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Nanosponges: a potential nanocarrier for novel drug delivery-a review

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ABSTRACT

The ideal delivery system will solubilize the drug, lead the therapy to the target site, and release the therapy to fulfill the individual need of the patient and disease stage. Nanosponges are one of such effective drug carriers which conquer the problems of drug toxicity and poor bioavailability as they can load both hydrophilic and hydrophobic drugs. Nanosponges are tiny in size with a 3-dimensional network and a nanometric cavity size. Nanosponges are highly porous having unique ability to entrap active moieties and offer a unique advantage of programmable release. They are biologically safe and simple to produce. Nanosponges can be prepared by cross linking different types of cyclodextrins with a carbonyl or a dicarboxylate compound as a cross linker. Nanosponge technology has been explored for various applications like enhancing the bioavailability of drug molecules and delivery of drugs into the oral, topical as well as parenteral routes. Nanosponges can also be used as a carrier for biocatalysts in the delivery and release of enzymes, proteins, vaccines and antibodies.

1. Introduction

Nanosponges are tiny mesh-like structures (Figure 1) in which a large variety of substances can be encapsulated[1,2]. They have a proven spherical colloidal nature, reported to have a very high solubilization capacity for poorly soluble drugs by their inclusion and non-inclusion behavior[3]. Nanosponges have recently been developed and proposed for drug delivery. Nanosponges can solubilize poorly water soluble drug and provide prolonged release as well as improving drugs bioavailability[4]. Nanasponges are able to load both hydrophilic and hydrophobic drug molecules because of their inner hydrophobic cavities and external hydrophilic branching, thereby offering unparalleled flexibility[5]. Nanosponges are more like a three-dimensional network or scaffold. The backbone is a long length of polyester which is mixed in solution with small molecules called crosslinkers that act like tiny grappling hooks to fasten different parts of the polymer together[6].

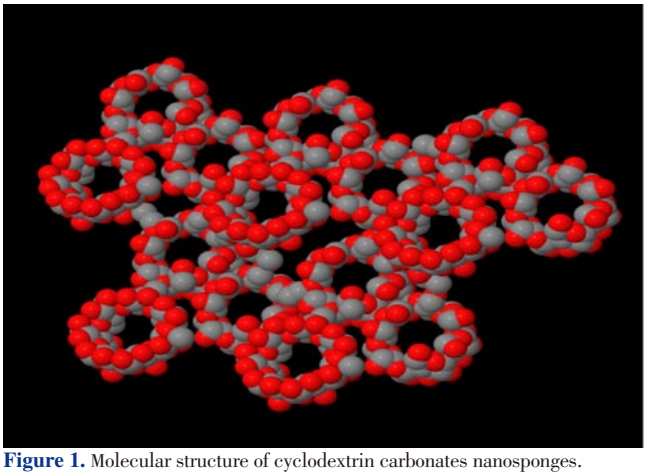

Figure 1. Molecular structure of cyclodextrin carbonates nanosponges.

It has been reported that, by reacting cyclodextrins (cyclic oligosaccharides) with suitable cross-linking reagents, a novel nanostructured material consisting of hyper-cross-linked cyclodextrins can be obtained, known as nanosponges[7-9]. Nanosponges can be synthesized as neutral or acid and can be swellable according to the agent used as crosslinker[10]. The net effect is to form spherically shaped particles filled with cavities where drug molecules

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can be stored[11].

The cross-linking-to-cyclodextrin ratio can be varied during preparation to improve the drug loading and to obtain a tailored release profile[12-14]. Their highly porous nanomeric nature enables drug molecules to orient themselves in nanosponge's inclusion as well as interact in a non-inclusion fashion, which offers higher drug loading compared with the parent cyclodextrin molecules[12].

Nanosponges show a remarkable advantage in comparison with the common nanoparticles. Indeed, they can be easily regenerated by different treatments, such as washing with eco-compatible solvents, stripping with moderately inert hot gases, mild heating or changing pH or ionic strength. For all these characteristics, nanosponges have been already employed in different applied fields, such as cosmetic and pharmaceutical sectors[15,16].

