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NANOSPONGES: A NOVEL COLLOIDAL CARRIER FOR TARGET DRUG DELIVERY

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ABSTRACT
Targeted delivery of drugs and enhancement of bioavailability of poorly water soluble drugs are the two main challenges encountered by the researchers for the design of safe and effective dosage form. Nanotechnology has gained significance in the design of controlled drug delivery with minimum side effects and achieves maximum therapeutic action at low doses. Nanosponges are novel colloidal carrier particles with a 3-dimensional network and a nanometric cavity size that can entangle drugs and then be formulated into a suitable dosage form based up on the route of administration. After administration of nanosponges, they circulate along with blood around the body until they come across the specific target site and bound to the surface and liberate the drug in a controlled and predictable manner. Both lipophilic and hydrophilic drugs can be encapsulated in nanoporous cavities of nanosponges. The advantages of nanosponges includes they avoid degradation of drugs and proteins; provide sustained drug release at the target site, increase aqueous solubility of low water-soluble drugs. Particle size of nanosponges depends upon proportion of the amount of crosslinker to the polymer. They are prepared by different methods such as solvent diffusion method, emulsion solvent diffusion method, ultrasound-assisted method and quasi emulsion solvent diffusion method. They possess the ability of delivery of active ingredients into oral, topical, parenteral, and nasal routes make them a superior candidate for targeted and local action. It can be used as a carrier for biocatalysts and in delivery of enzymes, proteins, vaccines and antibodies.

KEYWORDS: Nanosponges, Crosslinkers, β- Cyclodextrin and Emulsion diffusion method.

INTRODUCTION
Delivery of targeted drugs to the human body is a major problem for scientists for a long period due to difficulties arise in the design of dosage form that should deliver the drugs at the target site in the body and provide controlled release of the drug to prevent overdoses.[1] Novel drug delivery systems are designed to achieve a continuous delivery of drugs at a predictable and reproducible kinetics over an extended period in the circulation, in order to improve its performance in terms of patience compliance, safety and efficacy. The major advantages include minimization of drug related side effects due to controlled therapeutics blood levels instead of fluctuating blood levels, improved patience compliance due to reduced frequency of dosing and the reduction of the total dose of drug administered.[2,3]

Nanotechnology is defined as design and fabrication of substances at nanoscale level to create dosage form that shows novel properties. Nanoparticles are small discrete particles with a size range of 1-1000 nm having a surrounding interfacial layer. Nanoparticles are available in different forms like polymeric nanoparticles, niosomes, nanocochleates, solid-lipid nanoparticles, nanosponges, carbon nanotubes, nanocrystals, nanofibres, micellar systems, dendrimers and nanoemulsions etc.[2,3,4]

Nanosponge is one of the novel and emerging nanotechnology which plays a vital role in targeting drug delivery in a controlled release, in which the system uses a nanoparticles-sized system to deliver the drug payload.[5,6] Nanosponges are novel colloidal structures derived from a new category of hypercrosslinked polymers, and comprising of solid colloidal nanoparticles with nanosized cavities. These are discrete solid three-dimensional porous polymeric network or scaffold small spherical particles with large porous surface with a narrow cavity of few nanometers in which a large variety of hydrophilic and lipophilic drug substances can be capsulated or suspended, and then be incorporated into a dosage form.[6,7] The size of nanosponge is below 1 µm (250 nm-1µm) but fractions below500 nm is acceptable. Nanosponges are better than microsponges due to its smaller size, and reduce the side effects and protect drug from degradation because the diameter of the microsponge is 10-25µm, with the void size around 5-300µm^2. They are composed of biodegradable hyper-cross-linked polymers with superior drug absorption/complexation properties. The long length

polymer strands in solution form are mixed with small molecules called "cross-linkers", which acts like a small seizing lock to hold different parts of the polymer together, thereby forming roughly spherical particles with cavities to store the drug molecules.[3] These particles are injected directly into the body by using water as a transport fluid, this limits the prevalence of side effects associated with other nanoparticulate drug delivery system where a chemical transporter is used. After entering into the body, the polyester breaks down gradually in the body, owing to its biodegradable nature, and as a result, it releases the drug molecule in a predictable fashion. They resemble red blood cells, and has the ability to circulate around the body until they encounter the specific target site and stick on the surface and began to release the drug at a predetermined rate. These particles are capable of carrying both lipophillic and hydrophilic substances by forming inclusion and non-inclusion complexes with them and improve the solubility of poorly water soluble molecules.[2,3] Their molecular architecture generally contains different polymer chains which can form specific microdomains suitable for the co-encapsulation of two drugs with a different chemical structure. They can be used to mask unpleasant flavors, to convert liquid substances to solids. They can be designed in a particular size by varying the ratio of polymer to cross-linker or they can be magnetized and release drugs over a predetermined time.[1,2]

Nanosponges was originally developed as a tiny mesh-like nanoporous colloidal carriers for topical delivery of poorly water soluble drugs and provide prolonged release as well as improving drugs bioavailability.[3] They are used for the passive targeting of cosmetic agents or drugs to skin, there by achieving major benefits such as bypass first pass metabolism, reduction of total dose, avoid varied conditions of absorption, like pH changes, the presence of enzymes, gastric emptying time retention of dosage form on the skin, avoidance of risks and inconveniences of intravenous therapy and improving patient compliance by prolonging dosing intervals.[3] They exhibit high capacity of entrapping wide ranges of substances such as emollients, fragrances, essential oils, sunscreens, used as a topical carrier system. But in the 21st century, they can be administered by oral as well as intravenous (IV) route. The major applications of this delivery system being the oral, topical and parenteral delivery of various drugs, proteins, and peptides.[2,3]

Fig. 1: a) Structure of nanosponges. b) Nanosponges as novel drug delivery.

