shell of the material; furthermore, a continuous exchange of molecules occurs between the surface and the pore.

Starting from these assumptions, it follows that the relaxometric behavior of the water molecules bound to the pore surface and of the ones flowing in the channels is different (of course, there is a continuous exchange of molecules between the two populations). Hence, the longitudinal relaxation kinetics data can be subjected to regression analysis by means of a double-exponential model, according to the relationship:

$$M(\tau) = M_{\infty} + M_1 \cdot \exp(-R_f \tau) + M_2 \cdot \exp(-R_s \tau) \quad (14)$$

where $M(\tau)$ is the magnetization of the sample, R_f and R_s are the relaxation rates of the two water molecules populations (in particular, “*surface*” molecules relax faster than “*in channels*” ones, see later). Correspondingly, the Inverse Laplace transform of the relaxation kinetics data (which can be obtained by means of the UPEN algorithm, a typical example is depicted in Figure 3.6 left) features a bimodal distribution.

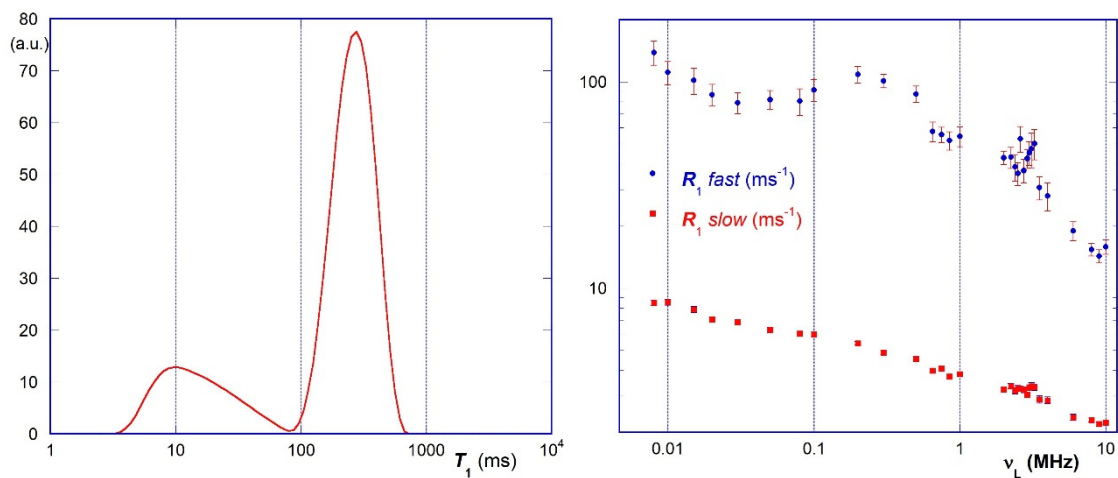


Figure 3.6. Typical Laplace transform (left, 1 MHz) and NMRD curves (right; water saturated material C19)

Therefore, two different NMRD curves can be obtained for the sample (Figure 3.6 right), one relevant to the fast relaxation of the ^1H nuclei present in the surface water molecules (and in the case of nanosponges, also of the ^1H nuclei of the material scaffold), the other one relevant to the slow relaxation of water molecules flowing in the pores.

More in detail, because of population exchange, the relaxation rate of in-pore water molecules can be expressed as weighted average between rates for the molecules close to the surface and the bulk pure solvent, i.e.: $R_s = f_s R_f + f_p R_w$, where f_s and f_p indicate the molar fractions of surface and pore water molecules respectively, R_s indicates the relaxation rate of surface water molecules and R_w that of free water. Because in general $R_s > R_w$ (free water moves faster and relaxes slower than bound water) and because $f_s + f_p = 1$, then the expression for R_f can be rearranged as: $R_f = R_w + f_s \cdot (R_s - R_w)$, from which:

$$f_s = \frac{R_f - R_w}{R_s - R_w} \quad (15)$$

This, in turn, enables to lead back to the texture properties of the material according to the following relationships:

$$\begin{aligned} R_s &= R_w + f_s \cdot (R_f - R_w) \\ f_s &= (R_f - R_w) / (R_s - R_w) = \lambda \cdot (S/V) = \lambda \cdot m/D \\ R_s &= R_w + \lambda \cdot (m/D) \cdot (R_f - R_w) \\ R_s &= R_w + \rho \cdot (S/V) = R_w + \rho \cdot (m/D) \end{aligned} \quad (16)$$

where D is the average pore diameter, S is the specific surface area and V the specific pore volume; λ indicates the thickness of the hydration sphere of the pores and m is a shape parameter whose value depends on the geometry of the pores. The coefficient ρ is referred to as the "*surface relaxivity*" and quantifies the relaxometric behavior of the protons consequent to the interaction with the surface of the porous medium. Based on this approach, in principle it would be possible to obtain the statistical distribution of the pore diameters from the inverse Laplace transform of the relaxation kinetics, once provided that reliable values are available for parameters λ , m and ρ . Attempts in this sense have been made in the case of inorganic materials. Unfortunately, however, this is frequently not the case. For cyclodextrin nanosponges, having a swellable and very disordered microscopic structure, it is hardly possible to unambiguously identify sensible values for parameters λ and m (considering also that water molecules can clusterize within the cyclodextrin cavities). In similar cases, in particular in soil science, it has been found convenient using the

relaxometric results to describe the functional mobility of water in porous systems by defining suitable "*connectivity indexes*".^{215,256-257} In the particular case of nanosponges, a "*Pore Connectivity Index*" (*PCI*) has been proposed as a suitable parameter to define the functional mobility of water within the pore network, according to the relationship:

$$PCI = \frac{\left(\int_{T_{A,fast}}^{T_{B,fast}} P(T_{fast}) T_{fast} dT_{fast} \right)^{-1} - R_W}{\left(\int_{T_{A,slow}}^{T_{B,slow}} P(T_{slow}) T_{slow} dT_{slow} \right)^{-1} - R_W} \quad (17)$$

where the functions $P(T_{fast})$ and $P(T_{slow})$ are the distribution functions normalized for the values of the relaxation times, while the integration limits for the two integrals are chosen in such a way as to take into account 95% of the time distribution of relaxation, leaving out the two outer tails by 2.5% each. Noticeably, equation (17) can be led back to inverse of the expression for the molar fraction (f_s) of surface water molecules provided by equation (15), with the two integrals replacing the values of R_f and R_s . Therefore, larger *PCI* values are expected, in principle, in correspondence of lower f_s values.

3.5 FFC-NMR relaxometric characterization of the materials: Results

The whole of the relaxometric results (namely, the R_1 vs v_L data, from which NMRD curves were built) is collected in Appendix to this chapter. As a general observation, it must be preliminarily noted that acquiring the ^1H NMRD curves of dry materials is by far more difficult than for water-saturated ones. This is likely due to the microscopic structural inhomogeneity of the samples and the relatively scarce abundance of ^1H nuclei in comparison to the overall sample mass, resulting in low magnetization values to detect, and poor quality of the relaxation kinetics trace as well. Moreover, it must be kept in mind that the stiffer the material, the larger R_1 values are expected. However, due to instrumental limitations, estimated relaxation rate values larger than 350 ms^{-1} should be considered poorly reliable. This fact severely limits the Larmor frequency range that can be explored, and consequently the correlation times range that can be sampled. Anyway, all the relaxation kinetic runs for dry materials fit well into a simple mono-exponential trend. Conversely, for water-saturated materials the relaxometric experiments were much more viable to perform.

The relaxation kinetic runs generally resulted as the superimposition of two independent mono-exponential processes, a *fast* and a *slow* one, in perfect agreement with the previous discussion (calixarene-only materials constituted an exception, see later). The former process cumulatively relates to the behavior of the nanosponge backbone H atoms and the water molecule directly interacting with the surface, whereas the latter one relates to the in-pore water molecules. Hence, two different NMRD curves could be built. However, it was observed that the slow kinetic component in nearly all cases accounts for more than 80% of the overall magnetization variation, whereas less than 20% is accounted for by the fast component. As a consequence, the determination of the relevant R_f value is often affected by very large indeterminations, up to get unreliable particularly at the lowest magnetic field values.

As long as dry materials are concerned, owing to the aforementioned reasons, their study was limited to few selected cases. In particular, the cyclodextrin-only materials C1 and C3, the calixarene-only materials K1 and the mixed materials CK1, CK2, CK3 and CK5_c were considered. In general, the curves show quite well the typical tips due to the quadrupolar interaction effect with the ^{14}N atoms of the urethane (and ureidic) groups. This makes the R_{NH} term in equation (12) non-negligible, complicating the heuristic analysis of the NMRD curves. For this reason, and because of the relatively narrow Larmor frequency range explored, it was chosen not to apply in these cases the MonteCarlo approach, limiting the analysis to the C^{H} distribution functions only. This analysis led to reliably identify for each material two short correlation time ($\tau_{\text{c},1}$ and $\tau_{\text{c},2}$) dynamic domains. The existence of at least a third long correlation time domain is undoubtedly apparent too (with $\tau_{\text{c},\text{long}} > 1 \mu\text{s}$); however, it was not possible to get any reliable estimation of the relevant τ_{c} values from the experimental results collected, owing to the sampling problems mentioned above. The latter slow dynamic regime domain might be tentatively attributed to the slow motion of the mass centers of the cyclodextrin or calixarene monomer units; conversely, the faster domains may be related to conformational rearrangements involving the linker units or the mutual reorientation of the glucose (for cyclodextrins) or benzene (for calixarenes) subunits. The results obtained are collected in Table 3.5.

Table 3.5. R_0 and correlation time ($\tau_{c,1}$ and $\tau_{c,2}$) values.

NS	composition	R_0 (ms ⁻¹)	$\tau_{c,1}$ (ns)	$\tau_{c,2}$ (ns)
C1	C/H 1:2	2.90	7	50
C3	C/H 1:4	3.72	8	56
K1	CA/H 1:2	3.07	25	181
CK1	C/K/H 1:1:8	4.33	30	362
CK2	C/K/H 1:1:4	0	23	139
CK3	C/K/H 0.5:1:8	2.57	14	85
CK5_c	C/K/H/A 0.5:1:8:3	0.06	76	228

It is interesting to notice that the two fast domains relate to quite short-ranged interactions (an average r value of 3.4 ± 0.2 Å can be calculated). As long as the two cyclodextrin-only nanosponges C1 and C3 are concerned, shorter $\tau_{c,1}$ and $\tau_{c,2}$ values (i.e. faster motions) are consistently found for the less reticulated material. Conversely, the possible presence of the calixarene component complicates the analysis. Mixed materials CK1, CK2 and CK3 all show much larger τ_c values than the cyclodextrin-only ones; however, apparently there is no straightforward relation between the correlation times and the host/linker combination ratio. The latter observation might be explained as a consequence of the formation of highly structured hydrophobic microdomains (involving the occurrence of a significant microscopic inhomogeneity). This hypothesis is consistent with the results from the titrimetric approach discussed in Section 3.3. Noticeably, the calixarene nanosponge K1 shows τ_c values consistent with those of the mixed nanosponges. Finally, comparison between materials CK3 and CK5_c shows that the presence of the amine chain extender makes the reticulated structure much more rigid, as accounted for by the neat increase of τ_c values, probably due to extensive hydrogen bond formation, caused by the presence of the ureidic groups in the linker chains.

As we mentioned previously, the FFC relaxometric study of water-saturated materials was much easier; therefore, it was possible to analyze a larger set of systems. In particular, materials C1, C3, C9, C10, C11, C18, C19, CK1, CK2, CK3, CK5_c, K1 and K2, obtained with either the “*thick paste*” or the “*solution*” synthetic procedures, were first considered; subsequently, the four highly swellable materials C2_[125], C14, C15 and C16 obtained with the peculiar “*smart dilution*” procedure described in Section 2.5 were investigated. Keeping in mind that for each material two different NMRD curves can be retrieved, it must be stressed that the most interesting and informative ones are those related to the slow component of the relaxation kinetics, i.e. the one related to the behavior of in-pore water molecules. Conversely, the analysis of the NMRD curves for the corresponding fast

component posed significant difficulties (similarly as for dry materials) for the reasons discussed previously. Good quality curves could be obtained only in a limited number of cases; therefore, everything considered, it was chosen to not proceed with their heuristic analysis. Rather, according to literature,²¹⁵ kinetic runs at $\nu_L = 1.0$ MHz were subjected to Inverse Laplace transform analysis, in order to retrieve the *PCI* index values. Noticeably, the presence of water causes the almost complete disappearance of the quadrupolar tips (that can be hardly detected, occasionally, in the NMRD curves of the fast component). This can be easily explained considering that in the presence of water quick proton exchange occur, by a hydrogen bond formation-disruption mechanism. Consequently, the quadrupolar contribution R^{NH} in equation (10) is negligible; thus, the fitting heuristic analysis is largely simplified, and the MonteCarlo approach can be viably exploited to obtain the IIT distribution curves. This enabled a much more reliable individuation of the dynamic regimes. The results obtained are collected in Table 3.6.

Table 3.6. R_0 and correlation times ($\tau_{c,1}$, $\tau_{c,2}$ and $\tau_{c,long}$), <i>PCI</i> and f_s values.							
NS	Composition	R_0 (ms⁻¹)	$\tau_{c,1}$ (ns)	$\tau_{c,2}$ (ns)	$\tau_{c,long}$ (μs)	<i>PCI</i>	f_s
C1	C/H 1:2	0.74	18	103	(3)	87.5	0.033
C3	C/H 1:4	0.74	17	210	(5)	27.8	0.068
C9	C/H/P 1:2:2	2.19	17	76	0.61	9.72	0.090
C10	C/H/P 1:4:2	1.81	5	51	1.48	3.1	0.145
C11	C/H/P 1:4:4	1.81	26	180	1.36	1.5	0.210
C18	C/H/P 1:8:4	3.02	19	340	1.77	18.7	0.065
C19	C/H/A 1:8:4	1.61	15	245	5.75	14.1	0.151
CK1	C/K/H 1:1:8	1.79	31	297	1.56	31.2	0.029
CK2	C/K/H 1:1:4	0.45	6	79	1.03	83.2	0.034
CK3	C/K/H 0.5:1:8	1.15	21	271	1.41	47.5	0.140
CK5_e	C/K/H/A 0.5:1:8:3	2.07	20	166	0.75	4.5	0.220
K1	CA/H 1:2	1.16	21	171	1.25	-	-
K2	CA/H/A 1:8:6	0.96	14	106	1.03	-	-

As a preliminary observation, all materials feature two main dynamic regimes, a first one with $\tau_{c,1}$ values ranging from ca. 5 up to 30 ns, and a second slower one featuring $\tau_{c,2}$ values from ca. 50 up to 340 ns (an additional “*ultrafast*” regime, with $\tau_{c,a} \approx 2$ ns, could be individuated for materials C1 and C3, due to the fact that in these two particular cases, availability of instrumental resources enabled to extend the relaxometric determinations up to 40 MHz). All the materials unambiguously feature also at least one further dynamic regime at longer $\tau_{c,long}$ values, from 0.5 μ s up (an accurate determination of the relevant $\tau_{c,long}$ values was not possible in two cases, an approximate value has been anyway indicated in

brackets). In comparison to dry materials, it is interesting to notice that these regimes account for significantly longer ranged interactions. In fact, average r values as large as $6.0 \pm 1.2 \text{ \AA}$ and $8.4 \pm 1.8 \text{ \AA}$ can be calculated for the first and the second regime, respectively (even larger values can be found for the $\tau_{c,\text{long}}$ regimes). Careful inspection of the data collected in Table 3.6 allows to outline some interesting trends. For instance, on comparing C1 and C3, the most reticulated material shows the longest $\tau_{c,2}$ and $\tau_{c,\text{long}}$ values. Insertion of a polyamine (material C19) or a diol (material C18) chain extender causes a significant increase of $\tau_{c,2}$ and a decrease of $\tau_{c,\text{long}}$. Conversely, significant shortening of $\tau_{c,2}$ and $\tau_{c,\text{long}}$ values is achieved as the pyromellitic units are present as co-linkers (materials C9, C10, C11). This suggests a favorable effect on molecular dynamics from the ionizable carboxyl groups of the co-linker. The apparent exception constituted by material C12 is justified by the much larger presence of the hydrophobic H linker. The effect of the different extent of reticulation is also confirmed by comparing the mixed cyclodextrin-calixarene materials CK1, CK2 and CK3. For the mixed nanosponges, however, insertion of the polyamine chain extender (comparison between CK3 and CK5_c) has a complex effect, because it decreases $\tau_{c,2}$, but complicates the $\tau_{c,\text{long}}$ regime. Therefore, everything considered, in a tentative way the dynamic regimes individuated by $\tau_{c,1}$ and $\tau_{c,2}$ may be identified, respectively, as the rotational and translational motions of the water molecules within the pores. Conversely, the $\tau_{c,\text{long}}$ regime might be identified with the motions relevant to the pore-surface exchange or the apparent motions of the water molecules in the pore consequent to the slow motions of the swelled nanosponge backbone (Figure 3.7).

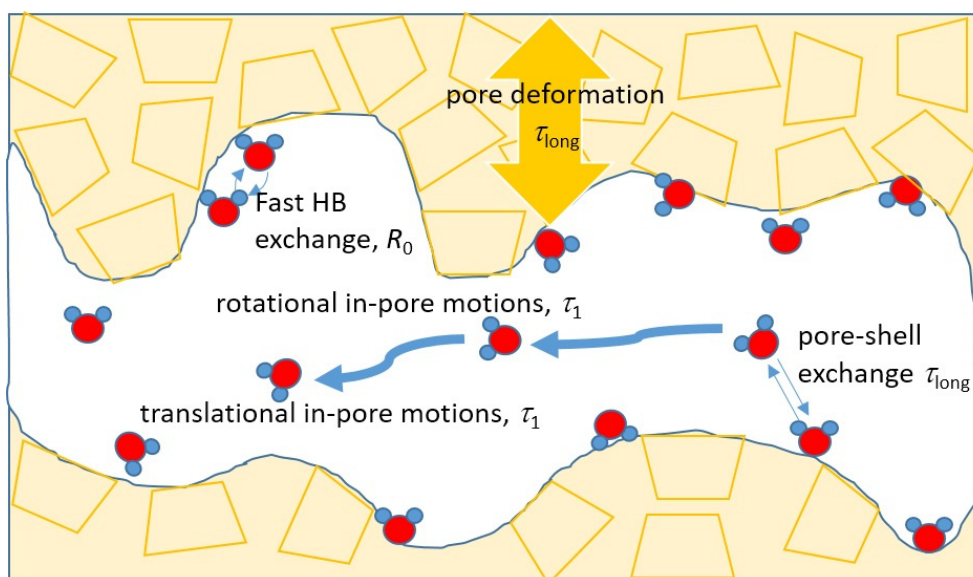


Figure 3.7. Possible attribution of τ_c values.

A particular discussion is needed regarding the behavior of calixarene-only nanosponges. Materials K1 and K2 show a significant difference with respect to all the other materials, because their relaxation kinetics do not follow the expected double-exponential trend described above, but rather fit a stretched mono-exponential trend, according to the equation:

$$M(\tau) = M_{\infty} + M_1 \cdot \exp[(-R_1 \tau)^n] \quad (18)$$

where n is a “*stretch*” parameter, the value of which is generally comprised between 0.75 and 1. From a mathematical standpoint, the occurrence of this particular trend can be justified assuming that the relaxation kinetics is described by the integral forms defined in equations (6) and (7), according to a log-normal distribution function $D(T_1)$ of the relaxation times (see Chapter 6 for a more detailed discussion). This, in turn, implies a Gaussian distribution of the activation energies for the molecular motions. Such a peculiar behavior must be undoubtedly related to the exceptionally high hydrophobic character of these materials. It can be reasonably hypothesized that the water molecules can hardly interact with the pore surface, having the (few) urethane units as the only hydrogen bond donor-acceptor groups; consequently, water undergoes extensive clusterization because of the well-known hydrophobic effect. Hence, differently from hydrophilic cyclodextrin-based materials, lack of effective water-surface interactions involves no neat split of the water molecules in two different populations. Finally, as long as τ_c values are concerned, comparison between K1 and K2 confirms the effect of the polyamine chain extender discussed for material CK5_c.

As previously mentioned, the highly swellable materials C2_[125] and C14-C16 obtained with the “*smart dilution*” method deserved a particular analysis, for suitable comparison purposes. The relaxometric determinations were carried out on samples saturated not only with water, but also with DMF. The relevant results are collected in Table 3.7.

Table 3.7. R_0 and correlation time ($\tau_{c,1}$, $\tau_{c,2}$ and $\tau_{c,long}$), PCI and f_s values for highly swellable materials.										
NS	DMF				Water (slow)				PCI	f_s
	R_0 (ms ⁻¹)	$\tau_{c,1}$ (ns)	$\tau_{c,2}$ (ns)	$\tau_{c,long}$ (μs)	R_0 (ms ⁻¹)	$\tau_{c,1}$ (ns)	$\tau_{c,2}$ (ns)	$\tau_{c,long}$ (μs)		
C2 _[125]	0.96	13	91	1	1.70	19	145	0.83	6.31	0.104
C14	0	4.8	93	8.1	0.73	11	91	0.83	16.5	0.121
C15	0.77	3.9	100	5.2	1.05	7.6	63	0.83	5.6	0.072
C16	0.74	13	93	0.5; 2.7	1.48	11	158	1.3	23.8	0.030

Regarding the DMF saturated systems, also in this case the relaxation kinetics fitted with a stretched mono-exponential model, indicating the occurrence of weak surface-solvent interactions only, consistent with the lower polarity of DMF with respect to water and with its inability to act as a hydrogen bond donor. Also in this case, three main dynamic regimes can be individuated from the IIT distribution curves. The first one, with $\tau_{c,1}$ correlation times value increasing from 3.9 up to 13 ns on decreasing the average linker length (and an average r value of 6.0 ± 1.2 Å), may be reasonably attributed to the in-pore motions of the DMF molecules. The second regime, with an almost constant $\tau_{c,2}$ value of ca. 93 ns (average r value of 8.4 ± 1.8 Å), can be likely related to the pore-surface exchange. A third long-correlation time regime might be again tentatively related to the apparent DMF motions due to the swelled backbone movements.

Water saturated systems constitute an intriguing problem, in comparison with the materials examined previously. Also in this case, the IIT curves for the slow relaxometric component feature three main domains, perfectly comparable with the previous materials. Noticeably, the $\tau_{c,1}$ correlation time values are perfectly comparable with those found previously (though with a slightly smaller average r value of 5.3 ± 0.5 Å). Conversely, $\tau_{c,2}$ values appear relatively smaller, ranging from 63 to 158 ns (average r value of 7.7 ± 0.8 Å), with a trend fairly consistent with the linker length. Curiously, $\tau_{c,long}$ values are identical in 3 cases (0.83 ms, average r of 9.6 ± 1.5 Å) out of 4. The latter finding seems to rule out any effect from the backbone movements; therefore, it might tentatively suggest the movements relevant to the pore-surface exchange.

Finally, a short discussion is deserved to parameter PCI values (equation 17) and surface water molar fraction values f_s (equation 15). It is worth recalling here that the PCI parameter was proposed as a suitable descriptor of the functional mobility of an aqueous medium within the pore network. In principle, as mentioned previously, its value should show an opposite trend with respect to the fraction f_s of surface water molecules. From the values reported on Table 3.6 and 3.7, some peculiar trends can be envisaged. Indeed, PCI values decrease on increasing the extent of reticulation, as clearly indicated by the comparisons between C1 and C3, and between CK1, CK2 and CK3 as well. Noticeably, the presence of the pyromellitic co-linker in materials C9, C10 and C11 causes a strong decrease in PCI values, likely due to the strong solvation of the ionizable carboxyl functional groups. This idea is confirmed by material C19 and CK5_c containing the polyamine chain extender. A decrease in PCI values can be observed also with the diol chain extender (material C18). At this purpose, the highly swellable materials C2_[125] and C15 (data in Table 3.7) provide an

interesting case of study. In fact, they all show *PCI* values even smaller than C3; moreover, along the series C14-C15-C16, *PCI* unexpectedly decreases on increasing the length of the linker chains. This counterintuitive result can be explained considering that on increasing the length of the linker chains, the latter ones will experience more and more effective hydrophobic effects in the presence of the aqueous medium. This causes a sort of collapse of the pore network, which strictly resembles the occurrence of the highly hydrophobic microdomains evidenced by the titrimetric characterizations. Furthermore, from the data reported it can be easily shown that, quite surprisingly, the strict inverse correlation expected between f_s and *PCI* values does not occur. This is partly due to the different way the two parameters are calculated: the former parameter is obtained from two single relaxation rates, whereas the latter one requires the preliminary fitting analysis of the Inverse Laplace transform distributions (in any case, the relaxometric results obtained at $\nu_L = 1$ MHz are conventionally used). Moreover, it should be kept in mind that f_s takes into account all the solvent molecules directly interacting with the nanosponge backbone, irrespective of the particular interaction involved. In other words, it cumulatively considers the water molecules clusterized inside the host cavities, those interacting with the hydroxylated cyclodextrin rims or with the urethane groups via hydrogen bonding (first and, possibly, second solvation shell), and so on. Therefore, f_s can be rather considered a measure of the extent up to which the nanosponge backbone is solvated, and its values are not strictly related to either the porosity or the solvent mobility within the pore network. Anyway, it is very interesting to notice that the largest f_s values occur for the materials bearing ionizable groups (i.e. those containing the triamine A or the pyromellitic P subunits), in agreement their particularly effective tendency to solvation.

3.6 Concluding highlights

The FT-IR and the ^{13}C CP-MAS solid-state NMR characterizations of the materials allowed to verify the actual occurrence of the reticulation process and to confirm the presence of the various constituent moieties. At the same time, some nanosponge materials were subjected to titrimetric analysis in order to establish the amount of cross-linking agent present. In this

case, the reliability of the results strictly depends on the nature of the material. In detail, in the case of the more hydrophilic materials, the quantification of polyamine chains affords results reasonably similar to the theoretical expected. Conversely, for the more hydrophobic materials, the amount of ionizable groups found is largely lower than the theoretical.

The FFC-NMR relaxometric characterization allowed to clarify some aspects of the interaction between the nanosponge structure and a possible solvent medium. This analysis performed on water-saturated materials led to reliably identify two different populations of water molecules, i.e. in-pore and surface solvation molecules. For the in-pore-population heuristic analysis of the NMRD curves allowed to identify the existence of two short correlation time ($\tau_{c,1}$ and $\tau_{c,2}$) dynamic domains, which can be associated with the rotational and translational motions of free water molecules, and a third long correlation time domain ($\tau_{c,long}$), which can be likely attributed to the pore-surface exchange. The functional mobility of the solvent within the pore structure was assessed by studying the trends of the PCI and f_s parameters. All data trends were suitably correlated with the structure of the materials, in terms of constituents and preparation procedures.

3.7 Experimental section

The FT-IR spectra (nujol) were acquired using an Agilent Tech. CARY630 FT-IR spectrometer. Solid-state CP-MAS NMR spectra were acquired using Bruker Avance II 400 MHz instrument, equipped with 15 kHz rotating MAS probe.

The potentiometric titrations are carried out as follows: an accurately weighed amount, generally 40.0 ± 0.05 mg of the solid was placed in a jacketed vessel thermostated at 25 °C, and suspended in 12 mL of double-distilled water. Then, 3.0 mL of a solution of Standard HCl 0.1 M was added into the beaker. The suspension is placed under magnetic stirring and titrated with NaOH 1 M introduced by means of a Chemetron-type microsyringe, recording the pH value following each addition of titrant. The obtained titration curve was subjected to regression analysis using the appropriate equation obtained analytically for titration of a mixture of a certain number of virtual weak acids:

$$\frac{v_i}{V_0} = \frac{\frac{n_{HX}}{V_0} + \frac{K_W}{10^{-pH}} - 10^{-pH} - \sum_{i=1}^a \left(\frac{n_i}{V_0} \cdot \frac{10^{-pH}}{10^{-pH} + K_{A(i)H^+}} \right)}{C_{NaOH} + 10^{-pH} - \frac{K_W}{10^{-pH}}}$$

where n_{HX} are the moles of strong acid, a is the number of virtual acids (which must be established case by case), n_i and $K_{A(i)H^+}$ are the apparent number of equivalents and the apparent dissociation constant of each acid, respectively. All determinations were made in triplicate. For the alkaline hydrolysis, an accurately weighed amount (generally, 25.0 ± 0.05 mg) of material is placed in a small beaker and a solution of Standard NaOH 1 M (2.0 mL) was added and it is made to react for 4 h at 60 °C. Then, the system is cooled to r.t. and it is placed under magnetic stirring adding 5.0 mL of Standard HCl 0.5 M and 8.0 mL of H₂O. Finally, the suspension was titrated with NaOH 1 M introduced by means of a Chemetron-type microsyringe, recording the pH value following each addition of titrant. The obtained titration curve was subjected to regression analysis using the same equation reported above.

FFC-NMR measurements were conducted on a STELAR SmarTracer 0.25 Tesla Bench-top NMR Relaxometer instrument. The materials (about 400 mg) are placed in NMR tubes with an internal diameter of 8.5 mm and placed overnight to equilibrate with 1.5 mL of buffer solution at pH 4.4. Before measurement, any excess supernatant liquid is carefully spitted out and weighed. The sample thus prepared is then subjected to instrumental analysis. The obtained relaxometric data were analyzed using the commercial software packages Excel 2016, Kaleidagraph 4.0 and UpenWin 1.6 β 1, the latter kindly made available by Prof. Villiam Bortolotti (Univ. of Bologna). The relative MonteCarlo analysis is carried out using the software package GUI_ModelFreeFFC (beta version). To obtain the IIT curves, the integration interval was set between 10 ns and 10 μ s, and divided into 100 logarithmic steps, while for the Monte Carlo analysis, 401 simulation runs were performed.

Systems in DMF. The measurements are performed on a portion of the nanosponge sample in gelatinous form, directly from the synthesis with quenching during the *gel point*.

Systems in water. The solid nanosponge (in such amount as to fill the bottom of the NMR tube to the correct extent) is suspended in deionized water and allowed to equilibrate overnight. The excess water is then carefully removed until the surface of the sample is apparently free of stagnant water.

APPENDIX

Relaxometric source data

Dry materials

C3 dry		C1 dry		K1 dry	
ν_L (MHz)	R_1 (ms ⁻¹)	ν_L (MHz)	R_1 (ms ⁻¹)	ν_L (MHz)	R_1 (ms ⁻¹)
35	7.08 ± 0.03	35	6.86 ± 0.04	10.00	11.8 ± 0.8
30	8.16 ± 0.04	30	7.92 ± 0.07	8.00	13.2 ± 1.0
25	9.68 ± 0.05	25	9.5 ± 0.1	6.00	16.2 ± 2.5
20	11.95 ± 0.09	20	11.2 ± 0.1	5.00	18.7 ± 2.0
17	13.41 ± 0.09	17	13.3 ± 0.2	4.00	27.9 ± 1.7
15	15.1 ± 0.2	15	14.2 ± 0.2	3.50	41.5 ± 3.4
14	16.4 ± 0.2	14	15.3 ± 0.2	3.35	57.2 ± 5.6
13	17.1 ± 0.2	13	17.4 ± 0.4	3.25	69.1 ± 5.0
12	18.8 ± 0.3	12	18.8 ± 0.7	3.15	66.0 ± 5.0
11	19.8 ± 0.3	11	21.3 ± 0.9	3.00	56.7 ± 3.9
10	21.1 ± 0.2	10	22.8 ± 0.6	2.90	52.8 ± 4.0
9	24.3 ± 0.5	9	25.6 ± 0.8	2.80	49.7 ± 3.1
8	26.9 ± 0.4	8	28.4 ± 0.7	2.70	50.6 ± 4.1
7	31.2 ± 0.5	7	34.1 ± 0.6	2.60	81.1 ± 3.8
6	34.7 ± 0.6	6	37.0 ± 0.7	2.50	68.5 ± 2.7
5	41.8 ± 0.5	5.0	45.4 ± 0.9	2.40	59.6 ± 3.9
4	56.0 ± 0.5	4.0	57.0 ± 1.0	2.20	45.6 ± 2.7
3.4	75.7 ± 0.5	3.7	61.3 ± 0.9	2.00	51.3 ± 2.9
3.3	93.9 ± 0.9	3.5	66.9 ± 1.2	1.75	62.9 ± 4.2
3.2	103.9 ± 0.9	3.4	79.4 ± 1.4	1.50	81.0 ± 2.8
3.15	109.3 ± 0.8	3.3	97.5 ± 1.0	1.00	83 ± 7
3.1	107.1 ± 0.9	3.2	114 ± 2	0.75	137 ± 11
3.05	101.0 ± 0.7	3.15	124 ± 3	0.50	232 ± 24
3	93.6 ± 0.7	3.1	117 ± 2	0.20	317 ± 32
2.9	88.2 ± 0.8	3	104.6 ± 1.7	0.10	364 ± 48
2.8	94.9 ± 1.0	2.95	95.7 ± 1.6	0.08	410 ± 69
2.7	101.6 ± 0.8	2.9	97.4 ± 1.7	0.05	468 ± 75
2.6	101.1 ± 0.8	2.8	97.2 ± 1.2		
2.55	103.8 ± 0.8	2.7	108 ± 2		
2.5	104.8 ± 0.8	2.6	115 ± 2		
2.45	103.4 ± 0.8	2.55	116 ± 2		
2.4	100.2 ± 0.9	2.5	113 ± 2		
2.35	96.6 ± 0.8	2.45	116 ± 2		
2.3	91.7 ± 0.6	2.35	106 ± 2		
2.2	90.3 ± 0.7	2.3	103 ± 2		
2.1	91.5 ± 0.6	2.2	97.9 ± 1.5		
2	92.0 ± 0.9	2.1	100.2 ± 1.2		
1.7	102.4 ± 0.8	2	102.3 ± 1.7		
1.5	113.9 ± 0.9	1.8	110 ± 2		
1.3	125.7 ± 0.9	1.7	115 ± 2		
1	150.4 ± 1.5	1.5	124 ± 3		
0.7	208.2 ± 1.6	1.3	147 ± 2		
0.3	348 ± 4	1	165 ± 3		
0.1	672 ± 10	0.7	236 ± 4		
		0.3	381 ± 10		
		0.1	689 ± 36		

CK1 dry		CK2 dry		CK3 dry	
v_L (MHz)	R_1 (ms ⁻¹)	v_L (MHz)	R_1 (ms ⁻¹)	v_L (MHz)	R_1 (ms ⁻¹)
10.00	13.9 ± 1.2	10.00	12.4 ± 1.5	10.0	10.6 ± 1.5
9.00	14.8 ± 0.7	8.00	13.6 ± 1.1	8.0	14.6 ± 2.3
8.00	17.7 ± 1.3	6.00	16.1 ± 1.4	6.0	19.0 ± 1.8
7.00	19.4 ± 1.4	5.00	18.9 ± 2.2	5.0	24.2 ± 4.0
6.00	22.7 ± 1.9	4.00	24.3 ± 2.6	4.0	37.8 ± 3.9
5.50	21.7 ± 2.3	3.50	35.4 ± 4.2	3.5	55 ± 10
5.00	27.5 ± 3.0	3.35	59.4 ± 5.0	3.5	64 ± 5
4.00	32.9 ± 4.0	3.25	74 ± 7	3.3	106 ± 8
3.50	52.5 ± 5.0	3.15	84 ± 8	3.3	120 ± 17
3.25	106 ± 8	3.10	80 ± 6	3.1	94 ± 11
3.00	92 ± 7	3.00	86 ± 11	3.0	74 ± 9
2.90	101 ± 8	2.90	97 ± 9	2.9	52 ± 6
2.80	96 ± 9	2.80	89 ± 6	2.9	91 ± 12
2.70	101 ± 8	2.70	71 ± 6	2.8	107 ± 18
2.50	95 ± 8	2.60	84 ± 5	2.7	113 ± 16
2.40	110 ± 13	2.50	75 ± 6	2.7	115 ± 17
2.30	90 ± 7	2.40	65 ± 4	2.6	85 ± 15
2.25	105 ± 7	2.20	79 ± 4	2.6	65 ± 10
2.15	96 ± 7	2.00	62 ± 5	2.5	70 ± 10
2.00	84 ± 12	1.75	73 ± 5	2.3	56 ± 12
1.75	102 ± 6	1.50	73 ± 6	2.0	86 ± 13
1.50	104 ± 9	1.25	111 ± 6	2.0	70 ± 9
1.00	148 ± 10	1.00	108 ± 7	1.8	94 ± 10
0.75	232 ± 22	0.75	167 ± 16	1.5	101 ± 17
0.50	291 ± 30	0.50	332 ± 23	1.3	132 ± 10
0.25	399 ± 66	0.30	515 ± 124	1.0	161 ± 12
0.10	782 ± 200	0.20	602 ± 151	0.8	191 ± 28
				0.5	275 ± 46
				0.3	347 ± 74
				0.2	320 ± 63
				0.1	443 ± 278

CK5 dry			
v_L (MHz)	R_1 (ms ⁻¹)	v_L (MHz)	R_1 (ms ⁻¹)
10.00	12.4 ± 1.5	2.60	84 ± 5
8.00	13.6 ± 1.1	2.50	75 ± 6
6.00	16.1 ± 1.4	2.40	65 ± 4
5.00	18.9 ± 2.2	2.20	79 ± 4
4.00	24.3 ± 2.6	2.00	62 ± 5
3.50	35.4 ± 4.2	1.75	73 ± 5
3.35	59.4 ± 5.0	1.50	73 ± 6
3.25	74 ± 7	1.25	111 ± 6
3.15	84 ± 8	1.00	108 ± 7
3.10	80 ± 6	0.75	167 ± 16
3.00	86 ± 11	0.50	332 ± 23
2.90	97 ± 9	0.30	515 ± 124
2.70	71 ± 6	0.20	602 ± 151

Wet materials (slow component)

