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Evolution of Cyclodextrin Nanosponges

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Evolution of Cyclodextrin Nanosponges

Abstract

Cyclodextrin-based nanosponges (CD-NSs) are insoluble, highly cross-linked 3D network polymers used in several scientific and technological fields, the main area of investigation concerns the pharmaceutical applications, in which CD-NSs have been mostly employed as drug delivery systems.

CD-NSs can be generally grouped into four consecutive generations, taking into account their chemical composition and properties. The 1st generation of NSs are plain nanosponges, subdivided into four main types: urethane, carbonate, ester and ether NSs, depending on the chemical nature of the functional group connecting the CD to the cross-linker. The 2nd generation of NSs are modified nanosponges characterized by specific properties, such as fluorescence and electric charge. The 3rd generation of NSs is represented by stimuli-responsive CD polymers, which are able to modulate their behavior according to external variations in the environment, such as pH and temperature gradients, oxidative/reducing conditions, and finally the 4th generation of NSs, a new family of molecularly imprinted CD polymers (MIPs), exhibiting a high selectivity towards specific molecules.

The following review focuses on the evolution of cyclodextrin nanosponges, listing some examples of each generation.

Keywords

Cyclodextrin nanosponges, stimuli responsive polymers, molecularly imprinted polymers, functionalized nanosponges

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Evolution of Cyclodextrin Nanosponges

1. Introduction

The term *nanosponge* (NS) refers to a class of insoluble materials with distinctive nanometric porosity and superior absorption/complexation properties. NSs can be synthesized using either organic or inorganic compounds.

Cylodextrins (CDs) are truncated cone-shaped cyclic oligosaccharides composed of glucopyranose units arranged around a slightly hydrophobic cavity, which can accommodate guest molecules through the formation of inclusion complexes. These nano-sized cavities render CDs suitable building blocks for organic NSs (Bilensoy, 2011; Szejtli, 1988).

Reactive hydroxyl groups oriented towards the exterior side of CDs allow them to act as polyfunctional monomers, able to be cross-linked using a wide variety of bi- or polyfunctional chemicals, including dianhydrides, diisocyanates, active carbonyl compounds, epoxides, carboxylic acids, etc. Consequently resulting with insoluble three-dimensional covalent networks, namely CD-based NSs. Final characteristic properties of CD-NSs are strongly influenced by the nature of the cross-linker used and degree of cross-linking taking place (Trotta, 2011).

Cross-linking CDs brings significant benefits to CD-NSs compared to the respective native CDs used. In general, CD-NSs are able to form complexes with a wider series of molecules. This is due to the presence of interstitial spaces among CDs, which can host more hydrophilic guests. A further advantage, deriving from the use of NSs, is represented by the polymer network that surrounds the cavities and hampers the diffusion of entrapped guest molecules, thus promoting slower release kinetics. No less important is the fact that NSs are insoluble, hence they can be easily recovered from aqueous media and recycled.

The commonly used method of NSs synthesis consists in dissolving the chosen CD in a suitable solvent (usually an aprotic organic solvent), then introducing a catalyst (if required) and finally adding a cross-linker (Patel and Deshpande, 2014), under continuous stirring or sonication (Trotta, et al., 2006). A melt polymerization can also be performed if the cross-linker is in the liquid form and able to solubilize the CDs (Trotta et al., 2009a).

In some cases, an increase in temperature is necessary to initiate the cross-linking process. The overall reaction can be either a sol-gel process or a precipitation polymerization, respectively leading to the formation of a monolithic block or in the latter case, to a freshly formed polymer separated from the liquid phase by precipitation (Patel and Deshpande, 2014). Once the synthesis has been completed, the solvent, the catalyst, eventual unreacted monomers and byproducts are removed from the polymer by washing with water and other volatile solvents. At the end, a solid powder is collected.

Besides these procedures, different routes for the synthesis of NSs have been explored. Reported is the CD-based NSs interfacial polymerization where two immiscible solutions are mixed and stirred vigorously; a solution prepared by dissolving CDs in an alkaline solution and another by solubilizing the chosen cross-linker in a chlorinated solvent. Cross-linking occurs rapidly at the interface of the immiscible phases and a white insoluble precipitate is obtained (Trotta et al., 2012a).

Another example is the synthesis of CD-based NSs via simple dehydration reactions. In this case, the CD and the cross-linker (usually an acid, bearing two or more carboxylic groups) are solubilized in water, in the presence of a suitable catalyst. The solution is then heated (under low pressure in

some cases) to remove excess water that was introduced as solvent as well as the water released as byproduct of the cross-linking condensation reaction (Martel et al., 2005; Zhao et al., 2009).

Most of the CD-NSs, described in the literature, are prepared through step grow polymerization reactions; different synthesis mechanisms, such as radical polymerization, involve multistep procedures, as preliminary derivatization of CDs might be required (Lu et al., 2008; Demir et al., 2008).

The application of CD-NSs in several scientific and technological fields, including chemistry, agriculture, environment, food, cosmetics, has been widely explored. Nevertheless, the main area of investigation concerns the pharmaceutical and biomedical sphere (Tejashri et al., 2013; van de Manakker et al., 2009), in which CD-NSs have been mostly employed as drug delivery systems (Swaminathan et al., 2016; Trotta et al., 2012b; Trotta et al., 2014), owing to their outstanding encapsulation properties and low toxicity. As a matter of fact, animal studies have been directed to evaluate the effect of plain NSs on physiological parameters and no evidence of either acute or repeated dose toxicity has been reported (Shende et al., 2015a; Garcia-Fernandez et al., 2016; Blanchemain et al., 2007).

CD-NSs can be generally grouped into four consecutive generations, taking into account their chemical composition and properties. NSs synthesized by simply reacting CDs with a cross-linker can be ascribed to the 1st generation of NSs subdivided, depending on the chemical nature of the functional group connecting the CD to the cross-linker, into four main types: urethane, carbonate, ester and ether NSs. In the course of the years, this class of materials has been used as scaffold for the preparation of more complex NS polymer architectures. These functionalized NSs belong to the 2nd generation group and are characterized by specific properties, such as fluorescence and electric charge. The 3rd generation of NSs is represented by stimuli-responsive CD polymers, which are able to modulate their behavior according to the surrounding environment. External variations, such as pH and temperature gradients, oxidative/reducing conditions, can trigger or increase the release of the loaded drug from this kind of smart materials. Lastly, a new family of molecularly imprinted CD polymers (MIPs), exhibiting a high selectivity towards specific molecules has been introduced. These materials can be assigned to the 4th generation of NSs.