Characteristics of nanosponges

1. They have a range of dimensions (1 µm or less).
2. They exist in paracrystalline form or crystal form, which depends on the process conditions of the nanosponges, which plays a vital role in their complexation with drugs. Para-crystalline nanosponges exhibit more drug loading capacity.[11,12]
3. They are nonmutagenic, nonallergenic, nonirritant, and nontoxic porous particles.
4. They are soluble in water and encapsulation of drug molecules in nanosponges allows the use of hydrophobic drugs, which are difficult to dissolve readily in water.
5. They form clear and opalescent suspension, when dissolved in water.
6. They are insoluble in most organic solvents and remain stable at high temperatures upto 130 °C.
7. Stable in pH range of 1 - 11.
8. They are able to capture, transport and selectively release of a vast variety of substances, all thanks to their 3D structure.
9. By varying the proportion of polymer to cross-linker, it is possible to control the size of nanosponge particles.
10. These are self sterilizing as their average pore size is 0.25 µm where bacteria cannot penetrate.[5]

Advantages of nanosponges

1. Enhance the aqueous solubility of the poorly water-soluble drugs.
2. Continuous and sustained action up to 24 hrs is possible with less irritation.
3. Incorporation of immiscible liquids and gases is possible while developing these systems.[4]
4. They help to remove the toxic and venom substances from the body.
5. Minimize side effects and adverse effects.
6. Increase formulation stability and enhance the flexibility of the formulation.

7. Better patient compliance by reducing dosing frequency.[5,7,8]
8. Easy scale up for commerical production.
9. Higher degree of material processing owing to the property of converting liquids into powders.
10. Better stability, enhanced flexibility, and higher elegancy.
11. These formulations are compatible with most vehicles and ingredients.[8]
12. These formulations are free flowing and can be cost effective.
13. Precise control of the drug release kinetics of the incorporated drugs by the fine tuning of the nanosponge matrix.
14. They could be easily regenerated via solvent extraction and thermal desorption by ultrasound, washing with eco-compatible solvents, stripping with moderately inert hot gasses, mild heating, or changing pH or ionic strength and microwaves.[5,8]
15. Targeted delivery of encapsulated substances can be achieved, due to the ability of nanosponges to link with different functional groups, which can be further improved through chemical linkers primarily binding to the target sites.
16. By loading magnetic properties in a system of nanosponges through addition of ferrite and other magnetic agents during synthesis, an external magnetic field can also be applied for targeted release.[9]

Disadvantages of nanosponges

1. Nanosponges have the capacity of encapsulating small molecules, not suitable for larger molecules.
2. Dose dumping maybe possible.[5,7,8]

Materials used in nanosponge preparation

1. **Polymers:** The type and nature of polymer determines the type of nanosponges to be fabricated and its pore size. The cavity size of nanosponge should be suitable to accommodate a dug molecule of particular size for complexation. The ability of the polymer to be cross linked depends on the functional groups and active groups to be substituted. The selection of polymer depends on the required release and the drug to be enclosed.[10,11] β-CD and its derivatives has been most commonly employed in the preparation of nanosponges due to their high complexation and encapsulation capacity.[4] Water-soluble or insoluble nanosponges structures can be obtained, depending upon the nature of different cross-linkers used, which may dramatically modify vital characteristics such as swelling ability and hydrophilicity/hydrophobicity of the polymer. Molar ratio of crosslinker and polymer also plays an important role in the formulation of nanosponges.[3,4]

Eg.: Hyper cross linked polystyrenes, Cyclodextrines and its derivatives like Alkyloxycarbonyl cyclodextrins, Methyl β- Cyclodextrin, 2-Hydroxy propyl β-cyclodextrins, Ethyl cellulose, Poly valerolactone, Eudrgit RS 100, Acrylic polymers.

2. **Copolymers:** Poly (valerolactone allylvalerolactone), Poly (valerolactone-allylvalerolactone oxepanedione), and Poly vinyl alcohol.
3. **Cross linkers:** Carbonyl diimidazoles, Diarylcarbonates, Epichloridin, Gluteraldehyde, Pyromellitic anhydride, 2,2-bis (acrylamido) acetic acid, Carboxylic acid dianhydrides, Diisocyanates, Di chloromethane, and Diphenyl carbonate.[4,7]

Classification of nanosponges

Nanosponges can be classified according to type of polymer and crosslinker used in the preparation.