C3			C1			C9		
ν_L (MHz)	R_1 (ms ⁻¹)		ν_L (MHz)	R_1 (ms ⁻¹)		ν_L (MHz)	R_1 (ms ⁻¹)	
35	1.38	± 0.01	35	0.94	± 0.04	10	2.65	± 0.13
30	1.44	± 0.01	30	0.97	± 0.03	8	2.62	± 0.3
25	1.53	± 0.01	25	1.01	± 0.03	6	3.46	± 0.22
20	1.62	± 0.01	20	1.04	± 0.01	5	3.06	± 0.23
15	1.74	± 0.02	17	1.08	± 0.01	4	3.7	± 0.4
10	1.93	± 0.02	15	1.09	± 0.01	3	4.4	± 0.4
9	1.98	± 0.03	14	1.11	± 0.01	2.5	4.5	± 0.4
8	2.05	± 0.03	13	1.13	± 0.01	2	6.5	± 0.3
7	2.07	± 0.03	12	1.14	± 0.01	1.5	5.9	± 0.5
6	2.18	± 0.04	11	1.16	± 0.01	1	6.8	± 0.4
5	2.26	± 0.03	10	1.18	± 0.01	0.75	10.5	± 0.7
4	2.38	± 0.03	9	1.19	± 0.01	0.5	11.2	± 1
3.5	2.48	± 0.04	8	1.22	± 0.01	0.3	11.7	± 0.5
3.4	2.51	± 0.04	7	1.23	± 0.02	0.2	12.9	± 1.9
3.3	2.55	± 0.03	6	1.27	± 0.02	0.1	16.8	± 1.1
3.2	2.56	± 0.04	5	1.32	± 0.02	0.05	24.1	± 2.1
3.15	2.61	± 0.03	4	1.35	± 0.06	0.03	25.6	± 2.2
3.1	2.63	± 0.04	3.7	1.37	± 0.02	0.02	33	± 3
3.05	2.65	± 0.04	3.5	1.33	± 0.02	0.01	32.7	± 4.1
3	2.60	± 0.04	3.4	1.39	± 0.02	C10		
2.9	2.64	± 0.04	3.3	1.41	± 0.02			
2.8	2.62	± 0.04	3.2	1.43	± 0.02	ν_L (MHz)	R_1 (ms ⁻¹)	
2.7	2.66	± 0.04	3.15	1.43	± 0.02	10	3.77	± 0.07
2.6	2.68	± 0.04	3.1	1.43	± 0.02	8	4.3	± 0.3
2.5	2.67	± 0.04	3	1.45	± 0.02	6	4.4	± 0.3
2.4	2.70	± 0.04	2.95	1.43	± 0.02	4	5.6	± 0.4
2.3	2.70	± 0.04	2.9	1.43	± 0.02	3	5.6	± 0.4
2.2	2.73	0.04	2.8	1.43	± 0.02	2	6.9	± 0.3
2.1	2.75	0.05	2.7	1.43	± 0.02	1.5	8.5	± 0.3
2	2.70	0.04	2.6	1.45	± 0.02	1	9.3	± 0.25
1	3.04	0.05	2.55	1.45	± 0.02	0.75	10	± 0.3
0.7	3.17	0.05	2.5	1.45	± 0.02	0.5	11.5	± 0.3
0.5	3.43	0.20	2.45	1.45	± 0.02	0.3	13.2	± 0.4
0.3	3.65	0.20	2.4	1.45	± 0.02	0.1	18.3	± 0.6
0.1	4.33	0.09	2.35	1.45	± 0.02	0.05	25.7	± 2
			2.3	1.47	± 0.02	0.01	34.4	± 1.2
			2.1	1.49	± 0.02			
			2	1.49	± 0.02			
			1.84	1.47	± 0.02			
			1.7	1.47	± 0.02			
			1.5	1.52	± 0.02			
			1.3	1.54	± 0.02			
			1	1.56	± 0.02			
			0.7	1.59	± 0.03			
			0.3	1.64	± 0.03			

C11			C19			C18		
v_L (MHz)	R_1 (ms ⁻¹)		v_L (MHz)	R_1 (ms ⁻¹)		v_L (MHz)	R_1 (ms ⁻¹)	
10	2.49	± 0.07	10	2.71	± 0.15	0.008	8.48	± 0.26
8	2.65	± 0.04	9	2.64	± 0.14	0.010	8.54	± 0.27
6	2.85	± 0.07	8	2.84	± 0.20	0.015	7.88	± 0.26
4	3.48	± 0.08	7	2.92	± 0.15	0.020	7.05	± 0.20
3	3.88	± 0.1	6	3.15	± 0.27	0.03	6.84	± 0.19
2	4.24	± 0.05	4	3.47	± 0.34	0.05	6.27	± 0.17
1.5	4.78	± 0.11	3.5	3.81	± 0.50	0.08	6.01	± 0.18
1	5.19	± 0.16	3.25	3.79	± 0.09	0.10	5.97	± 0.15
0.5	6.6	± 0.18	3.1	4.01	± 0.19	0.20	5.40	± 0.11
0.3	7.9	± 0.2	3	3.96	± 0.14	0.30	4.87	± 0.10
0.1	10.1	± 0.4	2.5	4.08	± 0.15	0.50	4.55	± 0.11
0.075	12.1	± 0.4	2	4.30	± 0.14	0.65	3.98	± 0.10
0.05	13.4	± 0.3	1.5	4.68	± 0.18	0.75	4.08	± 0.10
0.01	16.7	± 1.0	1.25	5.01	± 0.12	0.85	3.72	± 0.10
			1	5.11	± 0.18	1.00	3.83	± 0.10
			0.75	5.66	± 0.18	2.00	3.23	± 0.08
			0.5	5.93	± 0.15	2.25	3.35	± 0.10
			0.3	7.18	± 0.19	2.40	3.18	± 0.11
			0.2	7.5	± 0.3	2.50	3.27	± 0.10
			0.1	8.9	± 0.4	2.60	3.26	± 0.09
			0.05	8.9	± 0.5	2.75	3.22	± 0.10
			0.02	9.7	± 0.7	2.90	3.04	± 0.08
			0.01	10.5	± 0.7	3.00	3.29	± 0.10
						3.10	3.34	± 0.13
						3.25	3.31	± 0.12
						3.50	2.89	± 0.12
						4.00	2.86	± 0.11
						6.00	2.37	± 0.08
						8.00	2.29	± 0.05
						9.00	2.19	± 0.06
						10.00	2.23	± 0.05
CK1			CK2					
v_L (MHz)	R_1 (ms ⁻¹)		v_L (MHz)	R_1 (ms ⁻¹)				
0.008	5.77	± 0.31	10.00	0.81	± 0.01			
0.010	5.60	± 0.20	8.00	0.85	± 0.02			
0.020	5.69	± 0.17	6.00	0.88	± 0.03			
0.03	5.71	± 0.49	5.00	0.93	± 0.04			
0.05	4.82	± 0.23	4.00	0.99	± 0.07			
0.08	4.62	± 0.13	3.25	1.03	± 0.04			
0.10	4.46	± 0.13	3.00	1.03	± 0.03			
0.20	4.11	± 0.10	2.75	1.00	± 0.04			
0.30	3.35	± 0.07	2.50	0.96	± 0.02			
0.50	3.04	± 0.06	2.25	0.96	± 0.06			
0.75	2.90	± 0.06	2.00	1.04	± 0.03			
1.00	2.87	± 0.07	1.50	0.97	± 0.03			
1.50	2.53	± 0.05	1.00	1.20	± 0.03			
1.25	2.62	± 0.04	0.75	1.26	± 0.04			
1.75	2.42	± 0.05	0.50	1.20	± 0.11			
2.00	2.44	± 0.04	0.30	1.37	± 0.06			
2.50	2.35	± 0.07	0.20	1.36	± 0.11			
2.75	2.30	± 0.06	0.10	1.68	± 0.10			
3.00	2.25	± 0.05	0.08	1.81	± 0.09			
3.25	2.18	± 0.06	0.05	1.93	± 0.16			
3.50	2.29	± 0.06	0.03	2.16	± 0.09			
4.00	2.10	± 0.08	0.02	2.33	± 0.16			
5.00	2.00	± 0.04	0.01	2.33	± 0.17			
6.00	1.95	± 0.04	0.008	2.42	± 0.09			
8.00	1.93	± 0.02						
10.00	1.88	± 0.03						

CK3			CK5			K1		
v_L (MHz)	R_1 (ms ⁻¹)		v_L (MHz)	R_1 (ms ⁻¹)		v_L (MHz)	R_1 (ms ⁻¹)	
0.01	4.56	± 0.41	10.00	2.53	± 0.03	10.00	1.51	± 0.01
0.01	4.62	± 0.22	8.00	2.72	± 0.03	8.00	1.68	± 0.01
0.02	4.48	± 0.28	6.00	2.99	± 0.04	6.00	1.88	± 0.02
0.03	4.19	± 0.37	4.00	3.15	± 0.04	5.00	2.01	± 0.02
0.05	4.00	± 0.19	3.50	3.65	± 0.07	4.00	2.13	± 0.04
0.08	3.51	± 0.17	3.00	3.87	± 0.07	3.00	2.65	± 0.08
0.10	3.46	± 0.18	2.75	3.91	± 0.09	2.75	2.72	± 0.06
0.20	2.87	± 0.07	2.50	4.02	± 0.08	2.50	2.84	± 0.06
0.30	2.66	± 0.04	2.25	4.11	± 0.10	2.25	2.84	± 0.05
0.30	2.69	± 0.04	2.00	4.05	± 0.07	2.00	2.94	± 0.04
0.50	2.11	± 0.09	1.50	4.46	± 0.08	1.50	3.19	± 0.05
0.75	2.10	± 0.06	1.00	4.78	± 0.07	1.00	3.70	± 0.03
1.00	2.01	± 0.05	0.75	5.04	± 0.08	0.75	4.11	± 0.06
1.50	1.84	± 0.06	0.50	5.74	± 0.08	0.50	4.85	± 0.07
2.00	1.68	± 0.05	0.30	6.49	± 0.10	0.30	5.82	± 0.10
2.50	1.74	± 0.05	0.20	7.00	± 0.08	0.20	6.74	± 0.12
2.75	1.66	± 0.06	0.10	8.63	± 0.14	0.10	8.71	± 0.24
2.25	1.70	± 0.05	0.08	9.42	± 0.11	0.08	9.4	± 0.3
3.00	1.62	± 0.05	0.05	9.99	± 0.11	0.05	10.6	± 0.3
4.00	1.51	± 0.04	0.03	11.32	± 0.13	0.03	11.6	± 0.5
5.00	1.44	± 0.04	0.02	11.57	± 0.11	0.02	12.9	± 0.3
6.00	1.36	± 0.06	0.01	12.60	± 0.16	0.01	12.7	± 0.3
8.00	1.34	± 0.03				0.008	12.7	± 0.5
10.00	1.25	± 0.03						

K2		
v_L (MHz)	R_1 (ms ⁻¹)	
0.008	4.08	± 0.12
0.01	4.06	± 0.05
0.02	3.87	± 0.09
0.03	3.65	± 0.12
0.05	3.54	± 0.08
0.08	3.09	± 0.08
0.10	2.74	± 0.07
0.20	2.26	± 0.05
0.40	1.94	± 0.03
0.70	1.73	± 0.05
1.00	1.58	± 0.04
1.50	1.47	± 0.04
2.00	1.46	± 0.05
2.25	1.34	± 0.04
2.50	1.43	± 0.06
2.75	1.35	± 0.04
3.00	1.28	± 0.05
4.00	1.31	± 0.07
6.00	1.16	± 0.05
8.00	1.12	± 0.05
10.00	1.09	± 0.04

Highly swellable materials, DMF

C15		C16		C2 _[125]	
ν_L (MHz)	R_1 (ms ⁻¹)	ν_L (MHz)	R_1 (ms ⁻¹)	ν_L (MHz)	R_1 (ms ⁻¹)
10.0	0.88 ± 0.06	10.0	0.88 ± 0.02	10.0	1.10 ± 0.02
8.0	0.92 ± 0.02	9.0	0.94 ± 0.01	8.0	1.14 ± 0.02
6.0	0.97 ± 0.03	8.0	0.94 ± 0.02	6.0	1.21 ± 0.01
4.0	1.02 ± 0.03	6.0	0.99 ± 0.03	5.0	1.24 ± 0.01
3.0	1.05 ± 0.03	5.0	1.06 ± 0.02	4.0	1.27 ± 0.02
2.0	1.07 ± 0.01	4.0	1.12 ± 0.03	3.0	1.35 ± 0.01
1.5	1.13 ± 0.02	3.0	1.18 ± 0.04	2.0	1.43 ± 0.02
1.0	1.17 ± 0.02	2.0	1.29 ± 0.01	1.5	1.48 ± 0.01
0.75	1.14 ± 0.02	1.5	1.36 ± 0.03	1.0	1.59 ± 0.01
0.625	1.13 ± 0.01	1.25	1.41 ± 0.02	0.75	1.69 ± 0.02
0.50	1.20 ± 0.02	1.0	1.53 ± 0.02	0.50	1.77 ± 0.01
0.30	1.18 ± 0.02	0.80	1.61 ± 0.03	0.40	1.83 ± 0.03
0.20	1.22 ± 0.02	0.60	1.72 ± 0.02	0.30	1.89 ± 0.02
0.15	1.22 ± 0.01	0.40	1.95 ± 0.02	0.20	1.96 ± 0.02
0.10	1.20 ± 0.02	0.30	2.11 ± 0.02	0.15	2.04 ± 0.02
0.075	1.20 ± 0.02	0.20	2.35 ± 0.02	0.10	2.14 ± 0.01
0.050	1.25 ± 0.01	0.15	2.61 ± 0.02	0.075	2.18 ± 0.02
0.030	1.24 ± 0.02	0.10	3.07 ± 0.03	0.0625	2.24 ± 0.03
0.020	1.28 ± 0.02	0.080	3.35 ± 0.03	0.050	2.29 ± 0.02
0.015	1.28 ± 0.01	0.060	3.65 ± 0.04	0.040	2.33 ± 0.03
0.010	1.30 ± 0.02	0.050	3.90 ± 0.03	0.030	2.36 ± 0.04
		0.040	4.43 ± 0.07	0.020	2.42 ± 0.02
		0.030	4.88 ± 0.04	0.015	2.43 ± 0.01
		0.020	5.76 ± 0.10	0.0125	2.46 ± 0.02
		0.015	6.07 ± 0.03	0.010	2.47 ± 0.02
		0.0125	6.29 ± 0.10		
		0.010	6.45 ± 0.10		
C14					
ν_L (MHz)	R_1 (ms ⁻¹)				
10.0	0.88 ± 0.01				
8.0	0.97 ± 0.01				
6.0	1.04 ± 0.01				
4.0	1.09 ± 0.03				
3.0	1.12 ± 0.03				
2.0	1.17 ± 0.01				
1.5	1.18 ± 0.02				
1.0	1.24 ± 0.02				
0.75	1.27 ± 0.01				
0.50	1.34 ± 0.01				
0.30	1.35 ± 0.01				
0.20	1.37 ± 0.01				
0.15	1.41 ± 0.01				
0.10	1.41 ± 0.02				
0.075	1.44 ± 0.01				
0.050	1.43 ± 0.01				
0.030	1.45 ± 0.01				
0.020	1.49 ± 0.02				
0.015	1.49 ± 0.01				
0.010	1.53 ± 0.01				

Highly swellable materials, water (slow component)

C15		C16		C2 _[125]	
ν_L (MHz)	R_1 (ms ⁻¹)	ν_L (MHz)	R_1 (ms ⁻¹)	ν_L (MHz)	R_1 (ms ⁻¹)
10.0	1.73 ± 0.04	10.0	1.91 ± 0.03	10.0	2.6 ± 0.2
9.0	1.75 ± 0.04	9.0	1.96 ± 0.05	9.0	2.7 ± 0.2
8.0	1.83 ± 0.04	8.0	2.00 ± 0.07	8.0	3.2 ± 0.2
6.0	1.92 ± 0.05	6.0	2.21 ± 0.07	6.0	3.1 ± 0.2
5.0	1.98 ± 0.07	5.0	2.23 ± 0.07	5.0	3.5 ± 0.2
4.0	2.05 ± 0.09	4.0	2.27 ± 0.10	4.0	4.0 ± 0.3
3.0	2.21 ± 0.09	3.5	2.48 ± 0.13	3.25	5.1 ± 0.4
2.0	2.26 ± 0.10	3.0	2.48 ± 0.08	3.0	5.2 ± 0.4
1.5	2.41 ± 0.08	2.5	2.56 ± 0.14	2.75	5.1 ± 0.3
1.0	2.63 ± 0.06	2.0	2.68 ± 0.11	2.5	5.2 ± 0.3
0.80	2.76 ± 0.08	1.5	2.68 ± 0.12	2.25	5.3 ± 0.3
0.60	2.93 ± 0.10	1.0	3.03 ± 0.13	2.0	5.4 ± 0.3
0.40	2.98 ± 0.10	0.80	3.23 ± 0.09	1.5	5.7 ± 0.3
0.30	3.03 ± 0.10	0.60	3.34 ± 0.07	1.0	6.4 ± 0.3
0.20	3.32 ± 0.12	0.50	3.54 ± 0.07	0.80	6.7 ± 0.3
0.15	3.59 ± 0.08	0.40	3.80 ± 0.11	0.60	7.0 ± 0.5
0.10	3.74 ± 0.15	0.30	4.01 ± 0.09	0.40	8.0 ± 0.4
0.080	3.90 ± 0.14	0.20	4.38 ± 0.11	0.30	8.3 ± 0.5
0.060	4.27 ± 0.14	0.15	4.87 ± 0.16	0.20	10.0 ± 0.4
0.040	4.46 ± 0.14	0.10	5.14 ± 0.11	0.15	10.0 ± 0.5
0.030	4.38 ± 0.16	0.080	5.58 ± 0.20	0.10	12.0 ± 1.0
0.020	4.54 ± 0.12	0.060	5.91 ± 0.11	0.080	13.7 ± 0.7
0.015	4.55 ± 0.16	0.040	6.34 ± 0.11	0.060	15.0 ± 0.7
0.010	4.57 ± 0.18	0.030	6.46 ± 0.26	0.040	16.1 ± 1.0
		0.020	6.87 ± 0.12	0.030	16.1 ± 1.3
		0.015	7.01 ± 0.16	0.020	17.0 ± 1.2
		0.010	6.90 ± 0.19	0.015	17.0 ± 0.8
		10.0	1.91 ± 0.03	0.010	17.3 ± 1.0
		9.0	1.96 ± 0.05	10.0	2.6 ± 0.2
		8.0	2.00 ± 0.07		
		6.0	2.21 ± 0.07		
		5.0	2.23 ± 0.07		
		4.0	2.27 ± 0.10		
		3.5	2.48 ± 0.13		
		3.0	2.48 ± 0.08		
		2.5	2.56 ± 0.14		
		2.0	2.68 ± 0.11		
		1.5	2.68 ± 0.12		

C14					
ν_L (MHz)	R_1 (ms ⁻¹)	ν_L (MHz)	R_1 (ms ⁻¹)	ν_L (MHz)	R_1 (ms ⁻¹)
10.0	2.40 ± 0.25	2.0	4.13 ± 0.20	0.080	13.58 ± 0.69
9.0	2.62 ± 0.14	1.5	4.72 ± 0.27	0.060	13.43 ± 0.73
8.0	2.79 ± 0.31	1.0	5.21 ± 0.50	0.050	13.94 ± 0.82
6.0	3.01 ± 0.27	0.80	5.54 ± 0.50	0.040	15.13 ± 0.86
5.0	3.07 ± 0.26	0.60	6.88 ± 0.40	0.030	15.34 ± 1.10
4.0	3.49 ± 0.26	0.40	7.81 ± 0.40	0.020	15.84 ± 1.20
3.5	3.72 ± 0.47	0.30	8.40 ± 0.53	0.0175	15.27 ± 0.65
3.0	3.65 ± 0.44	0.20	8.50 ± 0.37	0.015	15.88 ± 1.13
2.5	3.88 ± 0.34	0.15	9.72 ± 0.54	0.0125	15.59 ± 0.91
		0.10	11.60 ± 1.10	0.010	16.04 ± 1.30

4 – Adsorption abilities of Nanosponges: preliminary assessment study

4.1 Introduction

As we have already mentioned in the previous Chapters, cyclodextrins have been widely used for the design and the production of nanoporous sorbent materials, due to their ability to adsorb organic molecules. Cyclodextrin-based nanosponges have been more frequently obtained by polymerization with *linkers* having bis-electrophilic properties, such as carbonic acid derivatives, epichlorohydrin, carbonyl-diimidazole, bis-isocyanates.²⁵⁸ In these cases, however, no functionalization able to respond to environmental changes is introduced. Therefore, no large variation of the adsorption properties for the materials might be reasonably expected on changing the environmental conditions, but for the minor effects onto the intrinsic binding properties of the cyclodextrin units (as reported in literature for the free cyclodextrins in solution²⁵⁹). Besides, responsivity to pH or, in general, any other environmental condition is expected whenever responsive functional groups are introduced by either a suitable choice of the linker or by post-modification of a preformed structure. The former case occurs for instance when bis-anhydrides are used, or electrophilic per-(6-halo)-(6-deoxy)-cyclodextrin are reacted with nucleophiles such as polyamines.¹⁸³ Another very smart example is constituted by the glutathione-responsive materials recently obtained by Trotta's group.¹⁹⁶ Conversely, post-modification has been performed on mixed CyCaNSs materials obtained by co-polymerization via *CuAAC* cycloaddition reaction between per-(6-azido)-(6-deoxy)-cyclodextrins and propargyloxy-calixarenes.⁸¹⁻⁸² Responsivity to pH conditions involves, indeed, the tunability of the properties of the material.

It is also worth recalling here that the interaction between a nanosponge material and a possible adsorbed guest is a somehow more complex processes than the simple host-guest interaction in solution. Indeed, several elements must be kept into account, namely: *i*) the reduced conformational flexibility of the host units due to the reticulation process, which

involves that the well-known induced-fit effect²⁶⁰ (i.e. the conformational adaptation of the host upon the guest structure for optimizing the energetics of the inclusion complex) cannot work properly; *ii*) the presence of microdomains of different hydrophobic character⁸⁴ within the structure (evidenced in the titrimetric experiments, see Section 3.3); *iii*) the possible existence within the microscopic structure of such tiny nanochannels to have a diameter comparable to the size of the host units cavities, able to work as additional binding sites. The latter possibility, (the occurrence of which can be assessed in principle by solid-state FSLG 2D-NMR techniques¹⁸³) involves that the potential maximum adsorption capacity of the material ($Q_{\max}$, in terms of the Langmuir or Sips isotherm adsorption models) cannot be predicted a priori simply on the grounds of the amount of host units per gram of material. Finally, it has been shown that the adsorption in nanosponges is mainly affected by electrostatic and (de)solvation effects.¹⁰⁵

A main goal of the present PhD work was the assessment of the pH-tunable abilities of the nanosponges whose synthesis and characterization has been described in the previous Chapters. Indeed, the panel of materials available made possible to consider the outcome on the adsorption performances deriving from different factors. The most evident ones are, of course: *i*) the nature and the amount of ionizable groups in the (co)linker units; *ii*) the different amount of the host units. It is worth recalling here that the deeply diverse nature of the two constituent hosts, cyclodextrin and calixarene, involves the occurrence of very different microscopic interactions with a possible guest inside the cavity, providing the two hosts with somehow complementary binding abilities. Moreover, their very different hydrophilic/hydrophobic character is obviously mirrored in the overall ability of the material to interact with an aqueous medium, as accounted for by the relaxometric characterization (see Section 3.5). This leads to consider a third factor, namely the overall microscopic structuration of the hyper-reticulated structure. As outlined in the previous Chapters, it is possible to modify up to certain extent the physical characteristic of the material by a smart choice of the experimental conditions for the synthesis. The case of the highly swellable materials obtained by the “*smart dilution*” method is particularly significant. A further case of study is provided by the material CK5, prepared in three different ways. It is important to stress that a detailed understanding of the possible pH dependent, or anyhow tunable abilities of the nanosponge materials in adsorbing organic species from solution constitutes an essential piece of information, in order to aid the rationale set-up of tailored or task specific sorbent systems. It is immediately evident that this, in turn, could be absolutely helpful in several contexts, such as the design of environment remediation devices.

Therefore, also on the grounds of previous works,^{81-82,84,88-89,183} the abilities of the nanosponges prepared in this PhD work to sequestrate, from buffered aqueous solutions at different pH values, some suitably selected pollutant model organic molecules (Figure 4.1) were systematically studied. In particular, four derivatives of *p*-nitroaniline (**1-4**) were chosen, as they have been excellent model molecules to study the inclusion equilibria in solution with the β CDs.¹⁰⁵⁻¹⁰⁶ Besides, some common organic dyes, namely Methyl orange (**5**), Toluidine blue (**6**), Naphthol blue-black (**7**), Malachite Green (**8**), Bromothymol blue (**9**) and Rhodamine B (**10**) were considered.

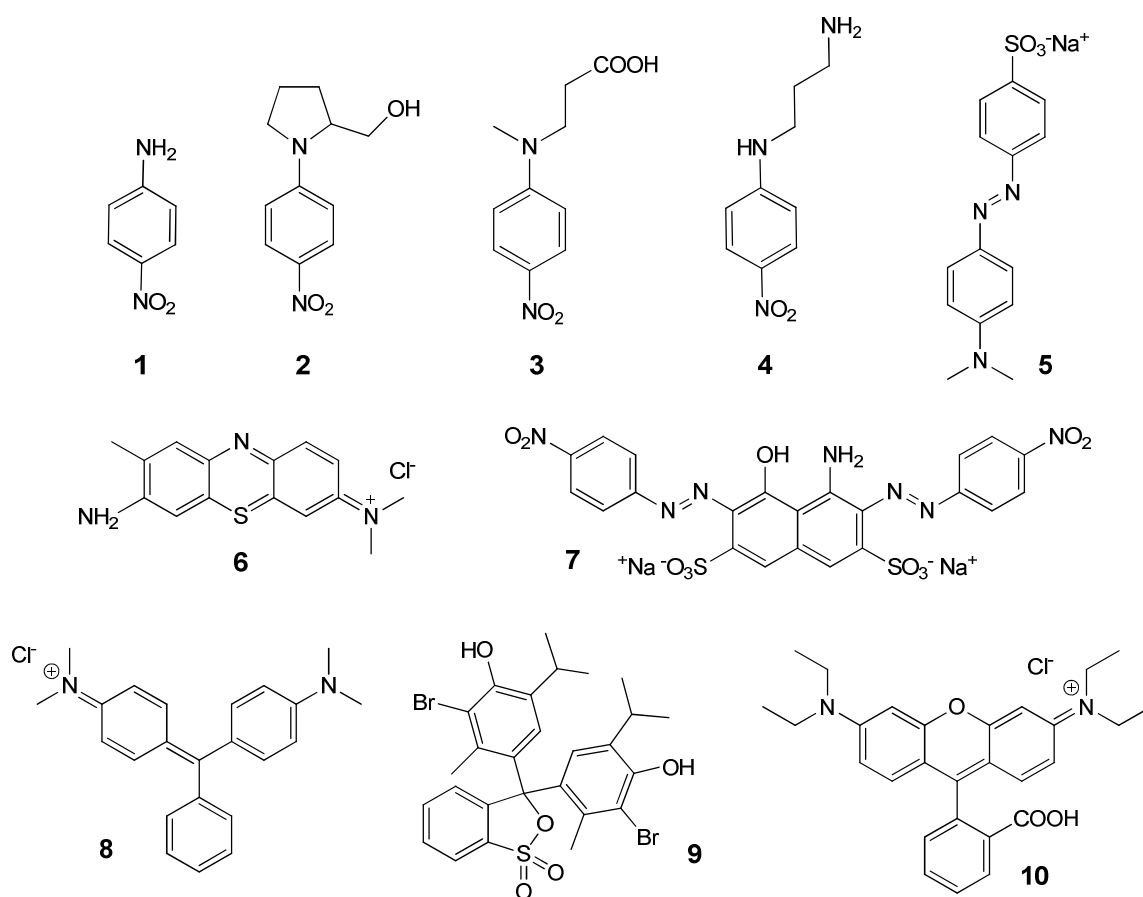


Figure 4.1. Structures of the guests **1-10**.

So much diverse molecular structures allow to test the response of the adsorbent materials to present large variations in the molecular properties of the guests, in particular, size, hydrophobic or hydrophilic character, possible electric charge due to the presence of some ionizable functional group. Tests were mainly carried out under nearly neutral (pH 6.7) or slightly acidic (pH 4.4) conditions, but some additional data were collected also in much stronger acidic (pH 1.0) or alkaline (pH 10.7) solution. The choice of the pH values was basically related the possibility to change the protonation status of the amine or carboxyl groups present on either the linker units of the nanosponge or the structure of the guest. In

particular, **3** ($pK_A = 3.2$) and **9** ($pK_A = 6.3$) pass from neutral to anionic; **4** ($pK_{BH^+} = 10.2$) from cationic to neutral, **5** ($pK_A = 3.5$) from zwitterionic to anionic, and **10** ($pK_A = 4.2$) from cationic to zwitterionic.

4.2 Results and discussion

The sequestration abilities were in general estimated by equilibrating a weighted amount of each material with a fixed volume of a stock buffered solution of a guest (40 μ M) and, then, determining the residual guest concentration in solution by UV-vis spectrophotometry (see Experimental section); hence, the percent of adsorbed guest is obtained by trivial calculations. The results obtained are collected in Tables 4.1, 4.2 and 4.3 (for the sake of clarity, considering the experimental uncertainties, almost complete adsorption is conventionally indicated in the Tables as $> 98\%$; conversely negligible adsorption is indicated as $< 2\%$). In a first set of experiments a sub-panel of 21 “*bulk*” materials obtained by the “*thick paste*” or the “*solution*” methods were selected, and the behavior towards guests **1-7** was considered at pH 4.4. and 6.7 (Table 4.1); few complementary determinations were carried out also at pH 1.0 and 10.7 (Table 4.2). Then, the behavior of the four highly swellable materials obtained with the “*smart dilution*” method towards dyes **5-10** was analyzed (Table 4.3). It is worth noting that the whole of the results collected constitutes a dataset of more than 400 entries, which should be suitably subjected to statistical analysis prior to undergo more detailed examination.

Starting from data obtained at the intermediate pH values 4.4 and 6.7 (reported in Table 4.1), it can be preliminarily observed that on average the cationic species **4** (the *N*-butylamino-*p*-nitroaniline derivative, that is protonated at the pH values considered¹⁰⁵) and **6** are adsorbed less efficiently than the other neutral and anionic guests. A remarkable exception occurs for the materials C11 and C12 decorated with the carboxyl groups of the pyromellitic P co-linker. In fact, the latter ones are partly or almost completely deprotonated at the pH values considered; therefore, strong Coulomb interactions may likely occur with the cationic guests. On the opposite side, decoration of the nanosponge scaffold with cationic groups from the possible presence of the triamine A has only a modest effect on the adsorption of anionic guests **5** and **7** in the case of the cyclodextrin-only material C19. Conversely a striking effect

occurs in the case of the mixed materials CK5 and CK6, and the calixarene-only material K2. The latter observation is quite interesting, because it implies that the largest effects from the possible presence of the cationic groups occur for the less hydrophilic materials. In turn, this also implies a sort of levelling effect as the hydrophilic character of the nanosponge scaffold increases.

Table 4.1. Percent adsorption data at pH 4.4 and 6.7^[a]

NS	1		2		3		4		5		6		7	
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
	4.4	6.7	4.4	6.7	4.4	6.7	4.4	6.7	4.4	6.7	4.4	6.7	4.4	6.7
C3	42	37	48	42	12	10	10	16	37	41	54	32	8	10
C5	27	27	27	28	19	26	11	4	17	23	51	29	6	8
C11	34	50	44	46	48	28	97	97	6	10	96	91	3	4
C12	65	65	41	46	28	<2	>98	>98	5	10	91	93	<2	<2
C13	20	15	29	31	28	25	4	4	20	15	11	8	14	9
C17	16	15	23	24	33	27	6	6	16	9	8	6	6	4
C18	13	11	17	21	16	16	4	5	30	28	12	12	24	20
C19	3	17	21	29	17	26	4	7	97	>98	4	6	>98	>98
K1	41	33	5	11	58	59	5	5	2	3	11	8	6	<2
K2	28	20	53	43	57	47	6	6	>98	97	3	0	>98	>98
K3	5	12	11	13	14	2	3	7	2	5	9	5	4	5
K4	15	14	7	10	25	22	4	8	8	<2	6	6	3	<2
K5	12	17	18	18	32	36	<2	7	<2	3	13	13	8	3
CK1	34	25	19	21	6	3	5	9	6	5	13	12	4	6
CK2	50	26	8	7	3	3	3	5	20	17	24	56	8	4
CK3	27	38	27	30	11	4	5	7	3	6	6	7	4	3
CK4	65	39	52	53	55	57	14	26	25	14	26	39	13	5
CK5_a	29	16	40	35	49	45	2	<2	>98	94	4	9	>98	74
CK5_c	44	23	65	47	70	47	5	5	97	91	5	5	>98	>98
CK6	46	36	62	60	65	63	8	7	>98	92	8	16	>98	95
CK7	13	5	<2	4	6	6	<2	5	<2	8	7	8	5	3

^(a) All data are given with ± 3 % indetermination.

As long as the composition of the material is concerned, a more articulated discussion is needed. Comparison of the average results for non-functionalized cyclodextrin materials C3, C5, C17 and C18 clearly indicates an increase in the adsorption abilities on increasing the amount of host subunits. Indeed, comparison between materials K1, K4 and K5 leads to the same conclusion. By contrast, mixed materials CK1, CK2, CK3 and CK4 show anomalously

large values for the latter one. The latter finding is not easy to explain, but could be tentatively justified in terms of a particularly favorable optimization of the microscopic arrangement of the host subunits; anyway, this particular case will need to be more deeply re-examined in future studies. Indeed, the possible outcome from the preparation procedures, that unambiguously reflect on the overall product structurization, are undoubtedly apparent in the comparison between CK5_a and CK5_c. In fact, the latter product, which was expected to possess a more ordered structure, showed on average better performances. Notably, materials C17-C19 containing the non-functionalized diol chain extender D adsorb on average less than materials C3 and C5 prepared without the chain extender (the same applies for the materials of the K-series). So, everything considered, it does not seem that an increase in the overall hydrophobic character of the nanosponges results in a beneficial effect on adsorption.

A more detailed analysis disclosed other interesting insights. The inclusion of the smallest *p*-nitroaniline guest **1** seems the one most sensitive to pH variations, in particular in the case of mixed materials. In a similar way, the relatively small sarcosine derivative **3** is much better included in the materials C11 and C12 (bearing the pyromellitic subunits) at pH 4.4, i.e. under the conditions providing less unfavorable Coulomb interactions. Conversely, for the large anions **5** and **7** the effects from pH variations are almost negligible (within the experimental uncertainties). This is somehow surprising, because adsorption of small guests is supposed to be mainly driven by their interaction with the cavities of the host units (whereas guests having a large structure are more likely to protrude from the cavities and partly reside in the pores). However, it must be also kept in mind that cyclodextrins decorated with ionizable groups such as polyamines can experience significant modification of their conformational behavior in solution.²⁶¹ Probably, in the particularly constrained situation occurring within the nanosponge architecture, the outcome of this effect is somehow amplified.

In order to have a clearer picture of the effect of charge interactions as a function of pH, few complementary experiments were carried out (Table 4.2). In particular, adsorption tests at pH 1.0 were performed in all those situations expected to show significant variations due to protonation of anionic groups, i.e. with materials C11 and C12, and with guests **3**, **5** and **7** (parallel tests were performed also on the non-functionalized materials C3 and C5) for useful comparison. Conversely, tests at pH 10.7 were performed for guest **4** and for material CK5_c as representative of the nanosponges bearing polyamine A as the chain-extender.

Table 4.2. Percent adsorption complementary data at pH 1.0 and 10.7 ^[a]											
NS	1		2		3		4		5	6	7
	pH 1.0	pH 10.7	pH 1.0	pH 10.7	pH 1.0	pH 10.7	pH 1.0	pH 10.7	pH 1.0	pH 1.0	pH 1.0
C3	25	33	25	43	0	-	0	56	9	30	24
C5	16	-	22	-	20	-	8	-	7	26	21
C11	13	< 2	22	5	41	-	24	86	26	70	27
C12	41	-	43	-	40	-	14	-	17	31	27
CK5 _c	-	-	-	47	< 2	6	< 2	35	< 2	-	< 2

^(a) All data are given with ± 3 % indetermination.

Data immediately evidence how the loss of the favorable Coulomb effects for materials C11 and C12 towards cationic guests **4** and **6** causes a dramatic drop in the observed adsorption values. Conversely, a favorable effect can be observed for the guests **5** and **7**, and up to a lesser extent for guest **3**, i.e. for the ones that lose their negative charge upon protonation. Moreover, in a perfectly consistent way, adsorption falls down for the same guests towards CK5_c. At the same time, a significant unfavorable effect for neutral *p*-nitroanilines **1** and **2** is found, as well as for all *p*-nitroanilines **1-4** towards the non-functionalized comparison materials C3 and C5. This can be justified considering that under very acidic conditions nitroanilines start to undergo protonation; therefore, they become much more hydrophilic and less prone to interact with the sorbent. Tests at pH 10.7 allow to appreciate the effect of deprotonation for the polyamine chain extender A, when present. In fact, **4** results much better included under such alkaline conditions; by contrast, inclusion of **3** is much worse. Notably, the guest **4** is better adsorbed under alkaline conditions even in non-functionalized materials C3 and C11; again, this can be easily explained considering that deprotonation involves charge loss and an increase hydrophobic character.

The highly swellable nanosponges C2_[125], C14, C15 and C16 offered the opportunity to further consider the outcome of the textural structurization of the material. Notably, they mainly differ for the average length of the linker units, but otherwise they are not functionalized. Therefore, it was chosen to study their behavior specifically with the large dye molecules **5-10**. The experiments were carried out in buffered solutions only at pH 4.4 and 6.7; results obtained are summarized in Table 4.3.

On average, data in Table 4.3 appear substantially consistent with those for the previous materials. In particular, as long as dyes **5-7** are considered, the behavior of C2_[125] obtained with H as the only reticulating agent is perfectly comparable with the one for the homologous nanosponge C3. On passing to the materials C14-C16 containing the chain extender D, their average adsorption efficacies appear in line with homologous materials C18 and C19. In

detail, it is interesting to notice that the average adsorption efficacies decrease along the series C14 > C16 ($\approx$ C19) > C15 (> C18).

