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Nanosponges: An Emerging Carriers for Drug Delivery

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ABSTRACT

Pharmaceutical nanotechnology is an emerging tool for the delivery of drugs to the targeted site. They have significant applications in disease diagnostics and therapeutics. Nanosponge is a novel technology that delivers drug in a controlled manner for topical delivery. These are microscopic particles with nanosize cavities in which wide variety of drugs can be encapsulated. They are advantageous due to their increased stability and bioavailability. Nanosponges can be formulated by crosslinking CD(cyclodextrin) with carboxylate (cross-linkers). They exist in different types such as CD-based carbamate NS, CD-based carbonate NS, CD-based ester NS, Polyamidoamine NS, and Modified NS. They are used in oral as well as topical formulations. Various methods have been employed for the preparation of Nanosponges such as solvent method, emulsion solvent diffusion method, ultrasound-assisted synthesis, quasi-emulsion solvent diffusion, polymerization method etc., synthesis of nanosponges include polymer, co-polymer, cross-linkers, and the bioactive material. Nanosponges serve as an effective carrier for proteins, enzymes, antibodies and vaccines.

INTRODUCTION:

Drug delivery has been a challenge for researchers, i.e., delivering the drug to the right place in the body and controlling its release to prevent overdoses. As of now, the complexity of chemistry involved in developing new systems has largely been involved in development. This problem can be solved by the development of new and complex molecules using nanotechnology[1].

Nanotechnologies have so far been used to produce nanoparticles, nanocapsules, nanospheres, nanosuspensions, nanocrystals, nano-erythosomes, etc. Nanotechnology consists of materials that are developed in the nanoscale level to show novel properties. Nanomaterials are those whose dimensions are smaller than 100 nanometers. Various applications of nanoparticles are biocompatible materials, textile fictionalization, UV-blocking coatings, drugs delivery, DNA delivery, enzyme immobilization, etc.

In general, nanotechnology refers to the development of active ingredients in the nanoscale, as well as the design, production, characterization, and application of various nanoscale materials in various fields, mainly in the medical sector. Various pharmaceutical sectors have been impacted by nanotechnology including immunology, cardiology, endocrinology, ophthalmology, oncology, pulmonology, etc. In addition, it is widely used in specialized areas like brain targeting, tumor targeting, and gene delivery[2].

Nanosponges are new class of techniques comprised of microscopic particles with nonmaterial wide cavities in which they can encapsulate a wide variety of substances with a 'backbone' similar to the scaffold structure of naturally degradable polyester. Poorly water-soluble molecules can be enhanced by nanosponge particles because they carry liphophilic and hydrophilic substances[3].

Nanosponges are the system that can be used for targeted drug delivery of a drug for an extended period. These are nanometric size particles in which the drug or core material can be encapsulated within a polymeric coat. This system is advantageous because of the complex that enhances the dissolution rate, solubility and drug stability[4].

Nanosponges are three-dimensional network-like structure that is mixed with small molecule known as cross-linkers. This results in the formation of spherical shape particles with cavities for the storage of drugs. These are insoluble in water and organic solvents and are stable at

high temperatures up to 300°C. The drug can be released at a predictable rate in this system which is the major advantage of the nanosponges[1].

ADVANTAGES OF NANOSPONGE[3, 5, 6]:

- Nanosponge drug delivery system enhances the bioavailability of the drug.
- The solubility of poorly soluble drugs can be increased.
- This system provides increased stability of the formulation.
- They can be used as a controlled as well as targeted drug delivery system.
- Unpleasant flavours of drugs can be masked by encapsulating drug in the polymer.
- The aqueous solubility of lipophilic drug can be increased by this technique.
- Nanosponges are non-irritating, non-toxic and non-mutagenic in nature.
- Enhanced stability of the formulation**.**

TYPES OF NANOSPONGES[8]:

- CD-based carbamate Nanosponges
- CD-based carbonate Nanosponges
- CD-based ester Nanosponges
- PolyamidoamineNanosponges
- Modified Nanopsponges

CD-based carbamate Nanosponge:

This CD-based carbamate Nanosponge system can bind efficiently to the organic molecules. It has a loading capacity ranging from 20 to 40 mg per cm^3. This NS system is used in the water purification process. They are prepared by reacting CDs with appropriate diisocyanates like hexamethylene diisocyanate and toluene, 2, 4-diisocyanate in the presence of DMF solution under nitrogen atmosphere for 16 to 24 hours at 70◦C. Acetone can be used for removal of residual DMF. This results in the formation of a crosslinked polymer[2].