The engineering capacity of nanosponges is due to the relatively simple chemistry of its polyesters and cross-linking peptides, compared to many other nanoscale drug delivery systems[17]. Nanosponges are water soluble but does not breakup chemically in water. They mix with water and use it as a transport fluid. They can beused to mask unpleasant flavors, to convert liquid substances to solids. The chemical linkers enable the nanosponges to bind preferentially to the target site[17].

The nanosponges are solid in nature[18]. They have been found to be safe for oral and invasive routes, and thus they could serve as a potential carrier for drug delivery[14,15]. The tiny shape of nanosponges enables the pulmonary and venous delivery of nanosponges[19]. For oral administration, the complexes may be dispersed in a matrix 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 hydrogel[20,21]. Nanosponges are encapsulating type of nanoparticles which encapsulate the drug molecules within its core[22].

2. Chemicals used for the synthesis of nanosponges

2.1. Polymers

Polymers used for the synthesis of nanosponges are including: hypercross linked polystyrenes, cyclodextrins and its derivatives like Methyl β-cyclodextrin (β-CD), alkyloxy carbonyl cyclodextrins, 2-hydroxy propyl β-CDs and copolymers like poly (Valero lactone-allylvalero lactone) and poly (Valero lactone-allyl Valero lactone oxepanedione) and ethyl cellulose and PVA.

2.2. Crosslinkers

Crossslinkers used for the synthesis of nanosponges contain diphenyl carbonate, diarylcarbonates, diisocyanates, pyromellitic anhydride, carbonyldiimidazoles, epichloridrine, glutarldehyde, carboxylic acid dianhydrides, 2,2- bis(acrylamido) acetic acid and dichloromethane[3].

3. Methods of preparation of nanosponges

3.1. Solvent method

In this method the polymer was mixed with a suitable solvent, in particular in a polar aprotic solvent such as dimethylformamide, dimethylsulfoxide. This mixture was added to excess quantity of the crosslinker, preferably in crosslinker/polymer molar ratio of 4 to 16. The reaction was carried out at temperature ranging from 10 °C to the reflux temperature of the solvent, for time ranging from 1 to 48 h. Preferred cross linkers are carbonyl compounds (dimethyl carbonate and carbonyl diimidazole)[19].

After completion of the reaction, the solution was allowed to cool at room temperature, then the product was added to large excess of bidistilled water and recovered the product by filtration under vacuum and subsequently purified by prolonged Soxhlet extraction with ethanol. The product was dried under vacuum and grinded in a mechanical mill to obtain homogeneous powder[23].

3.2. Ultrasound-assisted synthesis

In this method nanosponges were obtained by reacting polymers with crosslinkers in the absence of solvent and under sonication. The nanosponges obtained by this method will be spherical and uniform in size[18]. The polymer was mixed and the crosslinker in a particular molar ratio in a flask. The flask was placed in an ultrasound bath filled with water and heated it to 90 °C. The mixture was sonicated for 5 h. Then the mixture was allowed to cool and the product was broken roughly. The product was washed with water to remove the non reacted polymer and subsequently purified by prolonged Soxhlet extraction with ethanol. The obtained product was dried under vacuum and stored at 25 °C until further use[18,23].

3.3. Loading of drug into nanosponges

Nanosponges for drug delivery should be pretreated to

obtain a mean particle size below 500 nm. The nanosponges were suspended in water and sonicated to avoid the presence of aggregates and then centrifuged the suspension to obtain the colloidal fraction. The supernatant was separated and dried the sample by freeze drying[23]. The aqueous suspension of nanosponges was prepared and dispersed the excess amount of the drug and maintained the suspension under constant stirring for specific time required for complexation. After complexation, the uncomplexed (undissolved) drug was separated from complexed drug by centrifugation. Then the solid crystals of nanosponges was obtained by solvent evaporation or by freeze drying[19,23]. Crystal structure of nanosponge plays a very important role in complexation with drug. A study revealed that paracrystalline nanosponges showed different loading capacities when compared to crystalline nanosponges. The drug loading is greater in crystalline nanosponges than paracrystalline one. In poorly crystalline nanosponges, the drug loading occurs as a mechanical mixture rather than inclusion complex[24].