Table 4.3. Percent adsorption data for the highly swellable materials^[a]

	5		6		7		8		9		10	
	pH 4.4	pH 6.7	pH 4.4	pH 6.7	pH 4.4	pH 6.7	pH 4.4	pH 6.7	pH 4.4	pH 6.7	pH 4.4	pH 6.7
NS												
C2 _[125]	42	43	21	18	23	26	32	48	26	16	<2	5
C14	37	32	20	20	23	32	21	52	37	29	3	7
C15	18	16	16	12	9	8	19	38	26	16	<2	3
C16	24	26	18	14	21	27	20	38	34	30	<2	4

[a] All data are given with ± 3 % indetermination

This observation is confirmed extending the analysis also to dyes **8-10**. Keeping into account the different average length of the relevant linker units (see Section 2.3), this result seems to indicate the existence of an optimum chain length, which likely represents the best compromise between relative amount of the host units, textural properties and functional mobility of the solvent medium. Finally, in regard to the single dyes, data evidence significant variations on changing the pH only for Malachite Green **8** and to a lesser extent for Bromotymol Blue **9**, i.e. the two triarylmethane derivatives. In particular, the latter one is included slightly better at pH 6.7. Considering its pK_A value of 6.3, and the fact that its deprotonation involves the opening of its lactone ring, best inclusion may be confidently attributed to these structural changes. Conversely, the datum for **8** is not easy to rationalize, and needs further investigations. Finally, the unsatisfactory adsorption of Rhodamine B **10** is quite surprising, because there is positive evidence of good adsorption into other nanosponges (see Chapter 8). Also this result will need further investigations.

4.3 Concluding highlights

The whole of the results collected and discussed outlines that the adsorption of organic guest species into nanosponges, in a perfectly parallel way as the formation of inclusion complexes with cyclodextrins, is a very complex process. Adsorption results indeed from a fine balance between diverse factors, and it is hard to predict a priori the prevailing one. A very important contribution to the overall process seem undoubtedly provided by the occurrence of favorable Coulomb interactions, whenever the material is decorated by ionizable functional

groups. In addition, the hydrophilic character of the guest and its overall texture properties have a significant outcome, but it is important that a good compromise is reached between linker size and host-to-linker combination ratio. In this regard, the structure of the guest, and in particular its size, has also to be considered. In fact, the conditions for the adsorption of a small species able to be entirely enclosed into the host cavity, do not seem to work the same way as for large guests, which in turn can more effectively interact with the nanosponge pores.

4.4 Experimental section

Absorption tests: Stock solutions ($3 \cdot 10^{-5}$ M) of the different guests were prepared in aqueous buffers, which in turn were prepared as follows. *i*) pH 1.0: standard HCl (0.1 M, Merck commercial product); *ii*) pH 4.4: 200 mg of NaCH₃COO were dissolved in 250 mL of freshly double-distilled water, then the proper amount of HCl 1 M was added up to reach the desired pH value; *iii*) pH 6.7: 232 mg of NaH₂PO₄ and 113 mg of Na₂HPO₄ were dissolved in 250 mL of freshly double-distilled water; *iv*) pH 10.7: 531 mg of K₃PO₄ were dissolved in 250 mL of freshly double-distilled water, then the proper amount of HCl 1 M was added up to reach the desired pH value. The actual pH of all the buffer stock solutions was checked by a CRISOM MicropH 2001 instrument equipped with a commercial glass electrode. Then, samples for the adsorption tests were prepared by mixing 2.0 mL of guest solution ($3 \cdot 10^{-5}$ M) with a carefully weighted amount (4.00 ± 0.05 mg) of materials. The samples were shaken at r. t. for 90 min, and, then, centrifuged for 15 min at 4000 rpm. The supernatant liquor was carefully pipetted after centrifugation, and, then, the percent amount of guest left in solution at equilibrium was simply estimated by means of UV-vis spectrophotometry, comparing the absorbances of the starting and final solutions.

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5 – Composite sorbents based on nanosponges

5.1 Introduction

As described in the general overviews chapter, over the past decades NSs have been largely used for pharmaceutical applications (smart delivery system, stimuli sensitive drug release, cytotoxicity potentiation, increase bioavailability etc.); however, massive work has been also devoted to explore their non-pharmaceutical applications, such as water purification, catalysis, gas storage, agricultural and industrial applications. In particular, the use as sorbents for pollutant species deserves great consideration, in view of their possible use in the design of devices for the sequestration of both organic and inorganic pollutants from wastewaters.

Application of nanosponges to the adsorption of organic pollutant species has been object of very extensive investigations in the last decades, and there is a massive literature on the subject, that is periodically reviewed^{119-120,202,204,262-270} (at least a dozen of reviews on the topic has appeared just in the last three years). Nanosponges have been also occasionally employed for the sequestration of inorganic species,^{87,271-276} and there are even a few reports on their possible use for the simultaneous adsorption of both inorganic and organic species.²⁷⁷⁻²⁷⁹ However, the adsorption of metal ions requires the possible presence of suitable donor groups decorating the nanosponge architecture, in order to provide effective ligand sites. Nevertheless, this could be not the only solution.

Fungi and yeasts are well-known biosorbents for heavy metals.²⁸⁰⁻²⁸³ Similarly, biochars have also been largely used for sequestering both organic and inorganic species.²⁸⁴⁻²⁸⁸ Both undoubtedly constitute a renewable resource. Therefore, it seemed interesting to associate these materials to nanosponges, in order to obtain composite sorbent materials with improved characteristics. The idea was pursued to verify, by combining diverse adsorbents such as nanosponges, yeast and biochar, the occurrence of a possible synergism between these components, in order to obtain materials able to simultaneously absorb both inorganic

and organic polluting species more effectively than the single counterparts. In particular, it could be reasonably hypothesized that the nanosponge is able to capture the pollutant species, exploiting the cyclodextrin host units, and transfer them to the other co-adsorbent, which in turn would act as a "storage". Materials of this type would have potential interest for large-scale environmental remediation applications. Two main possible advantages deriving from this association might be the improvement in the recoverability of the otherwise easily dispersible yeast, and the introduction of sensitivity to pH conditions thanks to the nanosponge matrix. Hence, before illustrating this part of the experimental work, it is appropriate to address and illustrate the characteristics of biochar and yeast.

5.2 General characteristics of Biochars and Yeast

Biochars²⁸⁹⁻²⁹⁰ are a class of porous carbonaceous materials obtained from the pyrolysis of biomass, characterized by a porous structure with a high surface area. According to the definitions from the European Biochar Foundation, it *"is applied in such a way that the contained carbon remains stored as a long-term C sink or replaces fossil carbon in industrial manufacturing. It is not made to be burnt for energy generation"*.²⁹¹ It differs from charcoal in its origin: because the latter one is extracted from mines.²⁹² Given its nature and the materials from which it comes, biochar can be identified as a biomaterial that has a low environmental impact. Hence, it can be exploited in different contexts, in particular as a feed supplement,²⁹³ as a soil additive and conditioner²⁹⁴, as a source of inorganic carbon and soil pH stabilizer, and for the slow release of species such as nitrates and phosphates, essential for the sustenance of plant species.²⁹⁵ Finally, it is used as an absorbent material for removal of pollutants.^{289,296-297}

The chemical structure of biochars is basically constituted by a mixture of graphene-like sheets of polycondensed aromatic rings and amorphous aliphatic and aromatic structures (Figure 5.1), both carbocyclic and heterocyclic.²⁹⁸ Of course, the chemical and the physical properties of a biochar can largely vary depending on various factors. First, the starting biomass must be considered, which can be either a ligno-cellulosic substrate (e.g. wood, straw, food processing residues such as shells, seeds, pomace, etc.) or also of animal origin (manure, bones and slaughterhouse residues). Second, it is necessary to consider the different operational parameters used in the preparation, such as the temperature and time of pyrolysis,

including any programmed gradients, as well as the "*quenching*" methodology (dry or wet, i.e. with or without air). In particular, the pyrolysis temperature and time affect the degree of porosity and graphitization of the material, with consequences on the specific surface area and on the overall hydrophobic character of the material obtained.

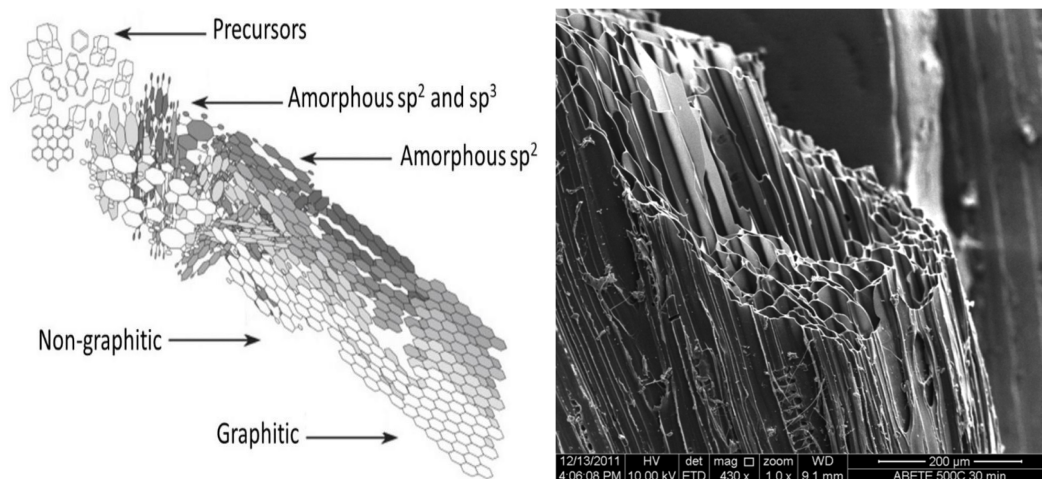


Figure 5.1. General structure of a biochar (left), and SEM micrograph of a typical biochar from vegetal origin.

Biomass treated pyrolytically at high temperatures generally return biochars having a higher hydrophobic character, and lower microporosity and surface area; such products are thus particularly suitable for absorbing organic hydrophobic pollutants. Conversely, biomasses treated in milder conditions allow to obtain less graphitized products, which are more suitable for absorbing inorganic and polar organic species.²⁹⁹

Various studies have reported²⁹⁰ that materials produced at relatively low temperatures ($< 350\text{ }^{\circ}\text{C}$) have a carbon percentage lower than 60% by weight, and show the simultaneous presence of alkyl and aromatic residues; those obtained in the temperature range between $400/500\text{ }^{\circ}\text{C}$ reach a carbon percentage of up to 80% and there is a progressive disappearance of the alkyl residues in favor of the aromatic ones. Finally, for temperatures higher than $500\text{ }^{\circ}\text{C}$, carbon percentages higher than 80% and the complete disappearance of alkyl residues are recorded (Figure 5.2). From a physical standpoint, all this coincides with the progressive modifications of the architecture of the material on a micrometric scale. In the case of materials deriving from cellulosic matrices, relatively low pyrolysis temperatures allow the framework of the cell walls to be kept relatively unaltered, a framework which undergoes a progressive collapse as the pyrolysis temperature increases.³⁰⁰ This causes a progressive increase in pore width of the material (Figure 5.3) shows a schematic representation of the coalescence process of the pores which occurs with increasing temperature.

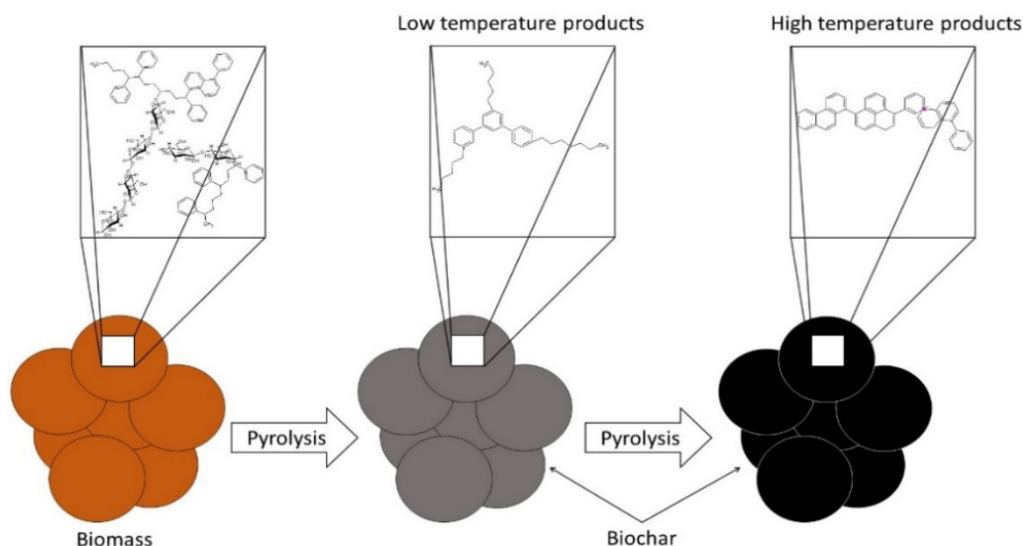


Figure 5.2. Schematization of the chemical transformations obtained with increasing temperature.

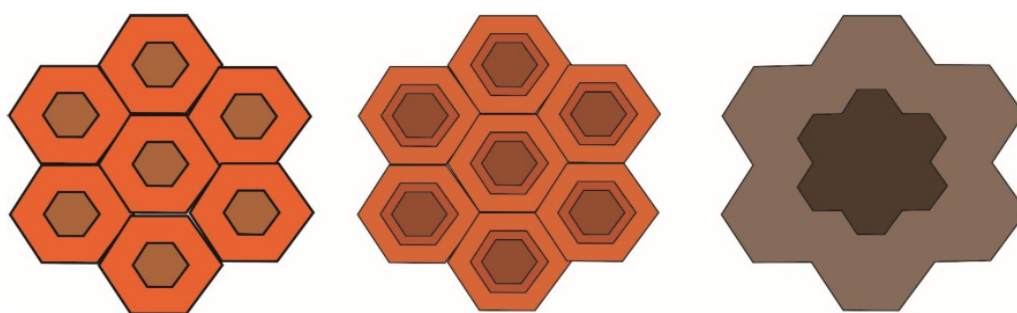


Figure 5.3. Representation of the pores formation during pyrolysis processes: porous structure of the material before pyrolysis takes place (left); increase in the surface area of the pores with increasing temperature (middle); collapse of the pre-existing structures resulting in the formation of larger channels (right).

Furthermore, increasing the percentage of carbon in the material corresponds to a decrease in the abundance of the content of N, O and H which constitute polar functional groups such as -NH_2 , -COOH , -OH , etc. The presence (or the absence) of these groups significantly affects the adsorbent capacity of the biochar: materials that abound in polar groups exhibit better adsorbent properties against heavy metals thanks to the possibility of ion-dipole, electrostatic, cation- π type interactions, whereas materials lacking the aforementioned functional groups are more prone to adsorb organic species, through Van der Waals, π - π and dipole-dipole interactions.³⁰¹ Thus, biochars obtained under milder conditions possess a greater amount of polar groups and greater porosity. The latter characteristics may favor the

absorption of heavy metals. Conversely, materials obtained at high temperatures have aromatic polycyclic structures (and therefore are more hydrophobic) and lower porosity, therefore they show excellent adsorbent properties towards organic species. Biochar, as already mentioned, is also used in agriculture and is used as a soil conditioner. Its use improves the structure of the soil, raises its pH, allows better water retention and, in the case of biochars rich in nitrogen and phosphorus, allows the slow release of nutrients containing N and P essential for seed germination and growth some plants. Finally, in the global carbon balance, the applications of biochar to soils make it possible to sequester carbon dioxide, a well-known greenhouse gas, in this environmental matrix, contributing to the mitigation of climate change on a global scale. Finally, the application of biochar as a soil improver reduces the use of nitrogenous fertilizers which, when applied incorrectly, can lead to pollution of groundwater, rivers, lakes and coastal waters. Their presence in these latter environments also favors the phenomenon of eutrophication with catastrophic consequences for all ecosystems.^{295,302} Biochar is therefore a very versatile material, which lends itself to many applications, with an extremely low; thanks to its characteristics and the growing demand for biocompatible materials, it is experiencing a "boom" in the field of research. Articles containing the word "*biochar*" have gone from a few dozens in 2009 to nearly 4.000 in 2020; among these many are focused on the preparation and study of composite materials able to increase the already excellent performance of this material.

Saccharomyces Cerevisiae, better known as brewer's yeast (Figure 5.4), is a unicellular eukaryotic organism belonging to the kingdom of *fungi*, which is included in the family of *Ascomycetes*.³⁰³ It is a facultative anaerobic organism, i.e. it can obtain energy through an aerobic process or through an anaerobic process depending on conditions.³⁰⁴ It is the main yeast of alcoholic fermentation in wine and beer. It is mainly used in the food and fermented products industry, and is easily available in small commercial distribution (in the form of compact loaves, Figure 5.4), being used, among other things, in home baking. It was the first unicellular eukaryotic organism to have its genome completely sequenced.³⁰⁵

In recent years, yeast has been object of growing interest as a bio-adsorbent material for metal pollutants. Its growing success is due to its non-toxicity, availability, biocompatibility, its low production cost and finally to its adsorbing capacity which allows it to remove metals in concentrations even in the order of ppb and ppt.³⁰⁶ Other factors that have contributed to the fortune of this material are its easy management and its modularity, moreover it is also able to carry out its functions regardless of whether the biomass is alive or not.



Figure 5.4. Commercial brewer's yeast block (left) and SEM micrograph of *Saccharomyces Cerevisiae* (right).

As regards the modularity of the yeasts, they can be chemically functionalized; moreover, it is possible to cultivate suitable strains of these *fungi* in suitable conditions in order to obtain materials able to express greater affinities in the adsorption of specific metal ions. A study by Ramsay *et al.*,³⁰⁷ for instance, focused on the selection of a particular type of vacuole-deficient yeast, which proved to be more selective in accumulating metals such as Zn, Mn, Co, Ni, compared to strains having a normal abundance of these organelles. The ability of yeasts to accumulate metals from aqueous solutions is the result of physico-chemical interactions such as adsorption and absorption. The first consists in the interaction between the cell walls and the ion; it is a fast, not very selective and reversible process which occurs above all with inactive biomasses;³⁰⁸ the second is a process in which the previously adsorbed ions manage to penetrate inside of the cell through some transport systems that require energy from metabolic processes; this is certainly a slower, more selective, toxic and less reversible process than the previous one. The dynamics of these processes are still being studied and have not yet been fully understood. However, it has been found that the effectiveness of absorption depends on various parameters such as the presence of functional groups on the surface of the cells, the nature of the metal ions (charge, electronegativity, ionic radius), the initial concentration, the co-presence of other ions, contact time, temperature and pH.^{306,309-310} Some of these factors may act simultaneously on different levels. For example, the pH of the solution affects the percentage of protonated groups on the yeast cell membrane and also the speciation of metal ions; at acid pH the amount of protonated functional groups on the cell wall increases and consequently the adsorbent capacity of the same decreases. On the contrary, at alkaline pH, metal hydroxides can precipitate, which cannot be adsorbed by the biomaterial, reducing the adsorbing ability. Also the presence of anions in solution such as carbonates, chlorides, phosphates, sulphates, etc., affects the adsorbent ability, in fact, these can complex the ions, reducing their

availability. The adsorbent ability of yeast can be traced back to the amino acid residues which make up the proteins contained in it; these are particularly abundant in lysine, tyrosine, aspartate, threonine, histidine, phenylalanine and proline.³¹¹ Thanks to the amide functional groups and to those present in the side chains of the single amino acids (groups -SH, -OH, -COOH, -NH₂), the metal cations can give rise to acid-base type interactions according to Lewis sufficiently strong to be able to coordinate toxic metals such as Cd, Hg, Pb, Zn; precious metals such as Au, Ag, Pd, Pt and even radionuclides such as Ce, Cs and U.³⁰⁶ The ability to adsorb a wide range of metals, even in trace amounts, means that these bioadsorbents are used not only to remove these metals from environment but also to recover and reuse them. For this reason, there are already several studies aimed at producing recyclable, low-cost and eco-sustainable composite materials able to enhance the properties of this absorbent and expanding its range of action. Among the materials potentially able to perform these functions we find the cyclodextrin-based nanosponges, which are discussed above.

5.3 Synthesis and characterization of the composite sorbents

In order to prepare the desired composite materials two different biochars were used, obtained from almond shells (Ba) and poplar wastewood (Bp). Biochars samples were kindly provided by prof. P. Conte, Dept. SAAF – University of Palermo (see Experimental section for details on their obtainment). Moreover, a sample of commercial baker's yeast (*Saccharomyces cerevisiae*, Y) was used, which obtained from a town food store, and inactivated prior to use. Five different cyclodextrin-only nanosponges, namely those labeled as C5, C8, C11 and C12 (see Section 2.1, Table 2.1) as supports for the co-sorbents. In detail, the former one is non-functionalized material prepared with di-isocyanate H as the sole linker (in a 1:8 mole-to-mole ratios with the cyclodextrin); C8 is prepared with the pyromellitic anhydride P (in a 1:7 mole-to-mole ratio); finally, C11 and C12 contain both the linkers H and P (in 1:4:4 and 1:8:4 ratios, respectively). The desired composites were easily obtained with the “*thick paste*” method. In brief, the all the solid components were previously homogenized in a mortar, and introduced into a vial; then a little amount of DMF (0.3 mL per g of solids) was added, together with di-isocyanate H if needed, and the system was vigorously mixed mechanically. The reaction crude was kept still at 60 °C for 18 h, and

the crude was then worked-up as described elsewhere (see Section 2.7). Some composites were prepared by using only one co-sorbent in a 75:25 w/w ratio; in other cases, tri-component composites were obtained by simultaneously using Y associated with either Ba or Bp. In this way, a panel of 15 different composites (summarized in Table 5.1) was produced. These composites were labeled as C20-C34, in prosecution with the C-series of materials previously reported on Table 2.1. For the sake of completeness, some samples were also prepared with the “*solution*” method, but the product obtained resulted to almost the same sorbent properties as the one obtained with the “*thick paste*” method.

Once the synthesis and isolation had been carried out, the materials were subjected to suitable characterization, in order to verify the actual occurrence of the reticulation process and to confirm the presence of the various constituent moieties. As well as for the materials described previously, FT-IR and ^{13}C CP-MAS solid-state NMR techniques were exploited.

Table 5.1. Complete panel of Nanosponge materials prepared with co-sorbent.

NS	linker		CD/linker mole-to-mole ratio	co-sorbent
C20	H	-	1 : 8	Y 25% w/w
C21	H	-	1 : 8	Bp 25% w/w
C22	H	-	1 : 8	Ba 25% w/w
C23	H	-	1 : 8	Y 25% w/w + Bp 25% w/w
C24	H	-	1 : 8	Y 25% w/w + Ba 25% w/w
C25	-	P	1 : 7	Y 25% w/w
C26	-	P	1 : 7	Y 25% w/w + Ba 25% w/w
C27	-	P	1 : 7	Y 25% w/w + Bp 25% w/w
C28	H	P	1 : 4 : 4	Y 25% w/w
C29	H	P	1 : 4 : 4	Ba 25% w/w
C30	H	P	1 : 8 : 4	Y 25% w/w
C31	H	P	1 : 8 : 4	Bp 25% w/w
C32	H	P	1 : 8 : 4	Ba 25% w/w
C33	H	P	1 : 8 : 4	Y 25% w/w + Bp 25% w/w
C34	H	P	1 : 8 : 4	Y 25% w/w + Ba 25% w/w

Again, FT-IR enabled to unambiguously identify the signals expected for the various functional groups originated in the reticulation reactions. However, in this case the presence was also checked of the signals relevant to the co-sorbents dispersed within the nanosponge matrix. The diagnostic signals whose presence was identified in the spectra to confirm the constitution of the composites are summarized in Table 5.2. Basically, with respect to the spectra of the plain uncharged materials it is possible to observe:

- i) a modification of the intense -OH str. band in the 3200-3500 cm⁻¹ region. This is mainly due to the reaction of these groups with the electrophilic reticulating agent. Moreover, it should be also kept into account that unreacted -OH groups experience significant environmental changes with respect to the pure reactant, which affect their abilities in hydrogen bonding formation.
- ii) the presence of new signals in the carbonyl region 1750-1500 cm⁻¹, accounting for the peptides present in yeast.
- iii) The modification of the fingerprint region, again mainly due to the presence of yeast, in particular its content in glucanes and mannanes. Indeed, biochars do not present any peculiar diagnostic signal. However, their (almost quantitative) incorporation into the materials can be unambiguously assessed by the fact that they cannot be detected in significant amount in the washing liquors.

Table 5.2. Diagnostic FT-IR signals expected for the various functional groups and moieties.

Band		$\tilde{\nu}$ (cm ⁻¹)
str. -OH	cyclodextrin	3350-3450
	carboxyl.acid (from P)	2500-2700
str. -NH-	urethane, urea (from H)	3320-3340
str. C=O	aroate ester (from P)	1710-1715
str. C=O (Amide I-type)	alkyl carbamate	1640-1690
bend. -NH- (Amide II-type)	alkyl carbamate	1535-1545
fingerprints:		
poly-urethane/urea		1257, 1222, 1144, 1076, 1055, 1005
Cyclodextrin		1156, 1079, 1030, 947
Yeast		1638 (Amide I), 1539 (Amide II), 1052, 971 911 (mannanes), 994 (β ,1-6 glucane), 1026, (β ,1-4 glucane), 1141, 1103, 1077 (β -1,3-glucane).

On passing to the ¹³C CP-MAS solid-state NMR characterization, spectra fully confirmed the presence of the constituents and the expected functional groups in the materials obtained. The complete attribution of NMR signals is summarized in Table 5.3. The yeast spectrum shows various signals which are undoubtedly attributable to both the protein and carbohydrate components. In particular, it is possible to find two signals centered respectively at approx. 174 ppm (amide C=O) and 55 ppm (C α), together with various low intensity signals in the aromatic carbon zone (157, 137, 129 and 115 ppm) which can be justified by the presence of aromatic amino acid residues. On the other hand, signals such as those at 103, 72 and 61 ppm are perfectly consistent with the presence of glucanes and

mannanes. In addition, the spectra of biochars present a main signal for aromatic C carbons at 124-128 ppm (with minor contributions at 137 and 160 ppm).

Table 5.3. Diagnostic signals in the ^{13}C CP-MAS solid-state NMR spectra		
Signal		ppm range
Cyclodextrin	C(1)	100-105
	C(2,3,5)	70-75
	C(4)	81-83
	C(6)	60-65
Yeast	C α aminoacid	54-55
	Glucanes, mannanes	61-72
	C=O amide	174-175
Biochar	C aromatic	124-126
Aliphatic linker/chain extender	-CH ₂ -	26-31
	-CH ₂ - (bound to O,N)	40-60
	urethane, urea	158-160
	aroate ester/carboxyl	160-170
	C aromatics (from P)	128; 133

In analogy with plain materials, the characterization of the composites should have been complemented by a relaxometric study. However, several difficulties were encountered on trying to perform the FFC-NMR experiments needed. In particular, for the dry materials it was absolutely impossible to obtain relaxometric kinetics of acceptable quality. This unfortunate finding can be tentatively attributed to the occurrence of significant paramagnetic effects from both the biochar and (unexpectedly) the yeast. Indeed, problems were encountered even for wet materials. Nevertheless, data of good quality could be collected for at least 5 wet materials, which enabled to carry out some interesting comparison with the plain uncharged nanosponges. The results are collected in Table 5.4 (complete relaxometric data are provided in Appendix to this chapter).

Table 5.4. R_0 and correlation times ($\tau_{c,1}$, $\tau_{c,2}$ and $\tau_{c,long}$), PCI and f_s values.							
NS	Composition	R_0 (ms ⁻¹)	$\tau_{c,1}$ (ns)	$\tau_{c,2}$ (ns)	$\tau_{c,long}$ (μ s)	PCI	f_s
C5	C/H 1:8	0.74	17	220	5.0	27.8	0.033
C12	C/H/P 1:8:4	3.02	19	340	1.77	18.7	0.065
C24	C/H 1:8 + 25% Y + 25% Ba	2.55	16	158	0.79	2.7	0.188
C30	C/H/P 1:8:4 + 25% Y	2.46	18	79	0.69	18.7	0.096
C31	C/H/P 1:8:4 + 25% Bp	2.63	17	151	1.74	19.3	0.145
C33	C/H/P 1:8:4 + 25% Y + 25% Bp	1.92	17	69	0.44	2.47	0.195
C34	C/H/P 1:8:4 + 25% Y + 25% Ba	0.98	13	132	1.15	1.28	0.130

Again, heuristic analysis of the NMRD curves for the slow relaxometric component (relevant to in-pore water molecules, see Section 3.5) show the presence of three main dynamic regimes. For the fastest one, the relevant $\tau_{c,1}$ values for the composites do not show significant variations with respect to the plain nanosponges; conversely a decrease can be generally noticed for $\tau_{c,2}$ and $\tau_{c,long}$ values. If the interpretation of correlation times proposed previously is correct, these results seem to indicate that no particular effect occurs on rotational motions, but rather translational and surface-pore exchange motions are faster in the composites. Nevertheless, significant changes occur also for PCI and f_s values. The former parameter undergoes significant decrease for the composites, whereas the latter increases. These combined trends indicate that the pore surface experiences the presence of a thicker solvation shell. This can be tentatively explained assuming that the co-sorbents strongly interact with, or at least exert a strong structuring effect on water molecules. In the case of yeast, strong interaction is fully justified by its glucidic and proteic components; conversely, for biochar this might be rather linked to its peculiar graphene-like porous structure.

5.4 Adsorption tests on organic pollutants

The possible pH dependent abilities of the composites in sequestering organic species from buffered aqueous solutions were assessed, in perfect analogy with plain nanosponges, by performing a set of sequestration tests on the same organic model pollutants **1-7** (Figure 5.5) already examined in the previous Chapter.

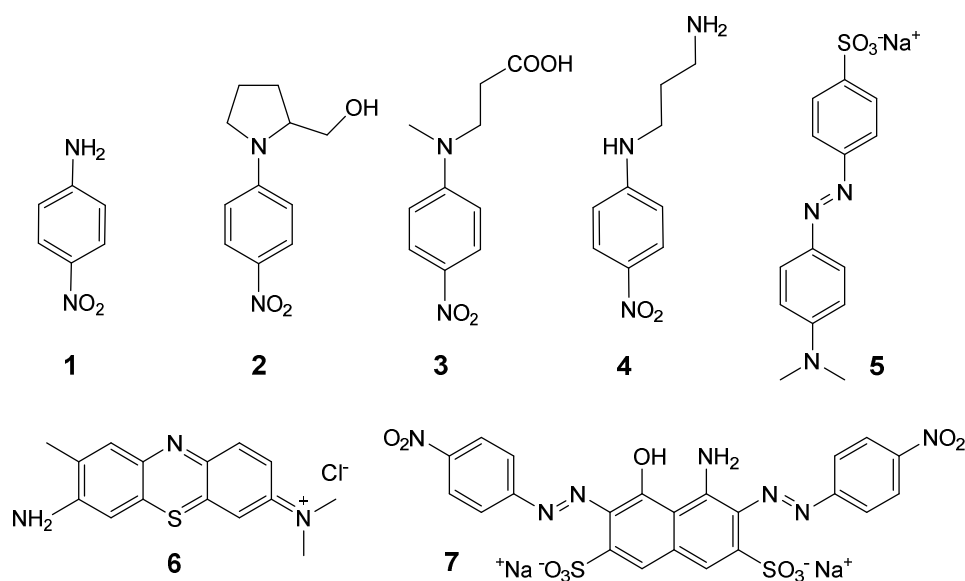


Figure 5.5. Structures of the guests.

For consistency, the tests were carried out at pH 1.0, 4.4 and 6.7. Notably, the most acidic condition was systematically explored in view of the fact that wastewaters treatment frequently requires to operate under relatively low pH. Complete data are collected in Table 5.5. It is important to stress that these experiments were designed for verifying a possible synergism between the nanosponge matrix and suitable co-sorbents such as yeasts and biochars. It is worth recalling here that association between the nanosponge matrix and the co-sorbents might be profitable because: *i*) the composite may improve the otherwise poor mechanical properties of the co-sorbent; in particular, yeasts are easily dispersible and difficult to recover from aqueous systems; *ii*) a sort of “*catch and store*” system might be obtained, where the nanosponge can quickly sequester the possible pollutants present, which in turn are transferred and stored by the co-sorbent. Of course, the sequestration abilities of plain biochars and yeast were evaluated in parallel. Again, a significantly large dataset has to be managed (462 entries), that is not straightforward to analyze. Therefore, the entire dataset was subjected to statistical suitably analysis. In particular, the average adsorption percentages of the composite materials were calculated, and then compared with the theoretical value obtained as the weighted average of the adsorptions for the plain components. The results obtained from this analysis are summarized in Table 5.6. This elaboration allows to individuate in an easier way some interesting trends. By considering first the composition of the materials, it must be preliminarily taken into consideration that yeast shows good adsorbing capacity only towards monocationic guest **6** at pH 4.4 and 6.7 and dianion **7** at pH 1.0, but otherwise appears as a very poor adsorbent. Conversely, biochars adsorb up to a significant (in some cases almost quantitatively) all the substrates regardless of the pH. For the composite materials data collected seem to indicate that the type of crosslinking agent critically affects the combined effect of the association between nanosponge matrix and co-sorbent. In the case of materials prepared with the crosslinker H alone, the association with the co-sorbent leads to materials that are more performing than the separate components. In particular, the C20 material shows an improvement in performance of nearly 20%, whereas fair improvement occurs in materials in which both yeast and biochar are present (C22 material prepared with only almond biochar, on the other hand, does not show significant improvements). Conversely, in all the cases in which P is present (both as the sole linker or as co-linker), a significant decrease in the performance of all materials containing only biochar is observed. In particular, the decrease in average affinities ranges from 14% for material C31 to a dramatic 26% for C29. The simultaneous association of yeast and biochar leads to less noticeable (-7.8% for C34) or negligible (-0.3% for C33) decreases in average activity. It is interesting to note how these trends are reflected

Table 5.5. Percent adsorption data for the composite sorbent materials.												
Guest	1			2			3			4		
NS	pH 1.0	pH 4.4	pH 6.7	pH 1.0	pH 4.4	pH 6.7	pH 1.0	pH 4.4	pH 6.7	pH 1.0	pH 4.4	pH 6.7
C3	<2	42	37	<2	48	42	<2	12	10	<2	10	16
C5	16	27	27	22	27	28	20	19	26	8	11	4
C20	15	29	29	25	28	38	19	16	23	2	21	44
C22	15	40	40	35	32	36	24	25	27	10	9	10
C23	42	48	31	32	27	23	41	47	31	13	43	82
C24	22	43	43	32	37	34	24	23	32	7	26	45
C8	4	21	21	54	43	37	16	<2	52	46	0	0
C25	25	38	38	49	36	40	25	15	35	59	71	68
C26	5	34	34	13	12	15	9	14	17	<2	14	13
C27	2	17	17	10	10	15	6	11	8	<2	12	8
C11	13	34	50	22	44	46	41	28	48	24	97	97
C28	14	29	29	28	44	40	24	16	26	10	90	92
C29	6	31	31	9	6	10	85	7	12	<2	4	96
C12	41	65	65	43	41	46	40	28	0	14	>98	>98
C30	19	37	37	16	32	53	11	14	31	4	89	97
C31	24	44	41	31	32	40	31	34	40	6	80	95
C32	18	22	43	24	15	21	26	20	42	4	81	49
C33	41	46	45	50	27	41	49	41	43	<2	74	41
C34	19	39	39	31	30	28	20	20	26	<2	22	48
Y	<2	<2	<2	3	4	9	<2	8	<2	<2	9	16
Ba	>98	>98	>98	88	84	67	96	97	>98	50	29	91
Bp	>98	>98	>98	88	84	67	99	88	80	49	69	90
Guest	5			6			7					
NS	pH 1.0	pH 4.4	pH 6.7	pH 1.0	pH 4.4	pH 6.7	pH 1.0	pH 4.4	pH 6.7			
C5	7	17	23	26	51	29	21	6	8			
C20	10	14	9	22	70	86	93	6	7			
C22	11	42	44	21	79	45	66	19	20			
C23	12	36	2	36	95	94	>98	33	39			
C24	11	38	25	38	81	90	95	34	35			
C8	41	47	36	89	19	<2	34	<2	<2			
C25	45	29	16	97	84	55	44	<2	<2			
C26	24	13	4	30	51	29	63	<2	<2			
C27	18	13	5	26	36	20	88	7	8			
C11	26	6	10	70	96	91	27	3	4			
C28	12	17	12	29	93	87	82	6	7			
C29	10	6	6	31	32	22	67	7	8			
C12	17	5	10	31	91	93	27	<2	<2			
C30	3	9	7	14	94	92	72	4	5			
C31	5	7	5	14	91	95	20	1	2			
C32	5	3	6	16	87	94	25	8	9			
C33	12	11	24	14	84	93	95	74	5			
C34	9	12	20	32	83	88	98	34	35			
Y	4	13	2	17	84	81	98	10	11			
Ba	43	33	40	83	68	84	60	45	45			
Bp	40	30	38	90	>98	97	70	55	50			

[a] All data are given with ± 3 % indetermination

Table 5.6. Difference between the observed and theoretical absorption percentages.					
NS	constituents and mole/mole ratio	pH 1.0	pH 4.4	pH 6.7	cumulative
C20	C/H 0.125:1 + Y 25% w/w	15.5	18.8	23.7	19.3
C22	C/H 0.125:1 + Ba 25% w/w	-5.4	1.9	-2.6	-2.0
C23	C/H 0.125:1 + Y 25% w/w + Bp 25% w/w	7.1	12.3	9.9	9.8
C24	C/H 0.125:1 + Y 25% w/w + Ba 25% w/w	1.2	8.2	10.1	6.5
C25	C/P 0.143:1 + Y 25% w/w	14.4	20.3	16.0	16.9
C26	C/P 0.143:1 + Y 25% w/w + Ba 25% w/w	-22.6	-10.3	-17.3	-16.8
C27	C/P 0.143:1 + Y 25% w/w + Bp 25% w/w	-21.6	-15.1	-22.1	-19.6
C28	C/H/P 0.25:1:1 + Y 25% w/w	0.2	4.5	0.5	1.7
C29	C/H/P 0.25:1:1 + Ba 25% w/w	-12.8	-36.1	-29.5	-26.1
C30	C/H/P 0.125:1:0.5 + Y 25% w/w	-7.3	0.0	8.2	0.3
C31	C/H/P 0.125:1:0.5 + Bp 25% w/w	-23.3	-12.8	-6.8	-14.3
C32	C/H/P 0.125:1:0.5 + Ba 25% w/w	-24.5	-17.9	-14.8	-19.1
C33	C/H/P 0.125:1:0.5 + Y 25% w/w + Bp 25% w/w	-1.3	4.1	-3.6	-0.3
C34	C/H/P 0.125:1:0.5 + Y 25% w/w + Ba 25% w/w	-8.1	-10.1	-5.0	-7.8

in the materials prepared with the sole crosslinker P. In fact, while the association with yeast alone leads to a more performing material (about 17% for C25), the simultaneous association of yeast and biochar leads to significantly less performing systems (with a decrease in activity for C33 close to 20%). Analyzing the data in more detail, the effect of the pH conditions can be considered. In the case of materials obtained with H as the only linker, this effect is quite modest and occurs essentially for the materials containing yeast. Conversely, in the case of materials containing P, it is possible to find a clear effect, in agreement with the presence of the –COOH functional groups on the linker units. In particular, in some cases there is a constant improvement of the average activities with increasing pH (C30, C31, C32 and C34), in others a bell-like trend with maximum performance at pH 4.4 (C25, C26, C27, C28 and C33). Trends of this type can be found in literature,⁸⁹ and can be explained by considering the average charge that the material assumes as a result of the progressive dissociation (as the pH increases) of the carboxyl groups present on the material. In fact, considering that the adsorption in nanosponge materials is driven by a fine balance between a whole complex of factors (electrostatic, dipolar and van der Waals interactions, desolvation effects, steric effects, etc., as already outlined in the previous Chapter), each material results to possess an optimal operating pH, which is not easy to predict *a priori*, but must be experimentally identified case-by-case. As a final remark, the distinctly negative effect due to the presence of P is interesting and requires a justification. It can be hypothesized that biochar, as a graphitic type material, can strongly interact with the aromatic units of the crosslinker, going to saturate its binding sites. However, it is not possible to exclude the occurrence of significant variations in the microscopic structure of the porous material at the same time, which however cannot be highlighted at this level.