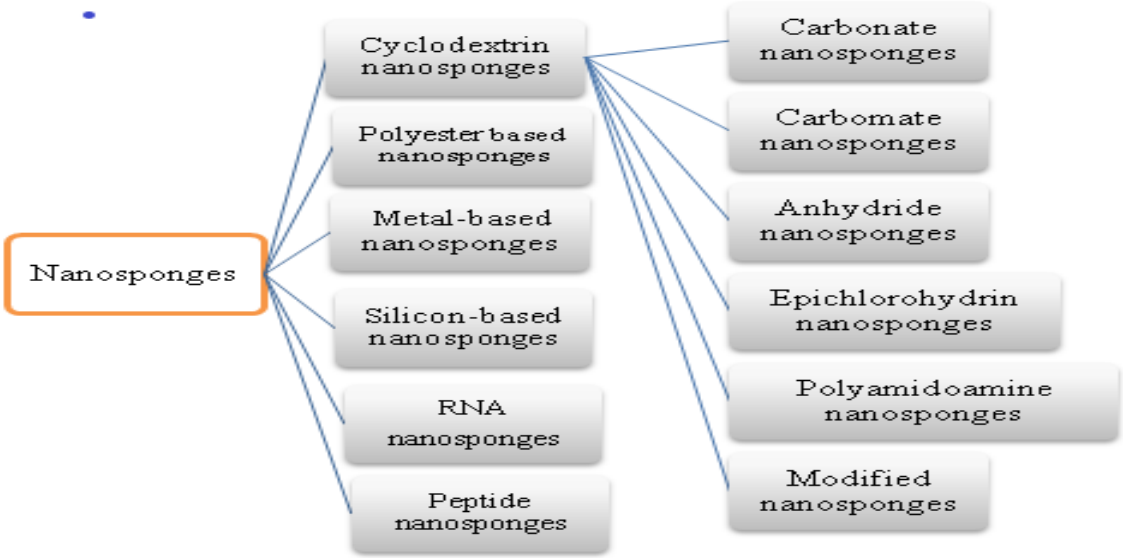

Fig. 2: Types of nanosponges.[6]

A. Cyclodextrin based nanosponges (CDNS)

In 1998, DeQuan Li and Min Ma were identified CDNS to indicate a cross linked β-CD with organic diisocyanates leading to the formation of an insoluble network which indicate high inclusion constant with drugs. Cyclodextrin (CD) is a polysaccharides obtained by enzymatic degradation of starch by bacillus amylobacter bacteria and composed of the binding of D-glycosupranoses with α-1,4-glycosidic bonds. These are water soluble, biocompatible and cyclic oligosaccharides with outer hydrophilic surface and lipophilic inner surface. These are shaped into a truncated cone or funnel with two open ends due to the sequence of glycopyranose units, with primary hydroxyl groups at one end and secondary hydroxyl groups at the other end. They are named α-CD, β-CD and γ-CD according to the number of glycosupranose units in their structure i.e, 6, 7, and 8 respectively.[11,12]

Natural CDs cannot form complexes with hydrophilic or high molecular weight drugs, and may exhibit toxic properties when injected intravenously. Chemical modification of natural CDs with crosslinkers such as carbonyl-diimidazole, diphenyl carbonate, hexamethylene diisocyanate and pyromellitic anhydride, form hyper-crosslinked cyclodextrins with nanoscale spongy pores and a three-dimensional network.[13]

β-CD has the highest complexity and stability due to the appropriate cavity size with crosslinkable polymers when compared to other two types of cyclodextrins and form more sites of interaction with drugs and obtain higher drug encapsulation complexes. Nanosponges are being explored as a promising nanocarrier system to improve drug solubility, prevent drug degradation, increase permeability, and control drug release.[14,15]

A. Cyclodextrin polymer with different cross-linkers: According to the type of cross- linking polymer materials used, they are classified into 6 types,

1. **Carbonate nanosponges:** These are prepared by solvent extraction, thermal desorption or microwave and ultrasound assisted synthesis methods using carbonyl crosslinkers such as diphenyl carbonate, carbonyl imidazole, dimethyl carbonate with CDs.[15] According to the method of preparation, they may be amorphous or crystalline in nature. Eg.: By using the melting method, a crystalline structure and by solvent method an amorphous nanosponge may be obtained. They are nonhygroscopic in nature and have the ability to retain crystal structure during absorption and desorption of moisture.[16] Examples of drugs incorporated are Paclitaxel, Tamoxifen, Reservertol, Telmisartan, Curcumin, Itraconazole, Camptothecin, Erlotinib, and Quercetin.[21,22,23]

2. **Carbomate nanosponges:** These are prepared by reacting cyclodextrins with cross-linkers such as hexamethylene diisocyanate and toluene- 2, 4-diisocyanate in a molar ratio of 1: 2 to 1: 8 in the presence of dimethylformamide solution at 70°C for 16 - 24 hrs by solvent method under an anhydrous / nitrogen atmosphere. Residual solvent is removed by thorough washing with acetone. These are used for the encapsulation of drugs such as steroids, dyes and dextromethorphan. The loading capacity for organic molecules ranges from 20-40 mg /cm^3.