CD-based carbonate Nanosponge:

This system posses important characteristics like changes in cavity dimensions, and adjustable polarity. Crosslinkers like active carbonyl compounds such as CDI, DPC and trifosgene are widely used for the preparation of CD-based carbonate-based Nanosponges. These Nanosponges exist in amorphous or semi-crystalline form based on different conditions. The solubility depends upon the degree of crystallinity. Carbonate-CD-based Nanosponges are used for encapsulation of many drugs such as paclitaxel, flurbiprofen, itraconazole, doxorubicin hydrochloride etc[2].

CD-based ester nanosponge:

Fabrications of nanosponges are done by using a suitable cross-linking agent known as pyromellitic anhydride. These Nanosponges contain a polar-free carboxylic acid group. In this process, DMSO can be used as a solvent in which CD and dianhydride are dissolved in presence of an organic base at room temperature[2].

Polyamidoamine Nanosponge:

The reaction for these forms of nanosponges can be done using water. - CD polymerizes with acetic acid 2, 20-bis (acrylamide) (i.e., 94 h at room temperature). They have acid and basic residues and swell in water. When the polymer comes into touch with water, it rapidly transforms into a translucent gel[2].

Modified Nanosponges:

Nanosponges have been modulated by varying the reaction conditions to better fit the application selected. Fluorescent derivatives were obtained by reacting carbonate nanosponges with fluoresce in isothiocyanate in DMSO at 90°C for a few hours. Fluorescent nanosponges can be used in biological studies such as cancer therapy. Similarly, carboxylated nanosponges can be obtained using a cyclic organic anhydride such as succinic anhydride or maleic anhydride. These nanosponges react with biologically important carriers such as biotin, chitosan, or proteins, possibly providing a promising specific receptor targeting activity for drugs[2].

MATERIALS USED IN THE PREPARATION OF NANOSPONGES[5]:

- Polymers
- Co-polymers
- Crosslinkers
- Apolar solvents

Polymers:

➢ Hyper cross-linked polystyrene

➢ Cyclodextrin and its derivatives like methyl β-cyclodextrin**,** alkyloxycarbonyl-cyclodextrin

Copolymers:

➢ Ethylcellulose

➢ Polyvinyl alcohol

Crosslinkers:

➢ Diphenyl carbonate

➢ Diarylcarbonate

➢ Di-isocyanates

➢ Dichloromethane

➢ Glutaraldehyde

➢ Carboxylic acid dianhydrides

➢ Acetic acid

Apolar solvents:

➢ Ethanol

➢ Dimethylacetamide

- Dimethylformamide

METHODS OF PREPARATION OF NANOSPONGES:

- Nanosponge prepared from hyper-cross linked β cyclodextrin
- Emulsion solvent diffusion method
- Quasi-emulsion solvent diffusion
- Ultrasound-assisted synthesis
- Polymerization

a) Nanosponge prepared from hyper-cross linked β-cyclodextrin

Dimethylformamide (DMF) and anhydrous -cyclodextrin (15.34 mmol) are dispersed in 100 mL of anhydrous dimethylformamide (DMF) in a round bottom flask. A solution containing 61.42 mmol of carbonyl di imidazole is added to 9.96 g of the solution, and it is allowed to react for 4 hours at 100°C. The translucent block of hyper-cross-linked cyclodextrin is roughly crushed after the condensation polymerization, and an excess of deionized water was added to remove DMF. Finally, leftover by-products or unreached reagents can be eliminated using ethanol-based Soxhlet extraction. The white powder is then subjected to drying overnight at 60°C in an oven before being pulverized in a mortar. Water was used to spread the fine powder that had been obtained. The colloidal component of the solution that remained suspended in water was extracted and lyophilized. The nanosponges identified had a spherical form and are sub-micron in size. The molar ratio of cyclodextrin to cross-linker can change (i.e. 1:2, 1:4, and 1:8). The molar ratio of the cross-linker utilized in the manufacturing of nanosponge can be categorized (i.e. Nanosponge, 1:4)[1].