Some final comments are needed to consider the guests. Data in Table 5.5 confirm the fact that the possible electric charge present on the organic species significantly affects the adsorption process. For instance, the cationic substrates **4** and **6** are adsorbed to a good extent at pH 4.4. and 6.7, with a sharp decrease on passing to pH 1.0. The tricyclic derivative **6** is adsorbed significantly better than the smaller *p*-nitroaniline derivative **4** (with the exclusion of a few cases at pH 6.7). This can be easily explained considering that in the former case the large molecule delocalizes the positive charge onto its entire structure, whereas the molecule is smaller and the positive charge is localized on the leading ammonium group. Probably, favorable effect from possible van der Waals interactions is act in synergism the effect of electrostatic interactions. The two monoanionic substrates **3** and **5** (the latter one indeed is zwitterionic at pH 1.0) are very poorly adsorbed. Conversely, the dianionic **7** appears interesting, because it shows an inverted behavior with respect to the cationic guests. In other words, it shows a relatively good tendency to adsorption at pH 1.0, which then collapses at pH 4.4 and 6.7. Finally, neutral guests **1** and **2** show an intermediate affinity for materials between that of cations and that of monoanions.

5.5 Concluding highlights

The results collected herein positively assess the possibility to associate nanosponges and different co-sorbents such as yeast and biochars. Besides the easy preparation of the composites, the association can be advantageous, provided that attention is paid to some caveats. In particular, the choice of the reticulating agent is a delicate issue, because it can affect the performance of at least one of the co-sorbents (as in the case of pyromellitic anhydride towards biochar). Moreover, association has undoubtedly an effect of the functional mobility of the solvent medium within the pore network, as accounted for by relaxometric evidences. Nevertheless, the rationalization of experimental data provides an overall picture that is perfectly consistent with the interpretation of data for plain nanosponges.

5.6 Experimental section

Biochars were prepared by gasification at 1200 °C for 6 hours and subsequent "dry" cooling in an ordinary atmosphere. The various samples came in fairly large flakes, and for this reason, they were ground by ball milling before use. The brewer's yeast (*Saccharomyces Cerevisiae*) used in the work was purchased at a common supermarket. The biomass as such was inactivated and dehydrated by treating it with a tenfold amount by weight of ethanol, sonicating for 10 min and centrifuging (this treatment was repeated three times). The precipitate was dried in a rotavapor and then dried overnight in a vacuum gun on P₂O₅ at 65 °C, and, finally, finely ground in an agate mortar.

5.6.1 Synthesis of materials

For the synthesis of composite materials two different methodologies were used. In the "thick paste" method, a given amount of β CD and all other reagents/solid components (biochar, yeast or pyromellitic dianhydride), calculated according to the various molar or weight ratios required for the particular material to be prepared, were carefully mixed in an agate mortar. The mixture of powders thus obtained was introduced into a sufficiently large glass vial and then mixed with 300 μ L of anhydrous DMF, in which the appropriate amount of H (if required by the synthesis) had possibly been dissolved. The reaction system is then vigorously mixed with a thin metal rod for a few minutes, trying to obtain a homogeneous mass. The system is then left to react in an oven at 60 °C for 18 hours. In the case of the "solution" method, a given amount of anhydrous DMF is placed in a sufficiently large glass vial, and a given amount of β CD is dissolved. The solid components (yeast, biochar) are then added to the solution, calculated on the basis of the weight ratios required for the particular material to be prepared. The suspension is sonicated for a few minutes, in order to ensure the best dispersion of the solids, then it is brought under magnetic stirring at 60 °C (heating plate) and the cross-linking reagents (H, P) are added in the appropriate calculated amounts. At this point, the reaction system is kept under magnetic stirring for about an hour, until gelation, and finally kept in the oven at 60 °C for 18 hours.

The isolation of the final product, for both methods, is carried out as follows. The reaction mass is recovered, mechanically crushed, and suspended in 40 mL of ethyl acetate. The suspension is sonicated for 15 min and the solid is isolated by decantation. The same washing with ethyl acetate is carried out two more times, then the recovered solid is resuspended in 40 mL of methanol, sonicated and left to stand overnight. The final product is filtered, dried

for 24 hours in a vacuum gun on P₂O₅ at 65 °C, and finally ground by ball milling and passed through a mesh sieve of 150 μ . The complete panel of the composite sorbents are reported in Table 5.7.

Table 5.7. Synthesis of composite sorbents.

NS	Host	Linker/chain extender		Co-sorbent			solvent	Yield (mg)
	C (mg)	H (mg)	P (mg)	Y (mg)	Bp (mg)	Ba (mg)	DMF (mL)	
C20	559	670	-	413	-	-	0.3	1612
C20	559	670	-	413	-	-	3.0	1510
C21	567	670	-	-	415	-	3.0	1213
C22	567	670	-	-	-	415	3.0	1213
C23	730	838	-	775	771	-	3.0	2323
C24	568	670	-	620	-	620	3.0	2231
C25	570	-	764	445	-	-	3.0	1240
C26	568	-	763	667	-	665	0.3	2004
C26	568	-	763	667	-	665	3.0	2311
C27	568	-	763	667	665	-	0.3	2004
C27	568	-	763	667	665	-	3.0	2311
C28	568	336	445	447	-	-	3.0	1396
C29	567	336	445	-	-	447	3.0	1133
C30	568	670	436	447	-	-	3.0	1732
C31	710	838	560	-	555	-	4.0	1918
C32	568	670	436	-	-	559	3.0	1766
C33	708	838	556	1057	1046	-	4.0	2658
C34	568	670	436	838	-	838	3.0	2884

5.6.2 Absorption tests

The adsorption tests were performed according to the procedure described previously in Section 4.4.

5.6.3 Relaxometric FFC-NMR analysis

FFC-NMR measurements were conducted on a STELAR SmarTracer 0.25 Tesla Bench-top NMR Relaxometer instrument. The materials (about 400 mg) are placed in NMR tubes with an internal diameter of 8.5 mm and placed overnight to equilibrate with 1.5 mL of buffer solution at pH 4.4. Before measurement, any excess supernatant liquid is carefully spitted

out and weighed. The sample thus prepared is then subjected to instrumental analysis. The obtained relaxometric data were analyzed using the commercial software packages Excel 2016, Kaleidagraph 4.0 and UpenWin 1.6 β 1, the latter kindly made available by Prof. Villiam Bortolotti (Univ. of Bologna).

APPENDIX

Relaxometric Data for water-saturated composite materials (slow component)

C5				C24				C30			
ν_L (MHz)	R_1 slow	(s^{-1})		ν_L (MHz)	R_1 slow	(s^{-1})		ν_L (MHz)	R_1 slow	(s^{-1})	
10.00	2.7	$\pm$	0.1	10.00	3.64	$\pm$	0.04	10.00	2.87	$\pm$	0.10
9.00	2.6	$\pm$	0.1	9.00	3.64	$\pm$	0.04	9.00	2.93	$\pm$	0.08
8.00	2.8	$\pm$	0.2	8.00	3.86	$\pm$	0.04	8.00	3.07	$\pm$	0.08
7.00	2.9	$\pm$	0.2	6.00	4.38	$\pm$	0.07	7.00	2.99	$\pm$	0.09
6.00	3.1	$\pm$	0.3	5.00	4.68	$\pm$	0.09	6.00	3.19	$\pm$	0.10
4.00	3.5	$\pm$	0.3	4.00	4.95	$\pm$	0.10	5.00	3.37	$\pm$	0.14
3.50	3.8	$\pm$	0.5	3.50	5.34	$\pm$	0.14	4.00	3.69	$\pm$	0.09
3.25	3.8	$\pm$	0.1	3.20	5.60	$\pm$	0.30	3.50	3.61	$\pm$	0.11
3.10	4.0	$\pm$	0.2	3.00	5.90	$\pm$	0.11	3.25	3.63	$\pm$	0.10
3.00	4.0	$\pm$	0.1	2.90	5.80	$\pm$	0.20	3.10	3.97	$\pm$	0.13
2.50	4.1	$\pm$	0.1	2.75	5.92	$\pm$	0.08	3.00	3.86	$\pm$	0.12
2.00	4.3	$\pm$	0.1	2.50	5.85	$\pm$	0.07	2.90	3.92	$\pm$	0.12
1.50	4.7	$\pm$	0.2	2.25	6.10	$\pm$	0.06	2.80	4.11	$\pm$	0.12
1.25	5.0	$\pm$	0.1	2.10	6.10	$\pm$	0.19	2.70	4.08	$\pm$	0.12
1.00	5.1	$\pm$	0.2	2.00	6.16	$\pm$	0.09	2.60	4.17	$\pm$	0.09
0.75	5.7	$\pm$	0.2	1.75	6.24	$\pm$	0.08	2.40	4.14	$\pm$	0.10
0.50	5.9	$\pm$	0.1	1.50	6.71	$\pm$	0.07	2.20	4.05	$\pm$	0.10
0.30	7.2	$\pm$	0.2	1.25	7.06	$\pm$	0.08	2.00	4.29	$\pm$	0.09
0.20	7.5	$\pm$	0.2	1.00	7.42	$\pm$	0.07	1.50	4.33	$\pm$	0.11
0.10	8.9	$\pm$	0.4	0.80	7.81	$\pm$	0.07	1.00	4.90	$\pm$	0.12
0.05	8.9	$\pm$	0.5	0.60	8.43	$\pm$	0.11	0.75	5.25	$\pm$	0.11
0.02	9.7	$\pm$	0.7	0.40	9.61	$\pm$	0.15	0.50	5.39	$\pm$	0.13
0.01	10.5	$\pm$	0.7	0.20	11.27	$\pm$	0.12	0.30	5.70	$\pm$	0.10
				0.10	13.45	$\pm$	0.13	0.10	6.88	$\pm$	0.10
				0.08	14.20	$\pm$	0.16	0.08	7.34	$\pm$	0.11
				0.06	15.25	$\pm$	0.17	0.05	7.79	$\pm$	0.13
				0.03	17.6	$\pm$	0.2	0.03	8.80	$\pm$	0.14
				0.02	19.0	$\pm$	0.2	0.02	9.18	$\pm$	0.14
				0.01	20.4	$\pm$	0.3	0.01	9.87	$\pm$	0.15
				0.008	20.9	$\pm$	0.3				
				0.006	21.5	$\pm$	0.4				
				0.004	23.2	$\pm$	1.2				

C31				C33				C34			
ν_L (MHz)	R_1 slow	$\pm$	(s ⁻¹)	ν_L (MHz)	R_1 slow	$\pm$	(s ⁻¹)	ν_L (MHz)	R_1 slow	$\pm$	(s ⁻¹)
10.00	1.52	$\pm$	0.06	10.00	3.0	$\pm$	0.2	10.00	2.4	$\pm$	0.2
8.00	1.60	$\pm$	0.06	8.00	2.9	$\pm$	0.3	6.00	2.6	$\pm$	0.3
6.00	1.71	$\pm$	0.08	6.00	3.5	$\pm$	0.7	4.00	3.1	$\pm$	0.2
4.00	2.10	$\pm$	0.15	3.50	5.2	$\pm$	0.4	2.95	3.2	$\pm$	0.3
3.00	2.40	$\pm$	0.10	3.30	5.0	$\pm$	0.4	2.90	3.6	$\pm$	0.2
2.90	2.49	$\pm$	0.11	3.20	5.9	$\pm$	0.4	2.85	3.5	$\pm$	0.2
2.80	2.46	$\pm$	0.08	3.10	5.0	$\pm$	0.4	2.80	3.4	$\pm$	0.3
2.70	2.61	$\pm$	0.14	2.95	5.2	$\pm$	0.4	2.75	3.0	$\pm$	0.2
2.65	2.53	$\pm$	0.12	2.90	4.7	$\pm$	0.3	2.70	3.3	$\pm$	0.2
2.60	2.58	$\pm$	0.12	2.85	5.7	$\pm$	0.4	2.60	3.3	$\pm$	0.2
2.55	2.60	$\pm$	0.13	2.80	5.7	$\pm$	0.3	2.50	3.9	$\pm$	0.2
2.45	2.74	$\pm$	0.12	2.70	6.0	$\pm$	0.3	2.40	3.2	$\pm$	0.2
2.40	2.66	$\pm$	0.08	2.60	6.1	$\pm$	0.4	2.20	3.9	$\pm$	0.2
2.35	2.70	$\pm$	0.15	2.50	5.9	$\pm$	0.3	2.00	3.5	$\pm$	0.2
2.25	2.91	$\pm$	0.12	2.30	5.9	$\pm$	0.3	1.80	3.2	$\pm$	0.3
2.15	2.71	$\pm$	0.13	2.05	6.3	$\pm$	0.4	1.60	3.2	$\pm$	0.2
2.10	2.89	$\pm$	0.12	2.00	6.0	$\pm$	0.4	1.40	3.8	$\pm$	0.2
2.05	2.61	$\pm$	0.08	1.90	6.1	$\pm$	0.3	1.20	4.1	$\pm$	0.2
2.00	2.72	$\pm$	0.08	1.80	6.4	$\pm$	0.4	1.00	3.9	$\pm$	0.2
1.95	2.65	$\pm$	0.11	1.70	6.6	$\pm$	0.4	0.75	3.8	$\pm$	0.2
1.90	2.63	$\pm$	0.12	1.60	6.2	$\pm$	0.4	0.50	4.7	$\pm$	0.3
1.80	2.67	$\pm$	0.10	1.40	6.3	$\pm$	0.5	0.30	4.5	$\pm$	0.3
1.60	2.63	$\pm$	0.09	1.20	6.5	$\pm$	0.5	0.20	5.0	$\pm$	0.4
1.40	3.05	$\pm$	0.09	1.00	7.3	$\pm$	0.4	0.10	6.7	$\pm$	0.4
1.00	3.33	$\pm$	0.09	0.80	7.9	$\pm$	0.5	0.08	6.5	$\pm$	0.4
0.50	3.80	$\pm$	0.13	0.60	9.7	$\pm$	0.5	0.05	6.5	$\pm$	0.4
0.30	4.4	$\pm$	0.2	0.40	10.8	$\pm$	0.5	0.03	6.5	$\pm$	0.4
0.10	5.5	$\pm$	0.2	0.20	12.5	$\pm$	0.5	0.02	7.9	$\pm$	0.6
0.08	5.9	$\pm$	0.2	0.10	15.9	$\pm$	0.9	0.01	8.6	$\pm$	0.4
0.05	7.0	$\pm$	0.3	0.08	18.8	$\pm$	0.9				
0.03	8.0	$\pm$	0.3	0.06	22.8	$\pm$	2.0				
0.01	9.0	$\pm$	0.4	0.04	25.3	$\pm$	1.4				
				0.02	33.6	$\pm$	2.5				
				0.02	28.7	$\pm$	2.6				
				0.01	33.3	$\pm$	1.5				

6 – Release abilities of Nanosponges: preliminary assessment study

6.1 Introduction

The ability to exchange (i.e. adsorb or release) guest molecules with a solvent medium is an essential property for sorbents materials, in view of any possible application. In particular, the ability to release drugs at a controllable rate is an essential property for designing effective therapeutic systems.^{7,77,85,132,147,172,312-316} In fact, the purpose of these systems is to maintain the concentration of the bioactive molecule more or less constant in the body for as long as possible, in order to optimize the action of the drug itself. Indeed, if an active principle is administered via a too rapid release, the concentration of the drug released is in the so-called therapeutic range for too short a time; it is therefore necessary to repeat the dosage for keeping the amount released constant over time.^{313,317} Hence, optimized release constitutes a prerequisite for the optimal absorption of the bioactive molecule, since it regulates both the rate of assimilation and the availability of the therapeutic agent for the organism.

Furthermore, it is worth stressing that the release kinetic profile perfectly mirrors the adsorption kinetics. Therefore, studying the release of a model guest can be helpful also for designing systems devoted to the adsorption or sequestration of organic species, for instance in view of environment remediation applications. At this purpose, it must be kept in mind that in both the adsorption and the release processes, an *equilibrium* actually occurs, i.e. a dynamic situation in which the guest undergoes a phase partition between the environment constituted by the solvent medium and the nanosponge adsorbent solid. At equilibrium the solvent-to-sorbent and the sorbent-to-solid processes proceed at the same rate. According to classical chemical kinetics, the apparent rate constant for both the adsorption or the release overall processes is simply the sum of the real constants of the two elementary processes. Notably, this situation indeed occurs for any equilibrium reaction and, in the case of

processes occurring in homogeneous solution, a simple first-order kinetic profile should be expected. Conversely, for non-homogeneous processes a more complex situation occurs.

The theoretical problem of the release from a sorbent medium has been addressed in literature³¹⁸⁻³²¹ exploiting various approaches. Different mathematical models for suitably describing the dependence of the concentration of a released bioactive molecule in time have been developed. These models undoubtedly constitute an important tool for extrapolating the physical parameters of the release, for instance the diffusion coefficient of the drug.³²²⁻³²³ It should be specified that the release of a drug from a system can take place in different ways, i.e. through dissolution, diffusion, osmosis, erosion, swelling.³¹⁸ Typically, when a hydrophilic drug is incorporated into a matrix, release occurs by diffusion, whereas the release of hydrophobic molecules is usually associated with swelling and/or erosion of the matrix.³²² Diffusion of the released molecule is a function of the structure of the matrix, for example of the morphology of the polymer.³¹⁵

It is therefore clear that the development of a mathematical model requires an understanding of all the phenomena that affect the release. The model can be indeed considered a "*mathematical metaphor*" of certain aspects of reality, which in this case is identified in the set of phenomena that govern the release kinetics of bioactive molecules.

6.2 Mathematical models for describing drug release: the state of the art

In this section the most common models used for rationalizing the time dependence of the concentration of a released guest molecule will be shortly presented. However, as it will be discussed later, the results obtained in this work required a more sophisticated physical approach, which involves non-trivial mathematical problems.

Zero-order kinetics.³²² Zero-order release kinetics are observed for therapeutic systems capable of releasing the bioactive molecule for a certain period at a constant rate. The release is described by an equation of the type

$$\frac{dM}{dt} = \frac{D \cdot S}{l} (C_s - C) \quad (1)$$

where M is the mass of solute dissolved up to instant t (considering $t_0 = 0$), dM/dt is the dissolution rate of the mass M , D is the diffusion coefficient of the solute in solution, S is the

area of the solute exposed, l is the thickness of the diffusion layer, C_s is the solubility of the solid at equilibrium and C is the concentration of the solute in solution at time t . Since the concentration is mass M on volume V of solution, we rewrite the equation (1) as

$$\frac{dC}{dt} = \frac{D \cdot S}{V \cdot l} (C_s - C) \quad (2)$$

known as the Noyes-Whitney equation, where dC/dt is the rate of dissolution.³²⁴

The dissolution of solutes included in systems that do not disintegrate upon release, considering a very low drug emission rate (at any instant C is much smaller than C_s), can be represented by the equation

$$W_0 - W_t = k \cdot t \quad (3)$$

where W_0 is the initial drug concentration, W_t is the concentration at time t and k is the constant of proportionality. Dividing equation (3) by W_0 we get

$$f_t = k_0 \cdot t \quad (4)$$

where f_t is the fraction of bioactive molecule dissolved up to instant t and k_0 is the apparent constant of the dissolution rate. The graphical representation is shown in Figure 6.1.

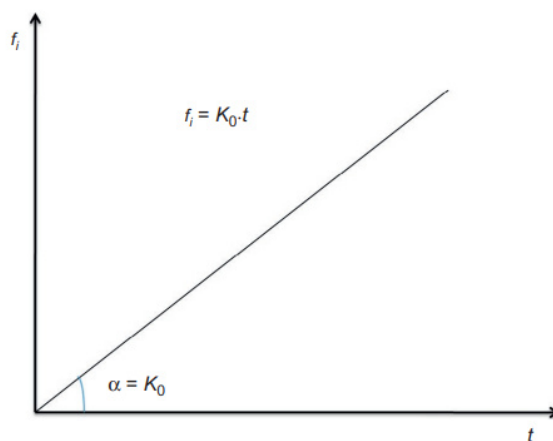


Figure 6.1. Graphical representation of zero-order kinetics.

Expressing the equation (4) in terms of concentration, one can write

$$C_t = C_0 + k_0 \cdot t \quad (5)$$

C_t is the drug concentration released up to time t , C_0 is the initial concentration (usually $C_0 = 0$), and k_0 is the zero-order kinetic constant. In zero order kinetics, the rate of release depends only on time and not on the concentration of the bioactive molecule in solution.

First order kinetics. ^{322,324} In the case of first order release kinetics (Figure 6.2), the rate depends linearly on the concentration of dissolved drug, according to the Hixson-Crowell equation:

$$\frac{dW}{dt} = KS(C_s - C) \quad (6)$$

where $W = (C \cdot V)$ is the quantity of drug solubilized up to instant t , dW/dt is the dissolution rate. Multiplying both sides by V , consider $K = k_l V$ and $k = k_l S$, the equation (6) becomes

$$\frac{dW}{dt} = \frac{KS}{V}(VC_s - W) = k(VC_s - W) \quad (7)$$

Assuming S constant, after integration, we obtain

$$W = VC_s(1 - e^{-kt}) \quad (8)$$

which can be rewritten as

$$C(t) = C_s(1 - e^{-kt}) \quad (9)$$

There are several therapeutic systems that feature this type of release. For active ingredients included in porous matrices, the quantity of drug released is proportional to that contained in the matrix; in fact, after some time, the release stops.

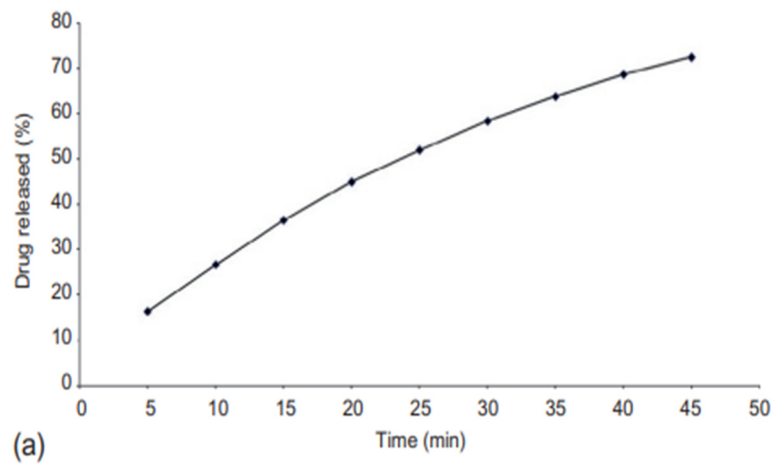


Figure 6.2. Graphical representation of first order release kinetics.

The equation (10) is a mono-exponential equation. Sometimes, it cannot accurately describe the profile of a release. In these cases, a good fit can be obtained by adding a new exponential term to the function, obtaining equations of the type

$$C(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \quad (10)$$

A , B , α and β are fitting parameters, with $\alpha > \beta$. When α is much larger than β , and when t is high enough, the first term ($Ae^{-\alpha t}$) is negligible with respect to the second, so $C(t)$ is well described by the monoexponential function.³²⁵

Other kinetic models.³²² In 1961, Higuchi published one of the most famous mathematical equations to describe matrix drug release.³²⁶ This model applies to both water-soluble and insoluble molecules, embedded in solid or semi-solid matrices. The simplified equation is:

$$f_t = Q = k_H \sqrt{t} \quad (11)$$

where f_t is the amount of drug released up to time t per unit area, while k_H is the Higuchi constant. The model in question can be used if the following conditions are met:

- The matrix contains an initial drug concentration much higher than the drug solubility;
- Diffusion is unidirectional;
- The thickness of the pharmaceutical form is much greater than the size of the bioactive molecule;
- Swelling or dissolution of the matrix is negligible;
- The diffusivity of the drug is constant.

The Higuchi equation³²⁶⁻³²⁸ has allowed the development of other models, including the Baker-Lonsdale³¹⁶ (12) and the Korsmeyer-Peppas³²⁹ (13). The first describes the controlled release of drugs from spherical matrices; the second was designed for the release of molecules from polymeric matrices, such as hydrogels.

$$f_t = \frac{3}{2} \left[1 - \left(1 - \frac{M_t}{M_\infty} \right)^{\frac{2}{3}} \right] - \frac{M_t}{M_\infty} = k \cdot t \quad (12)$$

$$f_t = \frac{M_t}{M_e} = k \cdot t^n \quad (13)$$

where f_t is the amount of drug released, M_t is the amount of drug released at time t , M_∞ is the amount of drug released at infinite time, M_e is the amount of drug at equilibrium, k is the release constant, n is the release exponent (relative to the mechanism of the same).

6.3 Kinetic tests for *p*-nitroaniline release

As discussed in the Introduction Chapter, a main goal of the present PhD thesis work was to clarify the possible mechanisms of interaction between nanosponge materials and organic species, specifically paying attention to the relationships between structure and release abilities. In perspective, this study aimed at collecting useful information, helpful for rationalizing the design of effective and versatile drug carrier/delivery systems. For this purpose, it was chosen to proceed stepwise. First, based on the results from the inclusion tests discussed previously, a panel of different materials was selected for studying the kinetics of release of a suitable structurally simple model guest. In particular, the *p*-nitroaniline was selected for this first part of the work, also because it has been a classical model guest for investigating the binding abilities of cyclodextrins and calixarenes in solution from a microscopic standpoint. These experiments constituted a preliminary screening in order to select the most promising materials for the second step, i.e. a study on the adsorption and release of structurally more complex model bioactive molecules. In particular, Rosmarinic acid, Quercetin and Folic acid were selected because of their interesting properties, as it will be discussed more in detail in Chapter 7. As long as the materials are concerned, representative nanosponges from the three main groups were chosen, in order to keep into account all the possible structural varieties provided by: *i*) the different host subunits (i.e. cyclodextrin-only, calixarene-only or mixed materials); *ii*) the different linker or chain extender (i.e. the non-functionalized H and D subunits, the acidic carboxyl group bearing P subunits, or the polyamine A subunits); *iii*) the host-to-linker combination ratio; *iv*) the variants of the synthetic procedure. Hence, the cyclodextrin-only materials C1, C2_[125], C3, C11_a, C12, C15_[125], C16, C18, the calixarene-only materials K1 and K2, and the mixed materials CK1, CK2, CK3 and CK5_c were selected. Together with these materials, for suitable comparison purposes, a further polyaminocyclodextrin-nanosponge was considered (hereinafter indicated as CA), obtained in previous works by reacting the *per*-(6-iodo)-(6-deoxy)- β -cyclodextrin with the *per*-(6-[3-methyl-3-(3-aminopropyl)]-propylamino)-(6-deoxy)- β -cyclodextrin (Figure 6.3). This particular material was chosen because of its basic character provided by the large presence of triamine linker subunits, which ensure a large sensitivity to pH conditions (the amine groups easily undergo protonation, making the nanosponge scaffold positively charged).

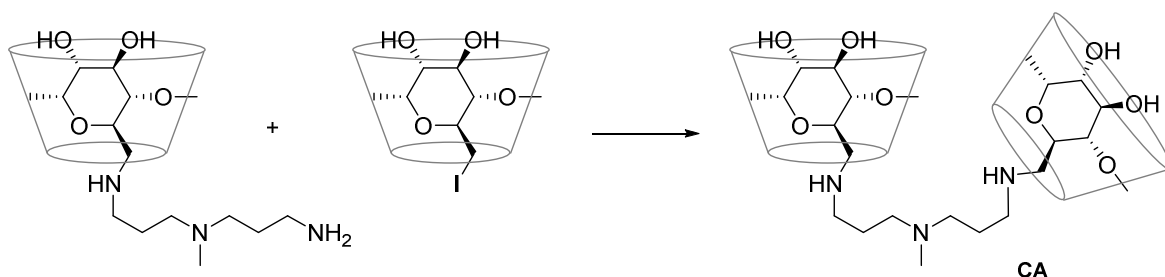


Figure 6.3. Synthesis of the polyamino-cyclodextrin nanosponge CA

In brief, from an experimental standpoint, the chosen materials were preliminarily loaded with the *p*-nitroaniline to obtain suitable composites, which in turn were used for performing the subsequent release kinetics experiments. Loading was basically performed (see Experimental section) by equilibrating at r.t. the material with a buffered (pH 4.4) aqueous, nearly saturated solution of the guest. The amount of actually loaded guest was subsequently determined by eluting it from the composite with methanol, and then determining spectrophotometrically the concentration of the eluted guest in the washing liquors. The release kinetics experiments were performed at 40 °C (for consistency with previous works) in buffered solution at pH 4.4 with the simple “tea-bag” method (see Experimental section). The results obtained are collected in Table 6.1.

Table 6.1. Release kinetics data for <i>p</i> -nitroaniline-loaded materials					
NS	constituents and mole/mole ratio	Loading % w/w	$k_{app} \cdot 10^4 \text{ s}^{-1}$	n	Guest conc. at equilib. (μM)
C1	C/H 1:2	3.0	2.8 ± 0.2	0.74 ± 0.04	37 ± 2
C2 _[125]	C/H 1:3	3.8	8.6 ± 0.7	0.72 ± 0.06	109 ± 2
C3	C/H 1:4	9.0	4.8 ± 0.3	0.76 ± 0.04	50 ± 1
C11 _a	C/H/P 1:4:4	3.7	5.9 ± 0.5	0.70 ± 0.05	97 ± 2
C12	C/H/P 1:8:4	7.0	1.7 ± 0.1	0.78 ± 0.02	121 ± 2
C15 _[125]	C/H/D 1:6:4	3.6	9.5 ± 0.4	0.81 ± 0.04	80 ± 1
C16	C/H/D 1:3:1	4.0	14.0 ± 0.7	0.85 ± 0.04	68 ± 1
C18	C/H/D 1:6:5	3.5	14.7 ± 0.9	0.67 ± 0.04	65 ± 1
CA	(PolyaminoCD)	3.0	18.3 ± 0.1	0.63 ± 0.04	59 ± 1
K1	K/H 1:2	7.1	1.4 ± 0.9	0.38 ± 0.04	91 ± 13
K2	K/H/A 1:8:6	3.1	2.6 ± 0.2	0.94 ± 0.07	66 ± 1
CK1	C/K/H 1:1:8	6.5	4.1 ± 0.2	0.80 ± 0.02	75 ± 2
CK2	C/K/H 1:1:4	3.4	5.6 ± 0.5	0.67 ± 0.05	20 ± 1
CK3	C/K/H 0.5:1:8	5.9	4.2 ± 0.2	0.75 ± 0.06	83 ± 1
CK5 _c	C/K/H/A 0:5:1:8:3	2.0	11.0 ± 0.2	0.94 ± 0.02	30 ± 1

As a preliminary observation, it was unexpectedly found that the experimental release kinetics data could be best modelled as apparent “*stretched-first-order*” kinetics trends, i.e. they could be adequately subjected to regression analysis by means of the equation:

$$C_{\text{released guest}}(t) = C_{\text{eq}} \cdot \{1 - \exp[-(k_{\text{app}} \cdot t)^n]\} \quad (14)$$

where the exponential “*stretching*” parameter n accounts for the deviation of the kinetic course from the standard first-order trend (n values range between 0 and 1; for $n = 1$ an ordinary first-order kinetic occurs). This surprising behavior, which is different from the usual release models described in the previous section, deserved a careful consideration. Indeed, the observation of a stretched exponential trend has been occasionally reported in literature³³⁰⁻³³² in various contexts (as discussed in Chapter 3, it has been observed in particular in the case of the relaxometric kinetics), and has been generically justified claiming for the occurrence of some “inhomogeneity” (whatever the term has to be intended) in the behavior of the system. Therefore, the collected kinetic data were also subjected to Inverse Laplace transform analysis. As a result, quite large but unimodal distributions of the kinetic constants were found. This result clearly indicated that the simple first-order mono-exponential trend was actually unable to adapt the experimental data, but at the same time, it ruled out the possibility that fitting analysis could be performed by means of a double exponential model.

In order to clarify the point, and taking inspiration from the discussion of the FFC-NMR technique reported in Section 3.4, one can consider that, due to its highly disordered microscopic structure, the nanosponge material might be considered as a *continuum* of microphases (rather than a single phase) having different release rate constants. Hence, the release (or, better, the equilibration) kinetics can be expressed as an integral form, i.e.:

$$C_{\text{released guest}}(t) = C_{\text{eq}} \left(1 - \int_{k=0}^{\infty} e^{-kt} D(k) dk\right) \quad (15)$$

where C_{eq} is the final equilibrium concentration and $D(k)$ is a suitable distribution function of k values. Then, to verify that this hypothesis is consistent the peculiar kinetic trends observed, a numeric analysis approach was exploited. In particular, a series of simulated kinetic datasets (“*synthetic*” datasets) were generated based on the previous integral form, by assuming a “reasonable” form for the distribution function $D(k)$, and attributing

“reasonable” numerical values to all the needed parameters, in such a way that the obtained synthetic datasets resulted comparable with true experimental data. The synthetic datasets were then subjected to regression analysis (pretending them to be true experimental data), using both the simple exponential and the stretched exponential fitting models. Interestingly, it turned out that, if $D(k)$ is a simple Gaussian distribution, i.e. if k values are normally distributed around an average k_0 value, according to the relation:

$$D(k) = \frac{1}{\sigma\sqrt{2\pi}} \exp \left[-\frac{1}{2} \left(\frac{k-k_0}{\sigma} \right)^2 \right] \quad (16)$$

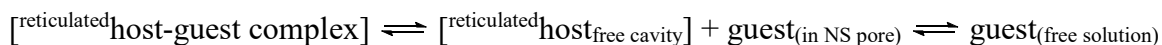
then the trend of the synthetic dataset is almost indistinguishable from a simple exponential trend, with an apparent rate constant k_{app} nearly equal to k_0 , even if the distribution is relatively large (i.e. for k_0/σ values up to 0.35 ca). Conversely, a stretched exponential trend is achieved in the case of a normal distribution of the $\ln k$ values, i.e. for:

$$D(k) = \frac{1}{\sigma\sqrt{2\pi}} \exp \left[-\frac{1}{2} \left(\frac{\ln(k)-\ln(k_0)}{\sigma} \right)^2 \right] \quad (17)$$

This finding is very interesting, because such a distribution, in turn, implies a Gaussian distribution of the relevant activation energies, i.e.:

$$D'(E^\ddagger) = \frac{RT}{\sigma'\sqrt{2\pi}} \exp \left[-\frac{1}{2} \left(\frac{E^\ddagger-E_0^\ddagger}{\sigma'} \right)^2 \right] \quad (18)$$

The latter result is perfectly sensible. In fact, it must be kept in mind that each single host unit constituting the hyper-reticulated polymer does not benefit of the conformational flexibility occurring in free solution, but is constrained in a particular distorted and relatively stiff conformation. Consequently, the interaction between the guest and the host units of the nanosponge does not provide a single value for the binding energy, but rather a (likely Gaussian) distribution of the binding energies. Considering that the release phenomenon can be reasonably depicted as a multistep equilibrium, in which the guest is distributed between the host cavities, the nanosponge pores and the free external aqueous medium, i.e.:



it is immediately apparent that the binding energies provide a significant contribution to the overall activation energy of the release process. Hence, the existence of a distribution for binding energies must imply a specular distribution of the activation energies.

A deeper examination of the numerical results from the study of the synthetic datasets provided further interesting insights. The fitting analyses showed that, if the standard deviation σ' of the activation energies is relatively small (less than 0.4 kJ mole⁻¹), then the stretching parameter n is large enough (> 0.92) to make the data trend barely distinguishable from a simple non-stretched exponential (differences between k_{app} and k_0 values are less than 5%). As σ' value increases, n value decreases, and the discrepancy between the fitted k_{app} and the theoretical k_0 values becomes larger and larger. The synthetic dataset can be still reasonably regressed as a stretched exponential up to relatively large σ' values, ca. 2.0 kJ mole⁻¹; for σ' values exceeding this limit the quality of the stretched exponential fitting is no longer acceptable.

On passing to consider now the experimental results, it can be first observed that most of the stretching parameter n values range from 0.63 up to 0.85, suggesting an average standard deviation for activation energies roughly evaluable around 0.7 kJ mole⁻¹. As long as k_{app} values are concerned, data overall suggest that the possible presence of ionizable groups in the nanosponge scaffold plays an important role in facilitating the release of the guest. For instance, the polyamine-cyclodextrin material CA releases much faster than the polyurethane-cyclodextrin materials C1 and C3. Similarly, the beneficial effect from the presence of ionizable linker or chain extender units is apparent as faster release occurs for C11_a compared to C12, for K2 compared to K1, and for CK5_c compared to CK3. The effect of ionizable groups can be ascribed to the consequent highly hydrophilic character of the material. Indeed, it can be noted that on average cyclodextrin-only materials release faster than mixed materials, which in turn averagely release faster than calixarene-only materials. The latter observation positively indicates an effect from the hydrophilic character of the host units, and outlines the fact that, along with the bare host-guest interaction, the mobility of the solvent medium within the pore network of the nanosponge is also very important. This leads to consider more in detail the physical/textural features of the materials. Experimental results provide interesting indications also in this sense. For instance, the highly swellable material C2_[125] obtained with the “*smart dilution*” releases much faster than C1 and C3, despite the fact that in these three cases no chain extender has been used.

Consistently, these materials show a slower release than C15, C16 and C18 prepared with the use of the diol D as linker chain extender.