3. **Anhydride nanosponges:** They can be prepared by reacting cyclodextrins with cross-linkers such as pyromellitic dianhydride ethylene diamine tetra acetic acid dianhydride in the ratio of 1: 2 to 1: 8 by solvent method in the presence of a base such as pyridine or triethylamine to accelerate the polymerization at room temperature. They can host both apolar organic molecules and cations simultaneously since it contains a polar free carboxylic acid group. Eg.: Doxorubicin, meloxicam, ibuprofen and acetylsalicylic acid.[15,16]

4. **Epichlorohydrin cyclodextrin nanosponges:** These are prepared by dissolving cyclodextrins in a basic medium such as sodium hydroxide using cross-linking agents such as epichlorohydrin in 1:10 ratio. They exhibit high chemical resistance, more hydrophilic in nature and adjustable swelling capability has been used to encapsulate drugs such as creatinine and captopril, enalapril, silazapril.[12,14]

5. **Polyamidoamine nanosponges:** These are prepared by polymerizing β- CD with acetic acid 2,20-bis(acrylamide) at room temperature for 94 hrs. It forms a translucent gel instantly on contact with water. Time-dependent swelling studies in bio relevant media confirmed the stability of the gel for up to 72 hrs.[12,13]

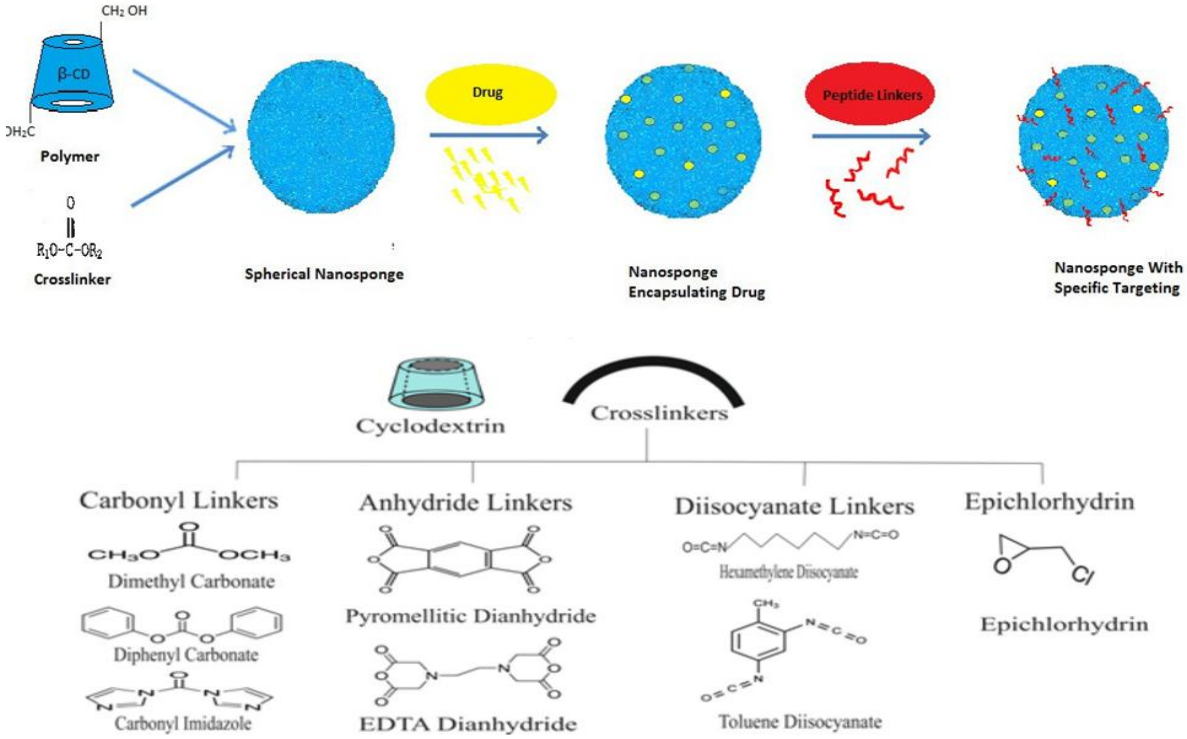

Fig. 3: Preparation of CDNS with different crosslinkers.

6. **Modified nanosponges:** Classical carbonate based nanosponges have been modulated with fluorescein isothiocyanate in dimethyl sulfoxide at 90°C for a few hrs. 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.[14,16]

B. Polyester based nanosponges: Intermolecular crosslinking reaction occurs between polymethacrylic acid (PMAA) and poly(N-isopropyl acrylamide) (PNIPAAM) and the formed nanosponges used for separation of peptides using gold substrates with a "grafting from" AIBN-type free-radical initiator. The developed 3D Nanosponges was significantly adsorbed more peptides than other 2D substrate and shown pH triggered the release of adsorbed peptide due to the squeezing effect.[12,14]

C. Metal-based nanosponges: Porous metals with sponge-like nanostructures are utilized for their specific properties such as increased surface area, low density, high gas permeability and thermal conductivity which had shown the immense application in catalysis, fuel cells, membranes, sensors, electrodes, and actuators. Firstly synthesize titanium coated nanoparticles and calcinate the dried particles, then core-shell particles turned into nanosponges.[12,13]