b) Emulsion solvent diffusion method

Two phases can be utilized in this approach, each with a distinct percentage of organic and aqueous components (ethyl cellulose and polyvinyl alcohol). The ethylcellulose and medicine in the dispersed phase are dissolved in dichloromethane (20 ml) and a specific amount of polyvinyl alcohol is added to 150 ml of the aqueous continuous phase. The mixture is then thoroughly agitated for 2 hours at 1000 rpm. The needed Nanosponge is gathered through the filtration process and dried in a 40°C oven for 24 hours. Desiccators are used to preserve dried nanosponges and ensure the removal of residual solvent[9].

c) Quasi-emulsion solvent diffusion

The polymer is used in various proportions to make the Nanosponge. Eudragit RS 100 is used to make the inner phase, which is then mixed with a suitable solvent. The drug was supplied in a suspension and dissolved at 35°C using ultrasonication. When this inner phase is mixed with the PVA-containing external phase, it acts as an emulsifier. At room temperature, the mixture is agitated at 1000-2000 rpm for 3 hours before being dried inside an air-heated oven at 40°C for 12 hours[9].

d) Ultrasound-Assisted Synthesis

By interacting polymers with cross-linkers in the total absence of solvent but under sonication, nanosponges can be made. The nanosponges produced will be spherical, homogenous in size, and less than 5 microns in diameter. The cross-linker in this approach is di-phenyl carbonate (or) pyromellitic anhydride. In a flask, combine the polymer and cross-linker. Heat the flask to 90°C in an ultrasonic bath filled with liquid and sonicate for 5 hours. The solid was then crushed in a mortar, and impurities (or) residual polymer are removed by soxhlet extraction with ethanol. They are then stored at 25°C after purification[10].

e) Polymerization

In the method, a non-polar drug solution is prepared, to which an aqueous phase is added, usually adding surfactant and dispersion to aid suspension. Once the suspension with discrete droplets of the required size has been created, the monomers are activated either by catalysis or by increasing the temperature. The polymerization process results in the construction of a reservoir system with pores that open at the surface[2].

MECHANISM OF DRUG RELEASE FROM NANOSPONGE:

The sponge particles are open, allowing bioactive ingredients to easily flow in and out of the pores and into the vehicle until they find equilibrium. In the case of topical distribution, the bioactive that is already in the vehicle will be incorporated into the target tissue once the finished product is applied to it. The balance is disrupted by diminishing the vehicle, which will become unsaturated. This will cause the activity to flow from the sponge particle into the medium and then to the targeted site until the vehicle is dry or absorbed. Even after that, the sponge particles that remain on the tissue's surface will gradually release the active, providing a long-term release[3].

FACTORS THAT INFLUENCE NANOSPONGE SYNTHESIS:

a) **Polymer and cross-linkers**: The variety of polymers employed in nanosponges can have an impact on their formulation and performance as well as an effective cross-linker converts molecular nanocavities into a three-dimensional nonporous structure.

- Epichlorohydrin is used as a crosslinker to create hydrophilic nanosponges. Even in immediate-release formulations, hydrophilic nanosponges can change the pace of drug release and improve drug absorption across cellular membranes, making them an effective drug carrier.

- As a crosslinker, phenoxy carbonate, pyromellitic anhydride, diisocyanates, and carbonyl diimidazole can be used to make hydrophobic nanosponges. They act as long-acting carriers for water-soluble medicines such as peptides and proteins[2].

b) **Types of drugs and media involved in interactions:** The medicinal molecule employed in the nanosponge composition should have the properties listed below.