The purpose of this review is to provide a brief and non-exhaustive overview on the four generations of CD-NSs and highlight some of their potential applications, with a particular attention to the pharmaceutical field. Neither CD-grafted polymers nor composite materials, in which CD is a minor component, will be discussed here.

2. **First generation of cyclodextrin nanosponges**

2.1 Cyclodextrin-based urethane nanosponges

Urethane (or carbamate) CD-NSs are mainly synthesized using diisocyanates. They are characterized by a rigid structure, high resistance to chemical degradation and negligible swelling extent in both aqueous and organic media.

Carbamate CD-NSs were first developed by Li and Ma, by reacting β-CD with hexamethylene diisocyanate and toluene-2,4-diisocyanate, and used to treat wastewaters. Compared with activated carbons, these NSs gave remarkable performances in the removal of some organic molecules, like p-nitrophenol, which was absorbed even at low concentrations (10^{-7}-10^{-9} M) and reduced to ppb levels. Considering the low surface area of these NSs (1-2 m^2/g, two orders of magnitude lower than activated carbons), it is believed that organic molecules can diffuse through the surface and be absorbed also in the bulk of NSs (Li and Ma, 1999; Li and Ma, 2000).

Mamba et al. employed urethane CD-NSs for the removal of organic pollutants (volatile compounds, dissolved organic carbon and total organic carbon) from feed waters of power generation plants. Significant results were achieved with volatile compounds and dissolved organic carbon fractions, which were retained up to 90 % and 84 %, respectively (Mamba et al., 2008).

Carbamate CD-NSs have also showed a good affinity for biologically important compounds, such as amino acids and bilirubin. The absorption of aromatic amino acids by a hexamethylene diisocyanate CD-NS was evaluated in comparison with the absorption of branched-chain amino acids. The NS was able to incorporate up to 24 % of the aromatic amino acids contained in the mixture, whereas the amount of absorbed branched-chain amino acids was negligible (< 2 %) (Tang et al., 2006). In a previous work, the same NS was also used for bilirubin absorption. As observed, the initial concentration of bilirubin (40 mg/L) was reduced up to 92.6 % after the addition of the NS (Zheng et al., 2004).

In addition, hexamethylene diisocyanate CD-NSs have been studied as heterogeneous catalysts for organic synthesis (Kiasat, et al., 2014) and as solid phase extraction resins for the analysis of carcinogenic aromatic amines (Bhaskara et al., 2004).

2.2 Cyclodextrin-based carbonate nanosponges

CD-based carbonate NSs are synthesized using active carbonyl compounds, like 1,1'-carbonyldiimidazole, triphosgene and diphenylcarbonate. These NSs present short cross-linking bridges, reduced swelling ability and good stability to acidic and slightly alkaline solutions. Similar to urethane NSs, carbonate NSs also show a low surface area (around 2 m^2/g) and a marked affinity for some organic molecules (Trotta, 2011). In some cases, absorption performances are comparable with or even better than those given by activated carbons. Trotta and Cavalli, investigated the ability of a β-CD carbonate NS to remove chlorinated persistent organic pollutants (POPs) from wastewaters, in comparison with activated carbon. For all the tested POPs, the absorption efficiency of the NS was higher than the efficiency exhibited by activated carbon and in the case of hexachlorobenzene, the NS was capable of removing up to 99.5 % of the pollutant (Trotta and Cavalli, 2009b).

Castiglione et al. developed a powerful method to evaluate the degree of cross-linking of carbonate β-CD-NSs by means of infrared and Raman spectroscopy. By cross-checking spectroscopic analyses data with quantum chemical computation, a correlation between the intensity of carbonyl absorption peak and the degree of cross-linking was pointed out. The degree of cross-linking increased with the content of cross-linker, in the considered range of molar ratios 1:2 – 1:8 (β-CD : cross-linker). This method was proved to be an advantageous alternative to X-ray diffraction

analysis, which cannot provide useful information in the study of predominantly amorphous materials, like sugar-based NSs (Castiglione et al., 2012a).

A study, conducted by Rossi et al., was focused on the investigation of the mechanical properties of carbonate β-CD-NSs as a function of CD/cross-linker molar ratio used for the synthesis. Raman and Brillouin scattering experiments, demonstrated that stiffness and elastic properties of the NSs were strictly dependent on the content of cross-linker. In other words, the study proved that the mechanical features of a NS can be easily tuned by changing the molar ratio CD/cross-linker. Conversely, the kind of CD used for the synthesis was observed to have no effect on the mechanical properties of the final NS (Rossi et al., 2012).

Carbonate CD-NSs have been successfully employed to encapsulate and release a wide series of drugs. Most of anticancer drugs show reduced efficacy due to low solubility, instability and dose-limiting side-effects. After inclusion in carbonate CD-NSs an improvement of bioavailability and pharmacokinetic was observed in the case of tamoxifen (Torne et al., 2012), curcumin (Darandale and Vavia, 2013), camptothecin (Swaminathan et al., 2010a; Gigliotti et al., 2016), paclitaxel (Ansari et al., 2011a; Mognetti et al., 2012), erlotinip (Dora et al., 2016) and doxorubicin (Cavalli et al., 2006). In addition, carbonate CD-NSs have been able to increase the solubility and stability to degradation of nutraceuticals and dietary anti-oxidant agents, such as quercetin (Singireddy and Subramanian, 2014; Singireddy and Subramanian, 2016), resveratrol (Ansari et al., 2011b), gamma-oryzanol (Sapino et al., 2013), rutin, phloridzin and chlorogenic acid (Ramírez-Ambrosi et al., 2014), antihypertensive drugs, like telmisartan (Rao et al., 2013), antifungal agents, such as itraconazole (Swaminathan et al., 2007) and analgsesic anti-inflammatory agents, such as flurbiprofen (Cavalli et al., 2006) and dexamethasone (Swaminathan et al., 2013a; Swaminathan et al., 2013b). Rao and Bhingole employed CD-NSs, obtained from diphenyl carbonate, not only to achieve a controlled release of gabapentin, a drug used for the treatment of seizures in pediatric and geriatric patients, but also as taste-masking agents, to reduce the bitter flavor of the drug (Rao and Bhingole, 2015). Different applications of carbonate CD-NSs include gas storage and delivery, support for enzymes and preparation of smart fabrics. The ability of carbonate CD-NSs to entrap oxygen was evaluated by adding an aqueous dispersion of NS, saturated with oxygen, to a hypoxic NaCl solution. After an initial burst effect, the release of oxygen from the tested NSs was slow over approximately 60 minutes. By using a carbonate α-CD-NS, a concentration of oxygen of almost 9 mg/L in the receiving solution was obtained (Cavalli et al., 2010). Carbonate CD-NSs have also been employed as support to host and stabilize lipase (Boscolo et al., 2010) and dioxygenase (Di Nardo et al., 2009) enzymes. Owing to the protective effect of the NS, the activity of the enzyme at lower pH values and higher temperature was observed to be increased and prolonged in time. Finally, functionalized cotton fabrics were prepared by physical adsorption of melatonin-loaded carbonate NS particles among cotton fibers and used for the transdermal delivery of melatonin (Mihailiasa et al., 2016).