D. Silicon-based nanosponges: Silicon nanosponges are prepared from a metallurgical grade silicon and used for applications in drug delivery, tissue engineering, cell-based and molecular biosensing.[11,19]

E. RNA nanosponges: They are composed of double-stranded RNA (dsRNA) and exhibit several sites to be cleaved by cytoplasmic dicer and degrade the mRNA. This method requires two complementary circular DNA. One of them is designed to target mRNA which then forms dsRNA and by the Dicer activity mRNA digested to siRNA called library siRNA and then these could be employed to develop RNA nanosponges. Such type of RNA nanosponges helps to manage gene expression.[7]

F. Peptide nanosponges: They can entrap almost any macromolecule (therapeutic) either by chemical bond or by linking with lipophilic or lipophobic nanosponges. These peptide Nanosponges form clear dispersion and are very stable at high temperatures (300°C) and wide pH range, unlike other nanosponges.[10]

Formulation of nanosponges[6,8]

The prepared nanosponges are solid in nature and formulated as oral, parenteral, topical, nasal or inhalation dosage forms. For the oral administration, they can be formulated as capsules, tablets, granules, pellets, suspensions and solid dispersions by mixing or dispersing drug complexes with suitable excipients such as diluents, lubricants, glidants and anticaking agents.

For parenteral administration, the complex dispersed in sterile water or saline or other aqueous solutions. For topical administration they can be effectively incorporated into topical hydrogel with the help of gelling agents.

Factors influence nanosponge formation

A. Type of drug molecules[5,9]

Drug molecules should possess precise characteristics to become effectively entrapped in nanocavities.

1. Molecular weight of drug should be in between 100 - 400 Daltons.
2. Drug molecule consists of < 5 condensed rings.
3. Solubility in water should be < 10 mg/ml.
4. Melting point of the substance should be < 250 °C. Compounds with higher melting points do not hold higher stability constant values after loading in the nanosponges and lower loading of drug can be observed, which can be ascribed to the structural rigidity of the compound.[10,11]

B. Nature of polymer and cross-linkers

Polymer: The type of polymer used can influence the formulation as well as performance of nanosponges. The size of the cavity of a nanosponge should be large in order to entrap a drug molecules for complexation. Three dimensional nanoporous structure of nanosponges can be obtained by the use of efficient cross-linker.[12,14]

1. **Hydrophilic nanosponges:** They are formed by using epichlorohydrin as cross linker. They modify the rate of drug release and enhance drug absorption across biological barriers, serves as a potent drug for immediate release formulation.
2. **Hydrophobic nanosponges:** They can be synthesized by using diphenyl carbonate, pyromellitic anhydride, diisocynates, and carbonyldiimidazole as crosslinker. They serve as sustained release carriers for water soluble drugs including peptide and protein drugs.

C. Complexation temperature: The stability constant of a complex is inversely correlated to temperature changes. At increased temperature, apparent stability constant decreases due to reduction in drug/nanosponge interaction forces such as vanderwaal forces and hydrophobic forces. Hence, temperature should be maintained when nanosponges are prepared.[16,17]

D. Method of preparation: The method of loading the drug into the nanosponge can affect drug/nanosponge complexation. Effectiveness of a method depends on the nature of the drug and polymer. Freeze drying is the most effective method for drug complexation.

E. Degree of substitution: The number, type and position of the substituent on the polymeric molecule affect the complexation ability of nanosponges. Higher number of substituents increases degree of cross-linking, which yields highly porous mesh-type network nanosponges. The position of substitution depends on the production conditions i.e, change in the production parameters will yield materials with different physicochemical properties.[8,10]

Methods of preparation of nanosponges

1. Solvent method: Nanosponges are prepared by mixing polar aprotic solvents like dimethyl sulfoxide (DMSO), dimethylformamide (DMF) with the polymer. A crosslinker is then added to this mixture in the ratio of 1:4 and the reaction is refluxed at temperature 10˚C for a period ranging from 1 to 48 hrs. Once the reaction has completed, the solution is cooled down to room temperature and it is added to bi-distilled water and filter the product. Then purify by prolonged soxhlet extraction with ethanol and dry the product. Then the sponges grind in mechanical mill to get homogenous powder.[24]

