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Review

Cyclodextrin based nanosponges for pharmaceutical use: A review

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Nanosponges are a novel class of hyper-crosslinked polymer based colloidal structures consisting of solid nanoparticles with colloidal sizes and nanosized cavities. These nano-sized colloidal carriers have been recently developed and proposed for drug delivery, since their use can solubilize poorly water-soluble drugs and provide prolonged release as well as improve a drug's bioavailability by modifying the pharmacokinetic parameters of actives. Development of nanosponges as drug delivery systems, with special reference to cyclodextrin based nanosponges, is presented in this article. In the current review, attempts have been made to illustrate the features of cyclodextrin based nanosponges and their applications in pharmaceutical formulations. Special emphasis has been placed on discussing the methods of preparation, characterization techniques and applications of these novel drug delivery carriers for therapeutic purposes. Nanosponges can be referred to as solid porous particles having a capacity to load drugs and other actives into their nanocavity; they can be formulated as oral, parenteral, topical or inhalation dosage forms. Nanosponges offer high drug loading compared to other nanocarriers and are thus suitable for solving issues related to stability, solubility and delayed release of actives. Controlled release of the loaded actives and solubility enhancement of poorly water-soluble drugs are major advantages of nanosponge drug delivery systems.

Keywords: nanosponges, solubility enhancement, cyclodextrin

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Nanosponges are a novel class of hyper-crosslinked polymer based colloidal structures consisting of solid nanoparticles with colloidal sizes and nanosized cavities. Well--known examples of nanosponges are titanium-based nanosponges (1), silicon nano-

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sponge particles (2), hyper-crosslinked polystyrene nanosponges (3) and cyclodextrin--based nanosponges (4).

The fundamental appeal of the nanosponge technology arises from the difficulty experienced with conventional formulations in releasing active ingredients over an extended period of time. Relatively high concentrations and short duration of action are typical features of conventional dermatological and personal care products. This might lead to a cycle of short-term overmedication followed by long-term undermedication. Side effects such as rash or other serious forms (due to active ingredient penetrating the skin) are the major drawback of such conventional dermatological and personal care products. Thus an alternative technology circumventing these drawbacks was the need of the hour. In recent decades, nanoparticles as drug delivery systems have gained plenty of attention. Nanotechnology permits even sustained release of actives, compounds and agents, reducing irritation while maintaining efficacy, thus making nanoparticles a versatile drug delivery vehicle (5, 6). However, only a few nanopreparations, like abraxane, have reached the market (7). Proteins, peptides, genes, anti-cancer agents and biomolecules have been loaded in nanoparticulate delivery systems and are widely studied so that the untoward effects may be lowered and efficacy may be improved (8–10). Nanoparticles provide controlled release of entrapped drugs and during this action offer protection to the drug particle against body conditions maintaining the bioactivity of the compound. Nanoparticles can be used to localize drugs in the liver, spleen and lungs since they are readily taken up by the macrophage system (11–15). Tailored coatings over nanoparticles help overcome reticulo-endothelial uptake of nanoparticles and thus deliver the drug across various body barriers like BBB (16). Hence, advantages of nanoparticulate drug delivery systems include protection of the trapped drugs, improved efficacy, lowering of untoward effects, controlled release and drug targeting. Nanoparticles come in the form of polymeric nanoparticles, solid lipid nanoparticles, nanoemulsions, nanosponges, carbon nanotubes, micellar systems, dendrimers, *etc*. Amongst these, nanosponge (NS) looks like a three-dimensional scaffold possessing a long length polymer backbone. The polymer in solution form is combined with small molecules called crosslinkers that act like tiny grappling hooks to fasten different parts of the polymer together. The net effect is formation of spherical particles with hydrophobic cavities where drug molecules can be entrapped (17). A single nanosponge system consists of a myriad of interconnecting voids within a non-collapsible structure capable of holding a wide variety of substances. The outer surface is typically porous, allowing a sustained flow of substances out of the particle (18). These nano-sized colloidal carriers have been recently proposed for drug delivery, since their use can solubilize poorly water-soluble drugs and provide prolonged release, as well as improve the drug's bioavailability by modifying the pharmacokinetic parameters of actives (19).

Advantages of nanosponge based drug delivery systems

- This technology offers entrapment of ingredients and reduces side effects;
- Improved stability, increased elegance and enhanced formulation flexibility;
- Extended release with continuous action up to 12–24 hours;
- Incorporation of immiscible liquids is possible;
- Improved material processing since liquids may be converted to powders.

Characteristic features of nanosponges

- Nanosponges exhibit a range of dimensions (1 μm or less) with tunable polarity of the cavities. Nanosponges of specific size and adjustable polarity can be synthesized by varying the crosslinker to polymer proportion (20).
- They could be either para-crystalline or in crystalline form, depending on the process conditions. Crystal structure of nanosponges plays a very important role in their complexation with drugs. The drug loading capacity of nanosponges mainly depends on the degree of crystallization. Para-crystalline nanosponges have shown various drug loading capacities (4).
- They are nontoxic, porous particles insoluble in most organic solvents and stable at high temperatures up to 300 °C (17).
- Nanosponges as formulations are stable over the pH range of 1 to 11 and temperature up to 130 °C (36).
- They form clear and opalescent suspensions in water and can be regenerated by simple thermal desorption, extraction with solvents, by the use of microwaves and ultrasounds (21).
- Their 3D structure enables capture, transportation and selective release of a vast variety of substances. They can be targeted to different sites due to their ability to be linked with different functional groups. Chemical linkers enable nanosponges to bind preferentially to the target site. They form inclusion and non-inclusion complexes with different drugs (22). Magnetic properties can be also imparted to nanosponges (by adding magnetic particles into the reaction mixture).

Factors influencing the formation of nanosponges (23)

Polymers and crosslinkers. – The type of polymer used can influence the formation as well as the performance of nanosponges. Efficient crosslinkers convert molecular nanocavities into three-dimensional, nanoporous structures. By tuning the degree of crosslinking, either hydrophilic or hydrophobic components that can trap targeted compounds are formed. Depending upon the nature of crosslinkers, water soluble or insoluble nanosponge structures are formed.

Hydrophilic nanosponges are formed when epichlorohydrin (24) is used as crosslinker. Hydrophilic nanosponges can modify the rate of drug release, and can be used to enhance drug absorption across biological barriers, serving as a potent drug carrier even in immediate release formulations. Hydrophobic nanosponges can be synthesized using diphenylcarbonate (4, 19, 20, 28, 29, 34) or pyromellitic anhydride (32), diisocyanates (31, 55), carbonyldiimidazoles (19, 30, 35) and other crosslinkers and may serve as sustained release carriers for water-soluble drugs including peptide and protein drugs (20). A list of polymers with their applications is given in Table I.

Apart from the nature of polymer and crosslinker, the type of the drug to be entrapped and solvent medium used for the method of preparation affect the formation of nanosponges.

Table I. Polymers and their potential applications

Polymer	Use	Method of preparation	Particle size	Reference
Cyclodextrins and their derivatives	Solubility enhancement, cytotoxicity, hemolytic activity	Simple thermal desorption, extraction with solvents and/or use of microwave and ultrasound techniques; Diphenylcarbonate or pyromellitic anhydride is used as crosslinker.	Below 500 nm	4, 19, 20, 28, 30, 33, 34, 46, 84
Titanium dioxide	Coating of polystyrene microspheres	Copolymerizing polymerizable surfactants with styrene	100–130 nm	1
β-Cyclodextrin & copolyvidonum	Saturation solubility study	Simple thermal desorption, extraction with solvents and/or use of microwaves and ultrasounds	Not reported	32
Ethylcellulose Polyvinyl alcohol	Irritation study	Emulsion solvent diffusion method	230–470 nm	80, 81
Poly(valero lactone allylvalero lactone) and poly(valerolactone--allylvalerolactone – oxepanedione)	Drug release study	Crosslinking using targeting units, *e.g.*, peptides	Not reported	85

Types of drugs and medium used for interaction. – Drug molecules to be complexed with nanosponges should have certain characteristics to be successfully entrapped in nanocavities. Molecules possessing molecular mass between 100 and 400 Da and having less than five condensed rings can be easily trapped in the nanocavity of sponges. Also, these molecules should be less than 10 mg mL^{-1} soluble in water with a melting point below 250 °C (23). Compounds with high melting points do not have high stability constant values when loaded into nanosponges. Thus, stable complexes between the drug and nanosponges are not obtained when they are loaded into the drug. Drug loading is also affected if the drug has a higher melting point. Lower loading of the drug would be observed with compounds melting at higher temperature. These low loading values could be attributed to the structural rigidity of the compound. The interaction between NS cavities and targeted compounds strongly depends on the medium; namely, a hydrophilic medium will drive the organic guest molecules into hydrophobic cavities, while an organic solvent tends to release the organic molecules trapped in nanosponges. These strong attractions between host and guest molecules depend on optimized chemical and physical interactions such as mutual matching of polarity, size, hydrophobic environment and structural properties (25).

Complexation temperature. – The stability constant of a complex is dependent on temperature changes. The stability constant and temperature rise are inversely correlated.

At increased temperature, the magnitude of apparent stability constant decreases due to reduction in drug/nanosponge interaction forces (17, 26). Hence, a thorough control over the temperature should be maintained when nanosponges are prepared.