As discussed in Chapter 3, the mobility of the solvent medium can be assessed by the FFC-NMR technique. However, a comparison between relaxometric results (collected in Tables 3.5, 3.6 and 3.7) and release rates is not straightforward, just because of the concomitant contribution to the overall process provided by binding phenomena. Nevertheless, careful analysis provides some further interesting insights. For instance, as long as the strictly related materials C1, C2_[125] and C3 are compared, release rates do not apparently correlate with the correlation times $\tau_{c,1}$ and $\tau_{c,2}$ relevant to the fastest dynamic regimes, but rather with the value $\tau_{c,long}$ relevant to the slowest regime. As long as the latter one may be interpreted as accounting for the motions related to the surface-pore exchange of water molecules, this somehow surprising result again suggests that the overall release rate is mostly affected by the first host-guest complex dissociation equilibrium step, rather than by the second in-pore diffusion step. Therefore, in order to accomplish a correct comparison between relaxometric and release rate results, it is convenient to split out preliminarily the systems considered into structurally homogeneous subsets, namely: *a*) the cyclodextrin-only materials C1, C2, C3 and CA bearing the short-chain linker units H or A; *b*) the cyclodextrin-only materials C11 and C12 prepared with the pyromellitic linker P; *c*) the cyclodextrin only materials C15, C16 and C18 bearing the long-chain linker units deriving from H with the diol chain extender D; *d*) the mixed cyclodextrin-calixarene materials CK1, CK2, CK3 and CK5; *e*) the calixarene-only materials K1 and K2. Cross data analysis shows interesting regularities. In particular, as long as short-chained materials subsets *a*), *b*), *d*) and *e*) are separately considered, the fastest release is always found for the materials (CA, C12, CK5 and K2, respectively) showing the shortest $\tau_{c,long}$ value and the largest hydrophilic character (accounted for by the water surface fraction parameter f_s). Noticeably, the materials CA, K2 and CK5 are those bearing the ionizable polyamine subunits. Moreover, considering in particular the *a*) subset, it is interesting to notice the intermediate position of C2, which is perfectly mirrored in the f_s values trend. Notably, in all these cases data trends do not show any apparent correlation with the pore connectivity index *PCI* values. All these findings provide full confirmation for the idea that release is more strictly controlled by host-guest dissociation than by in-pore mobility. Interestingly, for the long-chained materials subset *c*) a different behavior occurs, because in this case no correlation at all with relaxometric data is apparent. More in detail, these materials release faster than the short-chained ones of subset *a*). The slowest release is found for C15, showing the lowest *PCI* value. Conversely, C16 and C18 show similar release

rates; however, the former one has the largest *PCI* value, whereas the latter one has the lowest affinity (according to the results from the inclusion tests reported on Section 4.2, Table 4.1) for the guest. Therefore, a sort of compensation occurs between in-pore mobility and affinity. Of course, these differences with respect to the other subsets may be specifically attributed to the ability of the longer linker units to provide the material with widest pores. In addition, it is worth stressing that comparison between different subset is made difficult by the non-strict structural homogeneity. In general, cyclodextrin-only materials of subsets *a*) and *c*) release averagely faster than mixed materials, which in turn release faster than calixarene-only materials, once again outlining the importance of the hydrophilic character of the nanosponge. The exceptionally low release rate of C12 (perfectly mirrored by its large $\tau_{c, \text{long}}$ value) can be likely justified by its exceptionally large hydrophobic character attributable to its peculiar combination ratio: the large presence of the hydrophobic H constituent probably causes extensive association between the linker chains and clusterization of water molecules due to hydrophobic effects.

6.4 Concluding highlights

The kinetics of the release for the model *p*-nitroaniline guest considered were studied in order to clarify the role of the nanosponge structure. An unexpected stretched first order kinetics profile occurs in all the case examined, which can be rationalized assuming the materials as a continuum of microsystems with a Gaussian distribution of the activation energies for the release process. This, in turn suggests that a main role in the release, which can be suitably modelled as a two-step process, is assumed by the egress of the guest from the host cavity. Indeed, host units experience a significant structural distortion and a large decrease of its conformational rearrangement freedom because of reticulation, resulting in a Gaussian distribution of binding energies that provide a contribution to the release activation energy. The whole of the results collected outlines how the hydrophilic character of the materials plays an important role in determining the release rates. The effect of ionizable groups can be ascribed to the consequent highly hydrophilic character of the material. Along with the bare host-guest interaction, the mobility of the solvent medium within the pore network of the nanosponge is also very important. A comparison between relaxometric results and release rates confirmed that the overall release rate is mostly affected by the first

host-guest complex dissociation equilibrium step, rather than by the second in-pore diffusion step. Notably, in some cases data trends do not show any apparent correlation with the pore connectivity index *PCI* values.

6.5 Experimental section

6.5.1. *Preparation of loaded composites*

The composites used for the release tests were prepared as follows. The nanosponge (80 ± 0.05 mg) was placed in a screw-cap vial and 19.0 mL of a buffer solution at pH 4.4 (see Section 4.4) were added. A solution of *p*-nitroaniline is prepared separately by dissolving 200.0 mg of substance in 10 mL of methanol; then 1.0 mL of solution is loaded into a Chemetron-type screw microsyringe. The methanol solution is then added to the aqueous suspension of the material at a rate of approx. $20 \mu\text{L min}^{-1}$. The system was allowed to shake mechanically overnight at room temperature. The supernatant liquor was carefully pipetted and the solid was collected by filtration and dried in vacuum over P_2O_5 overnight.

6.5.2. *Determination of the loading percentage*

A carefully weighed small amount (ca. 4.00 ± 0.05 mg) of composite was placed in a vial and suspended with 2.0 mL of methanol; the suspension was sonicated and the solid was centrifuged for 5 min at 5500 rpm. This washing procedure was iterated more times, unless the washing liquor was colorless. The collected extracts were placed in cuvettes and the concentration of the solution obtained was determined spectrophotometrically. The loading is determined by considering the additivity of the absorbances of all the washings, known the ϵ in methanol of the molecule included ($\epsilon = 16600 \pm 300$), estimated through the construction of the appropriate calibration lines.

6.5.3. *Release kinetics (“tea-bag” method)*

Release kinetic profiles were determined as follows. A carefully weighed amount (10.00 ± 0.05 mg) of each composite was placed in a dialysis membrane (Medicell International Ltd MWCO 12–14 000, 21.5 mm diameter), dispersed with 1.0 mL of the chosen extraction medium (i.e. an aqueous buffer at pH 4.4). The membrane was then put in a round bottom flask containing 20.0 mL of the release medium thermostated at 40 °C under magnetic stirring. At given times, 1.0 mL of the release medium was withdrawn and analyzed

spectrophotometrically, to determine the concentration of released guest. To keep the volume of the release medium constant, 1.0 mL of fresh extraction medium was added each time to replace the withdrawn one.

7 – Release of model bioactive molecules

7.1 Introduction

The preliminary study on the release of *p*-nitroanilines presented in the previous chapter provided the necessary bases to address the exploitation of nanosponges for the design of advanced drug carrier/release systems. Indeed, one of the main difficulties encountered when designing pharmaceutical products is ensuring that the biologically active molecules are released continuously into the body for a certain period of time. The sudden action of a drug, unless it is desired, leads to its excessive intake in a short time, and to the total absence of treatment in the following period; this can be followed by a series of unwanted side effects for the body.⁶⁹ Other obstacles in the pharmaceutical formulation could be:⁷

- The bioactive molecule is not soluble in water, but only in apolar solvents which cannot be introduced directly into the body, such as for example paclitaxel, camptothecin and tamoxifen;
- The drug irritates the mucous membranes, tissues or skin;
- The drug has an unpleasant taste (when taken orally);
- The drug is sensitive to light, oxygen, water;
- The drug is a volatile liquid, has a foul odor, or is a hygroscopic solid;
- The bioactive molecule is immiscible with the rest of the ingredients in the formulation.

Encapsulating the drug can be an excellent strategy to overcome the drawbacks listed above. For this purpose, Nanosponges (NSs) are excellent candidates. In fact, their porous structure allows the bioactive molecules to be trapped and released in a controlled manner over a relatively long period of time (12-24 hours). This makes it possible to increase the stability of the molecules included, to prevent them from being dispersed in the body, but also, from an industrial and practical point of view, to make a drug that is difficult to handle solid (in

the form of powder).⁶⁹ The β -cyclodextrin-based nanosponges are particularly interesting in this context, because of their low toxicity, insolubility in most organic solvents and good thermal stability.⁷² These are all characteristics that make them interesting polymers to be used as drug carriers.^{142,150,333} According to literature, in order to be effectively included in nanosponges, the guest should preferably possess a series of characteristics, in particular a molecular mass between 100 and 400 Da and less than five condensed rings in its structure.⁶⁹ The first requirement derives from the practical need to obtain a pharmaceutical formulation having a “reasonable” weight (in the order of hundreds of mg). Considering the molecular masses of cyclodextrins (972, 1132 and 1297 Da respectively for α -CD, β -CD, γ -CD), 100 mg of drug-CD complex would contain only 5-25 mg of bioactive molecule³³⁴ (neglecting the crosslinker and the other constituent of the pharmaceutical formulation). Furthermore, it is desirable that the water solubility of these species is indicatively lower than 10 mg mL⁻¹ and that they have a melting point lower than 250 °C.⁷ Of course, depending on the particular bioactive molecule, a tailored design of the nanosponge could be suitably required.

The problem of the supplementation of antioxidant agents has been particularly addressed in recent times,⁸⁶ because it is considered an effective method to modulate various redox processes, in particular the ones associated with apoptosis, cell aging and the related pathological conditions.³³⁵ Increased oxidative stress relates to systemic inflammatory processes, which often occur in degenerative and cancer pathologies,³³⁶ and also in abnormal immune responses (those observed in COVID-19-related pneumonia are a typical example).³³⁷ Antioxidants from plant extracts are largely used in antioxidant therapies, and preferred to synthetic drugs, because they are already present in the human diet and are believed to be safe.³³⁸ Among the natural compounds used as potential modulators of the free radical-induced cellular processes, curcumin, resveratrol, phenolic acids and flavonoids in general have been particularly attentioned.³³⁸⁻³³⁹ Unfortunately, polyphenols suffer for their oxidative instability in water solution and their low bioavailability.³⁴⁰⁻³⁴¹ The use of nanodelivery systems has allowed to enhance the solubility and stability of bioactive phytochemicals,³⁴²⁻³⁴⁴ the bioavailability can be also 5-10 times higher than that of native formulation.³⁴⁵ Various examples of antioxidants in nanoformulations, constituted by lipidic or polymeric nanosystems, have been recently reported,³⁴¹ and showed promising efficacy in preclinical studies.³⁴⁴ Cyclodextrin-based nanosponges have been already employed to improve the bioavailability of diversely structured polyphenols, such as resveratrol,³⁴⁶⁻³⁴⁷ oxyresveratrol,³⁴⁷⁻³⁴⁸ curcumin,^{86,349} quercetin,^{86,344,350} ferulic acid,³⁵¹ rutin,³⁵² phlorizin,³⁵² phenetyl caffeate⁸⁶ and chlorogenic acid.³⁵² For instance, interesting cytotoxic activity against breast cancer cells (SUM 149, SUM 259, MDA-MB-231) is reported in literature for

composites obtained by absorbing quercetin in mixed cyclodextrin-calixarene nanosponges.⁸³ Furthermore, the same nanosponge has shown significant protective ability towards quercetin, curcumin and phenethyl caffeate, favoring and modulating their antioxidant properties.⁸⁶ The inclusion into the nanosponges protected these antioxidants and provided a controlled release in an aqueous medium.

Considering the results obtained in the studies mentioned above, and on the grounds of the information collected and discussed in the previous Chapters, it seemed interesting to perform an investigation on the interaction and the release abilities of the nanosponge materials synthesized in this work with some bioactive compounds. In particular, in view of their peculiar properties and potential applications, three molecules were selected, namely Rosmarinic acid (RA), Quercetin (Q) and Folic acid (FA), that will be briefly presented in the next section.

7.2 Properties of Rosmarinic Acid, Quercetin and Folic Acid

Rosmarinic acid (RA, Figure 7.1) is an ester of caffeic acid with 3,4-dihydroxyphenyl-lactic acid, hence biosynthetically deriving from both the phenylpropanoid and the shikimate pathways. It is abundant in various medicinal plants of the *Lamiaceae* family, such as rosemary (*Rosmarinus officinalis*), mint (*Mentha ssp.*), lemon balm (*Melissa officinalis*) and in certain species used in medicine traditional Chinese, such as *Perilla frutescens*, *Salvia miltiorrhiza* and *Rabdosia rubescens*.

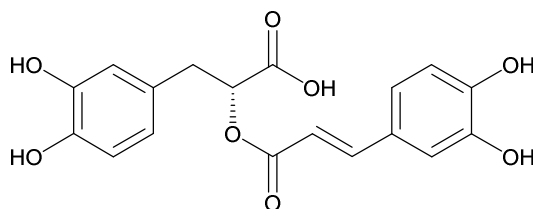


Figure 7.1. (R)-(+)-rosmarinic acid (RA).

Several extraction and separation protocols, as well as chromatographic methods, have been developed to optimize its extraction and identification.³⁵³ This compound shows excellent

antioxidant properties, which can be associated with the presence of the two catechol residues, which provide with the ability to penetrate lipid membranes, protecting them from oxidation.³⁵⁴ Other biological activities performed by this compound are anti-inflammatory, antimutagenic, cytotoxic, antimetastatic, neuroprotective, antimicrobial, immune-modulatory. To overcome the obstacles due to its hydrophobic character, which makes it difficult to use as such in the pharmaceutical and cosmetic fields, various strategies have been designed. In particular, its inclusion in polycaprolactone microspheres should be mentioned. The addition of zwitterionic and nonionic surfactants to the formulation improves the absorption capacity of the microspheres.³⁵⁵ Moreover, to increase the stability and water solubility of the RA, inclusion complexes in β -CD have been designed, by which also an increase in the antioxidant capacity of the molecule has been achieved.³⁵⁶⁻³⁵⁷ Rosmarinic acid has been proposed as an active compound against acne. In vivo experiments have been performed, via administration in the form of a gel, after including it in niosomes. The entrapment of the compound favors its slow release and bacterial multiplication was significantly reduced four days after applying the gel.³⁵⁸ Furthermore, Han *et al.*³⁵⁹ have proposed rosmarinic acid, inspired by oriental medicine, as a natural, less aggressive and cheaper alternative to the anticancer drugs currently employed. The cytotoxic action of RA was evaluated on HCT116, colon cancer cells. From in vitro studies, it was determined that rosmarinic acid has an IC₅₀ of 200 μ M after 48 hours. The bioactive compound acts on several fronts: it inhibits the cell cycle; causes apoptosis both extrinsically and intrinsically; prevents cells from migrating and adhering to tissues (through phosphorylation of AMPK, i.e. AMP-activated kinase), which causes metastasis.³⁵⁹ Furthermore, RA can improve the efficiency of radiotherapy by sensitizing to radiation cancer cells.³⁶⁰ From a technological point of view, rosmarinic acid has several other applications. For example, it is used as a bioprotective agent against fungal infections of crops.³⁶¹ It has been considered a good candidate for the partial replacement of monolignolol in lignin synthesis; this would improve the biodegradability of the polymer.³⁶² Finally, RA has been used to generate and stabilize gold and silver nanoparticles, acting as an antioxidant and surface protection agent.³⁶³

Quercetin (Q, Figure 7.2), whose name derives from the latin *Quercus* (oak), is a flavonoid known since the mid-19th century. It is found in various plants and fruits, which include apples, berries, capers, grapes, onions, tea and tomatoes, but also in many seeds, stones, flowers and leaves.³⁶⁴⁻³⁶⁵ The structure of quercetin largely justifies its antioxidant power, due to the presence of phenolic hydroxyls and double bonds. Its antioxidant and anti-inflammatory properties make it an interesting substance in the prevention and treatment of

cardiovascular diseases and cancer. Furthermore, quercetin also exhibits significant antibacterial activity.³⁶⁴

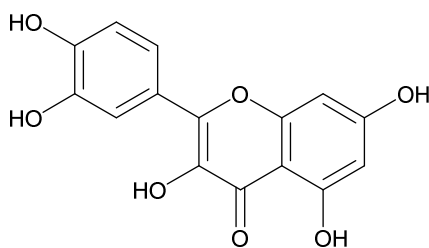


Figure 7.2. Structure of Quercetin (Q).

The antioxidant activity of quercetin is exerted in several ways. The first is the more direct one: Hanasaki *et al.* identified quercetin as the best free radical scavenger in the flavonoid family.³⁶⁶ Free radicals are produced by the body during metabolism and can cause a variety of problems, including damage to cell membranes, gene mutation, cardiovascular disease, liver damage and diabetes. The second mode of action of quercetin is to chelate a series of metal ions. One of the metals that controls the oxidative state of cells is certainly Fe^{2+} . Alcohol abuse leads to the presence of an excess of iron in the form of Non-Transferrin-Bound Iron (NTBI) in the blood, which can cause oxidative stress typical of alcoholic liver disease.³⁶⁷ Hence, the aforementioned flavonoid, by chelating Fe^{2+} , is able to inhibit the reactions induced by it, such as, for example, the Fenton reaction and the Haber-Weiss reaction.³⁶⁸⁻³⁷² These two processes lead to the formation of hydroxyl radicals, which, if generated near DNA, can cause its destruction. Similarly, the production of peroxy radicals via oxidative reactions can lead to lipid peroxidation, causing premature cell aging and cancer risk.³⁷³ Quercetin also exhibits antimicrobial properties; it has a good effect not only on bacteria, but also on fungi. Several experiments have shown that the molecule in question is effective in inhibiting the growth of pathogenic bacteria such as *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus* and *Aspergillus flavus*.³⁷⁴ Through TEM images, Wang *et al.* demonstrated that quercetin can destroy the cell membranes of *S. aureus* and that *E. coli* treated with quercetin undergoes cavitation and subsequent death.³⁷⁴

Among the applications of quercetin in pharmacology, its role as an anticancer is mentioned. It can prevent cell cycle, promote apoptosis and inhibit cell movement. Deng *et al.* found that quercetin induces apoptosis in MCF-7 (breast cancer cells) and inhibits their

proliferation; the effect is time and concentration dependent. In fact, cell viability decreases when the incubation time is extended, for the same dose. Furthermore, the speed of apoptosis when the dose is 40 mg/mL is much higher than that of 20 mg/mL.³⁷⁵ Another positive function of quercetin is that of anti-inflammatory and immunosuppressant; it is in fact a component of many anti-allergics. For example, comparing it with Cromolin (a well-known anti-allergic drug based on sodium cromoglycate), quercetin has a greater ability to inhibit IL-8 (interleukin 8); it also blocks the action of IL-6 and increases cytosolic calcium levels.³⁷⁶

Quercetin exerts beneficial effects in the treatment of cardiovascular pathologies, such as hypertension, atherosclerosis, ischemia and reperfusion damage. In fact, it is able to reduce arterial blood pressure; it limits the peroxidation of lipids in the heart and plasma and lowers the concentration of free fatty acids, phospholipids, cholesterol and triglycerides in the blood.³⁶⁴ Once again, given its antioxidant power, quercetin is able to inhibit the oxidation of LDL and endothelium-dependent vasodilation.³⁷⁷ As a further beneficial property of this flavonoid, it should be mentioned its ability to alleviate the toxic effects of mycotoxins.³⁷⁸

Folic acid (FA, Figure 7.3), more properly pteroylmonoglutamic acid, is a water-soluble vitamin, synthetic derivative of folate. In reality, the latter term refers to a series of pteroylglutamic acids with a variable number of glutamic acid chains. In plants, folates are present in which the glutamate chains have various lengths. For example, cauliflower flowers and tomato leaves contain mainly hexa- and hepta-glutamic folates; the tetra- and penta-glutamate forms are present in peas.³⁷⁹

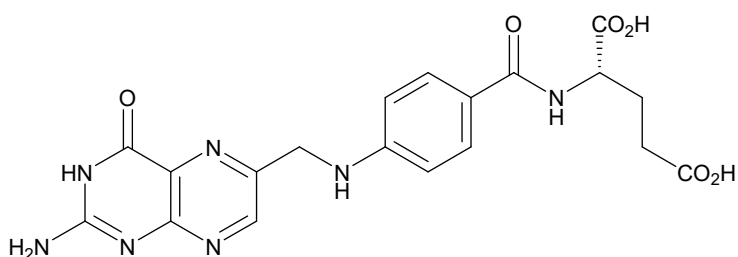


Figure 7.3. Structure of Folic acid (FA), or vitamin B₉.

The folate present in human body is the 5-methyl-tetrahydrofolate (5-MTHF). Therefore, the folic acid introduced from the outside into the body must be reduced and methylated in order to be converted into MTHF; this event does not occur if the dose of 400 µg of folic acid taken is exceeded, and it is therefore possible to detect it in the circulatory system.³⁸⁰

Exposure to this metabolite would not seem to cause health problems, but in any case it is useless if it is not converted into 5-MTHF. When designing a drug based on folic acid, it is therefore advisable to keep this physiological limit in mind.

Folic acid is more stable in pharmaceutical preparations and is better absorbed than other forms of folate; it is used as a fortifier and as a food supplement.³⁸¹ In addition to its various beneficial effects, it is able to ward off the risk of various pathologies, such as neural tube defects (NTDs, an example is spina bifida) and cardiovascular problems, thanks to its ability to lower blood homocysteine levels.³⁸² The involvement of folate in these events can be explained by recalling its intervention in human metabolism (Figure 7.4). Folic acid, as a cofactor, participates in single carbon transfer reactions; these are involved in the DNA biosynthesis cycle and in the methylation cycle.³⁷⁹ The function of the latter, in particular, is to continuously supply the methyltransferase enzymes with S-adenosylmethionine (SAM), which is used as a methyl donor. Methyltransferase, as the name suggests, transfers $-CH_3$ groups to a variety of substrates, such as proteins, lipids, hormones, DNA, etc. These enzymes are also involved in a number of mechanisms, such as the inactivation of the hormones DOPA and the stabilization of myelin, the lipid and protein layer that surrounds the axons of neurons.³⁷⁹

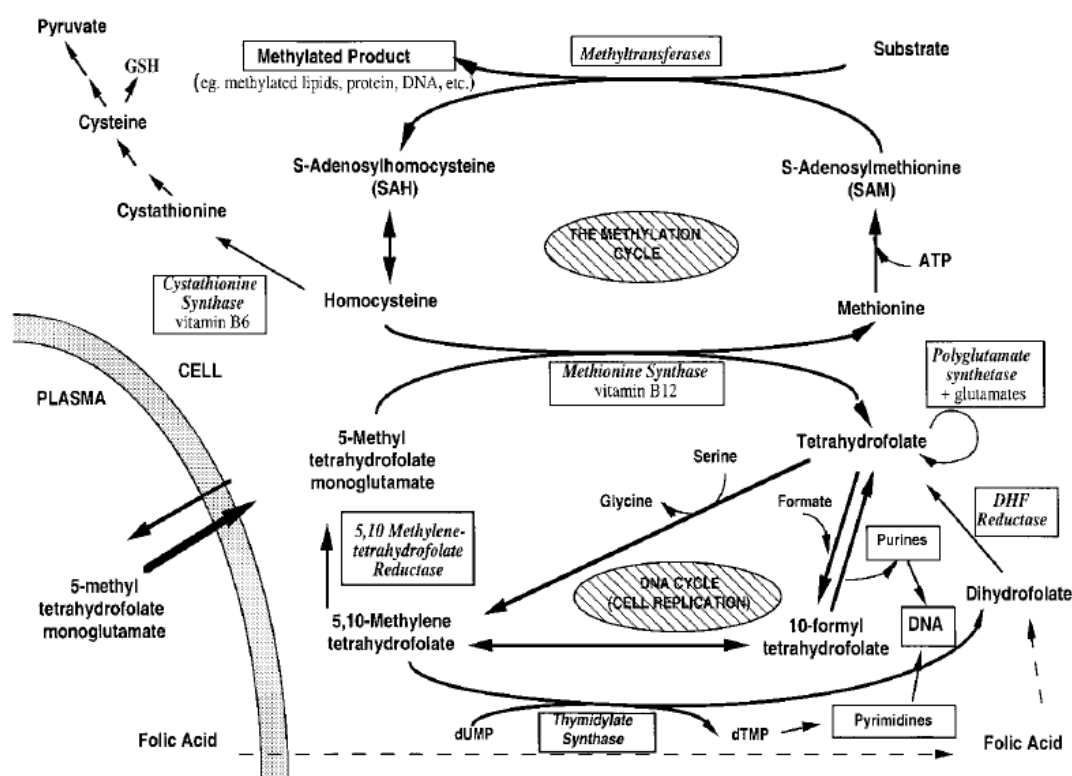


Figure 7.4. Metabolic pathway of folic acid in mammalian cells.

Folic acid is one of the best ligands for the selective targeting of certain types of cancer, such as breast cancer, due to its high affinity for folate receptors found on the surface of target cells.³⁸³ By functionalizing with it carriers of drugs, making them effective in the selective recognition of tumor cells. For example, Gholibegloo *et al.*³⁸⁴ have been involved in synthesizing nanosponges, on which folic acid is anchored and in which curcumin is included, to obtain theranostic systems for monitoring the action of the drug in real time. The inclusion of folic acid is carried out through the conjugation of the same with nanoparticles of magnetite fused on the surface of the polymer. The *in vitro* results show a remarkable inhibition of the viability of MCF10A and M109 tumor cells. These systems can be easily monitored through magnetic resonance imaging (MRI).³⁸⁴

7.3 Results and discussion

7.3.1 Adsorption tests

The interaction of the model bioactive molecules was preliminarily assessed towards a panel of 18 variously structured nanosponge materials, at two different pH values, namely 4.4 and 6.7. These two pH values were chosen by analogy with previous works.⁸⁵ It is worth mentioning here that, according to the reported pK_a values, guest RA is partly ionized at pH 4.4 and completely ionized at pH 6.7;³⁸⁵ conversely, guest FA passes from a mainly zwitterionic to a monoanionic form at the same pH values.³⁸⁶ Adsorption tests were accomplished, in perfect analogy with those described in Chapters 4 and 5; the relevant results are collected in Table 7.1. The results obtained constitute a dataset of 108 entries; so, once again a statistical approach was exploited for their analysis.

First, quercetin Q appears the guest that generally shows the greatest affinity for the materials, with an average inclusion percentage of around 74%. This can be easily justified considering that Q is clearly the most hydrophobic molecule between those considered. Conversely, the more hydrophilic molecules AR and AF show lower and similar average adsorption percentages, i.e. 28% and 22% respectively.

The general effect of the pH needs to be discussed in detail. For this purpose, the differences in the inclusion percentages at the two pH values for the same nanosponge/guest pairs can

be considered, taking into account the fact that, given the experimental uncertainties, differences of less than 6 percentage points should be considered non-significant.

Table 7. Percent adsorption data for materials ^[a]						
NS	RA		FA		Q	
	pH 4.4	pH 6.7	pH 4.4	pH 6.7	pH 4.4	pH 6.7
C3	15	17	54	41	68	91
C11	9	13	34	21	87	81
C13	51	10	6	6	> 98	84
C17	21	9	5	5	95	71
C18	48	23	15	< 3	> 98	74
C19	57	22	18	18	> 98	74
K1	10	< 2	4	37	79	55
K2	92	51	58	48	94	26
K3	32	11	8	5	69	75
K4	8	5	6	5	90	90
K5	8	< 2	4	< 2	75	70
CK1	6	4	5	3	60	42
CK2	60	6	< 2	< 2	61	55
CK3	14	8	7	3	56	63
CK4	50	16	64	5	71	53
CK5_a	94	56	70	76	91	79
CK5_c	> 98	51	70	74	94	96
CK7	3	8	8	5	67	49

[a] All data are given with ± 3 % indetermination

In the case of rosmarinic acid RA, better absorption at a more acidic pH is observed in 12 cases out of 18, while in the other 7 the differences are not significant. Particularly striking differences are observed in the case of materials containing the triamine A chain extender. This behavior is perfectly reasonable if the electrostatic effects are considered: compared to an anionic substrate,³⁸⁷ in fact, absorption is clearly favored in a material which has a positive charge density due to the protonation of the tertiary amino groups of the extender. Conversely, less marked or not significant differences are found in all those cases in which the material has a more hydrophobic character, such as those in which calixarene or the aliphatic extender is present.

Folic acid exhibits a quite different behavior. Indeed, a better inclusion at the most acidic pH is observed in only 5 cases out of 18, and in two cases a better absorption is found at pH 6.7. Moreover, these results do not apparently show a straightforward relationship with the structure of the materials. Hence, it must be concluded that, due to its zwitterionic structure³⁸⁵

and the presence of a rigid bicyclic moiety, the interactions between the molecule and the host sites of the material are less controlled by the mere interplay of electrostatic effects. Probably, specific hydrogen bonding and steric effects (the latter ones related to the possible inclusion modes of the rigid bicyclic pteridine moiety). Finally, in the case of quercetin, whose molecule is decidedly more hydrophobic than the other two, inclusion at pH 4.4 is once again favored (10 cases out of 18). Interestingly, the lower differences occur in the case of calixarene-only polymers, with the notable exception of the K2 material which bears the triamine extender. This fact can easily be related to the highly hydrophobic character of these materials (which is reduced in the presence of the protonated cationic extender) and to the possible establishment of strong π - π interactions between material and guest. The presence of cyclodextrin (CK materials) makes this factor less important, returning the absorption to them favored at the more acidic pH.

Finally, the effect of the presence of the chain extender should be briefly discussed. In the case that this is the triamine, it is undoubtedly linked to its ability to become cationic after protonation, strongly favoring the interaction with any anionic functional groups of the guest, as discussed above. The effect of any aliphatic extender consisting of 1,6-hexanediol, on the other hand, is less intuitively evident. In fact, in principle, it should make the structure of the material less hydrophilic. However, a greater amount of the extender with respect to the host units, as its molar ratio with the bis-isocyanate remains constant (materials C23-C25 and K3-K5) has also the effect of modifying the textural properties of the material, because it corresponds to an increase in the amount of linker units relative to host units. The combination of the two factors does not have easily predictable effects.

7.3.2 Preparation of inclusion composites

On the ground of the results of the preliminary absorption tests, some materials were suitably selected to prepare inclusion composites, to be used for the subsequent study of the release kinetics. Materials of the three different typologies were considered (cyclodextrin-only, calixarene-only and mixed), taking into account both the best affinity for the guests and the type of possible functionalization carried by the linker or the chain extender subunits. In detail, for the inclusion of rosmarinic acid the materials C19, K2 and CK5_c were selected, which presented the most interesting inclusion percentages. Furthermore, taking into account that C19 has the triamine extender, the non-functionalized material C3 and CK5_a, and the material C11 functionalized with carboxyl groups (from the pyromellitic co-linker) were

considered for useful comparison. In particular, comparison between CK5_a and CK5_c offered the opportunity to test the outcome of the different preparation protocol starting from the same reactant combination. Regarding the folic acid, the criterion of the most effective inclusion was again followed, on the basis of which the materials C3, C11, K2, CK4 and CK5_c were selected. Finally, for quercetin, which showed large affinities towards almost all materials; therefore, materials C3, C11, K2 and CK4 were taken into consideration by analogy with the other two guests.

The inclusion composites were prepared in a similar way as described previously in Chapter 6 for *p*-nitroaniline, i.e. the chosen nanosponges were loaded by equilibrating them with an almost saturated solution of the guest (see Experimental section for details). The actual resulting loadings were determined by evaluating the amount of guest eluted away from a given amount of composite, via repeated washing with methanol. The results are summarized in Table 7.2.

Table 7.2. Percentage loadings of inclusion composites^[a].			
NS	Loadings (w/w %)		
	RA	FA	Q
C3	9.9	7.7	5.0
C11	6.8	8.7	n.d.*
C19	n.d.*	-	-
K2	5.0	n.d.*	7.5
CK4	-	9.4	6.8
CK5_a	6.8	-	-
CK5_c	6.4	n.d.*	-

*Guest is not eluted from the material.
[a] All data are given with ± 3 % indetermination.

As it can be easily seen, loadings between 5% and 10% are obtained, but a strict correlation between the experimentally determined loadings and the inclusion percentages of the preliminary tests is not found. Just to give an illustrative example relating to rosmarinic acid, for the C3 material a load of 9.9% is obtained which is clearly higher than that of the CK5_c material (6.4%), when instead the inclusion percentages of the preliminary tests had given respectively 15% for the first material and almost quantitative for the second. These discrepancies can be explained by considering the profound difference between the two types of experiment. In the preliminary inclusion tests, in fact, the position of the inclusion equilibrium is obtained for a certain value of the initial concentration of the guest, without taking into account the effective load capacity of the material itself. In other words, assuming

for simplicity that the absorption equilibrium may be described by a model such as the well-known Langmuir isotherm:

$$q = \frac{q_{max} \cdot K_B \cdot C_{eq}}{1 + K_B \cdot C_{eq}}$$

at a given initial guest concentration, the equilibrium position, and therefore the residual concentration in solution C_{eq} , remains determined both by the binding constant K_B and by the maximum load capacity q_{max} . The latter depends on the nature and composition of the material; it cannot be known *a priori*, because even the narrowest nanopores can act as binding sites together with the host units. Consequently, q_{max} should be determined separately by constructing the entire adsorption isotherm (obtaining in this way also K_B , which expresses the intrinsic affinity of the material for the guest). Conversely, in the preparation of composites tried to force the conditions to saturate the material, ideally until the q_{max} is reached.

As it can be seen from Table 7.2, in four cases, namely RA/C19, FA/K2 and FA/CK5_c and Q/C11, the determination of the loading was not possible. In fact, even after repeated washings with methanol, the amount of eluted guest was negligible (< 0.1%). On the other hand, the presence of the guest in the composite is visually evident from its color (deep yellow). In the three cases cited, the color of the composite before and after washing clearly showed the presence of the guest included in the structure, which cannot be eluted even by aprotic dipolar solvents such as DMF or DMSO. Anyway, the presence of the guest was confirmed by IR spectroscopy, individuating some of the signals of the guest among the fingerprint signals of the nanosponge.

7.3.3 Release kinetics

The composites obtained were then subjected to controlled release tests at 40 °C, under the same pH conditions (i.e. 4.4 and 6.7) as in the previous experiments (Chapter 6), in order to study the relevant kinetics. The release was monitored by UV-Vis spectroscopy and the data were subjected to non-linear regression analysis, to retrieve the best fitting model describing the kinetic profiles and obtain the apparent kinetic constant for the release process. Then, for each kinetic experiment, from the equilibrium concentration value of the released guest and knowing the initial loading, the percentage of released guest with respect to the loaded was

also calculated, which can be useful information for a possible use of the nanosponge as a drug-carrier system.

In general, and in agreement with the behavior of *p*-nitroaniline discussed in the previous Chapter, most release profiles can be adequately treated as stretched first order exponential kinetics (except for few cases that will be specifically discussed), with an average value for the stretch parameter *n* of 0.83 ± 0.17 . The occurrence of a *n* value closer to 1 with respect to *p*-nitroaniline is interesting because indicates a behavior that is overall less influenced by the effect from the distribution of binding energies discussed in the previous Chapter (Section 6.3). Indeed, this finding is in full agreement with the discussion of the results for the adsorption tests on *p*-nitroaniline compared to those for dyes (Section 4.2). In fact, it may be ascribed to the ionic character of these guests, which decreases the intrinsic affinity for the cyclodextrin or the calixarene cavity.

Release of Rosmarinic acid. The results relevant to the release of rosmarinic acid are collected in Table 7.3.

Table 7.3. Kinetic data for Rosmarinic acid release.

NS	Load	pH 4.4				pH 6.7			
	(% w/w)	$k \cdot 10^4$ (s ⁻¹)	<i>n</i>	<i>C</i> _{eq} (μM)	Rls. %	$k \cdot 10^4$ (s ⁻¹)	<i>n</i>	<i>C</i> _{eq} (μM)	Rls. %
C3	9.9	4.5 ± 0.4	0.85 ± 0.03	90 ± 3	72	5.1 ± 0.4	0.85 ± 0.07	76 ± 3	55
C11	6.8	4.0 ± 0.5	0.80 ± 0.03	61 ± 2	64	3.5 ± 0.3	0.54 ± 0.04	54 ± 1	57
C19	8.5	2.6 ± 0.3	0.48 ± 0.06	50 ± 1	42	0.14 ± 0.01	(1)	29 ± 1	24
K2	5.0	0.70 ± 0.02	0.67 ± 0.07	25 ± 1	36	0.29 ± 0.02	0.69 ± 0.04	39 ± 1	56
CK5_a	6.8	1.10 ± 0.03	0.86 ± 0.04	22 ± 1	23	1.76 ± 0.18	(1)	30 ± 1	32
CK5_c	6.4	0.58 ± 0.02	(1)	9.4 ± 0.6	11	0.29 ± 0.05	0.92 ± 0.07	25 ± 1	28

As it can be immediately deduced from the data reported therein, the materials containing only cyclodextrin release on average quite more rapidly than those containing only calixarene, and release greater amounts of guest. Mixed materials are roughly placed in an intermediate position, but as can be clearly seen from the data, the method of preparation has a non-negligible effect, since there are significant differences between CK5_a and CK5_c. The non-functionalized material C3 and the one decorated with the carboxyl groups C11 show, within the limits of the experimental uncertainties, non-significant differences in the release rate. Conversely, release from the material C19 bearing the polyamine chain extenders is by far slower than the previous ones, in particular at pH 6.7. The latter finding

is clearly consequence of the strong Coulomb interactions between the nanosponge scaffold and the anionic guest. These trends can be also reasonably rationalized in terms of permeability of the nanosponge structure as a function of the protonation state of the functional groups present. In the case of C19 the extent of protonation of the tertiary amine groups of the chain extender decreases on passing from pH 4.4 to pH 6.7. Consequently, the structure undergoes a large decrease in its hydrophilic character, with a decrease in the mobility of the aqueous medium inside the nanochannels. It can be also noted that the equilibrium concentrations of the released guest vary accordingly. The behavior of the material K2 (bearing the amine chain extender) can be considered in analogy with the material C19 as long as the effect of pH variation is considered. Interestingly, lower n values than the average are found in this case (ca. 0.68), which can be explained in terms of the occurrence of effective π - π interactions between the aromatic guest and the calixarene units. Finally, mixed materials CK5_a and CK5_c provide the opportunity to compare the effect of the microscopic organization of the material. In fact, the synthetic procedure to obtain the latter one was designed to achieve a more ordered material. Consistently, it shows lower release apparent kinetic constants at both pH values. Again, this can reasonably be traced back to the fact that by varying the synthesis protocol, the differences in the degree of microscopic disorder are mirrored in the dimensions and distribution of the nanopores. It is worth noting the fact, highlighted in the literature,⁸⁴ that in the microscopic structure of mixed materials the formation of highly hydrophobic microdomains can occur, the distribution of which is obviously influenced by the synthesis methods. Notably, for these two materials the highest values of the n stretch parameter are found, indicating that the kinetic course is barely distinguishable from a simple first-order kinetic trend. This indicates that host-guest binding has more limited influence on the overall release kinetics, suggesting that diffusion phenomena within the pore network assume a greater importance.