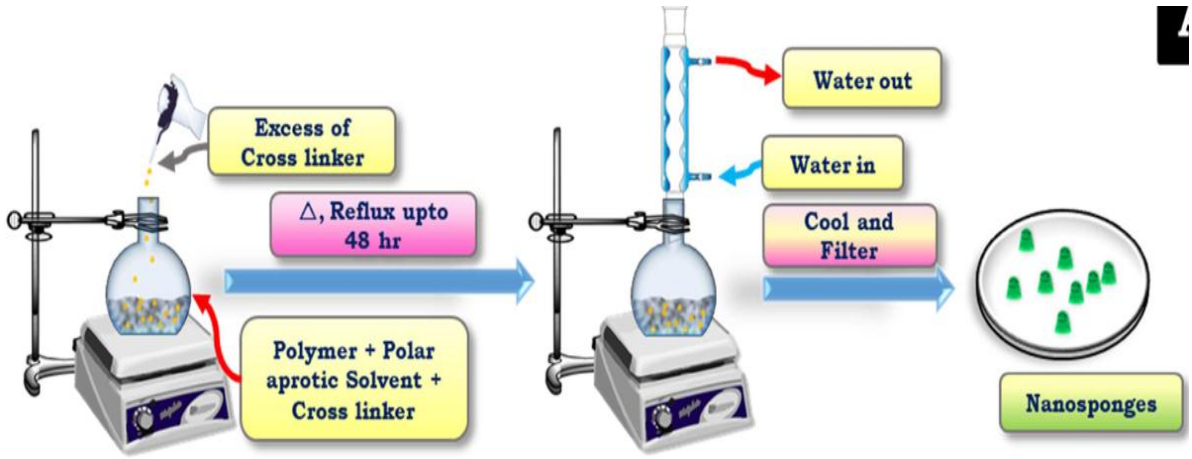

Fig. 4: Preparation of nanosponges by solvent method.

2. Emulsion solvent diffusion method: The dispersed phase containing ethyl cellulose and drug dissolve in dichloromethane and slowly added to aqueous continuous phase containing definite amount of

polyvinyl alcohol (emulsifier). The reaction mixture stirred at 1000 - 2000 rpm for 2 hrs at room temperature. Filter the nanosponges, then dried in an oven at 40 °C for 24 hrs and stored in vacuum desiccators to ensure the removal of residual solvent.[22,24]

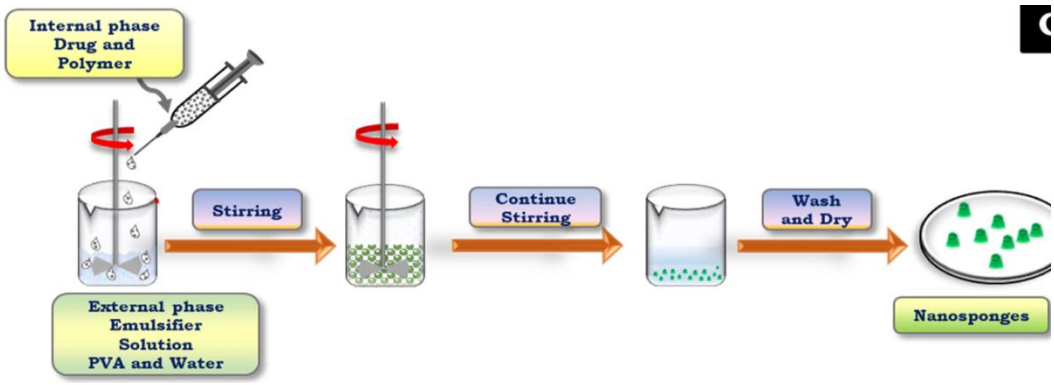

Fig. 5: Preparation of nanosponges by emulsion solvent diffusion method.

3. Quasi-emulsion solvent diffusion: The dispersed phase containing polymer (eudragit RS100) dissolves in a suitable solvent. Drug is added to polymer solution and sonicate under ultrasonication at 35°C. The dispersed phase is poured into the aqueous phase containing PVA and allowed for stirring for 1 hr. Then the mixture is filtered and dried in an oven at 40 °C for 12 hrs.[10]

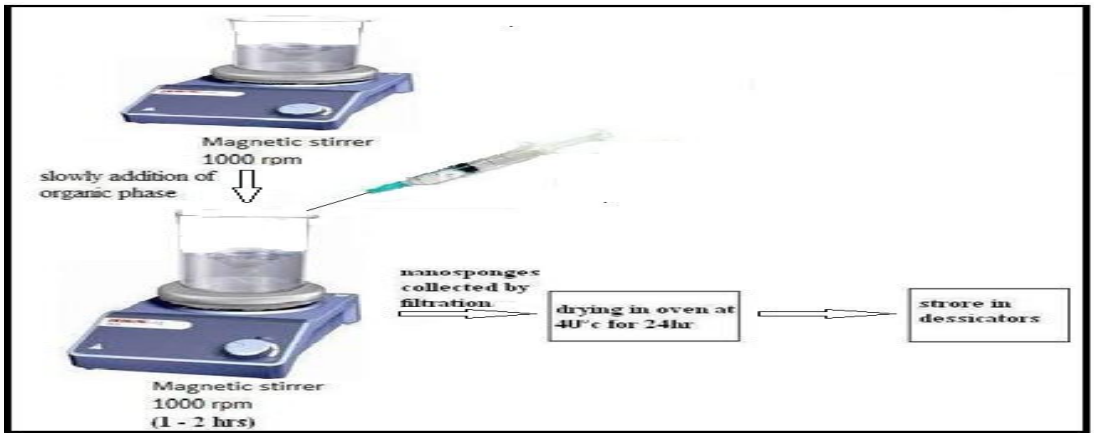

Fig. 6: Preparation of nanosponges by quasi-emulsion solvent diffusion.