4. Characterization of nanosponges

4.1. Solubility studies

The most widely used approach to study inclusion complexation is the phase solubility method described by Higuchi and Connors, which examines the effect of nanosponges on the solubility of drug. Phase solubility diagrams indicate the degree of complexation[19,25,26]. In this method the drug was added to an Erlenmeyer flask containing an aqueous solution of various percentages of nanosponges. The Erlenmeyer flask was stirred on a mechanical shaker at room temperature. When a steady state was reached, the suspension was filtered by centrifugation using a 3 000 Dalton molecular filter (MICRON YN 30, Millipore Corporation, Bedford MA 1730 U.S.A). The solution obtained was analyzed to determine the drug concentration by high performance liquid chromatography[22].

4.2. Microscopy studies

Scanning electron microscopy and transmission electron microscopy can be used to study the morphology and surface topography of the drug, nanosponges and the product (drug/nanosponge complex). The difference in crystallization state of the raw materials and the product observed under electron microscope indicates the formation of the inclusion complexes[24,26].

4.3. Thermoanalytical methods

Thermoanalytical methods determine whether the drug substance undergoes some changes before the thermal degradation of the nanosponge. The change of the drug substance may be melting, evaporation, decomposition, oxidation or polymorphic transition. The change of the drug substance indicates the complex formation. The thermogram obtained by differential thermal analysis and differential scanning calorimetry can be observed for broadening, shifting and appearance of new peaks or disappearance of certain peaks. Changes in the weight loss also can provide supporting evidence for the formation of inclusion complexes[25].

4.4. X-ray diffractiometry and single crystal X-ray structure analysis

Powder X-ray diffractiometry can be used to detect inclusion complexation in the solid state. When the drug molecule is liquid since liquid have no diffraction pattern of their own, then the diffraction pattern of a newly formed substance clearly differs from that of uncomplexed nanosponge. This difference of diffraction pattern indicates the 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[25]. A diffraction pattern of a physical mixture is often the sum of those of each component, while the diffraction pattern of complexes is apparently different from each constituent and leads to a "new" solid phase with different diffractograms. Diffraction peaks for a mixture of compounds are useful in determining the chemical decomposition and complex formation[25].

The complex formation of drug with nanosponges alters the diffraction patterns and also changes the crystalline nature of the drug. The complex formation leads to the sharpening of the existing peaks, appearance of a few new peaks and shifting of certain peaks[25].

4.5. Single crystal X-ray structure analysis

It is used to determine the detailed inclusion structure and mode of interaction. The interaction between the host and guest molecules can be identified and the precise geometrical relationship can be established[25].

4.6. Infra-red spectroscopy

Infra-red spectroscopy is used to estimate the interaction

between nanosponges and the drug molecules in the solid state. Nanosponges bands often change only slightly upon complex formation. If the fraction of the guest molecules encapsulated in the complex is less than 25%, bands which could be assigned to the included part of the guest molecules are easily masked by the bands of the spectrum of nanosponges. The technique is not generally suitable to detect the inclusion complexes and is less clarifying than other methods[25].

The application of infra-red spectroscopy is limited to the drugs having some characteristic bands, such as carbonyl or sulfonyl groups. Infrared spectral studies give information regarding the involvement of hydrogen in various functional groups. This generally shifts the absorbance bands to the lower frequency, increases the intensity and widens the band caused by stretching vibration of the group involved in the formation of the hydrogen bonds. Hydrogen bond at the hydroxyl group causes the largest shift of the stretching vibration band[25].

4.7. Thin layer chromatography

In thin layer chromatography, the R_f values of a drug molecule diminish to considerable extent and this helps in identifying the complex formation between the drug and nanosponge[25].