Degree of substitution. – The type, number and position of the substituent on the polymeric molecule affect the complexation ability of nanosponges (26). The type of substitution is important because β-CD derivatives are available in various forms differing in functional groups present on the surface of the cyclodextrin derivative. When complexed together with the help of a crosslinker, different functional groups would yield different types of complexed material (β-CD nanosponges, CD-carbamate nanosponges, CD--carbonate nanosponges, *etc.*)

There is a direct correlation between the number of substitutions present and the degree of crosslinking. The higher the number of substituents, the greater is the probability of undergoing higher crosslinking. Higher degree of crosslinking will yield highly porous nanosponges due to more interconnections between polymers forming a mesh type network.

The position of substitution depends on the production conditions. A change in the production process will yield materials with different physicochemical properties due to occupancy of some different position by the functional group on the parent compound. For example, when produced under different conditions, the physicochemical properties of HP-β-CD samples with the same degree of substitution may not be identical owing to the possible occupancy of hydroxypropyl groups at different positions on the parent CD molecule. Thus the purity of material can have a significant effect on the final quality of nanosponges, indicating the significance of the degree of substitution of the polymer.

As mentioned above, the type of polymer determines the type of nanosponges that can be fabricated. Thus, based on the polymer used, various types of nanosponges can be developed. The above listed factors have to be considered during the fabrication of nanosponges. A few well-known examples of nanosponges are titanium-based nanosponges (1), silicon nanosponge particles (2), hyper-crosslinked polystyrene nanosponges (3) and cyclodextrin based nanosponges (4). Amongst the various types of nanosponges, cyclodextrin based nanosponges have received great attention and are widely studied. Further, this review focuses on cyclodextrin based nanosponges for pharmaceutical use.

Cyclodextrin based nanosponges

Being a biodegradable entity, cyclodextrin breaks down gradually in the body. Once at the target site, other systems generally unload most of the drug (carried or entrapped by them) in a rapid and uncontrollable fashion. Unlike other systems, nanosponges offer controlled release of the actives, which is one of the major advantages of such systems compared to other nanoparticulate delivery systems under development. CD-based nanosponges were conventionally used for decontamination of water. However, they have recently been explored and employed as solubilizing agents or nanocarriers for drug delivery in the field of pharmaceuticals (27–29).

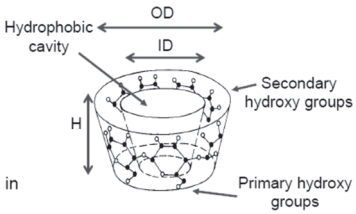
The basic physicochemical characteristics of cyclodextrins were discovered in the early 1950s; since then, their use is a practical and economical way to improve the physicochemical and pharmaceutical properties such as solubility, stability, and bioavailabi-

lity of administered drug molecules. The versatile pharmaceutical material cyclodextrins (CDs) are classified into hydrophilic, hydrophobic, and ionic derivatives (37). Cyclodextrins are a family of compounds made up of sugar molecules bound together in a ring (cyclicoligosaccharides). Enzymatic degradation of starch by cyclodextrin-glycosyltransferase (CGT) produces cyclic oligomers (38). CDs are non-reducing, crystalline, water soluble, cyclic oligosaccharides composed of 5 or more anhydrous α-D-glucopyranoside units (AGU) (39) linked together by a α-1,4-bond (40). Six, seven or eight AGU are generally present in CDs and are known as α, β and γ-CD, respectively. Due to the chair formation of glucopyranose units, cyclodextrin molecules are cone shaped with secondary hydroxyl groups at C2 and C3 positions extending from the wider edge and primary hydroxyl groups at C6 exposed from the opposite side of the narrow edge (38, 24). Their atomic arrangement is such that the inside cavity is hydrophobic, while the outside of the torus (truncated cone) is highly hydrophilic (31, 25).

CDs have a well-defined cylindrical cone defining a cavity of about 7.9–8 × 10^{-10} m deep and 5–10 × 10^{-10} m in diameter, depending on the number of glucose units (41). Certain characteristics of α, β and γ-CDs are given in Table II (23, 42).

Table II. Characteristics of α-CD, β-CD and γ-CD

Characteristics	α-CD	β-CD	γ-CD
Molar mass (g mol^{-1})	972	1135	1297
Depth-height cone (10^{-10} m)	7.9–8	7.9–8	7.9–8
Internal diameter (10^{-10} m)	4.9	6.2	7.9
External diameter (10^{-10} m)	14.6	15.4	17.5
Cavity volume	174	262	472
Water solubility (g per 100 cm^3) at 25 °C	14.5	1.85	23.2
Crystal water (*m*, %)	10.2	13.2–14.5	8.13–17.7



CDs have a well-defined cylindrical cavity exhibiting an apolar character, accompanied with high electron density and Lewis base properties (37). In essence, CDs have a cage like structure as shown in Table II. Due to their peculiar shape, CDs are able to form stable inclusion complexes with molecules of suitable polarity and size in aqueous solutions. The complexation mechanism does not involve covalent bonds and the main driving force of complex formation is the release of enthalpy-rich water molecules from the cyclodextrin cavity.

Many derivatives have been prepared to improve the characteristics of native CDs. Owing to their ability to complex with a wide range of compounds, CDs have found numerous applications as novel drug carriers (19). These CDs can serve as multi-functional drug carriers through the formation of inclusion complexes or by formation of CD/drug conjugates. More than 30 % of novel pharmaceutical products containing CDs are on the market worldwide (23). CDs and their derivatives have been used as solubilizers to enhance the loading capacity of liposomes, microparticles and nanoparticles (22).

The first studies of the role of CDs in preparation of microparticles were done by Loftsson and co-workers. Polymeric nanoparticles may also contain CDs: nanoparticles of poly(butylcyanoacrylate) and modified CDs have been used as matrices to obtain nanoparticulate systems. Nanospheres and nanocapsules have been also made from CDs modified on the secondary face with C6 aliphatic esters using the nanoprecipitation technique (20). Their solubilizing efficiency may be inadequate due to low intrinsic solubility of the drug or the low stability constant of a drug/CD complex; hence, research on CD complexes is now a challenging field, and innovative nanosponges are a solution to this challenge. Nanosponges are generally prepared from β-cyclodextrins because, among the natural (α, β, γ) CDs, β-CD has the highest complexing ability and stability with crosslinking agents. β-CDs have interesting characteristics, which are tuned to form nanochannels, wherein drugs may be incorporated in the cage as well as its complex network. Also, cavity dimensions, low cost of production, higher productive rates are some of the advantages offered by β-CD for the preparation of nanosponges (29). Reaction between different types of CDs and a crosslinker such as a carbonyl or dicarboxylate compound has been carried out (22, 43).

Thus, β-cyclodextrin is generally most preferred for nanosponge preparation. There are various techniques for the preparation of nanosponges from this material. The subsequent section discusses methods used for synthesizing and complexation of these nanoparticles.

Methods of nanosponge preparation

Cyclodextrin-based nanosponges (NS) may be synthesized by either a melt procedure or solvent method. Briefly, NS are obtained by the crosslinking of different types of CDs with a carbonyl or a dicarboxylate compound as crosslinker (22). The different crosslinking agents may dramatically modulate important parameters like swellability and hydrophilicity/hydrophobicity of the final nanoporous polymer. In the melt method, the crosslinker is melted along with CDs. All ingredients are finely homogenized and placed in a 250 mL flask heated at 100 °C and the reaction is carried out for 5 h under magnetic stirring. The reaction mixture is allowed to cool and the obtained product is broken down followed by repeated washing with suitable solvents to remove unreacted excipients and byproducts (Fig. 1).

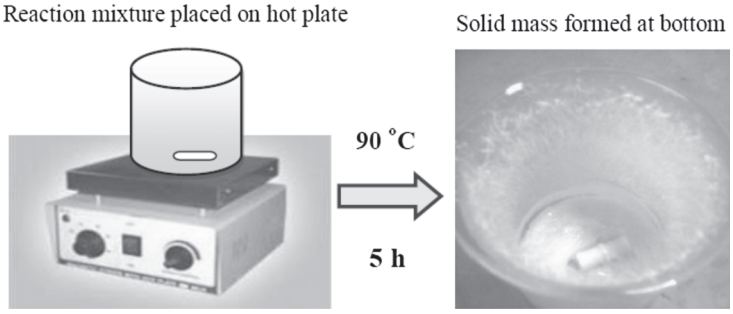


Fig. 1. Pictorial representation of the preparation of nanosponges (nanosponges designed in our laboratory).

In the solvent method, the melting step is eliminated and the crosslinker is solubilized in solvents like dimethylformamide or dimethylsulfoxide (DMF/DMSO). The polymer is generally mixed with a suitable solvent, particularly a polar aprotic solvent, followed by addition of this mixture to an excess quantity of the crosslinker. Optimization of the process is performed by varying the crosslinker/polymer molar ratio. The reaction is carried out at temperatures ranging from 10 °C to the reflux temperature of the solvent, for 1 to 48 h. Preferred crosslinkers for this reaction are the carbonyl compounds diphenyl carbonate (DPC), dimethyl carbonate (DMC) or carbonyldiimidazole (CDI). The product is obtained by adding the cooled solution to a large excess of bidistilled water. Recovery of the product is done by filtration under vacuum and the product is further purified by prolonged Soxhlet extraction (43, 29).