- Around 100 and 400 Daltons in molecular weight.
- A drug molecule is made up of fewer than five condensed rings.
- Water solubility is less than 10mg/ml.
- The substance's melting point is less than 250°C[2].

c) **Temperature:** Temperature fluctuations can affect the complexation of drugs with nanosponges. In general, as the temperature increases, the Drug/Nanosponge complex appears to have smaller apparent stability constant. This might be due to reduced van-der Waal and hydrophobic forces between the drug and nanosponge[3].

d) **Method of formulation:** Drug/Nanosponge complexation can be affected by how the drug is loaded into the nanosponge. However, because the success of a method is dependent on the nature of the drug and polymer, freeze-drying has been proven to be the most effective way for drug complexation in many circumstances[3].

e) **Degree of substitution:** The complexity of nanosponges is influenced by the number, type, and location of substituents on the polymeric molecule. The form of substitution is significant because β-CD derivatives come in a variety of forms with different functional

groups on the cyclodextrin derivative's surface. Distinct functional groups produce different types of complexed β -CD nanosponges when they are complexed together with the help of a crosslinker. The number of substitutions presents correlates directly with the degree of crosslinking. The more substituents present, the greater the likelihood of higher crosslinking. Due to additional linkages between polymers forming a mesh-type network, higher degrees of crosslinking will give highly porous nanosponges. The positioning of substitution is determined by the conditions of production. Due to the presence of various physicochemical qualities, a change in the manufacturing method will result in materials with varied physicochemical properties[2].

EVALUATION PARAMETERS OF NANOSPONGES:

a) Particle size and polydispersity index: In the optimization process, Nanosponge particle size is an important criterion. Dynamic light scattering, using a 90 Plus particle sizer with MAS OPTION particle sizing software, laser light diffractometry, or a Malvern Zeta sizer, can be used to estimate particle size[2].

Dynamic light scattering Instruments can also be used to determine the polydispersity index (PDI). The particle size distribution index (PDI) measures the width, spread, or variance of the particle size distribution. A lower PDI value implies a monodisperse sample, whereas a higher PDI value shows a wider particle size range and the polydisperse character of the sample. The following equation can be used to compute PDI:

$$PDI = \Delta d/davg$$

In the particle size datasheet, d is the width of the distribution, denoted as SD, and davg is the average particle size, denoted as MV (nm). The mean diameter and polydispersity index can be calculated using this information[1].

b) Zeta potential: The surface charge is measured by the zeta potential. It can be determined by employing an extra electrode in particle size measurement equipment. Nanosponges containing materials were extracted and dissolved with 0.1mol/l KCl before being placed in an electrophoretic cell with a 15V/cm electric field applied. After averaging the whole measurements, the mean hydrodynamic diameter and polydispersity index was calculated[2].

c) Resiliency: is viscoelastic characteristics of nanosponge can be altered to produce softer or stiffer beadles depending on the final formulation's requirements. The rate of release is

slowed by increased crosslinking. As a result, the resiliency of sponges will be investigated and optimized under the requirements, taking into account release as a function of cross-linking with time[2].

d) Microscopy Studies: Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) can be used to microscopic characteristics of the drug, nanosponges, and the resultant product (drug/nanosponge complex) can be studied using electron microscopy (TEM). The development of inclusion complexes is shown by the difference in crystallization condition of the ingredients and the result as viewed under an electron microscope[3].

e) X-ray diffractometry and single-crystal X-ray structure analysis: Inclusion complexation in the solid-state can be detected using powder X-ray diffractometry.

The diffraction pattern of a newly produced material varies from that of an uncomplexed nanosponge when the drug molecule is liquid, because liquids have no diffraction pattern of their own. When the drug compound is solid, the diffractogram of the presumed complex must be compared to the diffractogram of the mechanical combination of drug and polymer molecules. A physical mixture's diffraction pattern is frequently the sum of each component's, whereas complexes' diffraction pattern appears to be distinct from each constituent, resulting in a "new" solid phase with distinct diffractograms. The chemical decomposition and complex creation of a combination of substances can be determined using diffraction peaks.

The diffraction patterns are altered when a drug combination is formed with nanosponges. As well as altering the crystalline nature of the drug.

The exact inclusion structure and manner of interaction can be determined via single-crystal X-ray structural analysis. The interaction between the host and guest molecules can be determined, as well as their precise geometrical relationship[3].

f) Thermo-analytical methods: Thermo-analytical methods evaluate whether the drug substance changes before the nanosponge is thermally degraded. Melting, evaporation, breakdown, oxidation, or polymorphic transitions are examples of drug substance changes. The presence of a change in the drug substance implies the creation of a complex. Broadening, shifting, and the emergence of new peaks or the elimination of certain peaks can

be detected in the thermogram acquired by DTA and DSC. Changes in weight loss can also be used as evidence for the establishment of inclusion complexes[3].

g) Infra-Red Spectroscopy: The interaction between nanosponges and drug molecules in the solid-state is estimated using infrared spectroscopy.