4.8. Loading efficiency

The loading efficiency of nanosponges can be determined by the quantitative estimation of drug loaded into nanosponges by UV spectrophotometer and high performance liquid chromotography methods[2]. The loading efficiency (%) of nanosponges can be calculated according to the following equation[4].

$$\text{Loading efficiency} = \frac{\text{Actual drug content in nanosponge}}{\text{Theoretical drug content}} \times 100$$

4.9. Particle size and polydispersity

The particle size can be determined by dynamic light scattering using 90Plus particle size reequipped with MAS OPTION particle sizing software. From this the mean diameter and polydispersity index can be determined[24]. The measurements were made at a fixed angle of 90° for all samples. The samples were suitably diluted with Milli Q water for every measurement[3].

4.10. Zeta potential

Zeta potential is a measure of surface charge. It can be measured by using additional electrode in the particle size equipment[24]. For zeta potential determination, samples of the nanosponges were diluted with 0.1 mol/L KCl and placed in the electrophoretic cell, where an electric field of about 15 V/cm was applied. The mean hydrodynamic diameter and polydispersity index of the particles were calculated using the cumulated analysis after averaging of the total measurements[3].

5. Application of nanosponges

Due to their biocompatibility and versatility, nanosponges have many applications in the pharmaceutical field. They can be used as excipients in preparing tablets, capsules, pellets, granules, suspensions, solid dispersions or topical dosage forms[27]. They can encapsulate variety of drugs as shown in Table 1. Nanosponges can act as multifunctional carriers for enhanced product performance and elegancy, extended release, reduced irritation, improved thermal, physical and chemical stability of product. Following are the application of nanosponges which shows versatility of nanosponges.

Table 1
A list of drugs complexed by using nanosponges.

Drug	Therapeutic activity	Nanosponges vehicle	Attributes	Administration route	References
Itraconazole	Antifungal	β-CD, copolyvidonum	Enhanced drug solubility	Oral, topical	[12]
Dexamethasone	Anti-inflammatory	β-CD, diphenylcarbonate	Enhanced drug solubility	Oral, parenteral	[13]
Flurbiprofen	Anti-inflammatory	β-CD, diphenylcarbonate	Sustained drug release	Oral	[13]
Doxorubicin	Antineoplastic	β-CD, diphenylcarbonate	Sustained drug release	Parenteral	[13]
Nelfinavir mesylate	Antiviral	β-CD, dimethylcarbonate	Enhanced drug solubilization	Oral	[14]
Gamma-oryizanol	Antioxidant	β-CD, diphenylcarbonate	Enhanced stability, solubility, permeation	Topical	[39]
5-Fluorouracile	Antineoplastic	β-CD	Enhanced drug stability	Parenteral, topical	[49]
Tamoxifen	Antiestrogen	β-CD, carbonyldiimidazole	Enhanced bioavailability, solubility	Oral	[50]
Resveratrol	Antioxidant	β-CD, carbonyldiimidazole	Enhanced stability, permeation, cytotoxicity, controlled drug release	Oral, topical	[51]
Acetylsalicylic acid	Anti-inflammatory	β-CD, pyromellitic dianhydride	Prolonged drug release	Oral	[52]
Curcumin	Antineoplastic	β-CD, dimethylcarbonate	Enhanced activity, solubilization	Parenteral	[53]

5.1. Nanosponges as a sustained delivery system

Acyclovir is a widely used antiviral agent due to of its efficacy in the treatment of herpes simplex virus infections[28]. However, neither the parenteral nor the oral administration of the currently available formulations of acyclovir is able to result in suitable concentrations of the agent reaching at target sites. Acyclovir's absorption in the gastrointestinal tract is slow and incomplete, what's more, its pharmacokinetics following oral medication is highly variable. The *in vitro* release profiles of acyclovir from the two types of nanosponges showed a sustained release of the drug from the two types of nanosponges indicating the encapsulation of acyclovir within the nanostructures. The percentages of acyclovir released from Carb-nanosponges and nanosponges after 3 h *in vitro* were approximately 22% and 70%, respectively. No initial burst effect was observed for either formulation, proved that the drug was not weakly adsorbed onto the nanosponge surfaces[29].