2.3 Cyclodextrin-based ester nanosponges

Ester NSs are usually synthesized from CDs and dianhydrides or di/polycarboxylic acids, such as pyromellitic dianhydride, ethylenediamine-tetraacetic dianhydride (EDTA dianhydride) (Ferro et al., 2014), butanetetracarboxylic dianhydride (Kono and Nakamura, 2013), citric acid (Weltrowski et al., 2000), etc. Conversely to carbonate and urethane NSs, ester NSs are usually able to absorb

remarkable amounts of water (up to 25 times their own weight) and form hydrogels. The swelling ability of these NSs is usually dependent on the degree of cross-linking: the lower the density of cross-linking, the higher the water uptake. Concerning the chemical stability, ester NSs undergo hydrolysis in aqueous media more easily than carbonate and urethane NSs (Trotta, 2011).

Similarly to the study conducted on carbonate NSs, the combination of quantum chemical computations, infrared and Raman spectroscopy analysis allowed to detect a correlation between the vibrational properties of a pyromellitic β-CD-NS (Fig. 1) and its degree of cross-linking. The highest degree of cross-linking was observed in the sample prepared using a molar ratio of 1:6 (CD : pyromellitic dianhydride). Interestingly, higher contents of cross-linker, correspondent to the molar ratios 1:8 and 1:10, led to a decrease of the degree of cross-linking. This fact is probably caused by the steric hindrance generated by the pyromellitic units linked to CDs (Castiglione et al., 2012b).

The same work was repeated by the authors on another kind of ester β-CD-NS, prepared by reacting β-CD with EDTA dianhydride. Similarly, in the investigated range of molar ratios comprised between 1:2 and 1:10 (β-CD : cross-linker), the highest degree of cross-linking was observed for the 1:6 sample (Crupi et al., 2013).

As already described for carbonate NSs, a series of Raman and Brillouin scattering experiments allowed Rossi et al. to investigate the relationship between the mechanical characteristics of the ester β-CD-NS (obtained from pyromellitic dianhydride) and the molar ratios CD/cross-linker. The study demonstrated that stiffness and elasticity of the polymeric structure can be successfully modulated by varying the amount of cross-linker introduced in the synthesis reaction (Rossi et al., 2012).

The swelling properties of a pyromellitic β-CD-NS have been exploited by Conte and co-workers to prepare gels loaded with benzoporphyrin-derivative monoacid ring A, all-trans retinoic acid and diclofenac for drug delivery to the skin. In this study, the ester NS was proved to be able to act as multifunctional agent, allowing to increase the water solubility and stability to photo-degradation of the benzoporphyrin-derivative and retinoic acid and limit the diffusion of diclofenac to the epidermis, thus reducing its permeation through the skin (Conte et al., 2014).

A pyromellitic β-CD-NS was chosen also by Shende et al. for the delivery of acetyl salicylic acid. To this aim, an inclusion complex between β-CD and the anti-inflammatory drug was formed via precipitation method. The 1:1 complex was then recovered by filtration and freeze-dried. The acetyl salicylic acid-loaded β-CD was finally cross-linked with pyromellitic dianhydride. After purification, *in-vitro* and *in-vivo* release studies were performed (Shende et al., 2012).

Martel et al. reported the preparation of CD polymers, using citric acid, 1,2,3,4-butanetetracarboxylic acid and poly(acrylic acid) (average molecular weight = 2000 Da) as cross-linkers. The polycondensation reactions were carried out at a temperature comprised between 140 and 170 °C, under either atmospheric or low pressure. Depending on the experimental conditions, either soluble or insoluble polymers were obtained (Martel et al., 2005). In a subsequent study, some of the polymers synthesized from β-CD and citric acid were used as compression excipients for the preparation of tablets. The disintegration time of the tablets was found to be a function of the soluble/insoluble polymer weight ratio, i.e. the higher the amount of soluble polymer, the higher the resistance to crushing. Afterwards, Sprague-Dawley rats were administered (2 g/kg) with the so formed tablets, in order to evaluate the toxicity of plain β-CD-citric acid polymers. After 14 days, no significant variations in the physiological parameters of the rats were observed (Garcia-Fernandez et al., 2016).

A different method for the synthesis of citric acid-β-CD polymers was developed by Skiba and Lahiani-Skiba. Soluble and insoluble CD polymers were obtained in high yields through a melt polycondensation reaction by simply heating, at high temperature (140-150 °C) in a reactor, β-CD and citric acid in the presence of sodium phosphate dibasic (Skiba and Lahiani-Skiba, 2013). In a subsequent publication, these polymers were presented as efficient multi-cycle adsorbent materials for the removal of pharmaceutical residues from aqueous solutions (Moulahcene et al., 2015).