The particles obtained are solid nanoparticles with a spherical morphology that have a very high solubilizing power for poorly water-soluble molecules by either forming inclusion or non-inclusion complexes. Once the condensation polymerization is completed, the transparent block of hyper-crosslinked cyclodextrins can be incubated with the drug for loading.

Size reduction of synthesized nanosponges is carried out using the high pressure homogenization technique. In this technique, an aqueous suspension of nanosponges is initially homogenized with an Ultra Turrax for 10 min at fixed speed (rpm). The homogenized suspensions are then transferred into a high pressure homogenizer and subjected to cycles of homogenization. For example, a 2 % *m/V* aqueous suspension of swellable β-CD PAA nanosponges (PAA-NS) was homogenized using an Ultra Turrax (10 min at 24,000 rpm). The homogenized suspensions were then transferred into a high pressure homogenizer and subjected to 12 cycles of homogenization, that is, 5 cycles at 5,000 psi for 5 min, followed by 5 cycles at 7,000 psi for 5 min and 2 cycles at 5,000 psi in a recirculation mode. The PAA-NS (polyamioamines) aqueous nanosuspensions obtained were used for characterization and protein complexation studies. The obtained product could be safely stored in refrigerator at 4 °C without any aggregation (4). Nanosponges with narrow size distribution can be obtained by this technique.

Nanosponges may be also synthesized using the ultrasonication technique, wherein polymers are reacted with crosslinkers merely by keeping them under sonication in the absence of solvents. The method for fabrication of this type of nanosponges is discussed by Trotta *et al.* (20). Anhydrous β-CD and diphenyl carbonate were mixed in a 250 mL flask and the flask was placed in an ultrasound bath filled with water and heated to 90 °C with sonication for 5 h. Subsequent steps for production crystallization and purification were similar to those followed for the melt/solvent technique. Ultrasonication could be replaced by processes involving a high energy input like probe sonication. The advantage of this method is no use of organic solvents. Ultrasound assisted nanosponges have been synthesized by Trotta *et al.* using ultrasound probes (20).

Using the listed techniques, nanosponges can be synthesized, crystallized and purified. Purification of the obtained product is the most important and critical step. Depending upon the crosslinker used, byproducts of different nature and chemical structure are formed. Elimination of the formed byproducts is most essential because of the possible toxicity exerted by the byproduct residues in the final compound/product. Linkage between two cyclodextrin molecules mainly depends on the type of crosslinker used. Depending on the crosslinker used, different types of nanosponges (different linkages) are obtained.

Various types of cyclodextrin nanosponges depending on the crosslinker used for nanosponges

CD-based carbamate nanosponges. – CDs are reacted with suitable diisocyanates such as hexamethylene diisicyanate (HDI) and toluene-2,4-diisocyanate (TDI) in the presence of DMF solution at 70 °C for 16 to 24 hours under a nitrogen atmosphere. Residual DMF is removed by thorough washing with acetone and powder of the crosslinked polymer is obtained. These nanosponges have an ability to bind to organic molecules and have been largely employed for water purification. For example, nitrophenol is removed from its water solution even at very low concentrations. The loading capacity for organic molecules ranges from 20 to 40 mg per cm^3. Nanosponges can remove as much as 84 % of dissolved organic carbon (DOC) from wastewater (44). Owing to this property, they are used for removing undesirable taste and odorous compounds from water. Compounds such as geosmin and 2-methylisoborneol have been successfully removed by employing CD-based carbamate nanosponges (45).

Tang *et al.* studied CD-based carbamate nanosponges to adsorb aromatic amino acids (AAA) from phosphate buffer. L-tryptophane, L-phenylalanine and L-tyrosine were the AAA selected for further investigation. Adsorption efficiencies of AAA onto CD-based carbamate nanosponges were in the order L-tryptophane > L-phenylalanine > L-tyrosine (46).

CD-based carbonate nanosponges

The main crosslinkers used for preparation of this type of nanosponges are active carbonyl compounds such as CDI, DPC and trifosgene. The resulting CD nanosponges exhibit carbonate bonds between two CD monomers. The reaction can be carried out at room temperature (CDI) or at 80 to 100 °C (DPC) in the presence or absence of a solvent, *i.e.*, employing either the solvent technique or melt technique (28). Some of the important characteristics of carbonate-CD-based nanosponges are adjustable polarity and changeable dimensions of their cavities. They can be obtained in different forms, like amorphous or semicrystalline, by carrying out the reaction under different conditions. An amorphous crosslinked polymer is formed using the solvent method and a crystalline product is obtained when the melt technique is applied.

Carbonate-CD-based nanosponges have been used to encapsulate many drugs such as paclitaxel, camptothecin, dexamethasone, flurbiprofen, doxorubicin hydrocloride, itraconazole, 5-fluorouracil, cilostazol, progesterone, oxcarbamazepine, nelfinavir mesylate, resveratrol, and tamoxifen. Nanosponges particularly improve the wetting and solubility of these poorly water-soluble drugs. Carbonate nanosponges do not significantly affect the surface tension of water. They are non-hygroscopic in nature and they retain their crystal structure during absorption and desorption of moisture (28).

A unique feature of CD-based carbonate nanosponges is that their ability of solubility enhancement depends significantly on their degree of crystallinity. For example, the well-known anticancer drug dexamethasone is fourfold better solubilized by crystalline nanosponges, whereas the widely used antiviral compound acyclovir is solubilized twice as much by amorphous nanosponges (43).

CD-based ester nanosponges

A suitable dianhydride such as pyromellitic anhydride is used as a crosslinking agent for fabrication of these nanosponges. The exothermic crosslinking reaction is very fast (completed within a few minutes) and is carried out at room temperature, dissolving the CD and the dianhydride in DMSO in the presence of an organic base such as pyridine or triethylamine (to accelerate the reaction in a forward direction). This type of nanosponges can host both apolar organic molecules and cations simultaneously since it contains a polar free carboxylic acid group (28). The ionic moiety of NS-PYR complexes with a large number of heavy metal cations such as Al, Mn, Co, Ni, Cu, Zn, Cd, Pd and U at different pH values. Different pH values influence cation exchange and coordinate properties, enhancing the complexing activity of the native CD toward metal ions.

Mele and co-workers studied the inclusion abilities of NS-PYR using a high resolution magic angle spinning (HR MAS) NMR technique. Water diffusivity and the interaction of fluorescein in inner cavities of nanosponges were thus investigated (33).

Polyamidoamine nanosponges

These types of nanosponges are prepared by carrying out the reaction in water. β-CD polymerizes with acetic acid 2,20-bis(acrylamide) after long standing (*i.e.*, 94 h at room temperature). They swell in water (pH dependent behavior) and have both acid and basic residues. The polymer forms a translucent gel instantly on contact with water. Time-dependent swelling studies in biorelevant media confirmed the stability of the gel for up to 72 h. The studies were carried out using albumin as a model protein exhibiting very high encapsulation efficiency, around 90 %. *In vitro* drug release studies showed that protein release can be modulated up to 24 h. Sodium dodecyl sulphate (SDS PAGE) technique was used to investigate the stability of the product. Conformational stability of the protein, examined using the SDS PAGE technique, showed that the formulation was stable for as long as several months (33).

Modified nanosponges

Classical carbonate based nanosponges have been modulated by varying the reaction conditions to better fit the application selected. Fluorescent derivative has been obtained by reacting carbonate nanosponges with fluorescein isothiocyanate in DMSO at 90 °C for a few hours. Fluorescent nanosponges have found their use in biological studies such as cancer therapy. In a similar manner, carboxylated nanosponges can be obtained using a cyclic organic anhydride such as succinic anhydride or maleic anhydride (47). These nanosponges react with biologically important carriers such as biotin, chitosan, or proteins, possibly providing a promising specific receptor targeting activity for drugs. Powder XRD studies have shown that these nanosponges are amorphous in nature. They are also non-hemolytic and non-cytotoxic. For anti-cancer drugs such as camptothecin, carboxylated nanosponges appear to be promising safe carriers for drug targeting (4). Characteristics of various β-CD nanosponge fabrication methods are given in Table III.

Table III. Fabrication requirements for various types of β-CD nanosponges

Type of β-CD nanosponge	Crosslinker	Functional group formed	Temperature (°C)	Method	Solvent	Reaction time (h)	Nature of the material	Type of drug to be included
CD-based carbonate nanosponges	Carbonyl or dicarboxylate compound	Carbonate bond	100	Melt method	None	5	Crystalline	Any drug
	Diphenyl carbonate, dimethyl carbonate, carbonyldiimidazole	Carbonate bond	10 to the reflux temperature of solvent	Solvent method	DMF/DMSO	1–48	Amorphous	Any drug
CD-based carbamate nanosponges	Diisocyanates like HDI, TDI	Carbamate bond	70	Solvent method	DMF/DMSO	16–24	–	Any drug; chiral compounds can be separated
CD-based ester nanosponges	Dianhydride such as pyromellitic anhydride	Ester bond	r.t.	Solvent method	DMSO/DMF and a base as a catalyst	Completed within a few minutes		
CD-based polyamidoamine	Acetic acid 2,20-bis (acrylamide)	Polyamidoamine	r.t.	Solvent method	Water	96	Gel like material	Peptides and proteins to be separated
Modified type	Fluorescein isothiocyanate and carbonate nanosponges		90	Solvent method	DMF/DMSO	A few hours	Amorphous	Cancer therapeutics

Loading of drugs into nanosponges

Drug molecules can be inserted into the nanocavities of β-CD and due to further crosslinking, interactions of the guest molecules with more β-CD units might be observed. Moreover, the presence of a crosslinked network might also cause nanochannels to be formed in the NS structure. This peculiar structural organization might be responsible for the increased solubilization and protection capacities of NSs compared to the parent CD (4).