Release of Folic acid. The study of folic acid release has presented considerable experimental difficulties, also linked to the fact that in two cases, as previously mentioned, the included guest is retained by the nanosponge so strongly that it cannot be eluted. The kinetic data are collected in Table 7.4

Analyzing the data in detail, it can be noted again that polymers containing only-cyclodextrin release better than those containing calixarene, confirming that the overall hydrophilic character of the nanosponge and its permeability to the aqueous medium are important factors influencing the ease with which the guest is released. In the case of K2 at pH 4.4 the

release is so slow that it is not possible the usual exponential trend, but only an apparent linear rise of the guest concentration in solution.

Table 7.4. Kinetic data for Folic acid release.

NS	Load (% w/w)	pH 4.4				pH 6.7			
		$k \cdot 10^4 \text{ (s}^{-1}\text{)}$	n	$C_{\text{eq}} \text{ (}\mu\text{M)}$	Rls. %	$k \cdot 10^4 \text{ (s}^{-1}\text{)}$	n	$C_{\text{eq}} \text{ (}\mu\text{M)}$	Rls. %
C3	7.7	3.7 ± 0.3	0.94 ± 0.05	41 ± 1	47	3.3 ± 0.3	0.92 ± 0.04	41 ± 1	47
C11	8.7	0.75 ± 0.09	(1)	51 ± 1	52	1.21 ± 0.05	(1)	84 ± 2	85
K2	n.d.*	linear	-	n.d.*	n.d.*	3.7 ± 0.7	0.78 ± 0.05	n.d.*	n.d.*
CK1	9.4	0.10 ± 0.02	0.56 ± 0.06	38 ± 3	35	5.4 ± 0.4	(1)	57 ± 1	54
CK5_c	n.d.*	0.12 ± 0.02	0.79 ± 0.05	20 ± 1	n.d.*	0.16 ± 0.02	0.49 ± 0.05	20 ± 1	n.d.*

*Guest is not eluted from the material.

Conversely, in half of the cases the stretch parameter n values are so close to 1 that the release trend is almost a simple exponential. Furthermore, in all cases the variations in the release rate as the pH varies are significant, as they also reflect the different protonation state of the guest, passing from a zwitterionic to a monoanionic form.³⁸⁵ This heavily influences its affinity for the nanosponge, as already seen in the preliminary absorption tests. Notably, unlike Rosmarinic acid, the release occurs significantly faster at pH 6.7 in three cases (out of 5).

Release of Quercetin. It was in no way possible to obtain good quality kinetic data with quercetin, despite multiple attempts made with composites obtained from materials C3 and C11. Therefore, it was considered appropriate not to further investigate the behavior of this guest.

7.4 Concluding highlights

The data obtained from the study of the adsorption and the release of the three model bioactive molecules, at the two chosen pH values, are fully consistent with the results from the previous parts of the work. In particular, it is confirmed, once again, the importance of both Coulomb interactions and hydrophobic character of the guest in favoring the adsorption

by the nanosponge material. This is perfectly mirrored in the variations of the release rates on changing the operational pH value. Notably, consideration of kinetic data modelling (in particular the values of the n stretch parameter) suggests that the behavior of these molecules with an extensive rigid moiety, appears more comparable to the one of large dyes than to small *p*-nitroaniline.

7.5 Experimental section

7.5.1 Preliminary absorption tests

Stock solutions of known concentration ($3 \cdot 10^{-5}$ M) of the different guests (RA, FA and Q) were prepared in aqueous buffers, both at pH 4.4 (buffer made by solubilizing 200 mg of NaCH₃COO in 250 mL of water, then acidifying with appropriate amount of HCl), or at pH 6.7 (buffer made by solubilizing 232 mg of NaH₂PO₄ and 113 mg of Na₂HPO₄ in 250 mL of water). Sample was prepared by mixing 2.0 mL of guest solution ($3 \cdot 10^{-5}$ M) with a carefully weighted amount (4.00 ± 0.05 mg) of materials. The samples were shaken at r. t. for 90 min, and, then, centrifuged for 15 min at 4000 rpm. The supernatant liquor was carefully pipetted after centrifugation, and, then, the percent amount of guest left in solution at equilibrium was simply estimated by means of UV-vis spectrophotometry, comparing the absorbances of the starting and final solutions. In this way, it is possible to determine the percentage of absorbed guest, known its molar extinction coefficient (Table 7.5), estimated through the construction of suitable calibration lines. The percent absorbed is trivially obtained by difference.

Table 7.5. Molar extinction coefficients (ϵ) of guests at different pH values.

Guest	ϵ (pH 4.4)	ϵ (pH 6.7)
RA	17644 ± 520	16790 ± 430
FA	24400 ± 500	27700 ± 300
Q	14079 ± 210	13900 ± 170

7.5.2 Inclusion of rosmarinic acid (RA)

20.0 ± 0.05 mg of RA are dissolved in 400 μ L of methanol. 100.0 ± 0.05 mg of the selected nanosponge and 10 mL of distilled water are added to this solution. The system was allowed to shake mechanically overnight at room temperature. The solid was filtered under vacuum

and washed with a few drops of methanol; finally, the solid is dried in vacuum over P₂O₅ overnight at 60°C.

7.5.3 Inclusion of folic acid (FA)

60.0 ± 0.05 mg of nanosponge are suspended in 20 mL of aqueous buffer at pH 4.4 under magnetic stirring. In a glass vial 6.00 ± 0.05 mg of FA were weighed separately, and this was added to the polymer solution in small quantities over a period of five hours; and then the system is left under magnetic stirring overnight. After this time, the suspension was centrifuged and, after eliminating almost all the supernatant, the material was transferred into a balloon and subjected to freeze-drying overnight.

7.5.4 Inclusion of quercetin (Q)

60.0 ± 0.05 mg of the polymer to be loaded are suspended in 20 mL of pH 4.4 buffer under magnetic stirring. By means of a “Chemetron™” type micrometric screw syringe, 1.5 mL of a 10 mM solution of Q in methanol (approximately 5 – 10 µL/min) are slowly introduced into the suspension.

7.5.5 Determination of the loading percentage

A carefully weighed small amount (ca. 4.00 ± 0.05 mg) of composite was placed in a vial and suspended with 2.0 mL of methanol; the suspension was sonicated and the solid was centrifuged for 5 min at 5500 rpm. This washing procedure was iterated more times, unless the washing liquor was colorless. The collected extracts were placed in cuvettes and the concentration of the solution obtained was determined spectrophotometrically. The load is determined by considering the additivity of the absorbances of all the washings, known the ϵ in methanol of the molecules included (Table 7.6), estimated through the construction of the appropriate calibration lines.

Table 7.6. Molar extinction coefficients in MeOH.

<i>guest</i>	ϵ in MeOH
RA	18027 ± 500
FA	28500 ± 900
Q	20000 ± 200

7.5.6 Release kinetics

Release kinetic profiles were determined as follows. A carefully weighed amount (10.00 ± 0.05 mg) of each composite was placed in a dialysis membrane (Medicell International Ltd

MWCO 12–14 000, 21.5 mm diameter), dispersed with 1.0 mL of the chosen extraction medium (i.e. an aqueous buffer at pH 4.4 and 6.7). The membrane was then put in a round bottom flask containing 20 mL of the release medium thermostated at 40 °C under magnetic stirring. At given times, 1.0 mL of the release medium was withdrawn and analyzed spectrophotometrically, to determine the concentration of released guest. To keep the volume of the release medium constant, 1.0 mL of fresh extraction medium was added each time to replace the withdrawn one.

8 – Nanosponges functional supports for photocatalysis

8.1 Introduction

The use of nanosponges as support for metal nanoparticle catalysts has been largely reported in recent literature, and these are useful in organic reactions such as the reduction of olefins and nitroarenes, and the oxidative coupling of anilines.^{207,388-390} A brief overview on the use of nanosponges to support metal nanoparticles has been already presented in the introductory Chapter. In general, association of the catalyst with the nanosponge matrix can be advantageous because the matrix can both chemically and physically stabilize the catalyst, providing a suitable protective environment and avoiding its dispersion into the reaction medium. Moreover, the nanosponge can adsorb the substrate concentrating it close to the catalyst surface giving rise to an improvement of the catalytic performance. In the present PhD thesis work the idea was pursued to extend the use of nanosponges as functional supports for catalytic systems considering their possible exploitation in photocatalysis, and in particular in the photodegradative oxidation of organic pollutant species. Thus, in the following section a brief overview on the topic will be addressed, with particular reference to graphene-like materials, which were used in this work.

Photodegradation of organic pollutants constitutes a valuable tool in approaching environment remediation problems, in particular in the treatment of wastewaters, because it is cheap and effective. Hence, enormous research efforts have been paid in recent years, as accounted for by a massive literature: according to SCOPUS, more than 15000 research papers have been published on the photodegradation of dyes, and more than 3000 on drugs, along with at least 300 reviews. In general, these processes provide that air oxygen, as the sacrificial oxidant, is activated by the use of a suitable photocatalyst. The general mechanisms involved are quite well understood, and basically provide two main pathways. On one hand, O_2 can be excited to its singlet status by the excited photocatalyst as a sensitizer. On the other one, the excitation of an electron from the valence band to the conduction band, forms a hole-electron pair; then, the photosensitizer transfers the

accumulated energy to molecular oxygen, producing reactive species (ROS),³⁹¹ able of effectively initiating the oxidative degradation of the organic pollutant molecule, ultimately oxidizing it (possibly) up to CO₂ and H₂O.³⁹² Moreover, ROS species have the valuable ability to destroy undesirable microorganisms.

In terms of materials used as photocatalysts for the degradation of dyes and phenols, diverse semiconductor inorganic oxides have been exploited; in particular, titanium dioxide in its various crystalline forms has been widely used.³⁹³ For example, Khan *et al.* used mesoporous titanium oxides for the degradation of methyl orange, demonstrating the formation of various intermediates during the process, and confirming the intervention of the OH• radical in the economy of the process.³⁹⁴ However, the main problem encountered in the use of TiO₂ as a photocatalyst is its relatively large (3.2 eV) band-gap energy (defined as the difference between the conduction band energy and the valence band energy) which leads it to be activated exclusively by UV. For this reason, research has been directed towards the study of alternative heterogeneous photocatalysts, more selective and with satisfactory sustainability requirements. In particular, great attention has been attracted by 2D materials such as graphene (and its derivatives) and carbon nitride. These materials have been also largely tested as catalysts for oxidative photodegradation of dyes and phenolic pollutants, and several examples can be found in recent literature.³⁹⁵⁻³⁹⁹

Graphene is an atomically-thin, 2D sheet of sp² carbon atoms in a honeycomb structure. It has been shown to have many desirable properties such as high mechanical strength,⁴⁰⁰ electrical conductivity,⁴⁰¹ molecular barrier abilities⁴⁰². Unfortunately, the use of pristine graphene has proved challenging due to difficult bottom-up synthesis,⁴⁰³ poor solubility,⁴⁰⁴ and agglomeration in solution due to van der Waals interactions.⁴⁰¹ The oxidation of graphite in protonated solvents leads to graphite oxide, which consists of multiple stacked layers of graphene oxide (GO). GO has a similar hexagonal carbon structure to graphene but also contains hydroxyl (-OH), alkoxy (C-O-C), carbonyl (C=O), carboxylic acid (-COOH) and other oxygen-based functional groups (Figure 8.1).⁴⁰⁵

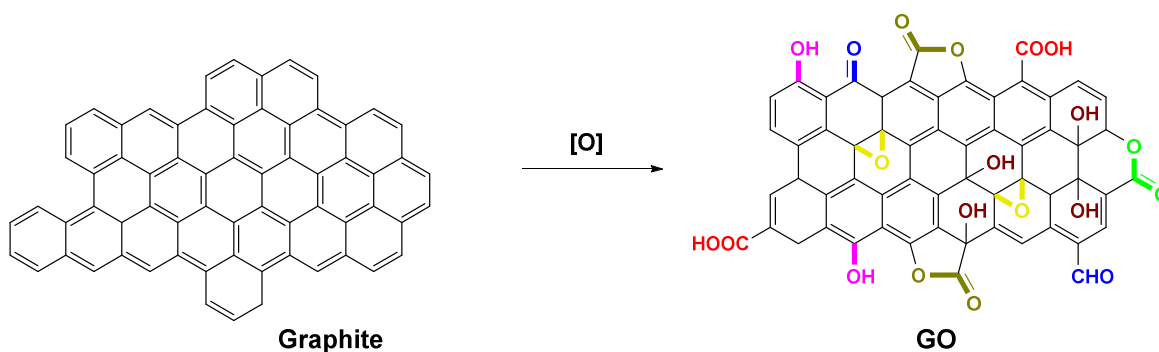


Figure 8.1. Graphite and Graphene Oxide (GO) structures.

Aside from the ease of synthesis, these oxygenated groups are responsible for many advantages over graphene, including higher solubility⁴⁰¹ and the possibility for surface functionalization which have presented many opportunities for use in nanocomposite materials. Furthermore, GO can be treated by a number of methods to synthesize reduced graphene oxide (RGO) in efforts to minimize the number of oxygen groups and achieve properties closer to those of pristine graphene.⁴⁰⁶ When considering the synthesis of graphene and its derivatives, the desired properties are largely dependent upon the size, shape, and functional groups attached to the surface of the material. The ideal structure is single layer graphene consisting of a single-atom-thin fully sp^2 hybridized carbon structure with minimal or no defects. However, due to the highly favorable stacking of graphene sheets, a multi-layered structure of graphene will also be generated. The synthesis of these structures with a bottom-up approach has proven difficult for industrial applications. As such, with a top-down approach, it is easier to generate the highly oxidized form of graphene, GO, with both sp^2 and sp^3 carbon containing abundant oxygen groups, which upon reduction (RGO) can eliminate most of the oxygen groups and sp^3 carbon to generate a more graphene-like material with much-improved properties. Because of the highly tunable nature of GO and RGO, a diverse range of mechanical, electrical, and thermal properties can be achieved and effectively incorporated into nanocomposite materials. GO and RGO can be used in a broad range of applications and show promise as an integral material to the progression of new and improved products (membranes/coating, stimuli-responsive materials, anticorrosion, and energy storage). The use of GO and RGO could also motivate the development and design of many other applications, including sensors,⁴⁰⁷⁻⁴⁰⁹ photoluminescence,⁴¹⁰ electronics,⁴¹¹⁻⁴¹² energy,⁴¹³⁻⁴¹⁴ and catalysis.⁴¹⁵⁻⁴¹⁶

At the same time, recently, the attention of researchers has been directed towards carbon nitride (C_3N_4), a cheap and non-toxic photocatalytic material which has appropriate thermodynamic requirements to be used as a semiconductor in heterogeneous photocatalytic processes of selective oxidation. Graphitic carbon nitride (g- C_3N_4) is a graphene-like layered material possessing appropriate thermodynamic requirements as photocatalyst to perform selective heterogeneous photocatalytic reactions.⁴¹⁷ Taking graphite as a model, for carbon nitride (C_3N_4) a structure consisting of s-triazine units⁴¹⁸⁻⁴¹⁹ or tri-s-triazine (heptazine) units connected via tertiary nitrogen atoms⁴²⁰ has been proposed (Figure 8.2). In both structures, it is possible to distinguish two different types of nitrogen atoms with different electronic characteristics, respectively of the pyridine type and of the aniline type; there are also vacancies, or pores, the edges of which are outlined by the N of the pyridine type, which are wider in the tri-s-triazine type structure.

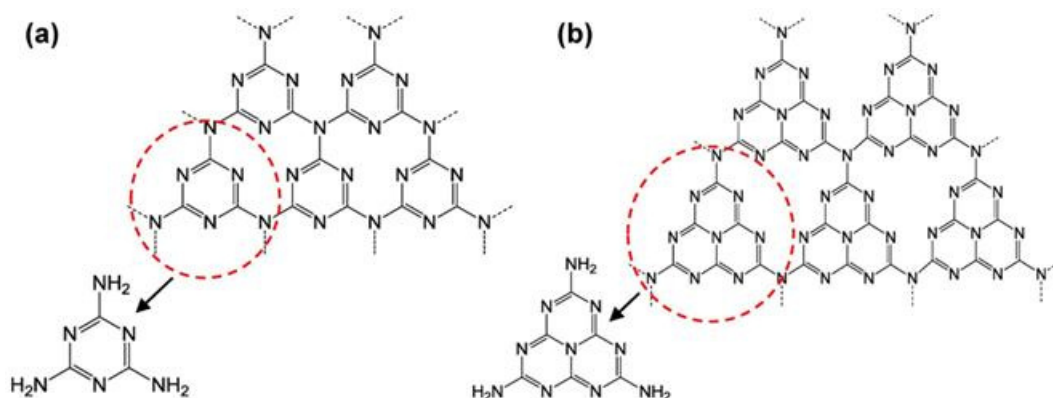


Figure 8.2. Carbon Nitride (C_3N_4) structures.

Computational studies⁴²¹ have shown that this second structure is favored.^{419,422–423} It has been thoroughly studied as photocatalyst for the partial oxidation of alcohols to the corresponding aldehydes in the presence of oxygen.⁴²⁴ The favorable energies of its conduction and valence bands, and the absence of hydroxyl groups on the surface, hamper the direct formation of the unselective $OH^\cdot$ radicals. It was previously observed that the thermal etching of the graphitic C_3N_4 having a low specific surface area (SSA, in the range $4\text{--}28\text{ m}^2\text{g}^{-1}$) affords an optimized thermos-exfoliated material (TE- C_3N_4 with $SSA > 150\text{ m}^2\text{g}^{-1}$), showing improved physicochemical features.⁴²⁵ TE- C_3N_4 was more active and selective photocatalyst than the g- C_3N_4 for the partial oxidation of different types of aromatic, aliphatic and furanic alcohols to the corresponding aldehydes⁴²⁶ also under natural solar illumination.⁴²⁷ The latter process has a significant industrial interest, because valuable organic synthons can be accessed.

Notwithstanding their interesting properties, graphene-like materials are not the ultimate solutions for photocatalysis, because of their own drawbacks, in particular their bandgap and HOMO/LUMO potentials, which limits the useful wavelength range for their photoexcitation. Therefore, they may largely benefit from the association with other semiconductors (including titania and similar inorganic oxides) or with metal nanoparticles. Indeed, these composite systems can absorb much more effectively the visible or near-infrared (NIR) radiation, enabling better photocatalytic performances. For instance, systems based on reduced graphene oxide (RGO) conjugated with silver nanoparticles (AgNPs) have been recently found very effective for this purpose.^{428–433} In particular, Divya,⁴³⁴ Naz,³⁹⁹ Li,⁴³⁵ Chandu⁴³⁶ and Pretheesia⁴³⁷ have reported very interesting results on testing these systems in the photodegradation of some model dyes such as Rhodamine B, Methylorange or Methylene blue (a pictorial representation is shown in Figure 8.3). The concomitant use of RGO and AgNPs is particularly intriguing, because very profitable association between

silver and nanosponges has been already well assessed, as mentioned previously. Conversely, association between cyclodextrins and graphenes⁴³⁸⁻⁴⁴⁰ or carbon nitride⁴⁴¹⁻⁴⁴³ has been reported only occasionally.

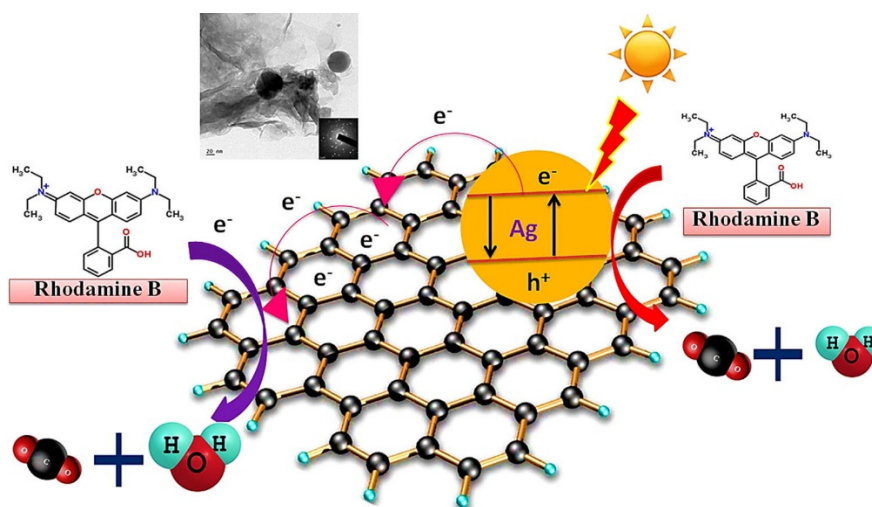


Figure 8.3. Diagram of the possible functioning of the catalyst RGO-Ag.

It was reasoned that an investigation on the association of graphene-like photocatalysts with nanosponges could have been particularly valuable, in order to further assess the advantages of the use of nanosponges as functional supports, in view of the possibility to new generation design photocatalytic systems with improved performances. These systems, in turn, could be particularly useful in view of possible applications in environment remediation devices. Therefore, two different studies were carried in parallel. On one hand, a RGO/AgNP photocatalyst (indicated hereinafter as RGOAg) similar to the ones used in the aforementioned studies^{399,434-437} was associated with the polyurethane-cyclodextrin material C3 and the polyamino-cyclodextrin material CA. Then these systems, indicated hereinafter as M1 and M2 (Figure 8.4), were compared with an unsupported RGOAg testing their photodegradation efficiency towards some model pollutants, namely Bisphenol A (BA), Rhodamine B (RB), Methylorange (MO) and Bromochresol Green (BCG) (Figure 8.5). On the other hand, the TE-C₃N₄ was embedded into four nanosponges, namely C3, CA, C8 and a further mixed cyclodextrin-calixarene material (CKT) obtained in previous studies⁸¹ via a *CuAAC*-type cycloaddition approach by reacting the heptakis-(6-azido)-(6-deoxy)- β -cyclodextrin with a tetra-propargyloxy-calix[4]arene (Figure 8.6). The latter material was selected because of its much higher hydrophobic character, ensured by its short triazole linker unit. These composite photocatalysts (indicated hereinafter as N1-N4) were tested for the photodegradation of *p*-nitrophenol (4NP) and for the selective photocatalytic oxidation

of the alcohol groups of 5-hydroxymethylfurfural (HMF) and veratryl alcohol (VA) into the corresponding aldehydes (Figure 8.7). It is worth mentioning here that this second study was accomplished in collaboration with the research group of prof. G. Marci (Dept. of Engineering, University of Palermo).

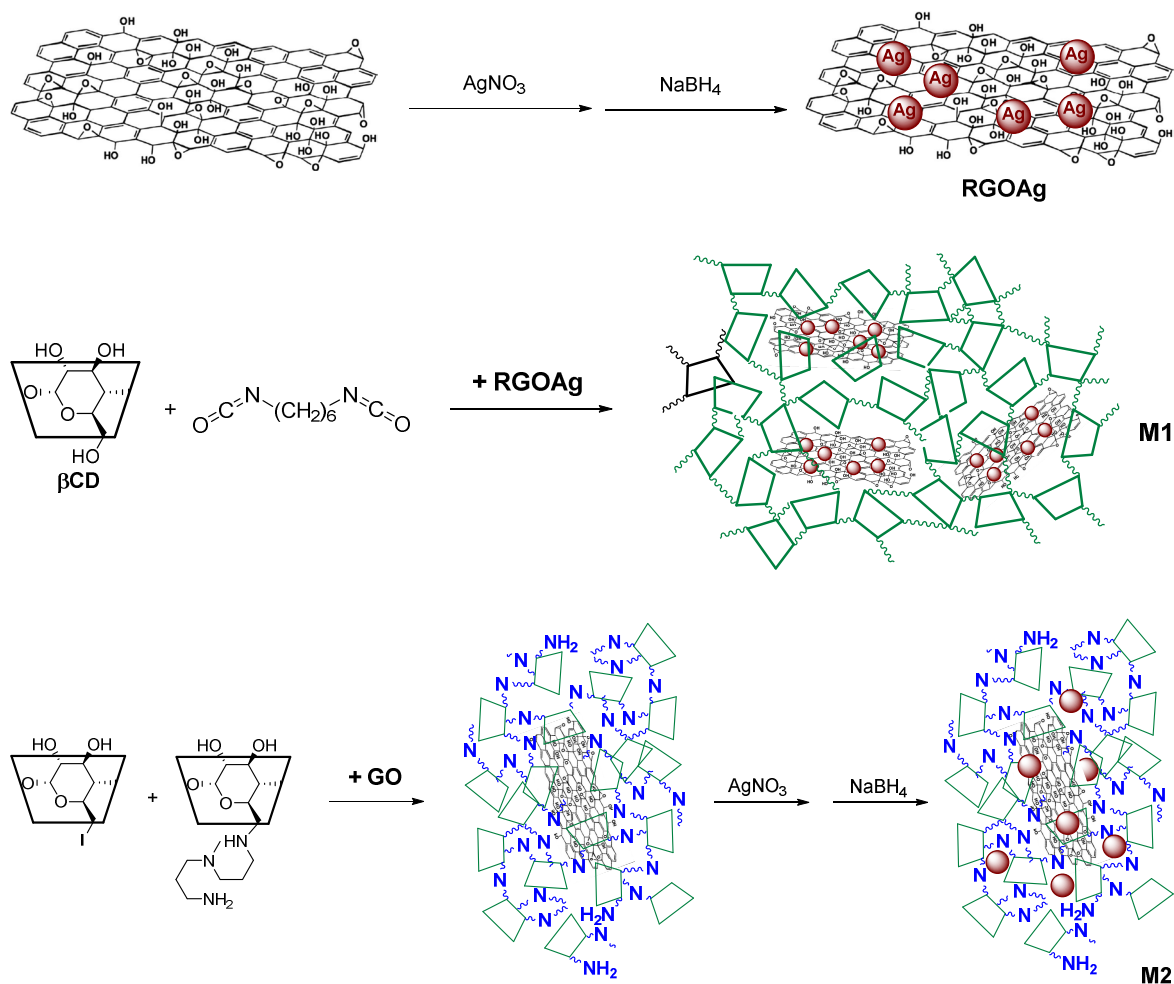


Figure 8.4. Structures of the β -cyclodextrins, the cross-linkers and the synthesized nanosponges, used for the preparation of the composite materials RGOAg, M1 and M2.

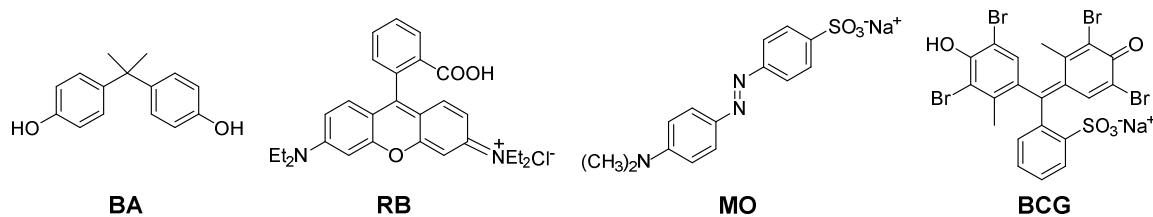


Figure 8.5. Structures of the model pollutants and dyes.

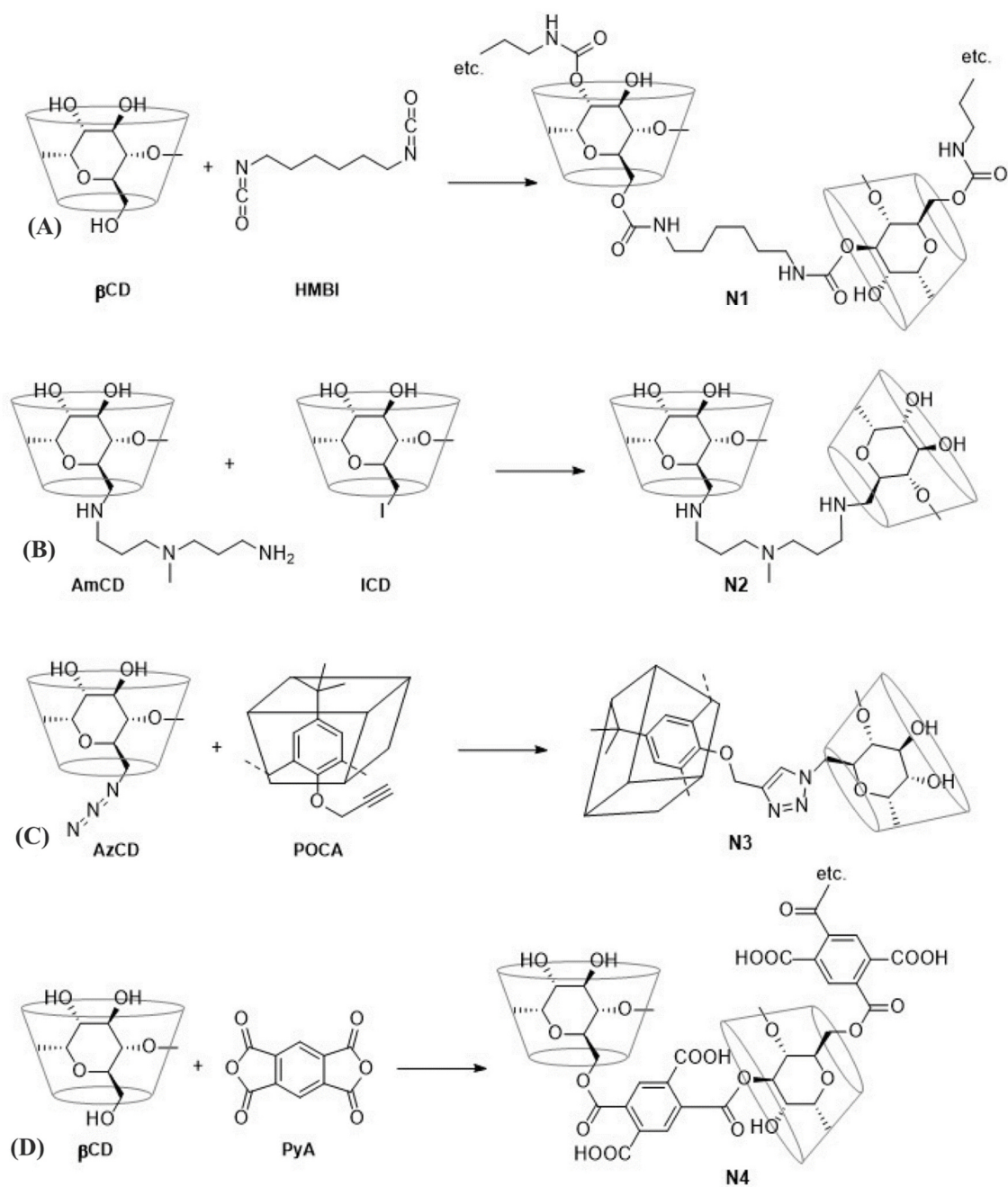


Figure 8.6. Structures of the β -cyclodextrins, the cross-linkers and the synthesized nanosponges, used for the preparation of the composite materials N1-N4.

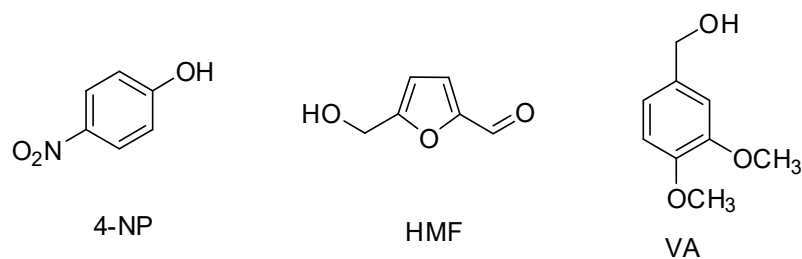


Figure 8.7. Structure of 4-nitrophenol (4NP), 5-hydroxymethylfurfural (HMF) and veratryl alcohol (VA).

8.2 Preparation and characterization of the photocatalysts

The complete synthetic procedures used to obtain the photocatalytic systems are described in detail in the Experimental section. In brief, a GO sample was prepared by a modified Hummers-like procedure (i.e. oxidation with KMnO_4 and H_2O_2 in H_2SO_4 , followed by treatment with conc. HCl and subsequent careful neutralization of the solid obtained). The RGOAg was obtained by equilibrating the GO with AgNO_3 and subsequent reduction with NaBH_4 . According to literature, a theoretical silver loading of 50% w/w was accomplished, because it is reported to be the one ensuring the best photocatalytic activity. A sample of reduced graphene oxide (RGO) was obtained by the same procedure (adding no Ag salt) for useful comparisons. The photocatalytic composite material M1 was prepared by the “*thick paste*” procedure, mixing the proper amounts of β -cyclodextrin, H and RGOAg in such a way that the obtained material contained a 10% in weight of the title photocatalyst. For material M2 a different approach was used. The “*thick paste*” method was first used to prepare a precursor material in which GO was dispersed (15% in weight) in the CA matrix; then, exploiting the affinity of material CA towards Ag^+ ion described in literature,¹⁸³ the precursor was loaded with AgNO_3 , followed by reduction with NaBH_4 . It is worth noting here that, whereas in M1 silver nanoparticles are specifically bound to the graphene surface, conversely in M2 such a strict association is not compelled. The $\text{TE-C}_3\text{N}_4$, obtained from melamine by a two-step thermolysis procedure,⁴⁴⁴ was kindly provided by prof. Marci's Group. The four materials N1-N4 were all obtained again via the “*thick paste*” method, by dispersing the title photocatalyst (5% w/w) into the reactants mixture.

The three silver containing materials RGOAg, M1 and M2 were preliminarily characterized by ICP analysis, in order to determine their actual metal loadings. In details, a silver content as large as 45 ± 2 % for RGOAg, 4.0 ± 0.4 % for M1 and 15.6 ± 0.5 % for M2 was found. Hence, the loading for the title RGOAg composite resulted slightly lower than the theoretical 50% silver content expected. It is worth noting that in the reference works⁴³⁴ no independent evaluation of the actual loading after the synthesis is reported. The occurrence of minor losses of silver during work up procedures is also accounted for by the reddish color of the first washing liquors, typical of nanosized silver because of the well-known surface plasmon resonance absorption band centered at ca. 400 nm.⁴⁴⁵⁻⁴⁴⁶ The lower-than-theoretical loading is confirmed by the analysis of material M1. In fact, physical embedding of the pre-formed RGOAg composite into the NS matrix involves a further little metal loss, hardly significant within the experimental errors. Regarding the material M2, the observed loading is

significantly lower than expected (i.e. ca. 20.8%, calculated considering that an analogous material not loaded with GO has been reported to have a 24.5% silver loading²⁰⁷). This can be partly attributed to the loss of AgNPs that occurs in the washing steps, as accounted for again by the fact that the first washing liquors showed the typical reddish color of nanoparticle silver. Further confirmations were obtained by means of a thermogravimetric (TGA/DTG) analysis, aimed also at verifying the thermal stability of the materials. The complete collection of TGA curves is depicted in Figure 8.8. The starting GO showed a complex TGA curve, with two main degradation steps at 177 °C and 495 °C, and a final 11.7% residue at 900 °C. The corresponding reduced product RGO shows significant variations in its degradation profile, with a much smoother curve, the slope of which significantly increases at 500 °C, with final 34.6% residue at 900 °C. For RGOAg the TGA curve features a main decomposition step at 458 °C, with a final residue at 900 °C as large as 72.1%. From these results, considering that the metal contributes no thermogravimetric loss, by trivial algebraic passages a 42.7% silver content can be estimated for RGOAg, which is in reasonable agreement with the results from ICP analysis.

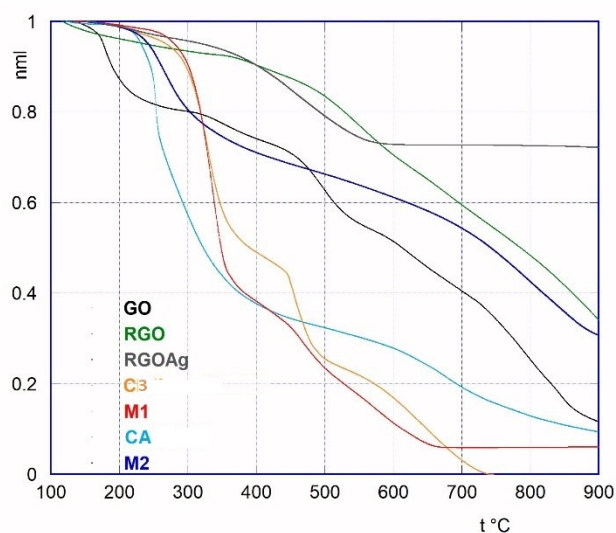


Figure 8.8. TGA curves of the photocatalysts and their precursors and reference materials.

For materials M1 and M2, comparison with the TGA curves of the relevant plain nanosponges again evidenced the effect due to metal loading. Material C3 shows a degradation profile with three main steps at 330 °C, 458 °C and 645 °C, and completely degrades above 750 °C. The M1 curve has a main apparent step at 335 °C (comparable to the first one for free NS), and a second smoother one up to the plateau above 680 °C, leaving

a final 6.0% residue. Again, the metal loading that can be calculated from these results, namely 4.3%, is in reasonable agreement with ICP. Similarly, the TGA profiles of CA and the relevant composite M2 show significant differences. The former one features two main decomposition steps at 253 and 286 °C, and a third shallow one centered at 678 °C, with a 9.7% final residue. Conversely, M2 shows a first decomposition step at 267 °C and a shallow second one at ca. 806 °C, with a 30.9% final residue. Keeping into account the theoretical RGO/CD weight ratio used in the preparation of the material, from the mass residues at 900 °C a silver content as large as 20.0% can be estimated, once again in reasonable agreement with the ICP result.

The analytical characterization of the pristine GO material was completed by a titrimetric determination of the present ionizable groups. The obtained titration curve was subjected to fitting analysis using the proper equation obtained algebraically^{88,181} for a mixture of two independent virtual bases. The regression parameters obtained confirmed the presence of two different types of ionizable group, having pK_a values as large as 5.16 ± 0.06 (0.93 ± 0.03 meq/g) and 9.13 ± 0.05 (1.11 ± 0.03 meq/g), which can be easily identified as carboxyl and phenolic groups respectively.