4. Ultrasound-assisted synthesis: In this method, polymers react with cross- linkers in absence of solvent and place the flask in an ultrasound bath filled with water. Heat the mixture at 90 °C and sonicate for 5 hrs. Wash the mixture with water to remove the unreacted polymer and purify with ethanol by prolonged soxhlet extraction. Dry the product under vacuum and store at 25 °C.[8]

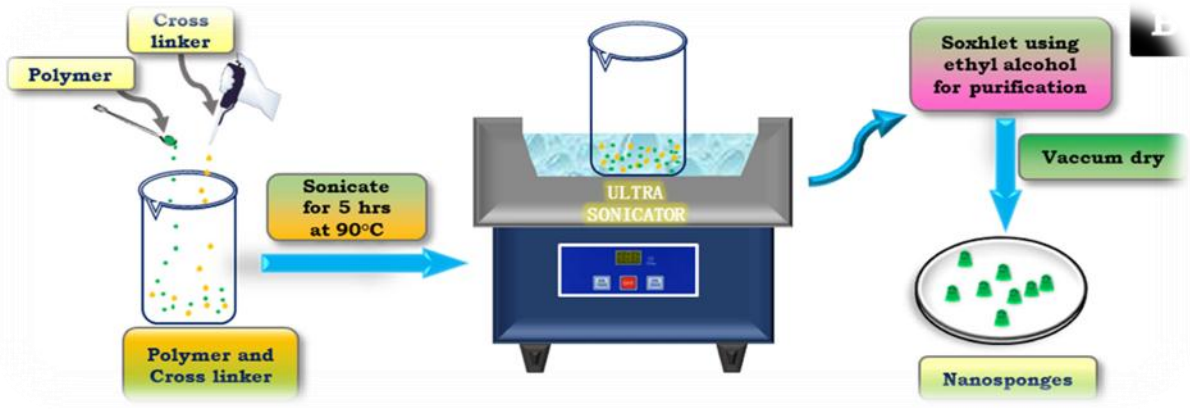

Fig. 7: Preparation of nanosponges by ultrasound-assisted synthesis.

5. Polymerization: A solution of non polar drug is made in the monomer, to which aqueous phase, usually containing surfactant and dispersant to promote suspension is added. By catalysis or increased temperature monomers activated and form suspension with the discrete droplets of the desired size. It leads to formation of a reservoir, which opens at the surface through pores.[6,8]

6. From hyper cross- linked β-cyclodextrin: Nanosponges can be obtained by reacting β-CD with a cross linker such as diisocianates, diary carbonates etc in neutral or acid forms. Sponges' size is controlled according to porosity, surface charge density for the attachment to poorly-water soluble drugs. They produce solid particles and converted in crystalline form.

7. Melting method: Nanosponges are prepared by reacting CD with a crosslinker in a 250 ml flask and heated at 100 ˚C for about 5 hrs by using magnetic stirrer. The mixture is cooled and washes the product with a suitable solvent to remove unreacted excipients and by products6.[12,15]

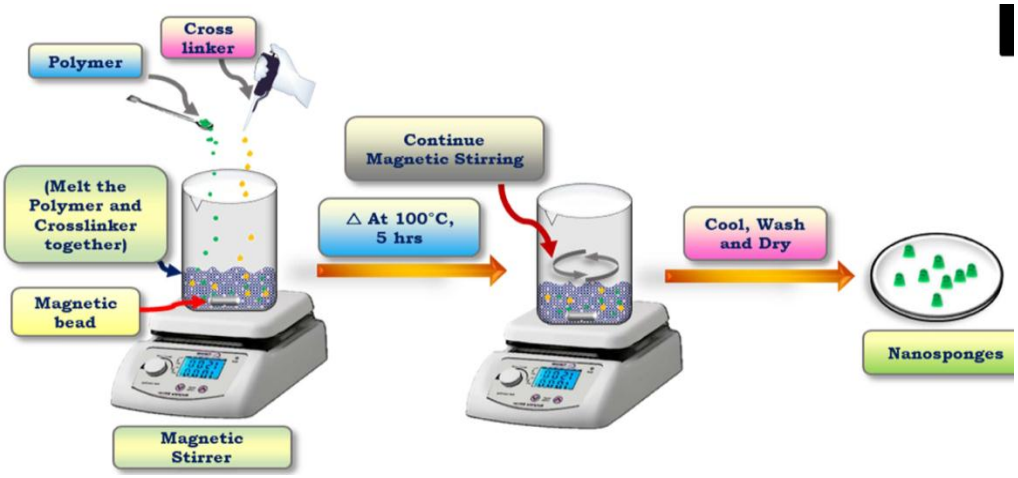

Fig. 8: Preparation of nanosponges by melting method.