Nanosponges so developed are suspended within drug dispersions and freeze--dried along with the drug candidate. Drug loading may be also carried out by the solvent evaporation technique, in which the drug is dissolved in a suitable organic solvent. Prepared NSs are added to the above drug dispersion and triturated until the solvent evaporates. Drug/NS ratio is determined based on the solubility of the drug. For example, itraconazole was dissolved in dichloromethane to form a solution. To this solution, nanosponges were added along with copolyvidonum and triturated until the solvent evaporated. The drug, nanosponges, copolyvidonum were added in a ratio of 1:1:1 (*m/m*). The obtained solid dispersion was dried in an oven overnight (at 50 °C and at atmospheric pressure) to remove any traces of dichloromethane. The CD/crosslinker ratio can be varied during the preparation to improve the drug loading and to obtain a tailored release profile.

A suitably developed, fully characterized, drug loaded nanosponge can be used for numerous purposes in the pharmaceutical industry. For suitable applications in pharmaceuticals, developed nanosponges need to be fully studied and characterized by various parameters.

Characterization of nanosponges

To study the interaction of nanosponges with loaded drugs and to understand the process of their synthesis, fabrication and design, nanosponges are characterized by the following parameters.

Phase solubility. – The effect of NSs on drug solubility is investigated by the phase solubility technique (48). To determine phase solubility constants, excess drug is added into suitable solvents to obtain saturated solutions. Saturated drug concentration is treated with varying concentrations of blank nanosponges, 1:1, 1:2, 1:3, *etc.* As there is an increase in their concentration, more of the drug interacts with NSs. The study is carried out until equilibrium is obtained. A plot of NS concentration *vs.* drug concentration is drawn and the type of plot is defined with the help of the Higuchi and Connors classification (49). Obtained stability constant values give an idea about the extent of interaction between nanosponges and the drug. Interaction of the drug with nanosponges can increase the solubility of poorly water-soluble drugs and thereby their dissolution rate. Such studies have been carried out for itraconazole loaded cyclodextrin nanosponges prepared by Shankar *et al.* Consequently, nanosponges could markedly increase the solubility of molecules with very low aqueous solubility such as anticancer drugs, steroids and anti-inflammatory drugs (31).

Saturation state interaction. – UV spectroscopy is used as a tool to carry out the saturated solution interaction study. Increasing concentrations of nanosponge solutions (1–80 ppm) are added to fixed concentrations of the drug. The samples are kept overnight for interaction and finally filtered solutions are scanned for λ_{max} and absorbance is measured. Drug loading is interpreted by taking scans of the formulation in the UV range and analyzing the shift of the absorbance maxima in the spectra compared to pure drug (31).

Porosity. – Porosity study is performed to check the extent of nanochannels and nanocavities formed. Porosity of nanosponges is assessed with a helium pycnometer, since helium gas is able to penetrate inter- and intra-particular channels of materials. The true volume of material is determined by the helium displacement method (50). Owing to their porous nature, nanosponges exhibit higher porosity compared to the parent polymer used to fabricate the system.

Percent porosity is given by equation *(1)*.

$$\% \text{ Porosity (E)} = \frac{\text{Bulk volume} - \text{True volume}}{\text{Bulk volume}} \times 100 \qquad (1)$$

Average diameter and polydispersity indices of nanosponges. – This is usually determined using a particle size analyzer applying the principle of dynamic light scattering (DLS) (also known as photon correlation spectroscopy, PCS) (51–59). With the help of the autocorrelation function, PCS correlates the intensity variation of scattered light to particle size (60). PCS/DLS measures the hydrodynamic diameter, *i.e.*, it considers all the particles under measurement as spherical. DLS/PCS gives the particle size taking into consideration the effective viscosity, temperature and refractive index of the dispersing medium. Hence, the measured particle size would be a parameter obtained after considering all the factors. It is always preferred to have qualitative results as well. Qualitative analysis of the particles can be carried out by performing scanning electron microscopy (SEM), transmission electron microscopy (TEM) or environmental scanning electron microscopy (ESEM) analysis of the sample. Particle size and morphology can be obtained by dispersing the sample in water or other suitable solvents and further assessing it using techniques like SEM/TEM/ESEM. For example, nanosponges loaded with paclitaxel were found to show mononodal particle size distribution of 350 ± 25 nm, with narrow distribution (polydispersity index, $p < 0.2$) (34).

Zeta potential. – Zeta potential of any system under investigation is a measure of the surface charge. Surface charge is the parameter that affects body distribution and interaction with the biological environment. Zeta potential measurement involves consideration of the electric potential, *i.e.*, diffusion coefficient and electrophoretic mobility (61). These values are transformed to zeta potential after calculating them from the Smoluchowski equation or Stokes equation. The pH and electrolyte concentration (62, 63) need to be considered while measuring the zeta potential. Stability of the formed nanoparticles can be estimated by zeta potential assessment. Zeta potential in water should be about ± 30 mV, which is sufficiently high to give stable nano-suspensions that do not undergo aggregation over time. Cavalli *et al*. measured the electrophoretic mobility and

zeta potential using a 90 PLUS instrument. To determine the zeta potential, samples of nanosponge dispersions were diluted with KCl solution (0.1 mmol L^{-1}) and placed in an electrophoretic cell, where an electric field of about 15 V/cm was applied (35).

SEM and TEM. – These tools are employed to evaluate the particle shape and size as described above (4) and to get morphological information related to the drug delivery system under investigation (64–72). SEM involves imparting conductivity to the developed particles under vacuum with a focused electron beam. Whenever moist samples are to be investigated, ESEM can be employed. TEM involves morphology investigation of particles suspended in liquids. TEM and SEM studies have been carried out by Ansari *et al.* for prepared nanosponges. TEM studies carried out by these authors (35) revealed a regular spherical shape and size of NS. The shape and size of the developed nanosponges remained unaffected even after drug encapsulation into the nanosponges.

Fourier transform-infrared spectroscopy (FT-IR). – It serves as a major tool to determine the presence of functional groups. After polymer synthesis, the appearance of functional group peaks in the FT-IR spectrum is an indication of the formation of bonds between two monomer units of the polymer. In FTIR for a crystalline structure, a vibrational spectrum is obtained (73). FT-IR spectra of dried nanosponges, pure drug and drug loaded nanosponges are taken to understand the interaction. The spectra are obtained in the wavenumber range of 4000 to 650 cm^{-1}. FT-IR also helps in determining the hydrophobic and hydrophilic parts of the developed system. Ansari *et al*. (35) carried out a comparison of FTIR spectra of resveratrol and the complex and showed that there were major changes in the fingerprint region, *i.e.*, 900 to 1400 cm^{-1} indicating drug loading in nanosponges.

Differential scanning calorimetry (DSC). – Thermo-analytical methods determine whether the drug substance undergoes some change before thermal degradation of the developed delivery system. Change in the drug substance may be in the form of melting, evaporation, decomposition, oxidation or polymorphic transition indicating complex formation. The thermogram obtained by DTA and DSC can be observed for broadening, shifting and appearance of new peaks or disappearance of certain peaks. Absence of the drug melting peak of the crystal structure in the DSC thermogram is a sign of molecularly dispersed drug within the polymer (74–76). Changes in weight loss can also provide supporting evidence for the formation of inclusion complexes (4).

Thermal analyses are carried out using a DSC instrument to assess the interaction pattern, crystallinity and nature of the developed nanosponges. Such studies have been carried out by Swaminathan *et al.* to analyze the formed nanosponges. DSC thermograms of the complexes did not show the melting peak corresponding to the drug; this indicates that the drug is no longer crystalline and confirms its interaction with the NS structure (28, 31).

Powder X-ray diffraction (P-XRD). – Diffraction peaks for a mixture of compounds are useful in determining chemical decomposition and complex formation. Complex formation of the drug with nanosponges alters the diffraction patterns and also changes the crystalline nature of the drug. XRD pattern of the sample is determined as a function of scattering angle (77). Complex formation leads to a sharpening of the existing peaks, appearance/disappearance of a few new peaks and shifting of certain peaks. Powder

X-ray diffractometry can be used to detect inclusion complexation in the solid state. When the drug molecule is liquid, since liquids have no diffraction pattern of their own, the diffraction pattern of a newly formed substance clearly differs from that of an uncomplexed nanosponge. This difference in the diffraction pattern indicates complex formation. When the drug compound is a solid substance, a comparison has to be made between the diffractogram of the assumed complex and that of the mechanical mixture of the drug and polymer molecules.

Thermogravimetric analysis (TGA). – These studies are carried out to understand the melting point, thermostability and crystalline behavior of the particles (78). Trotta *et al*. have shown that in a nitrogen atmosphere, β-CD decomposes in a single step, leaving a carbonaceous residue, which is thermally stable, decomposing at a slow rate at higher temperatures. On heating in air, the char is oxidized to volatile products below 600 °C, leaving a ceramic-like residue stable at 800 °C. The charring process involves opening of the cyclodextrin rings, followed by a chemical evolution similar to that of cellulose with loss of the glycoside structure and hydroxyl groups and buildup of unsaturation, carbonyl groups and aromatic structures. In an analogous manner, in both atmospheres, nanosponges decompose in a single step with a maximum rate of weight loss at 320 °C and form a char stable at 800 °C.