Spectroscopic characterization of the materials was first accomplished by FTIR and ¹³C solid-state NMR. In general, the IR spectra of the graphene-based materials (Figure 8.9) are difficult to acquire because of the strong background adsorption due to the low energy electronic transitions of the largely conjugated carbon backbone. Moreover, the additional presence of Ag nanoparticles also causes a strong broadband absorption in the IR region, making spectra almost unreadable. Hence, for these materials the ATR-FTIR technique was preferred. Conversely, carbon nitride-based materials did not present any of these difficulties, and ordinary transmittance spectra could be acquired (Figure 8.10). Considering the diagnostic 2000~1000 cm⁻¹ region, the spectrum of the precursor GO features a characteristic series of signals centered at 1722 (C=O str.), 1583 and 1383 (C=C str.), 1238, 1133 e 1067 (C-O str.) cm⁻¹. After borohydride reduction, the spectrum of RGO lacks the C=O and most of the C-O signals, showing only the 1577 and 1383 cm⁻¹ signals (the latter one very shallow) and a further peak at 1228 cm⁻¹. The spectra of the composites basically show the signals expected for the nanosponge backbone, which largely overlap with the relatively weak signals of the graphene derivatives. In particular, in the aforementioned spectral region the typical cyclodextrin finger band system (1155, 1078, 1028, 1005, 944 cm⁻¹) is apparent, together with the graphene band at 1572 cm⁻¹. Moreover, the spectrum of material M1 also features a signal at 1627 cm⁻¹ (with a shoulder at ca. 1700 cm⁻¹) accounting

for the presence of the urethane linker functional groups, whereas a signal at 1650 cm^{-1} (NH bending) can be found in the spectrum of M2.

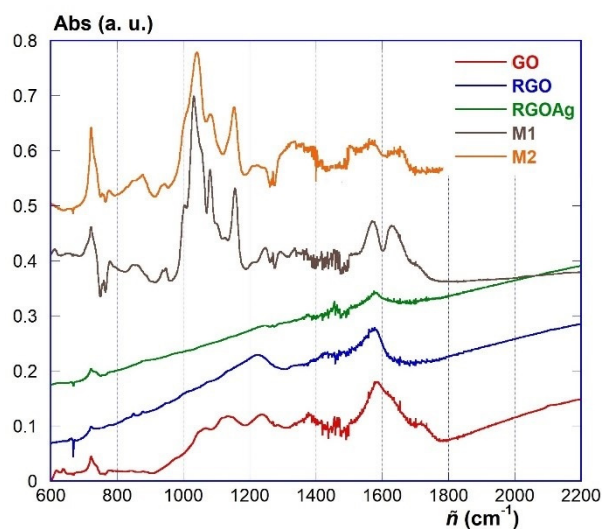


Figure 8.9. ATR-FTIR spectra (curves have been suitably offset for clarity).

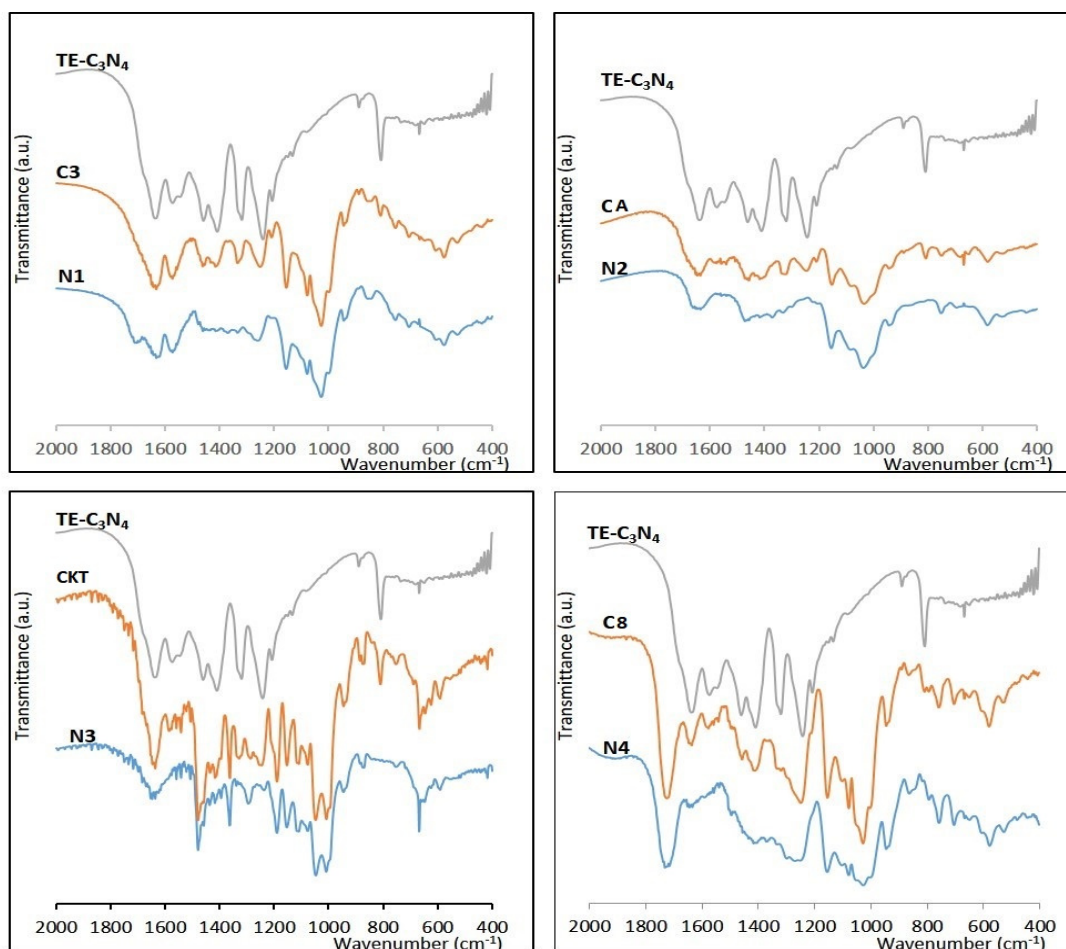


Figure 8.10. FTIR spectra of the NSs (C3, CA, CKT, C8) and the TE-C₃N₄/NS composites (N1 to N4) along with the pristine TE-C₃N₄ material.

For the materials based on carbon nitride a similar discussion can be done. All the signals expected for the functional groups of the nanosponge materials can be easily identified. The spectra of composites N1–N4 also feature some signals relevant to the various stretching modes associated to the carbon nitride, occasionally evidencing only minor shifts of few bands (Figure 8.10). The latter observation indicates the occurrence of no specific interaction for C_3N_4 with the nanosponge matrix, but occasional hydrogen bonding between the -OH groups of the CD moieties and the -NH- or the pyridine-like N atoms of the nitride. Thus, the TE- C_3N_4 is merely dispersed into the nanosponge matrix.

For the investigation of GO-based materials, Raman spectroscopy is a technique of choice. Therefore, Raman spectra of GO, RGO, RGOAg M1 and M2 (Figure 8.11) were also acquired, in order to provide further useful information. The spectra feature the well-known systems of bands in the ranges 1100–1700 cm^{-1} (given by the superimposition of the D^* , D, D'' , G and D' bands) and 2400–300 cm^{-1} (with the 2D and S3 bands). In particular, the amount of structural defects and the extent of the graphene-like domains, as well as the number of graphene layers,^{447–451} can be related to the intensity ratios between the D and G bands (I_D/I_G), the 2D and G bands (I_{2D}/I_G), and the 2D and S3 bands (I_{2D}/I_{S3}). These ratios can be obtained after the proper deconvolution analyses. In particular, the I_D/I_G value decreases from 4.11 for pristine GO to 3.51 for RGO, and then it increases to 6.08 and 6.27 for RGOAg and M1 respectively. A similar trend can be found for the I_{2D}/I_G value, passing from 0.74 for pristine GO to 0.69 for RGO, 0.97 for RGOAg and 1.64 for M1. Moreover, the I_{2D}/I_{S3} value increases from 1.02 for pristine GO to 1.13 for RGO, to 1.31 for RGOAg and to 1.79 for M1. Noticeably, the silver containing materials feature also a tiny signal centered at 611 cm^{-1} . These results suggest that the materials obtained are mixtures of few-layered rather than single-layered sheets; however, increasing exfoliation likely occurs, due to mechanical and chemical stress, on passing from the pristine GO to the silver-containing materials RGOAg and M1. Interestingly, deconvolution analysis could not be performed for material M2, because the spectrum appears poorly resolved and superimposed to a sort of wide absorption band. This anomalous finding might be related to the peculiar structure of the material, in particular to the possible presence of silver nanoparticles not bound to the graphene layers, but directly interacting with the N atoms present in the polyamine linker subunits of the CA nanosponge. Indeed, polyamino-cyclodextrins strongly bind the Ag^+ ion, forming complexes that easily undergo photoreduction to form silver nanoparticles,⁴⁴⁶ and this process is specifically promoted by green light (513 nm).⁴⁴⁵ Keeping into account that a 532 nm laser source was used for spectra acquisition, the occurrence of anomalous absorption effects of the excitation radiation seems then reasonable.

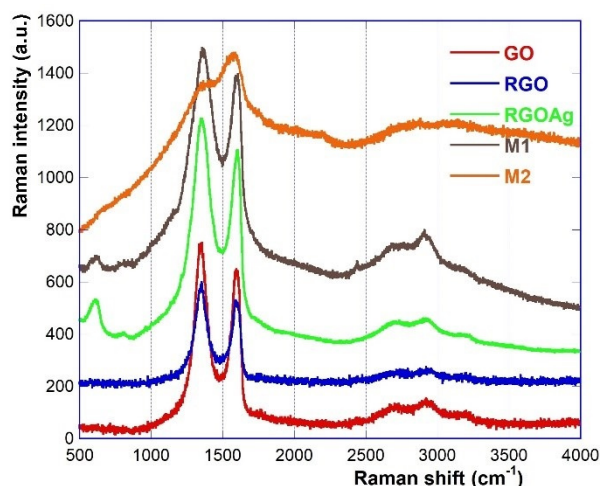


Figure 8.11. Raman spectra (curves have been suitably rescaled and offset for clarity).

Finally, $^{13}\text{C}(^1\text{H})$ CP-MAS Solid State NMR spectra of the various composite materials were also acquired. Indeed, all the spectra resulted almost superimposable to those of the plain nanosponges, confirming the existence of the cross-linked polymer network. In particular, spectra showed the signals expected for the cyclodextrin and the calixarene moieties (see Section 3.2), and feature also broad signals relevant to the linker chains centered at ca. 173 ppm (urethane C=O), 50 ppm (C bound to N) and 26 ppm (C not bound to N). However, possible signals relevant to the graphene or the carbon nitride C atoms are hardly detectable in the spectra. This can be easily explained considering that these C atoms cannot benefit from the cross-polarization enhancing effect, because they are not directly bound to H atoms. The characterization of M1 and M2 was complemented by SEM and XRD techniques. Some significant micrographs are reported in Figure 8.12. The pristine GO (Figure 8.12a) appears as a mass of microsheets tightly stuck together, which become densely covered in silver nanocrystals on passing to RGOAg (Figure 8.12b). Material M1 appears as a compact mass, the surface of which clearly reveals the presence of the graphene microsheets embedded into it (Figure 8.12c). Conversely, material M2 presents an even more compact appearance, but tiny silver nanoparticles can be spotted on the surface (Figure 8.12d; a SEM image of the material after reuse was also acquired, see later). SEM micrographs of the C_3N_4 -based materials were also acquired (Figure 8.13), and clearly show how the morphology of the nanosponges is different from that of the TE- C_3N_4 . However, the composites morphology was very similar to that of the plain nanosponges. This, considering the low amount of C_3N_4 (5% w/w), indicates its complete incorporation into the nanosponges matrix.

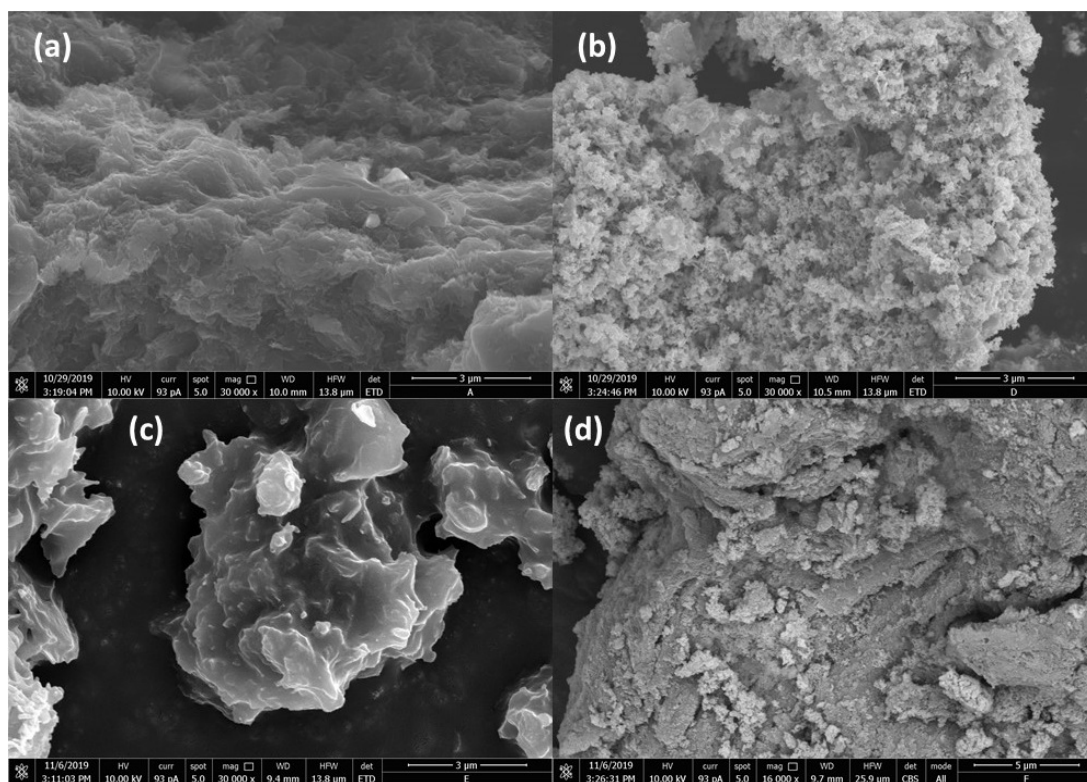


Figure 8.12. SEM micrographs of GO (a), RGOAg (b), M1 (c) and M2 (d). The reference line represents a 3μ length in (a-c) and 5μ in (d).

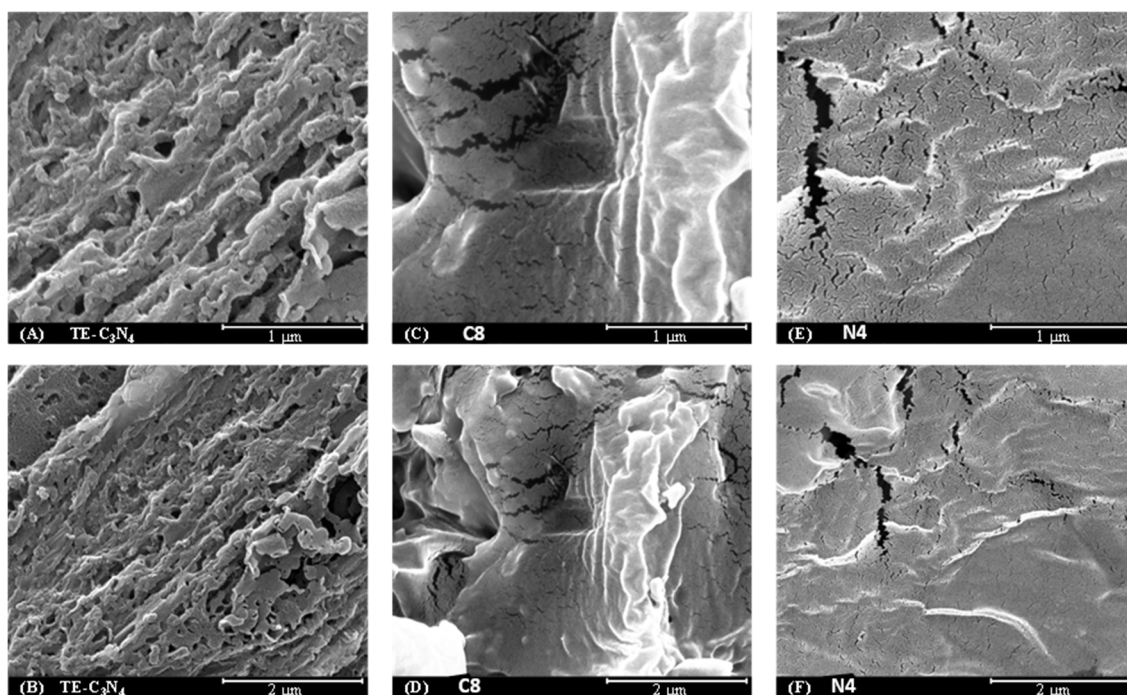


Figure 8.13. SEM micrographs of (A) and (B) bare TE- C_3N_4 ; (C) and (D) C8 nanosponge and (E) and (F) N4 composite at two different magnifications.

The characterization of GO, RGO and RGOAg was also complemented by XRD techniques. The XRD patterns of GO and RGO (Figure 8.14) show several peaks attributable to residual Na_2SO_4 (COD 900-4093) from the materials workup procedure. Besides, pristine GO features a broad diffraction peak centered at 12.3° corresponding to the (001) plane, providing further confirmation about the successful introduction of oxygen-containing functional groups in the graphene sheets.⁴⁵² Indeed, oxygen functionalities cause structural defects and surface roughness on the original graphene sheets, which, in turn, determines an enlargement of the inter-planar distance between GO sheets.⁴⁵³ After reduction, a shift of the 12.3° peak to 24.3° was detected in RGO, which refers to the (002) plane of graphene.⁴⁵³ This evidence corroborates the removal of oxygen-containing groups from GO and the reduction of the graphene sheets inter-spacing.⁴⁵² Specifically, the broadening of the RGO peak at 24.3° indicates the restoration of the conjugated sp^2 carbon network and the generation of randomly ordered graphene platelets.⁴⁵³ Moreover, GO and RGO XRD patterns display a peak corresponding to the (100) plane of the hexagonal carbon structure centered at 42.5° , which is sharper in the former and infers a short-range order of stacked graphene layers.⁴⁵⁴

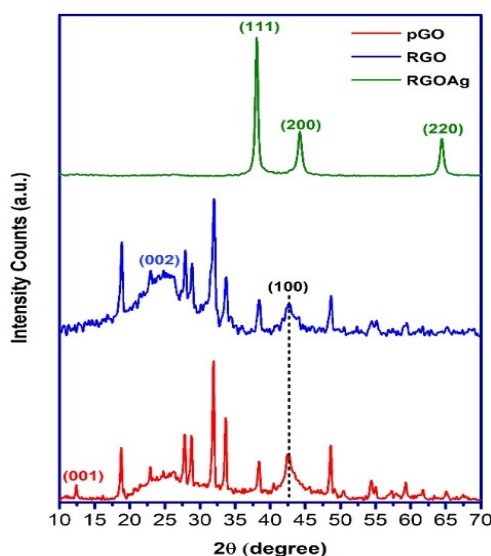


Figure 8.14. XRD patterns of GO, RGO, and RGOAg. Typical peaks of GO, RGO, and Ag (COD 9012961) within the RGOAg highlighted; in black is reported the (100) plane referring to the hexagonal graphitic carbon structure observed for both GO and RGO. The remaining peaks refer to Na_2SO_4 impurity (COD 9004092).

Finally, RGOAg shows three intense diffraction peaks at 38.1° , 44.2° , and 64.4° , which corresponds to the (111), (200) and (220) planes of face centered cubic crystal AgNPs (COD 9012961)⁴⁵⁰, confirming the formation of highly crystalline Ag on the RGO surface. Finally,

the absence of the RGO (002) peak suggest extensive exfoliation of GO deriving from the AgNP formation and growth on the graphene sheets, preventing their restacking.⁴⁵⁵ Finally, the optical properties of the samples were investigated by UV–Vis diffuse reflectance spectroscopy (DRS) as it is explained in detail in the Experimental section and reported in Figure 8.15. The estimated band gap value of the pristine TE-C₃N₄, obtained by extrapolating a linear fitting in the Tauc plot, results in two values, i.e., 2.8 and 3.0 eV, in agreement with previous reports.⁴²⁵⁻⁴²⁶ The Tauc plots of the composite materials (Figure 8.16) evidence some changes with respect to the bare TE-C₃N₄ photocatalyst. A red shift is observed, particularly for the composites N2 and N3 compared to the pristine TE-C₃N₄, indicating that these materials absorb in the visible part of the spectrum. However, a modification of the C₃N₄ bandgap resulted indeed very unlikely, also considering the low amount of the semiconductor in the composite materials. The decreasing of the onset on the Tauc plot can be then attributed to the medium and disorder effects provided by the nanosponge matrix (considered that specific nanosponge-C₃N₄ interactions are ruled out by FTIR evidence). In any case, although absorption occurs at lower energies (Figure 8.16) the band gap of C₃N₄ does not change significantly.⁴⁵⁶

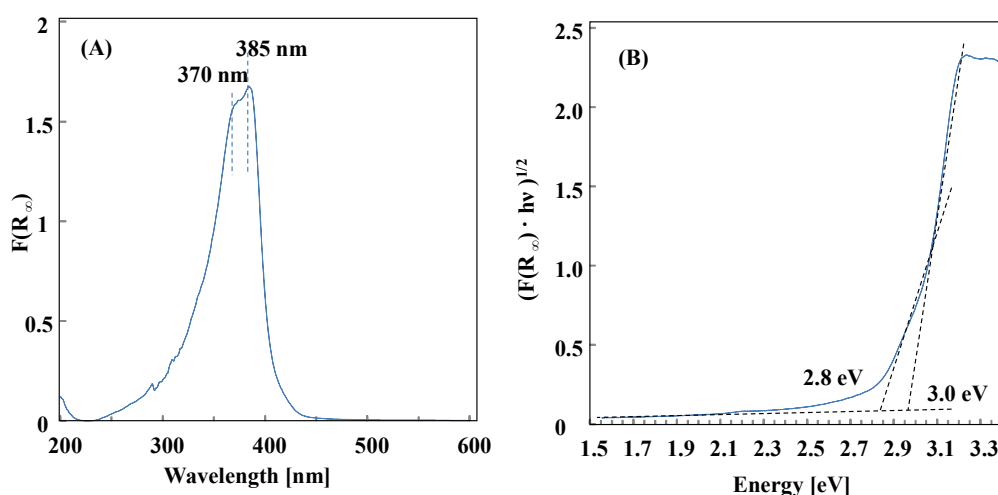


Figure 8.15 Absorbance of the TE-C₃N₄ pristine sample (A) and Tauc-plot of the same material (B).

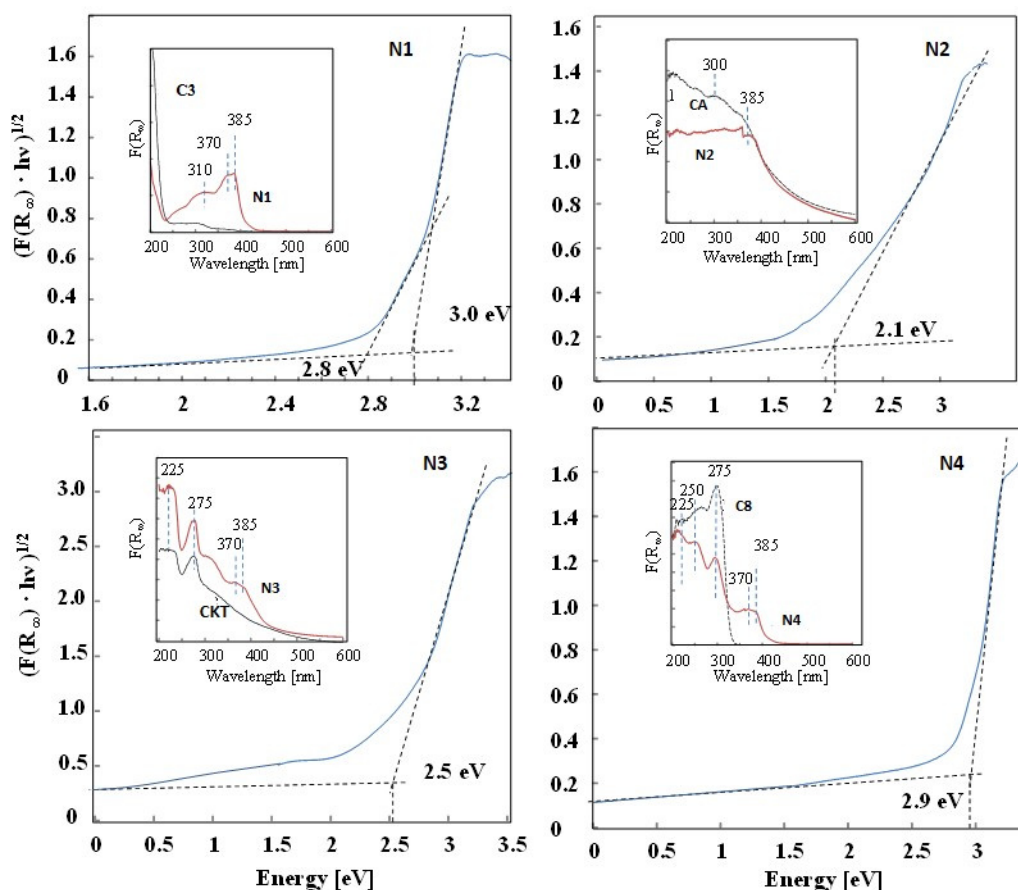


Figure 8.16. Tauc-plot of the composite materials N1 to N4 along with, in the inset, the absorbance of the NSs (C3, CA, CKT and C8) and composites (N1 to N4)

8.3 Photocatalytic activity of materials synthesized with GO and RGO

In order to provide a relevant comparison with the literature, in particular the work by Divya *et al.*,⁴³⁴ a preliminary efficiency assessment for the plain RGOAg photocatalyst was carried out towards Rhodamine B (RB). In fact, after two hours of irradiation under visible light in the presence of the RGOAg photocatalyst (20 mg per 100 mL of dye solution), an almost quantitative degradation for a 1 ppm solution of the dye is recorded. In this case, it was preferred to work with a more concentrated dye solution (5.0 ppm), using the same catalyst weight/solution volume ratio. According to ICP data, this corresponds to the presence of 0.78 mmol/L of Ag, compared with the theoretical 0.93 mmol/L of Ag of the reference work.⁴³⁴ The plain RGOAg composite prepared in this work apparently showed a similar activity as compared with Divya's results. In fact, after 2 h of irradiation, ca. 91% of the dye apparently disappeared. However, the potential existence of unreacted RB adsorbed onto the

photocatalyst was examined considering the well-known adsorption properties of graphene derivatives towards organic substances. Then, the catalyst was recovered by centrifugation after the photodegradation experiment, and then it was washed with methanol. In fact, it was actually found that ca. 74% of the initial dye could be retrieved unchanged. Thus, one can conclude that only a 17% amount of dye actually undergoes photodegradation under the operational conditions. It must be noted that the reference work did not include information on a comparable counter-check.⁴³⁴ Thus, the amount of unreacted material eluted (MeOH) following the photoirradiation experiment was taken into consideration in order to calculate the real neat photodegradation yields. Further tests with the title RGOAg composite revealed a fair activity only towards Bisphenol A, though under slightly modified conditions, i.e. starting from a 40 ppm solution, 4 mg of photocatalyst per 10 mL of solution and 300 min irradiation. Unfortunately, RGOAg was found inactive towards Bromochresol Green (BCG) and Methyl Orange (MO). It is important to note that RGOAg in the dark is able to adsorb quite effectively RB (88%) and MO (47%), whereas little adsorption of BA and BG is found. The whole of the results obtained with the three materials is summarized in Table 8.1.

Considering materials M1 and M2, operational conditions were set to have an amount of silver in the reaction sample roughly comparable with that present in the case of RGOAg. Therefore, 20 mg of photocatalyst per 10 mL of solution were generally used in the case of M1 and 10 mg per 10 mL in the case of M2. As regards M1, decreased activity towards BA (only 10% of photodegradation, under the same conditions) was observed if it is compared to the plain RGOAg. Conversely, activity towards dyes is quite interesting, because a significant degradation was observed after 2 h irradiation with both dyes BCG (22%) and MO (24%). Regarding RB, degradation after 2 h showed only a minor increase with respect to RGOAg. However, tests at different times showed that there is anyway a significant effect on reaction rates. In fact, after only 1 h of irradiation a 19% degradation was observed; moreover, it is possible to observe that after 3 h a slightly increased up to 21% occurs. Again, these results can be easily justified considering the ability of the material M1 to absorb the different substrates in the dark. In fact, under the same concentration conditions, for dyes RB, MO and BCG the adsorption percentages are 52%, 64% and 89%, respectively, but only 15% for BA. This positively outlines the favorable effect of the ability of the photocatalyst to interact with the organic substrate that can reasonably lead back to a local concentration effect, as mentioned in the Introduction.

Entry	Catalyst	Substrate	mg of catalyst per 10 mL	Initial conc. (ppm)	Time (h)	% of degraded substrate	light source
1	RGOAg	BA	4	40	5	32 ± 2	halogen lamp
2	RGOAg	RB	2	5	2	17 ± 1	halogen lamp
3	RGOAg	BCG	2	5	2	-	halogen lamp
4	RGOAg	MO	2	5	2	-	halogen lamp
5	M1	BA	40	40	5	10 ± 1	halogen lamp
6	M1	RB	20	5	1	19 ± 1	halogen lamp
7	M1	RB	20	5	2	20 ± 1	halogen lamp
8	M1	RB	20	5	3	21 ± 1	halogen lamp
9	M1	BCG	20	5	2	22 ± 1	halogen lamp
10	M1	MO	20	5	2	24 ± 1	halogen lamp
11	M1	RB	20	5	2	-	Blue LED 467 nm
12	M1	RB	20	5	2	16 ± 1	Green LED 513 nm
13	M1	RB	20	5	2	14 ± 1	Red LED 627 nm
14	M1	RB	20	5	2	15 ± 1	NIR LED 860 nm
15	M2	BA	10	40	5	-	halogen lamp
16	M2	RB	10	5	2	29 ± 2	halogen lamp
17	M2	BCG	10	5	2	95 ± 3	halogen lamp
18	M2	MO	10	5	2	77 ± 3	halogen lamp

Indeed, considering the adsorption percentages reported above, it can be roughly estimated that the concentration of the dye within the NS phase is ca. three orders of magnitude larger than in solution. The apparently unfavorable effect towards BA can be related to its scarce adsorption, which in turn leads back to its scarce affinity for the cyclodextrin cavity. This is consistent with the fact that shape and polarization complementarity plays a significant role in host-guest supramolecular interactions.^{103,106,457} Contrary to dyes, which present a flat, largely conjugated and polarizable molecular structure, the bent and almost apolar molecule of BA cannot effectively interact with the host subunits. The effect due to the NS matrix is even more apparent on passing to material M2. In fact, the NS backbone is positively charged, probably because of the presence of the polyamine chain linkers, which are partly protonated even under neutral pH conditions. This enhances the electrostatic effects affecting the adsorption properties of the material. It can be preliminarily observed that M2 adsorbs BA up to a negligible extent; conversely, the adsorptions for dyes RB, MO and BCG is equal to 68%, 78% and 77%, respectively. Consistently, no photodegradation was observed for BA, whereas a further improvement was observed for the degradation of dyes, resulting as large as 29% for RB, 77% for MO, up to an outstanding 95% for BCG. The latter

results must be evaluated carefully, because the actual amount of silver present in the system must be kept into account, i.e. 0.080 mg/mL and 0.156 mg/mL for M1 and M2, respectively. Hence, after suitable normalization of the results, it is interesting to notice that degradation of cationic RB is not enhanced at all, whereas significant enhancement occurs for anionic MO and BCG. These observations are in perfect agreement with the aforementioned cationic character of the backbone of the material M2, which, of course, favors the interaction with anionic species.

Further insights were gained by examining the effect of the light source. In particular, the degradation of RB with M1 was performed in the presence of some LED sources, under the same reaction and irradiation power conditions. Interestingly, no degradation was induced by blue radiation (467 nm); conversely degradation was observed in the presence of green (513 nm), red (627 nm) and NIR (860 nm) radiation, although slightly lesser performances (15%) occur in comparison with the use of polychromatic halogen lamp. These observations outline the existence of a lower wavelength limit for the induction of the electron transfer process involved in the photodegradation reactions. Noticeably, this limit corresponds to the wavelength at which the photoreduction of Ag^+ ion and the formation of silver nanoparticles is induced in the presence of polyaminocyclodextrins.⁴⁴⁵

Finally, the possible recyclability of the materials was verified; in particular, the reuse of material M2 in the photodegradation of RB was tested. This material was used up to nine reaction cycles. At each cycle, the catalyst suffered only minor mass losses during recovering (less than 3%). This led to an overall 80% mass recover at the ninth cycle. Normalized results obtained keeping into account these losses are summarized in Table 8.2.

Table 8.2. Recycling results of the M2 catalyst using RB.

Cycle	1	2	3	4	5	6	7	8	9
degradation %	29 ± 1	16 ± 1	20 ± 1	24 ± 1	30 ± 1	31 ± 1	30 ± 1	32 ± 1	23 ± 1

It is possible to note a peculiar trend. In fact, a significant drop in activity can be observed after the first use. Then, activity is progressively recovered in the subsequent reuses; however, it drops again at the ninth cycle. This complex trend can be justified in terms of wearing out of the material. In details, after the first use, a non-negligible amount of the silver loading is lost; in fact, the UV-vis spectrum of the aqueous solution, recovered after the photoirradiation experiment, presents the typical surface plasmon resonance band of nanoparticle silver centered at 420 nm. It can be reasonably hypothesized that relatively loosely bound AgNPs residing on the surface of the photocatalyst grains (which are well

visible in SEM micrographs) are mechanically lost during the process. The subsequent activity recover can be attributed to the gradual mechanical fragmentation of the material in the various reuses, which increases the surface exposed to the solution, thereby improving the permeability and the exchange ability of the nanosponge. However, this mechanism works until further loss of actual photocatalyst occurs. Comparison between the SEM micrographs of freshly prepared M2 (Figure 8.12d) and after nine reuse cycles (Figure 8.17), can be evidenced a loss of silver load. In fact, Ag nanoparticles on the surface appear much sparser on the surface of the nanosponge support, which in turn shows a fair mechanical wearing.

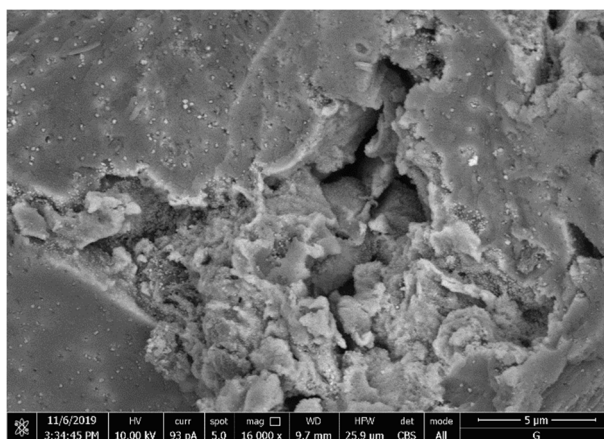


Figure 8.17. SEM micrographs M2 after nine reuse cycles (the reference line represents a 5 μ length).

8.4 Photocatalytic activity of materials synthesized with C₃N₄

The photodegradation of 4-nitrophenol (4NP), which was used as a probe reaction, served as a preliminary test to verify the photocatalytic activity of the materials. No appreciable degradation of 4NP occurred in the presence of the bare nanosponges. On the contrary, the N1, N2 and N4 composites successfully accelerated the photodegradation process, while the N3 composite was almost inactive. In Figure 8.18, the trends of the 4NP concentration during the photocatalytic experiments are reported. During the initial dark 30-minute equilibration phase, the 4NP was partially absorbed in the presence of all solids. Due to photothermal effects, the substrate might partially desorb at the start of the irradiation. However, at some point the photodegradation process becomes evident as the 4NP concentration decreases over time (Figure 8.18).

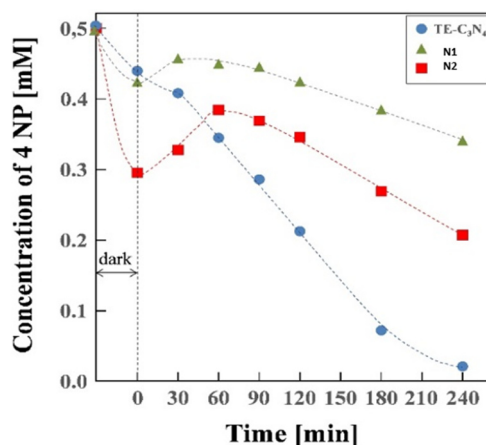


Figure 8.18. Concentration of 4NP versus time for run carried out in the presence of N1, N2 and TE-C₃N₄ photocatalyst.

The reaction course follows an apparent linear trend and the calculated initial reaction rates for the different experiments are collected in Table 8.3. The composites appear less effective than pristine TE-C₃N₄; however, taking into account the low content of TE-C₃N₄ (5% w/w), data normalization indicate that C3 and CA materials at both pH values, as well as the composite C8 at pH 5.5, were significantly more effective than the bare TE-C₃N₄. This confirms that embedding the C₃N₄ photocatalyst within the nanosponge enhances the photocatalytic activity, again due to the local concentration effect. Moreover, a slight increase of activity in the presence of CA (containing the nanosponge functionalized with basic amino groups) occurred by decreasing the pH. Conversely, the activity decreases dramatically by using N4 (containing the nanosponge functionalized with carboxylated groups) photocatalyst at pH 3.7. This behavior can be justified by considering the variations in the hydrophilic character of the nanosponge matrix, as the charge state of the ionizable groups present changes with the pH.^{84,87} The lack of activity of the N3 sample is justified considering the fact that a lower hydrophilic character can lead to a decreased mobility of the aqueous medium within the matrix and a less efficient mass transfer, both of which negatively influence photocatalytic performance. Based on the results obtained using 4NP, N1 and N4 composites have been chosen for further photocatalytic studies where HMF and VA were used as substrates. In fact, though the N2 photocatalyst gave the best results in terms of photoactivity, the occurrence of a strong initial adsorption of the substrate (ca. 40%) makes difficult the determination of the reaction rate.

Table 8.3. Initial reaction rate of 4NP degradation in the presence of the various materials prepared and the corresponding values normalized per gram of TE-C₃N₄ present in the photocatalyst.