5.2. Nanosponges in solubility enhancement

Swaminathan *et al*. studied a formulation of itraconazole in Nanosponges[12]. Itraconazole is a BCS Class II drug that has a dissolution rate limited poor bioavailability. Nanosponges improved the solubility of the drug more than 27-fold. When copolyvidonum was added as a supporting component of the nanosponge formulation, this exceeded to 55-fold. Nanosponges solubilize drug by possibly masking the hydrophobic groups of itraconazole, by increasing the wetting of the drug, and/or by decreasing the crystallinity of the drug[12].

5.3. Nanosponges in drug delivery

Nanosponges are nanomeric in size and have spherical shape, therefore, nanosponges can be prepared in different dosage forms like topical, parenteral, aerosol, tablets and capsules[2].

Telmisartan (TEL) is a BCS Class II drug having dissolution rate limited bioavailability. β-CD based nanosponges were formed by cross-linking β-CD with carbonate bonds. TEL was incorporated into the nanosponges. Saturation solubility and *in vitro* dissolution study of β-CD complex of TEL was compared with plain TEL and nanosponge complexes of TEL. It was found that solubility of TEL was increased by 8.53-fold in distilled water, 3.35-fold in 1 mol HCl and 4.66-fold in phosphate buffer pH 6.8 by incorporating $NaHCO_3$ in drug-nanosponges complex than TEL. The highest solubility and *in vitro* drug release was observed in inclusion complex prepared from nanosponges and $NaHCO_3$[30].

Paclitaxelis used for cancer chemotherapy having poor water solubility. β-CD based nanosponges to deliver paclitaxel is an alternative to classical formulation in cremophor EL because cremophor reduces the paclitaxel tissue penetration. The biological effect of paclitaxel *in vitro* is highly enhanced by nanosponges: not only its cytotoxicity is greatly increased after 72 h incubation, but even intracellular paclitaxel concentration is significantly enhanced when compared to plain paclitaxel[31].

Econazole nitrate, an antifungal agent used topically to relive the symptoms of superficial candidasis, dermatophytosis and skin infections available in cream, ointment, lotion and solution. Adsorption is not significant when econazole nitrate is applied to skin and required high concentration of active agents to be incorporated for effective therapy. Thus, econazole nitrate nanosponges were fabricated by emulsion solvent diffusion method and these nanosponges were loaded in hydrogel as a local depot for sustained drug release[32].

5.4. Nanosponges for protein delivery

Long term stability is a critical point in the successful development of pharmaceuticals, including macromolecular ones like proteins[33]. However, proteins can reversibly (or sometimes, even irreversibly) denature upon lyophilization and consequently adopt conformation markedly distinct from the native ones. Thus, a major obstacle in protein formulation development is the maintenance of the native protein structure both during the formulation process and upon the long term storage[34].

Swaminathan *et al*. reported new swellable cyclodextrin-based poly (amidoamine) nanosponges named nanosponges 10 and nanosponges 11, were synthesised by cross-linking β-CDs with either 2,2-bis-acrylamidoacetic acid or a short polyamido-amine chain deriving from 2,2-bis-acrylamidoacetic acid and 2-methyl piperazine respectively. The formulated β-CD based poly (amidoamine)-nanosponges were found to be stable at 300 °C and high protein complexation capacity was observed[35].

5.5. Nanosponges in enzyme immobilization

The issue of enzyme immobilization is particularly relevant for lipases, as it improves their stability and modulates properties such as enantio selectivity and reaction rates[36]. As a consequence, the demand for new solid supports, suitable for this family of enzymes is constantly growing.

For this Boscolo *et al*., reported high catalytic performances of *Pseudomonas fluorescens* lipase adsorbed

on a new type of cyclodextrin-based nanosponges[37].

5.6. Nanosponges as a carrier for delivery of gases

Gases play an important role in medicine, either for diagnostic or treatment purposes. The deficiency of adequate oxygen supply, named hypoxia, is related to various pathologies, from inflammation to cancer. It is sometime difficult to deliver oxygen in appropriate form and dosage in clinical practice.