Raman spectroscopy. – Raman spectroscopy is a useful tool for studying molecular structures because the width and the intensity, as well as the wavenumber of the Raman peaks, are sensitive to the environmental and conformational changes of the molecules and to intermolecular interactions. This explores the possibility of describing the behavior of CD-NS on passing from the dry to the swollen state. This further provides information on the state of water and the model solute dissolved in water inside the nanoporous network of swollen CD-NS, with particular emphasis on the diffusion phenomena in the gel-like state (32). Hydration dynamics was investigated by Mele *et al*. through the analysis of the vibration modes of O-H and C–H groups decoupled from the background of bulk water.

Drug loading and drug release kinetics. – The results of drug loading capacity prove that nanosponges may be used as drug carriers. For loading experiments, an excess of drug solution is incubated with a water dispersion of cyclodextrin nanosponges (10 %, *m/m*). After shaking for 24 h at room temperature, nanosponge aliquots are filtered and freeze-dried. The lyophilized product is used to determine the amount of drug present in systems with the help of suitable analytical methods (28). *In vitro* release kinetics experiments are performed using a multi-compartment rotating cell; an aqueous dispersion of nanosponges (1 mL) containing the drug is placed in the donor compartment, while the receptor compartment, separated by a hydrophilic dialysis membrane, is filled with phosphate buffer at pH 7.4 or pH 1.2. Each experiment is carried out for 24 h. At fixed times, the receptor buffer is completely withdrawn and replaced with fresh buffer. The amount of drug in the medium is determined by a suitable analytical method (29) and drug release is calculated to determine the release pattern.

Swelling and water uptake. – For swellable polymers like polyamidoamine nanosponges, water uptake can be determined by soaking the prepared nanosponges in aqueous solvent.

Swelling and water uptake can be calculated using equations (*2*) and (*3*).

$$\text{Percentage of swelling} = \frac{\text{Marking of the cylinder at a specified time point}}{\text{(Initial marking before soaking)}} \times 100 \quad (2)$$

$$\text{Percentage of water uptake} = \frac{\text{Mass of the hydrogel after 72 h}}{\text{(Initial Mass of dry polymer)}} \times 100 \quad (3)$$

As mentioned earlier, a fully characterized nanosponge formulation can have numerous applications in the pharmaceutical, water purification and cosmeceutical industries. A few important applications of nanosponges in pharmaceutical companies and sectors are listed below.

Applications of cyclodextrin nanosponges in pharmaceuticals

Nanosponges prepared from cyclodextrins are considered to be novel systems that can be used as drug carriers in pharmaceutical formulations. Molecular encapsulations of the drug and other modifications with appropriate cyclodextrin based nanosponges can overcome problems such as insolubility, permeability, sensitivity, *etc.*, and facilitate safe and efficient delivery of drugs (28). Nanosponges can advantageously carry water insoluble drugs and BCS (Biopharmaceutical Classification System) based class-II and IV drugs and can be also used to increase the dissolution rate, solubility and stability of such drugs. Nanoporous structures enable entrapment of flavors by adsorption and thus mask unpleasant flavors and also convert liquid substances to solids. Nanosponges can adsorb odorous material and thereby facilitate its removal from organic materials, water and other products. Some reports suggest that β-CD based nanosponges can deliver the drug to target sites three to five times more effectively than direct injections. Particularly good results were obtained by using carbonate based nanosponges in the delivery of some anticancer drugs such as paclitaxel and campothecin. Nanosponges are solid in nature and can be easily formulated as oral, parenteral, topical or inhalation dosage forms. For oral administration, the complexes may be dispersed in matrices of excipients, diluents, lubricants and anti-caking agents suitable for the preparation of capsules or tablets.

For parenteral administration, the complex may be simply carried in sterile water, saline or other aqueous solutions. For topical administration, they can be effectively incorporated into topical hydrogels. A few examples of β-CD based nanosponges used in the formulation of some drugs are given in Table IV.

Some specific applications of CD based nanosponges include the following:

Improvement of drug stability. – β-CD units are conjugated with a polymer, where a number of β-CD units are bound to the same polymer chain. Several β-CD units increase the stability of the drug complex (24). Further, the polymer may cooperate with the β-CD moieties in stabilizing the complexes. Such studies have been carried out for proteins and peptides because of their insufficient stability, costly production, immunogenic and allergic potential as well as poor bioavailability and sensitivity towards proteases (4). Bovine serum albumin (BSA) proteins in solution are not stable, they are stored in lyophilized state. However, proteins can get reversibly denatured on lyophilization and adopt conformation markedly different from the native structure. A major drawback in

Table IV. Examples of β-CD based nanosponges for pharmaceutical use

Crosslinkers	Use	Reference
Diphenyl carbonate (Diarylcarbonates)	Camptothecin for solubility enhancement, hemolytic activity and cytotoxicity Paclitaxel for bioavailability enhancement Tamoxifen for cytotoxicity, doxorubicin hydrochloride, dexamethasone for drug release studies and flurbiprofen for solubility enhancement	4, 28, 29, 20, 34
Diisocyanates	As water pollutants, itraconazole for saturation solubility studies	30, 31
Pyromellitic anhydride	As gelling agent	32
Carbonyldiimidazoles	Reseveratol for cytotoxicity, drug accumulation in the buccal mucosa of rabbit *ex vivo* studies and permeation studies through pig skin	19, 30, 35
Epichlorhydrine and carboxylic acid dianhydrides	As polymer (carrier) for maleic anhydride	24
2,2-Bisacrylamido acetic acid (BAC) or polyamido amine (PAA)	Bovine serum albumin (BSA)	22, 33

protein formulation and development is the need to maintain its native structure during processing and long-term storage. In the nanosponge based approach, proteins like BSA have been well encapsulated in swellable cyclodextrin based poly (amidoamine) nanosponges and have increased their stability (33).

Nanosponges as carriers for biocatalysts and in the delivery and release of enzymes, proteins, vaccines and antibodies. – Proteins, peptides, enzymes and derivatives thereof are used in the biomedical and therapeutic fields. Proteolytic enzymes are used to treat cancer or type I mucopolysaccharidosis, while DNA and oligonucleotides are used in gene therapy. Administration of these molecules presents various problems and limitations (79). Similarly to protein protection and stability concerns, there are concerns with enzyme, vaccine and antibody stability. Proteins and other macromolecules can be carried and delivered across a biological barrier, targeting them towards the site by adsorbing or encapsulating them in cyclodextrin nanosponges.

Modulating drug release. – Frequent administration is the major drawback of most of the conventional, commercially available delivery systems. However, a drug loaded in the nanosponge structure can be retained and released slowly over time. Hydrophilic CD NS can modify the rate of drug release, which can be used for enhancement of drug absorption across biological barriers, serving as a potent drug carrier in immediate release formulations. Hydrophobic CD NS may serve as sustained release carriers for water-soluble drugs, including peptide and protein drugs (23). Nanosponges can be also used as carriers of drugs such as doxorubicin (an anticancer drug), and they may protect the drug during its passage through the stomach. This drug is released very slowly at pH 1.1, whereas release is faster if pH is raised to 7.4.

Effective delivery carriers. – Cyclodextrin nanosponges have been used as vehicles for antitumor drugs such as paclitaxel, camptothecin and tamoxifen which present bioavailability problems because their solubility in water is low or non-existent. The drugs were incorporated into nanosponges and the study was carried out on various cell lines to investigate their antiproliferative effect. Complexes showed greater effect than that of the drug alone (19). Mean absolute bioavailability of paclitaxel increased after its loading in nanosponges and it was found to be 2.5-fold higher than the plain drug (34). Econazole nitrate, an antifungal agent used topically to relieve the symptoms of superficial candidasis, dermatophytosis, versicolor and skin infections, is available in cream, ointment, lotion and solution forms. Adsorption is not significant when econazole nitrate is applied to skin and thus high concentrations of active agents are required to be applied for effective therapy. To overcome this problem, econazole nitrate nanosponges were fabricated by the emulsion solvent diffusion method and were loaded in hydrogels to form a local depot for sustained drug release (80, 81).

Solubility enhancement. – The presence of cross-linking and cyclodextrin cavities in the structure favors interaction with active molecules. These characteristics enable several substances to be included and get solubilized in the formed cavities. Inclusion complexation or solid dispersions with CDs can improve drug solubility or rate of dissolution of poorly water-soluble drugs due to the reduction in drug crystallinity. The resulting complex hides most of hydrophobic functionality in the interior cavity of the CD while hydrophilic hydroxyl groups on the external surface remain exposed to the environment; the net effect is that a water soluble complex is formed (82). Among the various commercially available CDs, methylated CDs with relatively low molar substitution are the most powerful solubilizers. CD nanosponges can further enhance drug dissolution even when there is no complexation. CD nanosponges may also act as release enhancers; for example, β-CDs have been reported to enhance the release rate of poorly soluble drugs like naproxen and ketoprofen (23).