A further interesting feature of some ester NSs is the presence of free carboxyl groups, in their chemical structure, that can be exploited for the adsorption of heavy metal cations. The metal ions complexation ability of pyromellitic NSs has been studied for several cations, including Al^{3+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pd^{2+}, and U^{4+}. In most cases, pyromellitic NSs were found to be capable of adsorbing more than 70 % of the tested cation (Berto et al., 2007). Noteworthy results, in the simultaneous adsorption of metal ions and cationic dyes, were obtained also by using an EDTA-cross-linked β-CD-NS (Zhao et al., 2015) and 1,2,3,4-butanetetracarboxylic acid-cross-linked CD-NSs (Euvrard et al., 2016).

2.4 Cyclodextrin-based ether nanosponges

CD-based ether NSs are preferentially synthesized by reacting CDs with cross-likers bearing epoxide groups, such as epichlorohydrin (Solms, 1967), bisphenol A diglycidyl ether, ethylene glycol diglycidyl ether, etc. (Alvarez et al., 2008). This kind of NSs exhibits a high chemical resistance and a tunable swelling capability. Despite of epichlorohydrin toxicity, most of the studies that are reported in the literature on CD-NSs are focused on epichlorohydrin-cross-linked CD-NSs. Recently, an exhaustive review on epichlorohydrin-based CD polymers has been provided by Morin-Crini and Crini (Morin-Crini and Crini, 2013). The synthesis of CD-based ether NSs is usually performed in water in the presence of a base. According to the experimental conditions, either soluble or insoluble polymers can be obtained (Vélaz et al., 2007).

In the pharmaceutical field, epichlorohydrin CD-NSs have been used to deliver drugs, such as anti-inflammatory and antifungal compounds (Machín, et al., 2012) , to accelerate the healing process of wounds and to promote tablet disintegration in both dry compression and wet granulation systems (Fenyvesi, 1988). Additional applications include the removal of unwanted flavors in food processing (Shaw and Wilson III, 1983; Shaw et al., 1984) and the inhibition of enzymatic browning of apple, pear and selery juice (Hicks et al., 1996). Finally, several studies have been published on the ability of epichlorohydrin CD-NSs to catalyze chemical reactions, act as stationary phase for chromatographic separation and adsorb organic molecules, such as halogenated and aromatic pollutants, colorants, hormones, surfactants and essential oils in either static or flow processes (Morin-Crini and Crini, 2013).

The adsorption properties of a β-CD-based ether NS were described by Kono and co-workers to depend on both the cross-linker chain length and the degree of cross-linking. Insoluble β-CD polymers obtained by cross-linking with different amounts of polyethylene glycol diglycidyl ether (degree of polymerization = 7.2) were used to adsorb bisphenol A in comparison with a reference NS, prepared using a shorter cross-linking chain (i.e. ethylene glycol diglycidyl ether). The best performance was obtained by using loosely cross-linked NSs derived from polyethylene glycol

diglycidyl ether, whereas highly cross-linked NSs and NSs prepared from ethylene glycol diglycidyl ether gave lower results (Kono et al., 2015).

A CD-based aromatic ether NS exhibiting high surface area (35-263 m^2g^{-1}) was prepared by Alsbaiee et al. from a potassium carbonate-catalyzed reaction between β-CD and tetrafluoroterephthalonitrile. Permanent porosity and water regain ability were tuned by operating on the reaction conditions. When tested in the removal of organic pollutants, such as endocrine disruptive agents and pharmaceuticals, the polymer outperformed a leading activated carbon, with a surprisingly rapid adsorption kinetics (Alsbaiee et al., 2016).

CD-based ether NSs can be synthesized also using unsaturated cross-linkers. Ferruti and co-workers reported the polymerization of β-CD with acetic acid 2,2'-bis(acrylamide) in water, catalyzed by lithium hydroxide monohydrate. A precipitate was collected after 4 days at ambient temperature (Ferruti et al., 2008). The presence of carboxylic and amine groups in the cross-linking bridges provides a significant affinity for proteins, as demonstrated by Swaminathan et al. An encapsulation efficiency of nearly 90 % was observed in the adsorption of bovine serum albumin; in addition, sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis proved the ability of the NS to stabilize the conformational structure of the model protein up to several months (Swaminathan et al., 2010b).

Morales-Sanfrutos et al. described the use of divinyl sulfone as cross-linker for the polymerization of α- and β-CD in carbonate buffer solution (pH 12). NSs with a different degree of cross-linking were obtained and fully characterized. Finally, the polymers were used to adsorb aromatic pollutants (i.e. bisphenol A and β-naphthol) and pharmaceuticals (i.e. progesterone and curcumin). The inclusion kinetics of the tested compounds was observed to follow the Freundlich isotherm model, thus indicating an adsorption process onto a monolayer of homogeneous sites (Morales-Sanfrutos et al., 2015).

3. **Second generation of cyclodextrin nanosponges**

The addition of desired functionalities to the so far described CD polymers has allowed to extend their field of application and give rise to a new generation of NSs. Specific moieties can be introduced according to three strategies: post-cross-linking functionalization of a NS, pre-cross-linking functionalization of CDs or addition of the functionalizing agent simultaneously to the cross-linking step. In the first case, mostly the particle surface will be altered, whereas in the other cases a homogeneous concentration of chemical functionalities will be distributed throughout the entire NS particle. However, the insertion of pendant groups on CD molecules, prior to the polymerization reaction, might be detrimental to the degree of cross-linking or it might even prevent the formation of an insoluble network.

Hitherto, two main implementations have been achieved on the chemical structure of CD-NSs: the labelling with fluorescence active moieties and the introduction of either electrically charged or hydrophobic groups for tuning NS polarity.

Only in the past few years the synthesis of CD-NSs covalently tagged with fluorescent groups has been reported. Malanga et al. accomplished fluorescent labelling of an epichlorohydrin β-CD

polymer by introducing an anchoring group (i.e. azido moiety, subsequently converted to -NH_2) capable of reacting with the isothiocyanate form of fluorescent dyes, such as rhodamine and fluorescein. Similarly to a previous work, published by Ncube et al. (Ncube et al., 2014), this study was focused on soluble polymers, nevertheless, the methods described might be exploited also for the preparation of fluorescent NSs (Malanga et al., 2014).

Lembo and co-workers have reported the preparation of fluorescent carbonate and carboxylated NSs, by means of a one-step reaction between a pre-formed NS and fluorescein isothiocyanate, carried out in DMSO at 90 °C for 3 h. This functionalization allowed to track the NSs during biological studies (Lembo et al., 2013).