Photocatalyst	Initial reaction rate ($\mu\text{mol h}^{-1}$ per g of material)		Normalized initial reaction rate ($\mu\text{mol h}^{-1}$ per g of TE-C ₃ N ₄)	
	pH = 5.5	pH = 3.7	pH = 5.5	pH = 3.7
TE-C ₃ N ₄	116 $\pm$ 6	122 $\pm$ 6	116 $\pm$ 6	122 $\pm$ 6
C3	35.5 $\pm$ 1.5	32.2 $\pm$ 1.4	710 $\pm$ 30	664 $\pm$ 30
CA	56.5 $\pm$ 1.8	64.6 $\pm$ 1.9	1130 $\pm$ 40	1290 $\pm$ 40
CKT	negligible	Negligible	negligible	negligible
C8	35.3 $\pm$ 1.5	Negligible	710 $\pm$ 30	negligible

From an industrial perspective, the selective partial oxidation of HMF and VA is noteworthy. The partial oxidation products of HMF, schematized in Figure 8.19, are 2,5-furandicarboxyaldehyde (FDC), formylfuroic acid (FFA) and 2,5-furandicarboxylic acid (FDCA), an important building block for the synthesis of polymeric materials. Similarly, the photooxidation derivatives of VA (Figure 8.20), a natural degradation product of lignin, are useful in food and flavor chemistry.

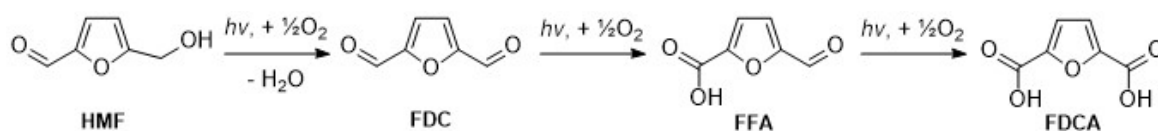


Figure 8.19. Partial oxidation products of 5-hydroxymethylfurfural (HMF).

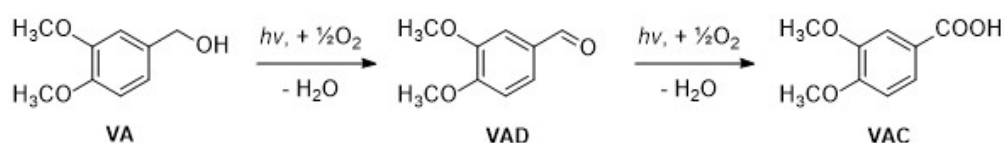


Figure 8.20. Partial oxidation products of veratryl alcohol (VA)

In particular, the corresponding veratryl aldehyde (VAD) is the most important one due to its high added value. A further oxidation of VAD gives rise to the formation of veratric acid (VAC). The photocatalytic experiments using HMF and VA as substrates were carried out at pH 5.5 with the four setups described in the Experimental section. The concentrations of HMF and FDC, its main partial oxidation product, during the photocatalytic experiments in

the presence of bare TE-C₃N₄ and N1 composite are reported in Figure 8.21(A) and 8.21(B), respectively. Noteworthy, traces of FFA were also detected during the tests. All the photocatalysts were preliminarily equilibrated with the HMF solution in the dark for 30 min, during which a partial adsorption occurred as in the case of 4NP, in particular on the composite materials.

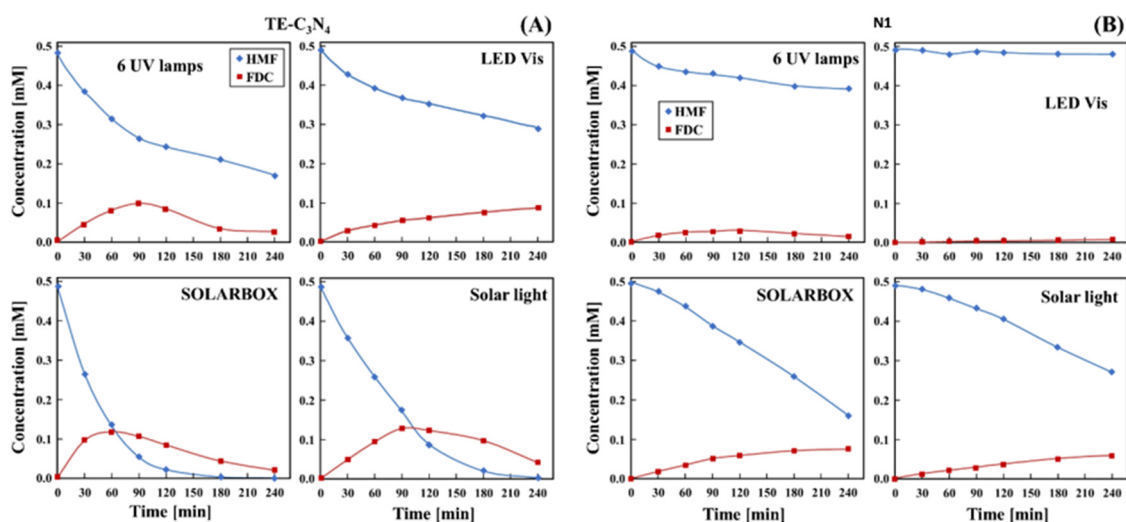


Figure 8.21. Concentration of 5-hydroxymethylfurfural (HMF) and 2,5-furandicarboxyaldehyde (FDC) versus time for runs carried out in the presence of TE-C₃N₄ photocatalyst (A) or N1 (B) composite under different irradiation sources: 6 UV lamps; Visible LED; SOLARBOX or natural sunlight.

After the start of irradiation, almost all the adsorbed organic molecules were released, likely due to the thermal effect. According to the initial conversion rate of the substrate and the corresponding values normalized per gram of TE-C₃N₄ present in the photocatalyst, the results obtained in all setups and in the presence of all the photocatalysts tested (TE-C₃N₄, N1 and N4) during the partial oxidation of HMF are summarized in Table 8.4. Inspection of the data in Table 8.4 indicates that bare TE-C₃N₄ was apparently more efficient than the composites for the photo-oxidation of HMF. Again, however, the initial reaction rate normalized per gram of TE-C₃N₄ delineates a large beneficial effect of the nanosponge presence, particularly for the N4 composite. As regards the radiation source role, the highest activity was observed using both the SOLARBOX or the natural solar irradiation containing the highest UV component, whilst the visible LED was the least effective irradiation source. The latter finding can be reasonably attributed to the lack of the UV component.

Table 8.4. Initial reaction rate of HMF conversion in the presence of the various materials prepared and the corresponding values normalized per gram of TE-C₃N₄ present in the photocatalyst. The amount of catalyst used in each run was 200 mg in the case of the UV lamp and the visible LED and 50 mg in the case of the SOLARBOX and sunlight. The TE-C₃N₄ content in the N1 and N4 composites was 5 wt.%.

Photocatalyst	Initial reaction rate ($\mu\text{mol h}^{-1}\cdot\text{g}_{\text{cat}}^{-1}$)				Normalized initial reaction rate ($\mu\text{mol h}^{-1}\cdot\text{g}_{\text{TE-C}_3\text{N}_4}^{-1}$)			
	UV lamp	Vis. LED	SOLAR BOX	Sunlight	UV lamp	Vis. LED	SOLAR BOX	sunlight
TE-C ₃ N ₄	150	94	668	386	150	94	668	386
N1	58	—*	60	47	1160	—*	1200	940
N4	125	60	147	212	2500	1200	2940	4240

*Almost inactive

The analytical study of the oxidation of HMF indicated that during the first steps of the reaction the selectivity towards FDC was approx. 50%, while it was less than 4% towards FFA. These values decreased for longer reaction times. Consequently, ca. 50% of the photo-oxidized HMF underwent mineralization through a series of unidentifiable by-products already during the first steps of the reaction. This result suggests that the degradation of HMF proceeds by at least two parallel reactions, one of which gives rise to the formation of FDC. Since only FDC and small amounts of FFA (resulting from further oxidation of FDC) were found as the products, the parallel and/or successive reactions resulted in the complete mineralization of HMF by the formation of adsorbed open-chain aliphatic compounds, as observed elsewhere.⁴²⁵ A careful inspection of the experimental data shows that in various cases the concentration of FDC follows a bell-shaped trend and that the selectivity (rarely higher than 50%) decreases by increasing the HMF conversion, in particular under sunlight or SOLARBOX irradiation conditions, due to the higher fraction of UV light and the thermal effect. The Figure 8.22(A) and 8.22(B) show respectively the time profiles for veratryl alcohol (VA) and its main partial oxidation products (VAD and VAC) obtained during the runs carried out in the presence of the bare TE-C₃N₄ and N4 composites. All photocatalysts were preliminarily equilibrated with the VA solution in the dark for 30 min, during which, as in the case of 4NP and HMF, a partial adsorption occurred in particular on the composite materials. After the start of irradiation, almost all the adsorbed organic molecules were released due to the thermal effect. For this reason, the preliminary adsorption step is not shown in Figure 8.22.

The results obtained in all the setups and in the presence of all the photocatalysts tested (TE-C₃N₄, N1 and N4) during the partial oxidation of VA are summarized in Table 8.5 in terms of the initial conversion rate of the substrate and corresponding values normalized per gram of TE-C₃N₄ present in the photocatalyst. For the photooxidation of veratryl alcohol (VA) the

beneficial effect due to the association of the photocatalyst with the nanosponge is even more evident than in the case of HMF. In fact, the photoactivity of N1 and N4 materials is in many cases even comparable to the pristine TE-C₃N₄ (Table 8.5).

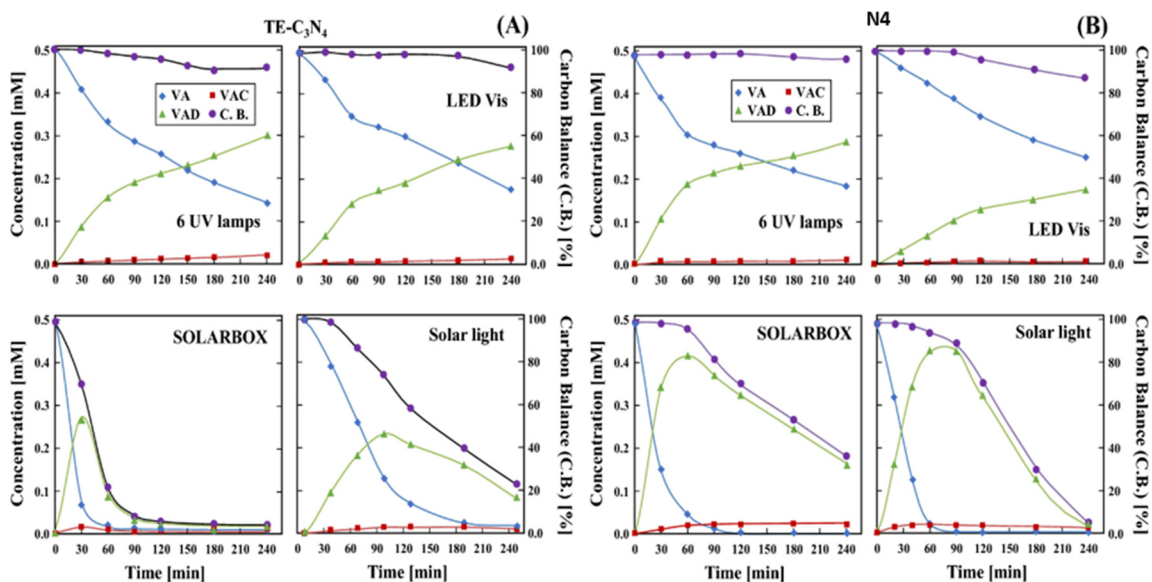


Figure 8.22. Concentration of veratryl alcohol (VA), veratryl aldehyde (VAD), veratric acid (VAC) and the carbon balance versus time for runs carried out in the presence of TE-C₃N₄ photocatalyst (A) or N4 (B) composite under different irradiation sources: 6 UV lamps; Visible LED; SOLARBOX or natural sunlight.

Table 8.5. Initial reaction rate of VA conversion in the presence of the various materials prepared and the corresponding values normalized per gram of TE-C₃N₄ present in the photocatalyst.

Photocatalyst	Initial reaction rate ($\mu\text{mol h}^{-1}\cdot\text{g}_{\text{cat}}^{-1}$)				Normalized initial reaction rate ($\mu\text{mol h}^{-1}\cdot\text{g}_{\text{TE-C}_3\text{N}_4}^{-1}$)			
	UV lamp	Vis. LED	SOLAR BOX	Sunlight	UV lamp	Vis. LED	SOLAR BOX	sunlight
TE-C ₃ N ₄	154	96	1278	327 1000 ^a	154	96	1278	327 1000 ^a
N1	135	53	480	345 660 ^a	2700	1060	9600	6900 13200 ^b
N4	138	59	1023	520 1520 ^a	2760	1180	20,460	10,400 30400 ^b

^a $\mu\text{mol}\cdot\text{kJ}^{-1}\cdot\text{g}_{\text{cat}}^{-1}$; ^b $\mu\text{mol}\cdot\text{kJ}^{-1}\cdot\text{g}_{\text{TE-C}_3\text{N}_4}^{-1}$

A different adsorption behaviour with the formation of species more or less destabilized interacting differently with the photons, can tentatively justify, in our opinion, the comparable photoactivity of N1 and N4 materials to that of pristine TE-C₃N₄. Furthermore, the fact that some molecules can be destabilized in different extent when adsorbed onto a photocatalyst can allow their partial oxidation even under light with lower energy giving rise to comparable activity by irradiating the system with UV or visible light. In the past, it has

been reported that some molecules, as for instance acetic acid, that are stable in gaseous phase even under UV-light irradiation, can be decomposed when adsorbed on TiO₂ and other insulating and semiconductor materials.⁴⁵⁸⁻⁴⁵⁹ Another example is the degradation of Rhodamine B (RB) in aqueous solution over BiOBr irradiated with light at 532 nm.⁴⁶⁰ Almost no degradation was observed in homogeneous system due to the low energy of photons. The bandgap of BiOBr semiconductor is 2.9 eV and consequently it cannot be excited by the photons at 532 nm. However, it was found that RB molecules were effectively removed and decomposed over BiOBr irradiated with light at 532 nm. These observations indicate a general behaviour in heterogeneous photocatalysis, i.e. the behaviour of some species under irradiation is completely different, when adsorbed, although the examples mentioned above are heterogeneous photocatalytic reactions different from those studied in this work.

According to the data in Figure 8.22, in which the carbon balance has also been reported, the mineralization of the organic matter occurs to a significant extent only at high alcohol conversions. In any case, VA was oxidized to veratryl aldehyde, which is subsequently transformed into veratric acid (VAC). The concentration of the latter remains always quite low, whereas the veratryl aldehyde follows a bellshaped trend, indicating that VAC was rapidly photomineralized. Furthermore, it was observed a slower photo-conversion of VA into VAD under irradiation by visible LED and UV lamps, for which the UV component was absent or lower with respect to the SOLARBOX or natural sunlight. In these cases, a very high selectivity towards the formation of aldehyde (> 90%) throughout the runs are observed, although in these cases the VA conversion never exceeded 70%. Conversely, in the SOLARBOX or under natural sunlight, the VA conversion was much faster than in the first two systems. The selectivity towards the aldehyde was ca. 85–90% only in the case of runs carried out in the presence of composites even when the VA was almost completely photo-converted (Figure 8.22 (B) SOLARBOX and solar light). Besides, in the presence of pristine TE-C₃N₄, the selectivity towards the aldehyde was very low after high VA conversions (Figure 8.22 (B) SOLARBOX and sunlight).

Consequently, the selectivity towards the aldehyde was greater than 90% even after ca. 80–90% alcohol conversion only in the cases of composite materials. This finding indicates that the nanosponge matrix not only causes an increase in the reaction rate per gram of photoactive material by increasing the local concentration of the substrate, but apparently protects the veratryl aldehyde (in particular for the N4 composite) from its further oxidation to veratric acid which is rapidly mineralized. This fact is clearly highlighted in Figure 8.23, which reports the evolution of VA and its derivatives concentration for experiments carried

out under natural sunlight versus the cumulative energy entering the photoreactor. Indeed, comparing the results obtained in the presence of bare TE-C₃N₄, and N1 and N4 composites (after 10-15 kJ·L⁻¹ of cumulative energy have entered the reactor), it is possible to observe that the VA is completely (N4) or almost completely photo-converted, but the selectivity towards veratryl aldehyde is 35%, 56% and 88%, respectively.

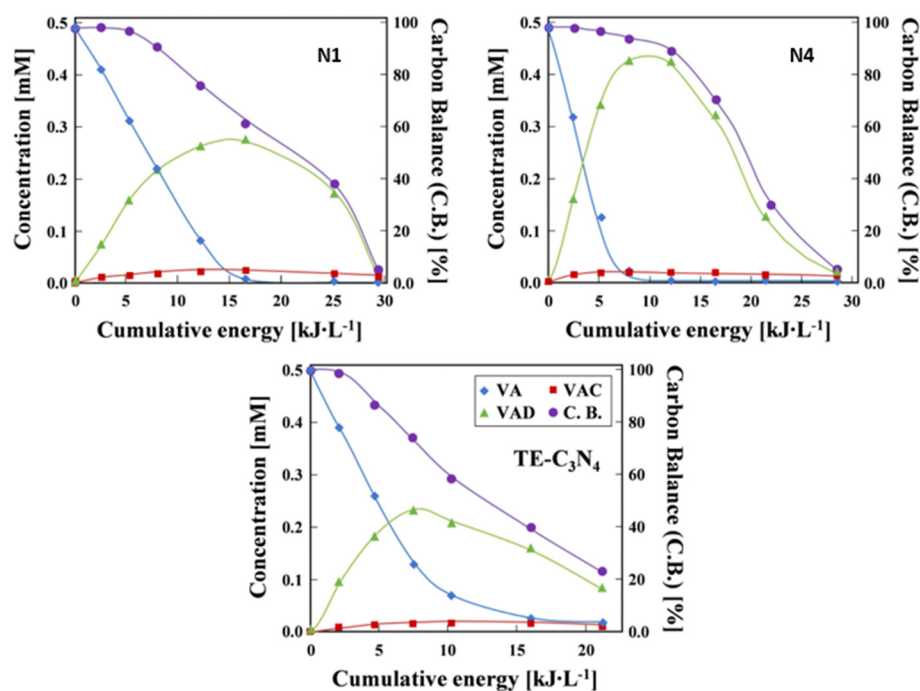


Figure 8.23. Concentration of veratryl alcohol (VA), veratryl aldehyde (VAD), veratric acid (VAC) and the carbon balance versus cumulative energy for run carried out under natural sunlight in the presence of N1, N4 composites and TE-C₃N₄.

8.5 Concluding highlights

Composite materials were prepared, consisting of a RGOAg or a TE-C₃N₄ photocatalyst embedded into a nanosponge architecture. The characterization of the composites suggested the occurrence of no specific interaction between the photocatalyst and the nanosponge matrix. Then, the photocatalytic activity of the diverse materials was explored. The composites were tested for the oxidative photodegradation of some model organic pollutants; moreover, materials loaded with carbon nitride were also tested for the selective oxidation of aromatic alcohols of industrial interest. The composite photocatalyst systems showed

enhanced performances with respect to the base photoactive species. Possible recyclability was also verified in one case. The better performances can be positively attributed to ability of the nanosponge to concentrate and capture the organic substrate in the proximity of the photocatalyst.

8.6 Experimental section

8.6.1 Preparation of GO and its derivatives

Pristine graphene oxide (GO). The pristine GO product was prepared according to a modified Hummers procedure. In details, graphene (1.04 g), NaNO_3 (0.52 g, 6.1 mmol) and conc. H_2SO_4 (23 mL, 429 mmol) are mixed at 0 °C under vigorous stirring, then solid KMnO_4 (3.02 g, 19.1 mmol) is cautiously added in small portions. The mixture is kept under stirring for 2 h at 0 °C and subsequently warmed at 35 °C in 30 min. Then, H_2O (46 mL) is added and the system is stirred at 90 °C for 50 min. After a short cooling, further water (140 mL) and 30% H_2O_2 (3 mL) are added, and the mixture is allowed to settle at r.t. for 3 days. The precipitated solid is decanted and treated with conc. HCl (20 mL) at r.t. overnight. The solid is separated and repeatedly washed with warm water (70 °C); then, it is suspended again in cold water (110 mL) and cautiously treated with NaHCO_3 up to neutrality. The final product is centrifuged, washed with methanol (twice) and diethyl ether (twice), dried overnight in vacuo over P_2O_5 at 60 °C, and crunched in a mortar. Yield 1.20 g.

Reduced graphene oxide (RGO). The pristine GO (200 mg) is suspended in water (15 mL) and treated with an excess NaBH_4 (200 mg, 5.28 mmol). The mixture is allowed to stir at r.t. for 30 min; then HCl 1 M is added dropwise up to neutrality. The solid is centrifuged, washed with methanol (twice) and diethyl ether (twice), dried overnight in vacuo over P_2O_5 at 60 °C, and finally crunched in a mortar. Yield 198 mg.

Reduced graphene oxide/silver nanoparticles composite (RGOAg). The pristine GO (214 mg) is dispersed in water (15 mL) by brief sonication, and solid AgNO_3 (339 mg, 2 mmol) is dissolved into the suspension. Then NaBH_4 (75 mg, 2 mmol) is added, and the mixture is kept under vigorous stirring at r.t. for 30 min. The system is allowed to settle overnight, then the supernatant liquor is decanted off. The solid is washed with methanol (twice) and diethyl ether (twice) and filtered off. Yield 401 mg.

Synthesis of Material M1. Anhydrous β CD (283 mg, 0.25 mmol) and RGOAg composite (40.5 mg) from the previous preparation are carefully mixed in a mortar. The obtained powder is introduced into a little vial, then a solution is added obtained by dissolving hexamethylene-bis-isocyanate (H, 120 μ L, 126 mg, 0.75 mmol) in DMSO (250 μ L). The system is mechanically mixed with a tiny metal rod to obtain a paste which is allowed to react still at 60 °C for 20 h. The crude solid obtained is grossly crunched and transferred into a centrifuge tube with methanol (30 mL), shortly sonicated and centrifuged. The product is again washed with methanol (30 mL) and then with diethyl ether (twice, 30 mL each). Finally, the solid is dried overnight in vacuo over P_2O_5 at 60 °C, crunched in a mortar and passed through a 150 μ sieve. Yield 282 mg. A sample of a polyurethane- β CD NS material analogous to M1 without the RGOAg composite was prepared according to literature²¹⁵ for useful comparison, using the same cyclodextrin/H mole-to-mole ratio.

Synthesis of Material M2. The *per*-(6-iodo)-(6-deoxy)- β -cyclodextrin (260 mg, 0.136 mmol), the *per*-{6-[3-methyl-3-(3-aminopropyl)]-propylamino}-(6-deoxy)- β -cyclodextrin (274 mg, 0.124 mmol) and the pristine GO (81 mg) are carefully mixed in a mortar. The powder mixture is transferred in a little vial, DMSO (300 μ L) is added, and the system is mechanically stirred with a tiny metal rod to obtain a paste, which is allowed to react still at 60 °C for 48 h. The gummy crude formed is then washed twice with methanol and twice with diethyl ether as described hereinabove, to obtain a precursor solid material that is dried, crunched in a mortar and passed through a 150 μ sieve. This material is subsequently suspended in water (3 mL), $AgNO_3$ (250 mg, 1.47 mmol) is added, and the system is allowed to stir in the dark for 1 h. Then, excess $NaBH_4$ (200 mg, 5.28 mmol) is added to the suspension, and the system is stirred for further 30 min. The solid product is centrifuged, washed with methanol and diethyl ether, and finally dried as described previously. Yield 518 mg. A sample of the polyamino-cyclodextrin NS material analogous to M2 was prepared according to literature^{183,207} for useful comparison.

8.6.2 Preparation of $g-C_3N_4$ and its derivatives.

Synthesis of $g-C_3N_4$ and TE- C_3N_4 . The polymeric carbon nitride $g-C_3N_4$ was prepared via thermal condensation of melamine as reported elsewhere.⁹⁰ In this procedure, melamine (10 g) was placed in a covered ceramic crucible heated at 2 °C min⁻¹ up to 520 °C and maintained at this temperature for 2 h. The $g-C_3N_4$ sample was then subjected to a second thermal treatment with the aim to obtain a thermal etched material. This process was carried out in an uncovered ceramic bowl heated at a 3 °C min⁻¹ rate and maintained for 4 h at 500 °C. The obtained light yellow powder was designated as TE- C_3N_4 . Yield 2.8 g.

Synthesis of composite materials (N1-N4). The synthesis of the bare nanosponges was accomplished according to literature procedures.^{88,103,260,461-462} In brief, the proper reactants were mechanically mixed in a vial with a suitable amount of dry DMSO, and the mixture was kept overnight at 60 °C. For the corresponding composites, TE-C₃N₄ was added to reaction mixture, in such a way that the amount in weight of photocatalyst was as large as 5% of the total amount of reactants. In all cases, after the reaction time was completed, the crude material was mechanically crushed and repeatedly washed with water, then methanol and finally diethyl ether. The product was finally dried at 60 °C overnight in vacuo over P₂O₅, finely crunched in a mortar and passed through a 150 µm sieve. In these conditions all products can be stored indefinitely in a desiccator at r.t. before use. The amount of the reactants used for the preparation of each composite are reported below.

N1: 710 mg (0.625 mmol) of C, 54 mg of TE-C₃N₄, 300 µL of hexamethylene-bis-isocyanate (H, 315 mg, 1.875 mmol) and 700 µL of DMSO; yield 733 mg.

N2: 460 mg (0.200 mmol) of *per*-{6-[3-methyl-3-(3-aminopropyl)]-propylamino}-(6-deoxy)-β-cyclodextrin, 275 mg (0.144 mmol) of *per*-(6-iodo)-(6-deoxy)-β-cyclodextrin and 300 µL of DMSO; yield 572 mg.

N3: 140 mg (0.107 mmol) of *heptakis*-(6-azido)-(6-deoxy)-β-cyclodextrin, 300 mg (0.375 mmol) of tetra-propargyloxy-calix[4]arene and 3 mL of DMSO in the presence of a catalyst constituted by 40 mg (0.16 mmol) of CuSO₄·5H₂O and 61 mg (0.31 mmol) of sodium ascorbate. Yield 396 mg.

N4: 611 mg (0.54 mmol) of C, 351 mg (1.61 mmol) of pyromellitic anhydride (P), 70 µL of triethylamine and 700 µL of DMSO; yield 765 mg.

8.6.3 FTIR, ¹³C CP-MAS NMR, SEM, TGA and XRD characterizations of materials

UV-Vis spectra were recorded on a Beckmann Coulter DU 800 spectrophotometer equipped with a Peltier thermostatic apparatus. ATR-FTIR spectra were acquired on a Bruker LUMOS instrument. Raman spectra were acquired on a Horiba LabRAM HR Evolution apparatus using a 532 nm laser source. Scanning electron microscopy (SEM) and EDX images/spectra observations were acquired on a JEOL JEMES-2100 instrument and performed on the samples after deposition by sputtering of a thin gold layer, using a FEI Quanta 200 SEM microscope operating in high vacuum at 20 kV. Infrared spectra of the samples in KBr (Aldrich) pellets were recorded with a 4 cm⁻¹ resolution and 256 scans by using a FTIR-8400 Shimadzu spectrometer. An integrated sphere was used to obtain diffuse reflectance spectra (DRS) in air at room temperature in the 200-800 nm wavelengths range by means of a

Shimadzu UV-2401 PC spectrophotometer, by using BaSO₄ as the reference material. The CP-MAS solid-state ¹³C-NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer. Thermogravimetric analyses were performed on a TA Instruments TGA Q5000 apparatus under a nitrogen flux; samples were heated up to 900 °C at a 2.5 °C/min rate. ICP-OES analyses were performed on a Perkin Elmer Model Optima 2100 apparatus. X-ray diffraction (XRD) patterns were collected using a Philips PW 1050/39 diffractometer (Philips Analytical, Almelo, The Netherlands) with Bragg Brentano geometry, equipped with a Ni-filtered Cu K α radiation source and operated at 40 kV and 30 mA (step 0.1°; counting time 5 s/step); patterns were collected in the 2 θ range between 10° and 70°. The obtained XRD patterns were processed and analyzed using the Match! Software (Crystal Impact) and the linked Crystallography Open Database (COD). AFM images were acquired on a Bruker FastScan Bio apparatus.

8.6.4 Potentiometric titration

A carefully weighed amount of GO (ca. 40 mg) was suspended by sonication in 12.0 mL of freshly double distilled and degassed water; then, 3.0 mL of standard HCl 0.1 M were added. The suspension was then titrated with a standard NaOH 1.0 M solution, introduced in small portions by means of a Chemetron screw microsyringe. The pH value of the suspension was determined, after each addition of the titrant, by means of a common pH-meter, and the obtained titration curve was subjected to regression analysis according to literature,^{181,261} using the proper equation obtained analytically for the mixture of one strong acid with two weak bases in solution, i.e.:

$$\frac{v_i}{V_0} = \frac{\frac{n_A}{V_0} + \frac{K_w}{10^{-pH}} - 10^{-pH} - \frac{n_{B1}}{V_0} \cdot \frac{10^{-pH}}{10^{-pH} + 10^{-pK_{B1H^+}}} - \frac{n_{B2}}{V_0} \cdot \frac{10^{-pH}}{10^{-pH} + 10^{-pK_{B2H^+}}}}{c_{NaOH} + 10^{-pH} - \frac{K_w}{10^{-pH}}}$$

where V_0 is the initial volume, v_i is the volume of titrant NaOH solution, n_A is the excess in moles of strong acid, n_{B1} and n_{B2} the mole amounts of the virtual weak bases, K_{B1H^+} and K_{B2H^+} the dissociation constants of the relevant conjugate acid forms. Relying on the values of the apparent dissociation constants found, it is reasonable to assume that n_{B1} and n_{B2} refer to the amounts of carboxyl and phenol groups, respectively, present in the sample of material weighed for the titration experiment. Hence, the equivalents (ne_1 and ne_2) of the two functional groups per g of material can be trivially calculated.

8.6.5 ICP analyses

A carefully weighed amount (ca. 5.00 mg) of silver containing material was placed in a small ceramic crucible and mineralized with HNO₃ (ca. 2 mL) at 90 °C up to complete decomposition. The residue was dissolved in freshly double distilled water and diluted up to 100 mL. The obtained solution was filtered through a 0.45 µm Millipore membrane before analysis by Inductively Coupled Plasma Emission Spectroscopy (ICP-OES) technique by using a PerkinElmer Model Optima 2100, equipped with an autosampler model AS-90.

8.6.6 Photodegradation experiments for materials synthesized with GO and RGO

The proper amount (Table 8.1) of photocatalyst was mixed in a vial with a solution (10 mL) of the organic substrate dissolved in Milli-Q grade water (18.2 MΩ·cm) at the proper concentration (namely, 5.0 ppm for RB, BCG and MO, 40.0 ppm for BA). The sample was allowed to equilibrate in the dark for 30 min; then, it was irradiated in a home-made apparatus constituted by a box, having the internal walls covered in tin foil, and a 50 W halogen lamp placed in such a way to provide an irradiation power as large as 275 W/m². Irradiation was maintained for the proper time while stirring was achieved by mounting the described apparatus on an orbital shaker plate. After the proper irradiation time, the sample was centrifuged to separate the photocatalyst, and the residual amount of substrate was determined by UV-Vis spectrophotometry. Experiments with LED irradiation were performed in a similar way, by simply substituting the light source and maintaining the same irradiation power. All the experiments were performed in the presence of no buffer, after having checked that pH variation was negligible. In order to gain the correct estimation of the catalytic activity, the amount of unreacted adsorbed dye was evaluated. In detail, the catalyst recovered from the photodegradation experiment was repeatedly washed with small portions (i.e. 1 mL) of methanol till washings appeared colorless (three or four passages were usually sufficient; at each passage, the photocatalyst was recovered by centrifugation at 13000 rpm for 10 minutes). The collected liquors were diluted up to 10 mL, and the obtained solution was analyzed by UV-vis spectrophotometry to determine the residual dye concentration. Finally, the overall amount of unreacted dye was obtained by trivial stoichiometric calculations. Photodegradation data reported in Tables 8.1 and 8.2 are averaged over three replicates.

For the reuse tests, the photocatalyst recovered from the previous experiment was dried in vacuo at 60 °C overnight, carefully weighed, and re-suspended in the suitable amount of

fresh dye solution, in such a way to keep constant the catalyst-to-solution (w/v) ratio, namely 1 mL of solution per 1 mg of catalyst.

8.6.7 Photocatalytic activity tests for materials N1-N4

For the tests carried out under natural solar radiation, however, to consider the variability of solar irradiance with atmospheric conditions, the photonic flux in the range 315-400 nm, which largely determines the reactivity of photocatalytic materials, was measured every 10 min throughout the test, and the cumulative energy (E) was estimated based on the data obtained. The E value allows the comparison of photoreactivity results obtained under natural sunlight irradiation at different meteorological conditions. It is given by:

$$E = \int_0^t I(t) dt \quad (1)$$

where $I(t)$ is the instantaneous photon flow and t the irradiation time. The values of $I(t)$ were calculated from the recorded values of irradiance, UVG (t), according to the relationship:

$$I(t) = \text{UVG}(t) \cdot A \quad (2)$$

where A is the total irradiated area of the photoreactor and UVG is the irradiance (315-400 nm wavelength range).¹⁰⁵ The aliquots of reacting suspensions were filtered and analysed by HPLC. The aromatic species were analyzed by a Beckman coulter HPLC with a Diode Array detector. The column was a Phenomenex KINETEK 5 μm C18 and the eluent (0.8 mL min^{-1}) a mixture of acetonitrile and 13 mM trifluoroacetic acid (20:80 v:v). HMF and its intermediates and reaction products were analysed by a Thermo Scientific Dionex UltiMate 3000 HPLC equipped with a Diode Array detector and a REZEK ROA organic acid column with a 2.5mM H_2SO_4 aqueous solution mobile phase (flow rate 0.6 mL min^{-1}). Standards purchased from Sigma-Aldrich with a purity >99% were used to identify the products and to obtain the calibration curves.

9 – Conclusions, final remarks and perspectives

This final chapter is devoted at summarizing the results obtained in the whole of this PhD work. Drawing a unitary picture of a complex work, that has proceeded through different parallel pathways, is not straightforward. However, some main points can be undoubtedly pointed out, in order to stress the most important achievements reached.

1) Exploring the synthesis of nanosponge materials, it was possible to get deep insights regarding the outcome on the overall characteristics of the obtained product deriving not only by the choice of the constituent reactants and their combination ratio, but also by the choice of the reaction conditions. In this work, more than 50 diversely structured different materials were prepared, characterized and studied. In detail, cyclodextrin-only, calixarene-only and mixed cyclodextrin-calixarene materials were obtained, using four different species (namely, hexamethylene di-isocyanate, pyromellitic dianhydride, *N*-metyl-di-(3-aminopropyl)-amine and 1,6-hexanediol) as linkers or chain extenders, in such a way to vary the average length of the linker chains and, possibly, to decorate the structure with ionizable pH-responsive functional groups. The syntheses were designed in such a way to gain a suitable tunability of the physico-chemical features of the materials obtained, in particular as long as responsivity to pH conditions and permeability to aqueous media is concerned. This study led to get interesting information mechanism of formation of the reticulated network, which provides both a chemical cross-linking covalent bonding, and a physical interconnection process. Hence, a smart choice of the reaction conditions enables to obtain products with significantly different physical/mechanical features (in particular hardness and swellability).

2) Besides the ordinary spectroscopic (FT-IR, CP-MAS NMR) characterization of the materials, aimed at confirming the chemical structure, the assessment of the texture properties of nanosponges is a challenge. The FFC-NMR relaxometric approach provided an extremely useful and versatile tool in this sense. Indeed, NMR relaxometry may appear as an old niche technique; however, it is a very valuable and informative tool for assessing the dynamic regimes at a microscopic level. Hence, the methodologically innovative

heuristic inverse integral transform analysis of the NMRD dispersion curves, which has been very recently introduced, enabled to obtain detailed information on the functional mobility of solvent molecules within the pore network of the nanoponges. Indeed, relaxometric determinations on the water-saturated materials enabled to make a distinction between in-pore and solvation shell water molecules. In turn relaxometric data were useful for aiding the rationalization of the release abilities of the materials.

3) The pH-dependent adsorption abilities of the materials towards organic guests were carefully explored. On the whole, the results of almost 900 adsorption tests were collected. Such a massive dataset required a statistical analysis approach, in order to identify the general trends, rather than a point-by-point examination of every single datum. This enabled to obtain in a simpler and more straightforward way the desired information. The desired pH-responsivity is of course provided by the possible ionizable groups present. However, the features of the guest must be considered in addition. On the whole, data confirm the idea that the nanosponge-guest affinity derives from a balance between different factors, although a main control is undoubtedly exerted by Coulomb interactions. Also the size of the guest matters, depending on whether it is entirely included into the host cavity or partly resides within the pores. The possible association of nanosponges with other co-sorbents such as yeast and biochars was considered, and the results obtained positively assess the possible occurrence of a synergism, at least under certain conditions.

4) The pH-dependent release abilities were also addressed. In this work 30 inclusion composites were prepared and the release kinetics were studied. The unexpected observation of a stretched exponential kinetic profile was led back to the occurrence of a Gaussian distribution of activation energies. This, in turn, suggested the idea (supported by relaxometric characterization data) that the release rate is more strictly controlled by the host-guest inclusion equilibrium rather than by in-pore diffusion. The possible release of bioactive molecules was also addressed.

5) The use of nanosponges as functional supports for photocatalysts was explored. Also in this case, synergism was demonstrated, allowing to obtain photocatalytic systems with improved activity in comparison to the plain photoactive species (namely, reduced graphene oxide decorated with silver nanoparticles, or carbon nitride). The activity improvement can be attributed to the ability of the nanosponge support to concentrate the substrate near the photoactive species. So, these systems appear very promising for pollutant degradation applications.

From a methodological point of view, it is important to stress how the use of smart mathematical approaches was important in order to manage the amount of numerical data collected. Statistical analysis was essential for the interpretation of the adsorption tests, the relaxation kinetics and the release kinetics. Indeed, in the latter cases the individuation of the correct fitting model provided an important piece of information in order to achieve the rationalization of the results in term of the microscopic features of the systems.

As a final remark, it must be stressed that this work, by far from being exhaustive, constitutes only a step in the advancement of the knowledge of nanosponges. The possibilities in the design of diverse systems, provided by the possible choices in the constituent moieties (host units, linker units, chain extenders) and the possible post-modification of a pre-formed material, are virtually countless, and limited only by fantasy. The more information we acquire on these fascinating materials, the more questions arise...!

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