CDs also increase the permeability of hydrophobic drugs by increasing drug solubility and dissolution and thus making them available at the surface of the biological barrier, wherefrom they partition into the membrane without disrupting the lipid layers of the barrier. The effect of CD nanosponges on drug solubilization in the case of dexamethasone, flurbiprofen and doxorubicin hydrochloride, with different structures and solubilities were investigated by Trotta *et al*. Dexamethasone and flurbiprofen are lipophilic drugs whose log *P* values are 1.9 and 4.1, respectively, while doxorubicin hydrochloride is a hydrophilic drug with a log *P* value of 0.25. Improved aqueous solubility of lipophilic drugs was compared to the hydrophilic drug doxorubicin after the drugs were loaded into nanosponges. This behavior could be ascribed to the higher number of lipophilic sites available for the complexation of lipophilic drugs in comparison with the hydrophilic sites on the cyclodextrin cage (19).

Swaminathan *et al.* have studied nanosponges with itraconazole, a drug with aqueous solubility of about 1 ng mL^{-1} at physiological pH. Incorporation of the drug in nanosponges improved the drug's solubility by 27 times and on adding PVP as an auxiliary component in the nanosponge formulation, the solubility was enhanced 55-fold. Nanosponges could thus increase the bioavailability of itraconazole (31).

Other applications of nanosponges

Nanosponges based on cyclodextrins can strongly bind organic molecules and are therefore used to remove organic matter, flavors and odors from water (30). Applying the principles of size exclusion chromatography, hyper-crosslinked nanosponges have been used in selective separation of inorganic electrolytes. CD-carbonate nanosponges have been used as supramolecular reaction media for sensitizing the enantiodifferentiating photoisomerization of cyclooctene and 1,3-cyclooctadiene compounds. Nanosponges exhibited unique photochirogenesis behavior, significantly different from the conventional sensitizer-modified CD nanoporous structure (86).

The ability to entrap organic molecules can be ascribed both to the pores generated by the polymerization reaction and to the CD cavities and the swelling property upon absorption of water (86). Three-dimensional nanosponges play an important role in the fractionalization of peptides for proteomic applications. Nanosponges can be used as carriers for gases such as oxygen and carbon dioxide and could be useful for many biomedical applications (28). Nanosponges can selectively soak up biomarkers for diagnoses of various disease conditions and can be also used for carrying fluorescent dyes and other agents (83).

CONCLUSIONS

In the light of such findings, it may be concluded that cyclodextrin-based nanosponges are a novel class of biocompatible, versatile crosslinked polymers that greatly enhance solubility performances of their parent CDs. They are able to include lipophilic as well as hydrophilic drugs and release them in a controlled and predictable manner at the target site, thus increasing their bioavailability. By controlling the polymer to crosslinker ratio, the particle size and release rate can be modulated to better fit the application. They could be used to improve the aqueous solubility of lipophilic drugs, and protect the active moieties from physicochemical degradation. They have been found to be promising materials for immediate technological use for drug entrapment and as novel drug carriers. Because of their small size and spherical shape, nanosponges have the potential to be formulated into a wide range of dosage forms such as parenteral, aerosol, topical, tablets and capsules.

Abbreviations: AAA – aromatic amino acid; AGU – α-D-glucopyranoside units; BSA – bovine serum albumin; CD – cyclodextrin; CDI – carbonyldiimidazole; CGT – cyclodextrin-glycosyltransferase; DLS – differential light scattering; DMF/DMSO – dimethyl formamide/dimethyl sulfoxide; DOC – dissolved organic carbon; DPC – diphenyl carbonate; DSC – differential scanning colorimetry; ESEM – environmental scanning electron microscopy; FTIR – Fourier transform infrared; HDI – hexamethylene diisicyanate; NS – nanosponges; PAA – polyaminoamide; PVA – polyvinyl alcohol; SDS-PAGE – sodium dodecyl sulphate polyacrylamide gel electrophoresis; SEM – scanning electron microscopy; SLN – solid lipid nanosuspension; TDI – toluene-2,4-diisocyanate; TEM – transmission electron microscopy; TGA – thermogravimetric analysis; XRD – X-ray diffraction

REFERENCES

1. L. Guo, G. Gao, X. Liu and F. Liu, Preparation and characterization of TiO2 nanosponge, *Mater. Chem. Phys*. **111** (2008) 322–325; DOI: 10.1186/1556-276X-6-551.
2. D. Farrell, S. Limaye and S. Subramanian, *Silicon Nanosponge Particles*, U.S. Pat 0,251,561A1, 9 Nov 2006.
3. V. Dakankov, M. Llyin, M. Tsyurupa, G. Timofeeva and L. Dubronina, From a dissolved polystyrene coil to intramolecularly hyper cross linked nanosponges, *Macromolecules* **29** (1998) 8398–8403; DOI: 10.1021/ma951673i.
4. S. Swaminathan, L. Pastero, L. Serpe, F. Trotta, P. Vavia, D. Aquilano, M. Trotta, G. Zara and R. Cavalli, Cyclodextrin-based nanosponges encapsulating camptothecin: Physicochemical characterization, stability and cytotoxicity, *Eur. J. Pharm. Biopharm.* **74** (2010) 193–201; DOI: 10.1016/j.ejpb.2009.11.003.
5. F. Melani, P. Mura, M. Adamo, F. Maestrelli, P. Gratteri and C. Bonaccini, New docking CFF91 parameters specific for cyclodextrin inclusion complexes, *Chem. Phys. Lett.* **370** (2003) 280–292; DOI: 10.1016/S0009-2614(03)00126-X.
6. P. Couvreur and C. Vauthier, Nanotechnology: intelligent design to treat complex disease, *Pharm. Res.* **23** (2006) 1417–1450; DOI: 10.1007/s11095-006-0284-8.
7. C. Zhang, N. Awasthi, M. A. Schwarz, S. Hinz and R. E. Schwarz, Superior antitumor activity of nanoparticle albumin-bound paclitaxel in experimental gastric cancer, PLoS One. **8** (2013) e58037; DOI: 10.1371/journal.pone.0058037.
8. Y. Fukumori and H. Ichikawa, Nanoparticles for cancer therapy and diagnosis, *Adv. Powder Technol.* **17** (2006) 1–28; DOI: 10.1163/156855206775123494.
9. M. Morishita and N. Peppas, Is the oral route possible for peptide and protein drug delivery, *Drug Discov. Today* **11** (2006) 905–910; DOI: 10.1007/s11095-006-0284-8.
10. H. Cohen, R. Levy, J. Gao, I. Fishbein, V. Kousaev, S. Sosnowski, S. Slomkowski and G. Golomb, Sustained delivery and expression of DNA encapsulated in polymeric nanoparticles, *Gene Ther.* **7** (2000) 1896–1905; DOI: 10.1038/sj.gt.3301318.
11. L. Grislain, P. Couvreur, V. Lenaerts, M. Roland, D. Deprez-Decampeneere and P. Speiser, Pharmacokinetics and distribution of a biodegradable drug-carrier, *Int. J. Pharm.* **15** (1983) 335–345; DOI: 10.1016/0378-5173(83)90166-7.
12. L. Illum, S. S. Davis, C. G. Wilson, N. W. Thomas, M. Frier and J. G. Hardy, Blood clearance and organ deposition of intravenously administered colloidal particles, The effects of particle size, nature and shape, *Int. J. Pharm.* **12** (1982) 135–146; DOI: 10.1016/0378-5173(82)90113-2.
13. P. Couvreur, B. Kante, V. Lenaerts, V. Scailteur, M. Roland and P. Speiser, Tissue distribution of antitumor drugs associated with polyalkylcyanoacrylate nanoparticles, *J. Pharm. Sci.* **69** (1980) 199–202; DOI: 10.1002/jps.2600690222.
14. J. Kreuter, *Nanoparticles*, in *Encyclopedia of Pharmaceutical Technology* (Ed. J. Swarbrick and J. C. Boylan), Marcel Dekker In., New York 1994, pp.165–190.
15. J. Xing, D. Zhang and T. Tan, Studies on the oridonin-loaded poly(D,L-lactic acid) nanoparticles *in vitro* and *in vivo*, *Int. J. Biol. Macromol.* **40** (2007) 153–158; DOI: 10.1016/j.ijbiomac.2006.07.001.
16. E. Garcia-Garcia, K. Andrieux, S. Gil and P. Couvreur, Colloidal carriers and blood-brain barrier (BBB) translocation: a way to deliver drugs to the brain, *Int. J. Pharm.* **298** (2005) 274–292; DOI: 10.1016/j.ijpharm.2005.03.031.
17. S. Subramanian, A. Singireddy, K. Krishnamoorthy and M. Rajappan, Nanosponges: A Novel Class of Drug Delivery System – Review, *J. Pharm. Pharmac. Sci.* **15** (2012) 103–111.