Zohrehvand and Evans have been able to prepare fluorescent 2-naphthol-tagged NSs by reacting β-CD and 2-naphthol with epichlorohydrin at 60 °C in alkaline conditions. Water insoluble polymers were obtained by using molar ratios epichlorohydrin/β-CD > 22 : 1. An increase of the fluorescence intensity was observed as the molar ratio epichlorohydrin/β-CD was increased up to 39 : 1. Experimental evidences confirmed that a fraction of 2-naphthol was not chemically bonded to epichlorohydrin units but entrapped in the NS structure by either physical adsorption or complexation (Zohrehvand and Evans, 2005).

Electrically charged CD-NSs have been developed to achieve efficient complexation of polar/ionic compounds. Moreover, electric charges generate high Z potential absolute values, hence stronger repulsion forces between NS particles and more stable suspensions. In some kind of first generation NSs, the cross-linker itself bears electric charges (e.g. polyester carboxylic NSs obtained from dianhydrides or polyfunctional carboxylic acids), in other cases, neutral NSs have been turned into ionic polymers by substitution with suitable pendant groups.

Fenyvesi et al. encapsulated several cationic disinfectant agents, such as methylene blue, gentian violet, brilliant green, fuchsin acid, ethacridine lactate and cetylpyridinium chloride, in carboxymethylated NSs to be used in the prolonged treatment of wounds. In this work, a neutral β-CD-based ether NS, prepared by cross-linking β-CD and polyvinyl alcohol with epichlorohydrin and ethyleneglycol bis(epoxypropyl) ether (content of β-CD: 55 %), was suspended in a chloroacetic acid solution and stirred for 24 h. Both the resulting anionic carboxymethylated NS and the neutral NS, used for comparison, were loaded with disinfectants and release studies were carried out. In all the cases, the drug dissolution rate that was observed from the anionic NS was lower than the one obtained from the corresponding neutral NS (Fenyvesi et al., 1996).

Negatively charged NSs have been obtained also by reacting carbonate NSs with succinic anhydride (Fig. 2) in DMSO at 90 °C for 3 h. Thanks to this carboxylation process, the ability of the NS to encapsulate a polar antiviral drug (i.e. acyclovir) was significantly enhanced. Furthermore, a slow and prolonged release kinetics of the loaded drug was observed (Lembo et al., 2013).

Junthip and co-workers reported the preparation of positively charged CD polymers, obtained by reacting β-CD with glycidyltrimethylammonium chloride and epichlorohydrin in alkaline solution at either 30 or 60 °C. The syntheses led to insoluble polymers preferentially, nevertheless, the authors preferred to quench the polymerization reaction, before the gelation point was reached, in order to collect soluble polymers, to be used in layer deposition on PET fabrics for drug delivery applications (Junthipa et al., 2015).

In contrast to ionic NSs, hydrophobic NSs have been developed to enhance the complexation efficiency towards apolar compounds. Mallard et al. described the preparation of polyether NSs, starting from a pre-cross-linking functionalized β-CD, more specifically a low methylated-β-CD

(CRYSMEB) and epichlorohydrin. Polymers with different degree of cross-linking were obtained and tested in the absorption of volatile organic compounds (Mallard Favier et al., 2011). Similarly, apolar NSs were synthesized also by cross-linking heptakis(2,6-di-O-methyl)-β-CD with isocyanates, such as 4,4'-methylenebis(phenyl isocyanate), 1,4-phenylene diisocyanate and hexamethylene diisocyanate and successfully employed for the removal of polychlorobiphenyls (Kawano et al., 2015).

4. Third Generation of Cyclodextrin Nanosponges

Stimuli-sensitive polymers respond to external changes by altering properties such as shape, permeability, or color. The ability to recognize a stimulus and react to it is derived from stimuli-sensitive processes on both molecular and supramolecular levels, as well as the morphology (Lendlein and Shastri, 2010). Stimuli-sensitive nano-carriers are known for their controlled target release upon initiation by stimulating signals or specific transport pathways, as well as their ability to enhance the therapeutic efficacy with minimal side effects (Zhang and Ma, 2013).

The usefulness and safety of CDs for drug formulation and gene delivery, is due to the reversibility of host–guest interactions between CDs and guest molecules easily achieving the chemical-responsiveness, besides the facility for CDs to serve as supports to functionalized groups for additional responsive moieties by supramolecular recognition or covalent conjugation to attain physio-chemical stimuli sensitivity (Zhang et al., 2011).

Controlled delivery and cargo release can be achieved using nano-systems stimulated by different signals ranging from temperature, electromagnetic fields, redox potentials, reactive species and pH levels (Jianxiang et al., 2013). CD-based NSs represent bio-responsive nano-vehicles exploiting the versatility of syntheses, they can act as stealth nano-carriers in order to increase the circulating life-time of the drug in the physiological environment.

The molecular mechanism responsible for the thermosensitive behavior exhibited by pH-responsive cross-linked CD-based hydrogels was explored to clarify some basic aspects of H-bond interactions, experimental evidence suggested that the dominant effects in the mechanism of solvation of CD-based hydrogels are due to the changes occurring, upon increasing of temperature, in the hydrophobicity character of specific chemical moieties of the polymer, as triggered by pH variations (Trotta et al., 2015).

Recently, Trotta et al. developed glutathione-responsive NSs (GSH-NS, Fig. 3) as innovative nano-carriers which release entrapped anticancer drugs in response to intracellular stimuli. GSH-NSs were designed with the aim to deliver anticancer drugs mainly in cells with high glutathione content, as an ideal internal stimulus for rapid destabilization of nanocarriers inside cells to obtain efficient intracellular drug release. Doxorubicin loaded in GSH-NSs showed remarkably higher effectiveness than free drug in cancer cells characterized by high GSH content both *in vitro* and *in vivo* (Pizzimenti et al., 2016).
Other pH responsive NS materials, namely aminocyclodextrin nanosponges (ACN) formed by joining (6-deoxy)-β-CD subunits by means of different aliphatic polyamine linkers were reported. Diamines and polyamines linkers with different lengths and hydrophobic characters were chosen, their adsorption abilities were evaluated towards a set of guest models, at different pH values to show the significant variations in molecular properties such as the hydrophobic/hydrophilic character, the charge status and the molecular volume (Lo Meo et al., 2016).
Wajs et al. also prepared modified CD-NSs for biosensor applications, particularly as a tool for celiac disease diagnosis and therapy. β-CD was cross-linked with pyromellitic dianhydride, then functionalized by anti-IgG antibody and later loaded with horseradish peroxidase, a label enzyme