Cavalli *et al*. developed nanosponges formulations as oxygen delivery systems for topical application which have the ability to store and to release oxygen slowly over time[38].

5.7. Nanosponges as protective agent from light or degradation

Gamma-oryzanol is a ferulic acid ester mixture, has recently attracted a great interest as natural antioxidant and usually employed to stabilize food and pharmaceutical raw materials, moreover as a sunscreen in the cosmetics industry. Its application is limited by its high instability and photodegradation. Gamma-oryzanol was encapsulated in nanosponges, showing a good protection from photodegradation. A gel and an O/W emulsion were formulated with the gamma-oryzanol-loaded nanosponges[39].

5.8. Earlier work done on nanosponges

Wong *et al*. reported that three dimensional nanosponges plays an important role in the fractionalization of peptides for proteomic applications[40].

Moura and Lago studied catalytic growth of carbon nanotubes and nanofibers on vermiculite to produce flotable hydrophobic "nanosponges" for oil spill remediation[41].

Arkas *et al*. reported that nanosponges have the property of encapsulating organic pollutants from water. Ceramic porous filters can be impregnated with these nanosponges resulting in hybrid organic/inorganic filter modules. These hybrid filter modules were tested for the effective purification of water, employing a variety of water pollutants. It has been established that polycyclic aromatic hydrocarbons can be removed very efficiently (more than 95%). Representatives of the pollutant group of trihalogen methanes, mono aromatic hydro carbons, and pesticides (simazine) can also be removed (>80%)[42].

Alongi *et al*. reported novel flame retardants containing cyclodextrin nanosponges and phosphorous compounds to enhance ethyl vinyl acetate copolymer combustion properties[43].

Alongi *et al*. studied the interaction between β-cyclodextrin nanosponges and two different ultraviolet stabilizers (namely, 2-hydroxy-4(octyloxy)-benzophenone and triphenyl phosphate) in the photooxidation of polypropylene exposed to UV light have been investigated. A significant decrease of the oxidation induction time has been observed in presence of β-CD nanosponges[44].

Lee *et al*. synthesized graphite-naofiber-supported porous Pt-Ag nanosponges and mesoporous platinum nanosponges as electrocatalysts for the oxygen reduction reaction[45,46].

The precise control of chiral photoreactions or photochirogenesis is one of the most challenging topics in current photochemistry. A supramolecular approach to photochirogenes provides a convenient and also promising tool to facilitate excited-state chirality transfer from chiral host toprochiral substrate.

Liang *et al*. developed the pyromellitate-linked cyclodextrin nanosponges, employed for the first time as supramolecular reaction media for sensitizing the enantio differentiating photoisomerization of (Z)-cyclooctene and (Z,Z)-1,3-cyclooctadiene exhibited unique photochirogenesis behavior significantly different from the conventional sensitizer-modified cyclodextrins[47].

Yang *et al*. developed non-cytotoxic scaffolds with a nanometer resolution through using silicon substrates as the backbone. This method was merged an optics-based approach with chemical restructuring to modify the surface properties of an IC-compatible material, switching from hydrophilicity to hydrophobicity. Through this nanofabrication-based approach, they synthesized hydrophobic oxidized silicon nanosponges. This study had demonstrated the potential application of using these silicon-based nanopatterns such as influencing cellular behaviors at desired locations with a micro-/nanometer level[48].

6. Conclusion

Nanosponges are versatile drug carrier system as they carry both hydrophilic and hydrophobic drugs by forming inclusion and non inclusion complexes. They can deliver drugs by various routes like oral, topical and parenteral in a predictable manner to the target site. Besides their application in the drug delivery field, potential applications exist for cosmetics, biomedicine, bioremediation processes, agro chemistry, and catalysis, among others. Drugs delivered by nanosponges can be proved safe and effective and the pharmaceutical industries will benefit greatly if

clinical studies can prove their potential for human use.

Conflict of interest statement

We declare that we have no conflict of interest.

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