18. A. Nokhodchi, M. Jelvehgari, M. Reza Siahi and M. Reza Mozafar, Factors affecting the morphology of benzoyl peroxide microsponges, *Micron* **38** (2007) 834–840, DOI: 10.1016/j.micron.2007.06.012.
19. F. Trotta and R. Cavalli, Characterization and application of new hyper-cross-linked cyclodextrins, *Compos. Interfaces* **16** (2009) 39–48, DOI: 10.1163/156855408X379388.
20. F. Trotta, R. Cavalli, V. Tumiatti, O. Zerbinati, C. Roggero and R. Vallero, *Ultrasound Assisted Synthesis of Cyclodextrin Based Nanosponges*, EP Pat 1786841A1, 23May, 2007.
21. S. Eki, T. Lei, L. Jingquan, J. Zhongfan, B. Cyrille and P. D. Thomas, Biodegradable star polymers functionalized with β-cyclodextrin inclusion complexes, *Biomacromolecules*, **10** (2009) 2699–2707; DOI: 10.1021/bm900646g.
22. S. Swaminathan, R. Cavalli, F. Trotta, P. Ferruti, E. Ranucci, I. Gerges, A. Manfredi, D. Marinotto and P. Vavia, In vitro release modulation and conformational stabilization of a model protein using swellable polyamidoamine nanosponges of β-cyclodextrin, *J. Incl. Phenom. Macrocycl. Chem.* **68** (2010) 183–191; DOI: 10.1007/s10847-010-9765-9.
23. A. Vyas, S. Saraf and S. Saraf, Cyclodextrin based novel drug delivery systems, *J. Incl. Phenom. Macrocycl. Chem.* **62** (2008) 23–42; DOI: 10.1007/s10847-008-9456-y.
24. T. Girek and W. Ciesielski, Polymerization of β-cyclodextrin with maleic anhydride along with thermogravimetric study of polymers, *J. Incl. Phenom. Macrocycl. Chem.* (2010) 1–7; DOI: 10.1007/s10847-010-9778-4.
25. D. Li and M. Ma, Nanosponges: From inclusion chemistry to water purifying technology, *Chem. Sci. Technol.* (1999) 26–28.
26. C. Rajeswari, A. Alka, A. Javed and R. Khar, Cyclodextrins in drug delivery: an update review, *AAPS PharmSciTech*. **6** (2005) E329-E357; DOI: 10.1208/pt060243.
27. A. Modi and P. Tayade, Comparative solubility enhancement profile of valdecoxib with different solubilization approaches, *Ind. J. Pharm. Sci*. **69** (2007) 427–430; DOI: 10.4103/0250-474X.33156.
28. R. Cavalli, F. Trotta and W. Tumiatti, Cyclodextrin-based nanosponges for drug delivery, *J. Incl. Phenom. Macrocycl. Chem.* **56** (2006) 209–213; DOI: 10.1007/s10847-006-9085-2.
29. F. Trotta, V. Tumiatti, R. Cavalli, C. Roggero, B. Mognetti and G. Berta, *Cyclodextrin-based Nanosponges as a Vehicle for Antitumoral Drugs*, WO 2009/003656 A1; 2009.
30. F. Trotta and T. Wander, *Cross-linked Polymers Based on Cyclodextrins for Removing Polluting Agents*, WO 2003/085002, US20050154198 A1, 14 July. 2005.
31. S. Swaminathan, P. Vavia, F. Trotta and S. Torne, Formulation of beta-cyclodextrin based nanosponges of Itraconazole, *J. Incl. Phenom. Macrocycl. Chem*. **57** (2007) 89–94; DOI: 10.1007/s10847-006-9216-9.
32. A. Mele, F. Castiglione, L. Malpezzi, F. Ganazzoli, G. Raffaini, F. Trotta, B. Rossi, A. Fontana and G. Giunchi, HR MAS NMR, powder XRD and Raman spectroscopy study of inclusion phenomena in β-CD nanosponges, *J. Incl. Phenom. Macrocycl. Chem*. **69** (2011) 403–409; DOI: 10.1007/s10847-010-9772-x.
33. S. Swaminathan, P. Vavia, F. Trotta, R. Cavalli, P. Ferruti, E. Ranucci and I. Gerges, Release modulation and conformational stabilization of a model protein by use of swellable nanosponges of b-cyclodextrin, First European Cyclodextrin Conference, Aalborg, Denmark 2009.
34. S. Torne, K. Ansari, P. Vavia, F. Trotta and R. Cavalli, Enhanced oral bioavailability after administration of paclitaxel-loaded nanosponges, *Drug Deliv.* **17** (2010) 419–425; DOI: 10.3109/10717541003777233.
35. K. Ansari, P. Vavia, F. Trotta and R. Cavalli, Cyclodextrin-based nanosponges for delivery of resveratrol: in vitro characterisation, stability, cytotoxicity and permeation study, *AAPS Pharm SciTech*, **12** (2011) 279–286; DOI: 10.1208/s12249-011-9584-3.

36. E. Patel and R. Oswal, Nanosponge and micro sponges: a novel drug delivery system, *Int. J. Res. Pharm. Chem.* **2** (2012) 237–244.
37. T. Loftsson and M. Brewster, Pharmaceutical applications of cyclodextrins: drug solubilization and stabilization, *J. Pharm. Pharmacol.* **85** (1996) 1017–1025; DOI: 10.1021/js950534b.
38. A. Radi and S. Eissa, Electrochemical study of indapamide and its complexation with β-cyclodextrin, *J. Incl. Phenom. Macrocycl. Chem.* **71** (2011) 95–102; DOI: 10.1007/s10847-010.9906-1.
39. H. Bricout, F. Hapiot, A. Ponchel, E. Monflier and S. Tilloy, Chemically modified cyclodextrins: an attractive class of supramolecular hosts for the development of aqueous biphasic catalytic processes, *Sustainability* **1** (2009) 924–945; DOI: 10.3390/su1040924.
40. H. Dodziuk, *Molecules with Holes – Cyclodextrins*, in *Cyclodextrins and Their Complexes* (Ed. H. Dodziuk), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim 2006, pp. 1–30.
41. D. Li and M. Ma, Nanosponges for water purification, *Clean Prod. Process*. **2** (2000) 112–16; DOI: 10.1007/s100980000061.
42. E. Bilensoy and A. Atilla, *Cyclodextrin-based Nanomaterials in Pharmaceutical Field*, in *Pharmaceutical Sciences Encyclopedia*: Drug Discovery, Development, and Manufacturing, John Wiley & Sons Inc. Publishers 2010; DOI: 10.1002/9780470259818.ch31.
43. R. Lala, A. Thorat and C. Gargote, Current trends in b-cyclodextrin based drug delivery systems, *Int. J. Res. Ayur. Pharm,* **2** (2011) 1520–1526.
44. B. Mamba, R. Krause, T. Malefetse and S. Sithhole, Cyclodextrin nanosponges in the removal of organic matter to produce water for power generation, *Water SA*, **34** (2008) 657–660.
45. B. Mamba, R. Krause, T. Malefetse, S. Mhlanga, S. Sithhole, K. Salipira and E. Nxumalo, Removal of geosmin and 2-methylisoborneol (2-MIB) in water from Zuikerbosch water treatment plant (Rand Water) using β-cyclodextrin polyurethane, *Water SA*, **32** (2007) 223–228.
46. S. Tang, L. Kong, J. Ou, Y. Liu, X. Li and H. Zou, Application of cross-linked β-cyclodextrin polymer for adsorption of aromatic amino acid, *J. Mol. Recogn. Macrocyclic Chem.* **19** (2006) 39–48; DOI: 10.1002/jmr.756.
47. F. Trotta, R. Cavalli, S. Swaminathan, C. Sarzanini and P. Vavia, Novel functionalized nanosponges: synthesis, characterization. Safety assessment, cytotoxicity testing and interaction studies. Proceedings of the 14th International Cyclodextrin Symposium, Kyoto 2008, pp. 338–342.
48. G. Yurtdas, M. Demirel and L. Genc, Inclusion complexes of fluconazole with β-cyclodextrin: physicochemical characterization and in vitro evaluation of its formulation, *J. Incl. Phenom. Macrocycl. Chem.* **70** (2011) 429–435; DOI: 10.1007/s10847-010-9908-z.
49. A. Rasheed, Cyclodextrins as drug carrier molecule: a review, *Sci. Pharmac.* **76** (2008) 567–598; DOI: 10.3797/scipharm.0808-05.
50. P. Sinko, Martin's *Physical Pharmacy and Pharmaceutical Sciences*, 5th ed., Lippincott Williams & Williams Publishers, Philadelphia 2006, p.466.
51. T. Govender, S. Stolnik, M. Garnett, L. Illum and S. Davis, PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug, *J. Control. Release* **57** (1999) 171–185; DOI: 10.1016/S0168-3659(98)00116-3.
52. S. Galindo-Rodriguez, E. Allémann, H. Fessi and E. Doelker, Physicochemical parameters associated with nanoparticle formation in the salting-out, emulsification-diffusion and nanoprecipitation methods, *Pharm. Res.* **21** (2004) 1428–1439; DOI: 10.1023/B:PHAM.0000036917.75634.be.
53. M. Leroueil-Le Verger, L. Fluckiger, Y. Kim, M. Hoffman and P. Maincent, Preparation and characterization of nanoparticles containing an antihypertensive agent, *Eur. J. Pharm. Biopharm.* **46** (1998) 137–143; DOI: 10.1016/S0939-6411(98)00015-0.
54. N. Santos-Magalhães, H. Fessi, F. Puisieux, S. Benita and M. Seiller, An in-vitro release kinetic examination and comparative evaluation between submicron emulsion and polylactic acid nanocapsules of clofibride, *J. Microencapsul.* **12** (1995) 195–205; DOI: 10.3109/02652049509015290.