generally used in biosensor assays. The possibility to use this NS as a signal enhancement tool in enzyme-linked colorimetric and electrochemical assays was evaluated. A 3.2-fold signal enhancement of the loaded NS in comparison to horseradish peroxidase-antibody conjugate was achieved (Wajs et al., 2014).
An earlier work on chemical responsive nano-vehicles by Ma et al., on nano-assemblies formed by double hydrophilic copolymers with one PEG block and β-CD containing block was demonstrated in the presence of various hydrophobic compounds. The study supports the potential applications of assemblies based on PEG-b-PCD copolymers as nano-platform for the delivery of hydrophobic bioactive compounds (Ma et al., 2010).

5. Fourth Generation Cyclodextrin Nanosponges

Molecular imprinting is a method of inducing molecular recognition properties to three dimensional polymers in response to the presence of a template molecule during formation of a polymer (Sellergren and Allender, 2005).

The synthesis of molecularly imprinted polymers (MIPs) is based on the formation of defined interactions between a template molecule and functional monomers in the presence of a cross-linking agent during a polymerization process (Martin-Esteban, 2004). The template molecule is later removed, leaving behind cavities complementary to the target in shape and size, enabling it to rebind selectively (Martín-Esteban and Turiel, 2010).

MIPs are formed by the non-covalent interactions of the template hosted within the polymer (Bikiaris et al., 2013). MIPs possess specific molecule recognition, high selectivity and affinity for target molecules. These polymers present wide recognition due to their stability, ease of preparation, and low-cost potential (Wulff and Liu, 2004).

MIPs can serve as biosensor materials, drug delivery systems, catalysts, antibody mimics for quantitative assay and molecular recognition (Shende et al., 2015). In biosensor applications, MIP synthetic materials are used in protein or enzyme recognition, cholesterol estimation, antibody isolation, glucose estimation, etc. (Ogoshi and Harada, 2008).

The introduction of CDs into MIPs is thought to be advantageous, compared to conventional methods, as it can increase the performance based on the selection and optimization of suitable polymerization conditions (cross-linkers, initiators, polymers) (Komiyama et al., 2000). As well as the ability to form guest-host inclusion complexes, under mild parameters, with high affinity binding constants between the target and the polymer, through various intermolecular interactions, such as van der Waals forces, dipole–dipole, electrostatic affinity and hydrogen bond interactions following simple polymerization processes (Liu et al., 2008; Roche et al., 2009).

The binging sites of CD MIPs arrange matching to template in the polymer (Fig. 4); it is worth noting that the template should hold hydrophobic sites in order to form inclusion complexes with the CDs.

A list of few examples of CD MIPs has been presented by Yazdani-Pedram et al. in an interesting review (Yazdani-Pedram et al., 2014). Egawa et al. (Egawa, et al., 2005) prepared CD-based molecular imprinted microspheres in dimethylsulfoxide/poly(dimethylsiloxane) emulsion to bind cholesterol and steroids; bovine serum albumin was also able to form an inclusion complex with modified CD pseudo-polyrotaxanes in the presence of copper ions (Guo et al., 2012). Other MIPs were obtained through radical polymerization of acryloyl-CD and used for imprinting antibiotics and oligopeptides in water (Alvarez-Lorenzo and Concherio, 2006).

Photonic MIPs were designed for sensing amino acid using maleic anhydride, acrylic acid and modified β-CD, even after swelling they retained the ability to sense L-phenylalanine (Yu et al., 2013); β-CD based MIPs exhibited specific creatinine recognition in a multicomponent of its analogues.

Ng et al. (Ng and Narayanaswamy, 2009) used N-phenyl-1-naphthylamine and dextromethorphan as template molecules for two different studies, the first showing better sensing signal by increasing the binding affinity and substrate-selectivity towards the template molecule, compared with the control polymer prepared in its absence, while the latter to identify the activity of an important pharmacological drug marker.

CD-MIPs cross-linked by toluene-2,4-diisocyanate with different template molecules, showed higher rebinding of dye than chitosan MIPs prepared in a study done by Bikiaris et al. The selectivity of MIPs was elucidated by their different rebinding capabilities in a trichromatic mixture. Regeneration/reuse of the dye-loaded polymers was evaluated via sequential adsorption–desorption cycles (Bikiaris et al., 2013).

Shende et al. (Shende et al., 2015b) developed biomimetics for glucose estimation using biocompatible molecularly imprinted polymers of pyromellitic dianhydride cross-linked CD-based NSs in anticancer drug delivery, proteins delivery and anti-inflammatory drugs delivery systems.

Trotta et al. was the first to report a paper on MIP CD-NSs applied to the study of an oral formulation for the delivery of L-DOPA for the treatment of neurodegenerative disease. MIP-NSs were synthesized through condensation polymerization by reacting β-CD with different amounts of 1,1'-carbonyldiimidazole (CDI) in the presence of a variable amount of drug (Fig. 5). CD cavity allowed the formation of the inclusion compound with unstable and reactive L-DOPA, shielding its reactive groups and preventing its degradation (Trotta et al., 2016).

6. Conclusions

CD-NSs are highly cross-linked polymers, that show remarkable encapsulation efficiency and find uses in several applications. In particular, CD-based NSs have been proposed as nanomedicine strategy to address challenging issues of drug delivery, such as solubility, stability and controlled release. Nanoscale size, polymer matrix properties and surface engineering can be exploited to improve the therapeutic treatment. CD-NSs offer the possibility to incorporate small molecules, macromoles and gases in a great extent.

The design of stimuli sensitive NSs permitted to control drug release in response to specific stimuli, either exogenous or endogenous. Positively charged NS can be used to form stable complexes for gene delivery overcoming limitations of nucleic acid delivery. Combined delivery of active molecules associated with hosting capacity to obtain more synergistic effects and achieve better therapeutic response is another NS possibility (Trotta et al., 2017).