When the fraction of the guest molecules enclosed in the complex is less than 25%, bands that may be assigned to include the portion of the guest molecules are readily obscured by the bands of the nanosponge spectrum. The methodology isn't always effective at detecting inclusion complexes, and it's less precise than other methods. The use of infrared spectroscopy is limited to medications with certain distinctive spectra, such as aldehyde or sulfonic groups. The presence of hydrogen in different functional groups can be determined via infrared spectral analysis. The stretching vibration of the component involved in the creation of hydrogen bonds often changes the absorbance bands to a particular wavelength, increases the intensity, and expands the band. The biggest shift in the vibrational modes band is caused by the hydrogen bond at the hydroxyl group[3].

h) Determination of loading efficiency and production yield: The un-entrapped drug is subtracted from the total amount of drug to determine the prepared nanosponge loading efficiency. Separating un-entrapped drugs calculated by any suitable method of analysis will be used to measure drug entrapment efficiency. Gel filtration, dialysis, and ultracentrifugation were utilized to separate the unentrapped medication[7].

The loading efficiency is calculated as:

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

The beginning weight of the raw materials and the final weight of the nanosponge obtained can be used to calculate the production yield of the nanosponge.

$$\text{Production yield} = \frac{\text{Practical mass of nanosponge}}{\text{Theoretical mass (drug+polymer)}} \times 100$$

i) Porosity: To determine the extent of nanochannels and nanocavities generated, a porosity analysis is carried out. Because helium gas may penetrate both intra and inter channels of materials, the porosity of nanosponges is measured with a helium pycnometer. The helium displacement method is used to determine the real volume of a substance. Nanosponges have

a higher porosity than the parent polymer utilized to make the system because of their porous nature. The equation gives percent porosity[7].

$$\%\text{Porosity} = \frac{\text{Bulk volume} - \text{True volume}}{\text{Bulk volume}} \times 100$$

j) Permeation studies: For analyzing the dissolution release of nanosponge across a cellophane membrane, diffusion studies of the manufactured nanosponge can be carried out in the Franz diffusion cell. The diffusion studies were carried out at 37°C using 250 ml of phosphate buffer (pH 7.4) as the dissolving medium and a nanosponge sample (0.5g) in a cellophane membrane. At 1, 2, 3, 4, 5, 6, 7, and 8 hours, 5ml of each sample can be withdrawn and replaced with an equivalent volume of new dissolving media. After that, using phosphate buffer as a blank, the samples can be tested for drug content[7].

k) *In-vitro* release studies: The dissolution characteristic of Nanosponge can be investigated using a modified basket made of 5m stainless steel mesh and the dissolution equipment USP xxiii. The rotational speed is 150 rpm. To achieve sink conditions, the dissolution media is chosen while considering the solubility of the actives. Samples from the dissolving media can be evaluated using an appropriate analysis method using a modified basket made of 5m stainless steel mesh and the dissolution equipment USP xxiii. The rotational speed is 150 rpm. To achieve sink conditions, the dissolution media is chosen while considering the solubility of the actives. An appropriate analytical method can be used to examine samples from the dissolution medium[7].

APPLICATION:

1. **Solubility enhancement:** Nanosponges can assist low-water-solubility chemicals to improve both wetting and solubility. Before getting released as molecules, the drugs can be chemically dispersed within the nanosponge structure, which benefits the requirement for dissolution. As a result, the drug's apparent solubility may be increased. Many formulation and bioavailability problems can be solved by enhancing a substance's solubility and dissolving rate, and nanosponges can help greatly[12].

2. **Sustained delivery system:** The design of a modified-release medication is frequently intended to assist in the improvement of the treatment regimen by administering the drug slowly and continuously throughout the dosing interval. This enables a decrease in the frequent dose administered, a change in the pharmacokinetic profile, and a decrease in side

effects. Drug release kinetics from nanosponges can be accomplished with a sustained release profile over time using the right polymers and cross-linking agents[12].