55. A. Layre, R. Gref, J. Richard, D. Requier, H. Chacun, M. Appel, A. Domb and P. Couvreur, Nanoencapsulation of a crystalline drug, *Int. J. Pharm.* **298** (2005) 323–327; DOI: 10.1016/j.ijpharm.2005.02.039.
56. D. Lemoine, C. Francois, F. Kedzierewicz, V. Preat, M. Hoffman and P. Maincent, Stability study of nanoparticles of poly(β-caprolactone), poly(D,L-lactide) and poly(D,L-lactideco-glycolide), *Biomaterials* **17** (1996) 2191–2197. DOI: 10.1016/0142-9612(96)00049-X.
57. Y. Jeong, Y. Shim, C. Kim, G. Lim, K. Choi and C. Yoon, Effect of cryoprotectants on the reconstitution properties of surfactant-free nanoparticles of poly (D,L-lactide-co-glycolide), *J. Microencapsul.* **22** (2005) 593–601; DOI: 10.1080/02652040500162659.
58. H. Redhead, S. Davis and L. Illum, Drug delivery in poly(lactide-co-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: in vitro characterisation and in vivo evaluation, *J. Control. Release,* **70** (2001) 353–363; DOI: 10.1016/S0168-3659(00)00367-9.
59. M. Bivas-Benita, S. Romeijn, H. Junginger and G. Borchard, PLGA-PEI nanoparticles for gene delivery to pulmonary epithelium, *Eur. J. Pharm. Biopharm.* **58** (2004) 1–6; DOI: 10.1016/j.ejpb.2004.03.008.
60. R. Pecora, Dynamic light scattering measurement of nanometer particles in liquids, *J. Nanoparticle Res.* **2** (2000) 123–131; DOI: 10.1023/A:1010067107182.
61. M. Santander-Ortega, A. Jódar-Reyes, N. Csabac, D. Bastos-González and J. Ortega-Vinuesa, Colloidal stability of Pluronic F68-coated PLGA nanoparticles: a variety of stabilisation mechanisms, *J. Colloid. Interf. Sci.* **302** (2006) 522–529; DOI: 10.1016/j.jcis.2006.07.031.
62. Y. Ishikawa, Y. Katoh and H. Ohshima, Colloidal stability of aqueous polymeric dispersions: effect of pH and salt concentration, *Colloid Surf.* B **42** (2005) 53–58; DOI: 10.1016/j.colsurfb.2005.01.006.
63. J. Shar, T. Obey and T. Cosgrove, Adsorption studies of polyether's- Part1: Adsorption onto hydrophobic surfaces, Colloid Surf A: *Physicochemical and Engineering Aspects* **136** (1998) 21–33, DOI: 10.1016/S0927-7757(97)00182-9.
64. E. Leo, B. Brina, F. Forni and M. Vandelli, In vitro evaluation of PLA nanoparticles containing a lipophilic drug in water-soluble or insoluble form, *Int. J. Pharm.* **278** (2004) 133–141, DOI: 10.1016/j.ijpharm.2004.03.002.
65. U. Bilati, E. Allemann and E. Doelker, Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles, *Eur. J. Pharm. Sci.* **24** (2005) 67–75; DOI: 10.1016/j.ejps.2004.09.011.
66. M. Bivas-Benita, S. Romeijn, H. Junginger and G. Borchard, PLGA-PEI nanoparticles for gene delivery to pulmonary epithelium, *Eur. J. Pharm. Biopharm*. **58** (2004) 1–6; DOI: 10.1016/j.ejpb.2004.03.008.
67. H. Fessi, F. Puisieux, J. Devissaguet, N. Ammoury and S. Benita, Nanocapsule formation by interfacial polymer deposition following solvent displacement, *Int. J. Pharm*. **55** (1989) R1-R4; DOI: 10.1016/0378-5173(89)90281-0.
68. M. Chorny, I. Fishbein, H. D. Danenberg and G. Golomb, Lipophilic drug loaded nanospheres prepared by nanoprecipitation: effect of formulation variables on size, drug recovery and release kinetics, *J. Control. Release* **83** (2002) 389–400; DOI: 10.1016/S0168-3659(02)00211-0.
69. V. Mosqueira, P. Legrand, H. Pinto-Alphandary, F. Puisieux and G. Barratt, Poly (D,L-lactide) nanocapsules prepared by a solvent displacement process: influence of the composition on physicochemical and structural properties, *J. Pharm. Sci*. **89** (2000) 614–626; DOI: 10.1002/(SICI)1520-6017(200005)89:5<614::AID-JPS7>3.0.CO;2-7.
70. J. Ren, H. Hong, J. Song and T. Ren, Particle size and distribution of biodegradable poly-D,L--lactide-co-poly(ethylene glycol) block polymer nanoparticles prepared by nanoprecipitation, *J. Appl. Polym. Sci.* **98** (2005) 1884–1890; DOI: 10.1002/app.22070.

71. M. Teixeira, M. Alonso, M. Pinto and C. Barbosa, Development and characterization of PLGA nanospheres and nanocapsules containing xanthone and 3methoxyxanthone, *Eur. J. Pharm. Biopharm.* **59** (2005) 491–500; DOI: 10.1016/j.ejpb.2004.09.002.
72. M. Tobìo, R. Gref, A. Sanchez, R. Langer and M. Alonso, Stealth PLA-PEG nanoparticles as protein carriers for nasal administration, *Pharm. Res.* **15** (1998) 276–279; DOI: 10.1023/A:1011922 819926.
73. H. Brittain, D. Bogdanowich, J. DeVincentis, G. Lewen and A.Newman, Physical characterization of pharmaceutical solids, *Pharm. Res.* **8** (1991) 963–973. DOI: 10.1023/A:1015888520352.
74. M. Hombreiro-Perez, J. Siepmann, C. Zinutti, A. Lamprecht, N. Ubrich, M. Hoffman, R. Bodmeier and P. Maincent, Non-degradable microparticles containing a hydrophilic and/or a lipophilic drug: preparation, characterization and drug release modeling, *J. Control. Release* **88** (2003) 413–428; DOI: 10.1016/S0168-3659(03)00030-0.
75. M. Guyot and F. Fawaz, Nifedipine loaded-polymeric microspheres: preparation and physical characteristics, *Int. J. Pharm.* **175** (1998) 61–74; DOI: 10.1016/S0378-5173(98)00253-1.
76. Y. Jeong, Y. Shim, K. Song, Y. Park, H. Ryu and J. Nah, Testosterone encapsulated surfactant-free nanoparticles of poly(D,L-lactide-co-glycolide): preparation and release behavior, *Bull. Korean Chem. Soc.* **23** (2002) 1579–1584.
77. R. Suryanarayanan, *X-Ray Powder Diffractometry*, in *Physical Characterization of Pharmaceutical Solids,* Vol. 70 (Ed. H. G. Brittain), Marcel Dekker Inc., New York 1995, pp.187–221.
78. J. Alongi, M. Skovi, A. Frache and F. Trotta, Novel flame retardants containing cyclodextrin nanosponges and phosphorus compounds to enhance EVA combustion properties, *Polym. Degrad. Stabil.* **95** (2010) 2093–2100; DOI: 10.1016/j.polymdegradstab.2010.06.030.
79. G. Gilardi, F. Trotta, R. Cavalli, P. Ferruti, E. Ranucci, G. Di Nardo, C. Roggero and V. Tumiatti, *Cyclodextrin Nanosponges as Carrier for Biocatalysts, and in the Delivery and Release of Enzymes, Proteins, Vaccines and Antibodies,* WO2009149883 A1, 17 Dec.2009.
80. S. Renuka, B. W. Roderick and P. Kamla, Evaluation of the kinetics and mechanism of drug release from Econazole Nitrate nanosponge loaded carbopol hydrogel, *Ind. J. Pharm. Edu. Res.* **45** (2011) 25–31.
81. S. Renuka and P. Kamla, Polymeric nanosponges as an alternative carrier for improved retention of econazole nitrate onto the skin through topical hydrogel formulation, *Pharm. Dev. Technol.* **16** (2011) 367–376; DOI: 10.3109/10837451003739289.
82. S. Baboota, R. Khanna, S. Agarwal, J. Ali and A. Ahuja, *Cyclodextrins in Drug Delivery Systems*: An update, Available from Pharma. info. net., 2003, accessed on 13/01/2011.
83. V. N. Wong, G. Fernando, A. R. Wagner, J. Zhang, G. R. Kinsel, S. Zauscher and D. J. Dyer, Separation of peptides with polyionic nanosponges for MALDIMS analysis, *Langmuir* **25** (2009) 1459–1465; DOI: 10.1021/la802723r.
84. A. Jenny, P. Merima, F. Alberto and T. Francesco, Role of β-cyclodextrin nanosponges in polypropylene photooxidation, *Carbohyd. Polym.* **86** (2011) 127–135; DOI: 10.1016/j.carbpol.2011.04. 022.
85. K. William, S. Benjamin and H. Eva, *Synthesis and Characterization of Nanosponges for Drug Delivery and Cancer Treatment*, www.Vanderbilt.edu, accessed on 20.12.2011.
86. L. Wenting, Y. Cheng, N. Masaki, F. Gaku, M. Tadashi, M. Andrea, C. Franca, C. Fabrizio, T. Francesco and I. Yoshihisa, Cyclodextrin nanosponge-sensitized enantiodifferentiating photoisomerization of cyclooctene and 1,3-cyclooctadiene, *Beilstein J. Org. Chem.* **8** (2012) 1305–1311; DOI: 10.3762/bjoc.8.149.