Molecularly imprinted NSs, aimed at enhancing drug loading capability, have been broadly considered. Indeed, in this types of NS the drug could be included in the cross-linked structure during the synthesis thereby leading to an increased payload and much slower drug release (Trotta et al., 2016).

7. References

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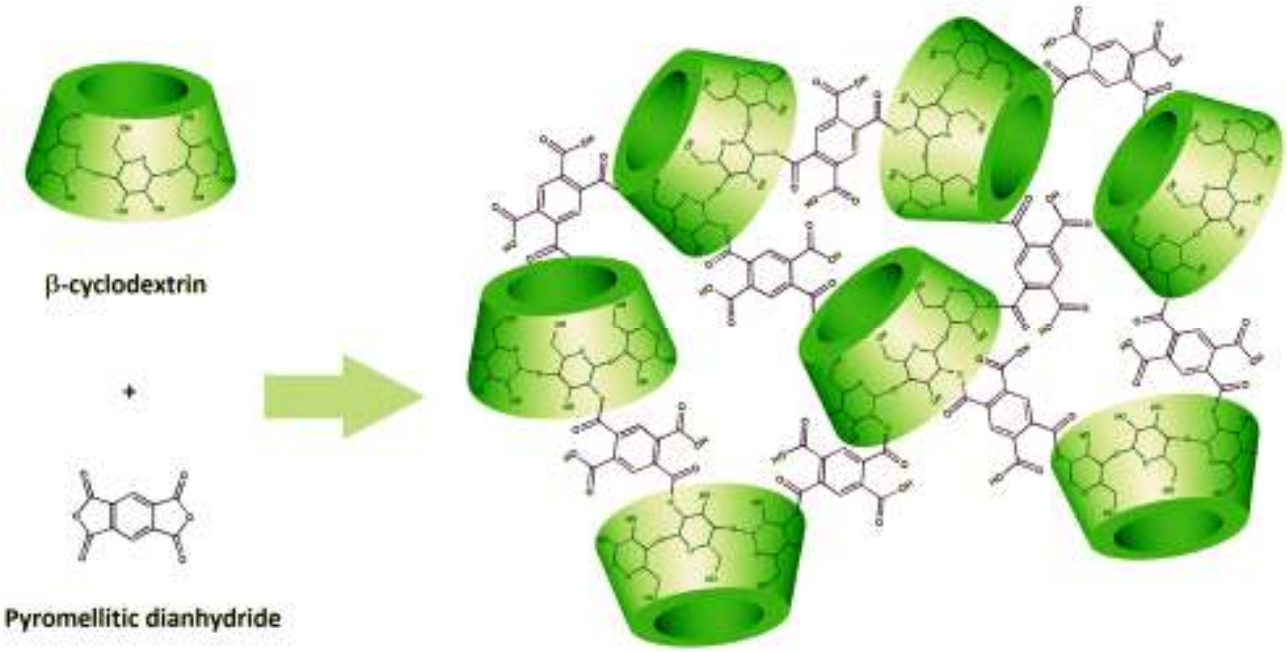


Fig. 1. Schematic representation of the synthesis reaction of pyromellitic β-CD NS.

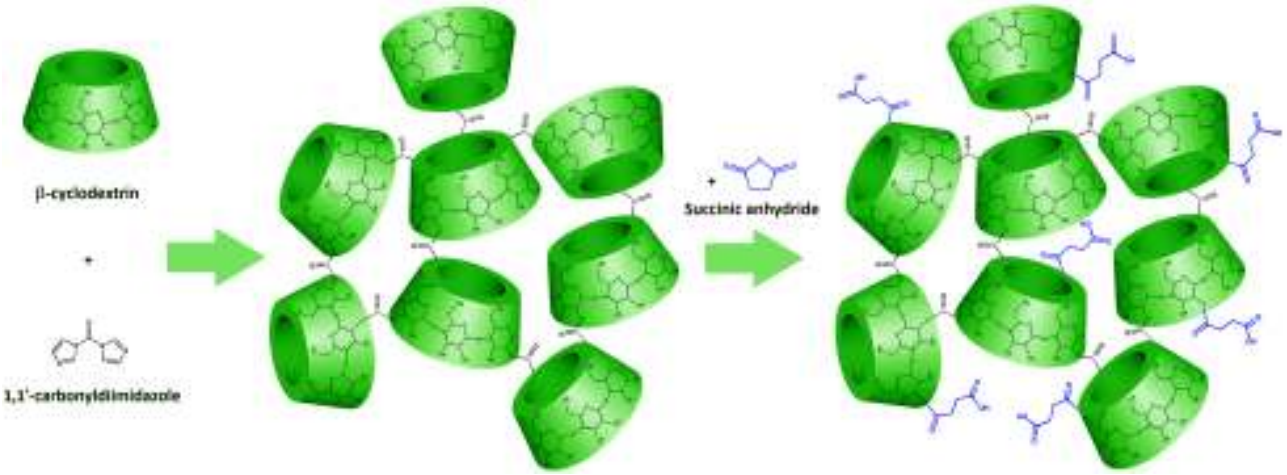


Fig. 2. Schematic representation of the two-step preparation of a carboxylated β-CD NS.