3. **Drug delivery:** Nanosponges are used in oral, topical, ocular and parenteral delivery of dosage forms.

- **Topical delivery:** Topical delivery Nanosponges is a unique system which is used for topical administration of drug. They release the dosage form in a controlled manner for prolonged period. Drugs like local anesthetics, antifungals, and antibiotics can be formulated for topical delivery. A wide variety of bioactive materials can be incorporated into a formulated product such as liquid, powder, lotion, cream, gel[6].

- **Oral drug delivery:** Capsules or tablets can be prepared by dispersing the complexes in a suitable matrix of excipients, lubricants and anticaking agents. The oral delivery of Nanosponge is more effective which results in increased dissolution rate, solubility and stability of drug. The solubility of poorly soluble drugs are increased due to the formation of pores[6].

- **Ocular delivery:** NS based ocular delivery increases the residence time by releasing drug in a controlled manner. The delivery of β cyclodextrin-based Nanosponges resulted in enhanced permeation than conventional Nanosponges[6].

4. **Oxygen delivery system:** Nanosponges can also be used for the treatment of hypoxic tissues. A silicone membrane is utilized with hydrogel system for the better permeation of oxygen[11].

5. **Nanosponges for cancer therapy:** The use of the Nanosponge system for cancer therapy is three times more efficient than direct injection. A targeting peptide binds strongly to the tumour receptor radiation-induced cell top layer. The Nanosponges stick to the surface and release the molecules which provide enhanced therapeutic effect with decreased side effects[12].

6. **Enzyme immobilization by nanosponges:** Boscolo et al., demonstrated that adsorption of pseudomonas fluorescence lipase on cyclodextrin-based nanosponges resulted in enhanced catalytic performance[12].

7. **Nanosponges as carrier for delivery of proteins, vaccines and antibodies:** Cyclodextrin-based nanosponges acts as a carrier for the proteins, vaccines and antibodies

that adsorbs these substances and also maintain their activity, and efficacy and extends their pH and temperature. This system protects the protein from breakdown and improves its stability in-vivo[2].

8. **Biomedical application:** The nanosponge system are used in both hospitals and the medical industry where the storage of oxygen is complicated at times. The formulation of carbonate Nanosponges based on cyclodextrin forms inclusion complexes with some of the gases like carbon dioxide, methyl cyclopropane and oxygen-carrying Nanosponges that is used to provide oxygen during hypoxic conditions[12].

9. **Carrier for calcium delivery:** Pravin Shende et al., developed enteric coated calcium carbonate-based Nanosponges that binds efficiently to free phosphate ions and releases the calcium in a regulated way. This crosslinking resulted in improved stability and helps in controlled of calcium without causing side effects[12].

10. **Sustained delivery system:** Sustained release of Nanosponges can be achieved by using appropriate polymers and a crosslinking agent. The release of volatile compounds like essential oils can be extended by encapsulation technique[12].

Table 1: Nanosponge based marketed formulation[13]

DRUG	NANOSPONGE VECHICLE	INDICATION
Paclitaxel	β-cyclodextrin	Cancer
Tamoxifen	β-cyclodextrin	Breast cancer
Camptothecin	β-cyclodextrin	Cancer
Econazole nitrate	Ethyl cellulose, polyvinyl alcohol	Antifungal
Itraconazole	β-cyclodextrin and copolyvidonum	Antifungal
Antisense	Sodium alginate	Cancer
Resveratrol	β-cyclodextrin	Inflammation, cardiovascular diseases, dermatitis, gonorrhea

CONCLUSION:

Nanosponges are developed for topical delivery of drug in a controlled and predictable manner. They can encapsulate either lipophilic or hydrophilic drugs. NS provides enhanced solubility of poorly soluble drug as well as increases bioavailability. These are nano-sized

colloidal particles that can easily penetrate through skin. NS offers entrapment of drugs and provides advantages like increased stability, elegance, formulation flexibility and reduction in side effects. Modulate Nanosponges can be developed as different dosage forms like topical, parenteral, oral, aerosol, tablet and capsule. The applications of Nanosponges are potential in the field of biomedicine, cosmetics, agrochemistry, solubility enhancement and catalysis.

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