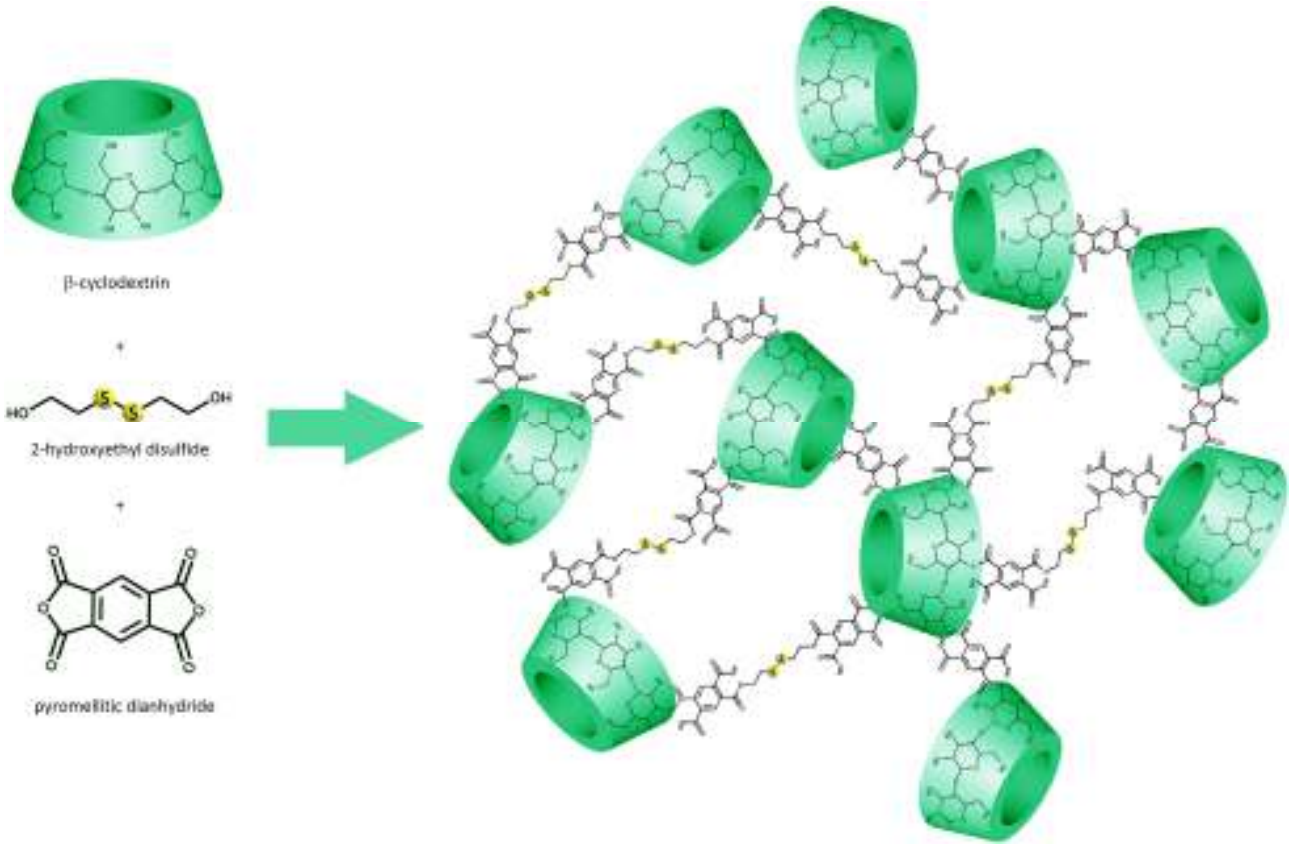
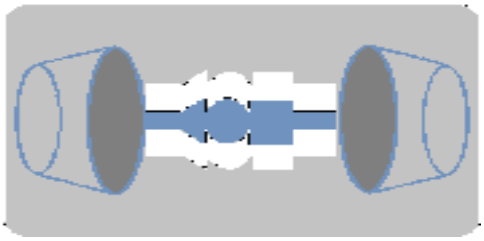


Fig. 3. Schematic representation of the synthesis of GSH-responsive CD-NS.



Schematic representation of a template molecule in a CD-based MIP.

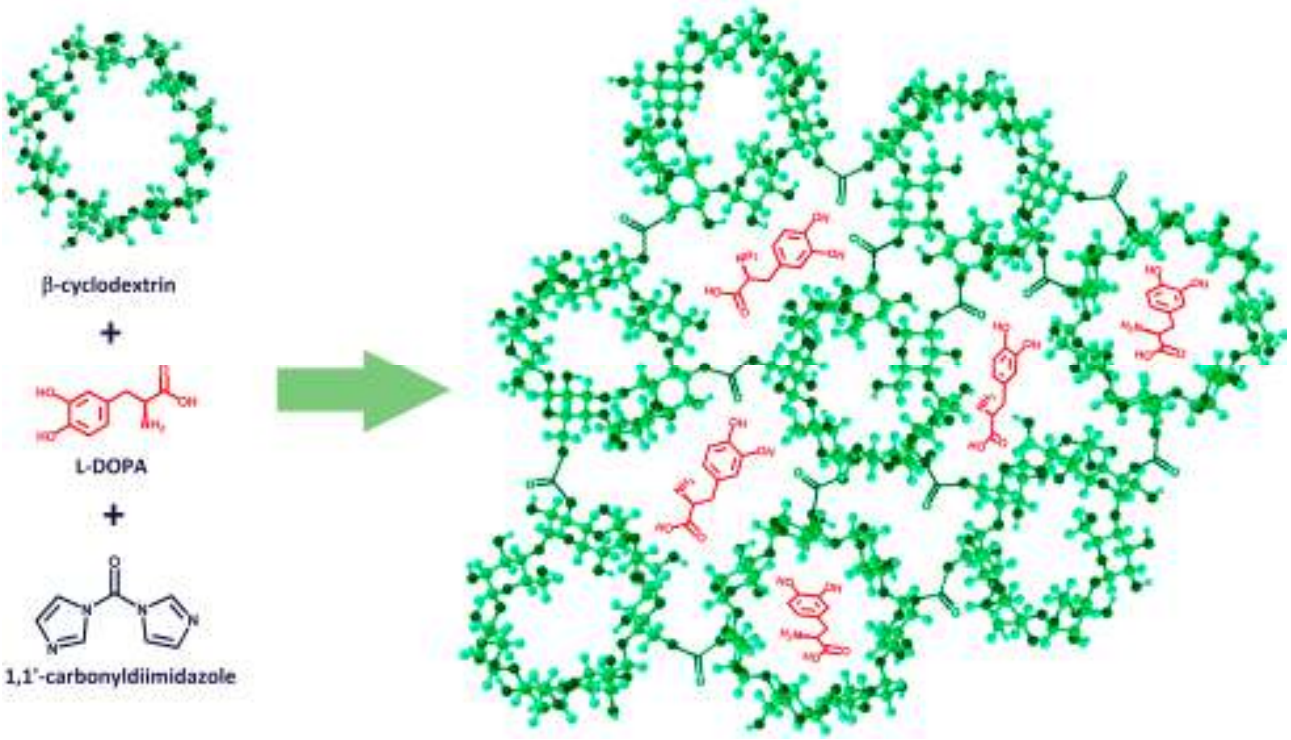


Fig. 5. Schematic representation of the preparation of L-DOPA molecularly imprinted CD-NS.