Loading of drug into nanosponges[6,7,8]

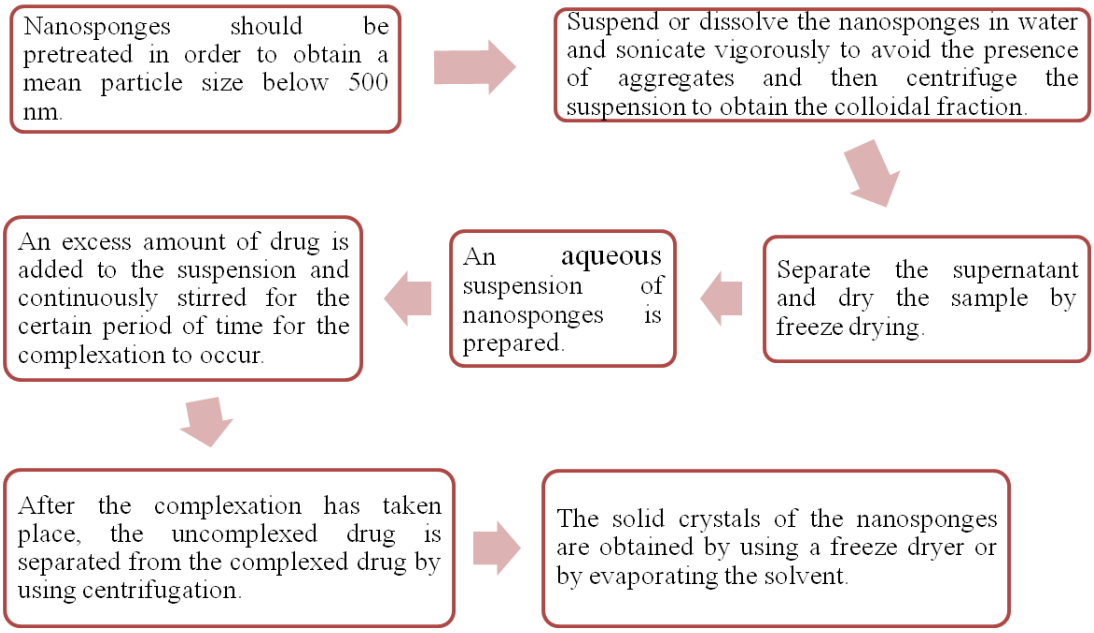

Fig. 9: Loading of drug into nanosponges.

Mechanism of drug release from nanosponges

Nanosponges have an open structure without any surrounding membrane; the active substance is added to the vehicle in an encapsulated form. They move freely from the particles into the vehicle until equilibrium is obtaied.[8]

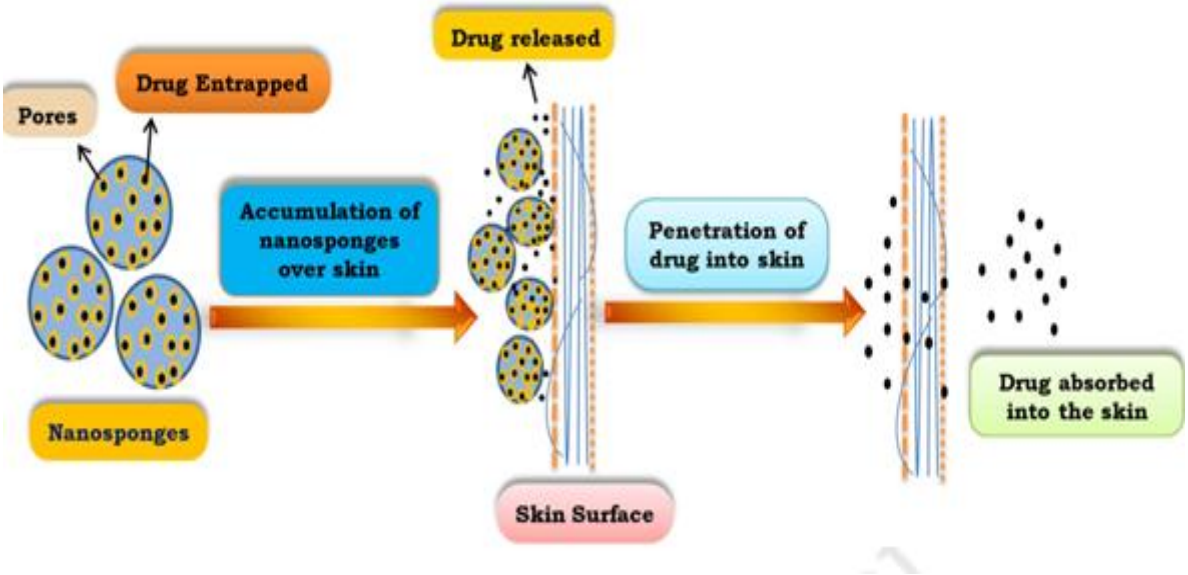

Fig. 10: Mechanism of drug release through nanosponges.

Approaches of Qbd in nanosponges formulation

Table 1: Qbd in formulation of nanosponges.[20]

Independent variables	Dependent variables
Amount of drug	Particle size
Amount of polymer	% cumulative drug release
Amount of plasticizers	Permeation rate
Amount of Surfactant	Zeta potential
Stirring time	Viscosity
Rotation speed	Drug content
Effect of temperature	Stability
Nature of substitution	% drug entrapment efficiency
Nature of solvent	Moisture content

Characterization of nanosponges

Several studies have revealed that nanosponges are highly branched, porous nanostructured which forms 3D meshwork compounds, available in both crystalline and paracrystalline form, all these properties need to be studied properly, therefore different characterization parameters are studied after its formulation.[15,16]

1. **Solubility studies:** Phase solubility technique described by higuchi and connors indicate the degree of complexation and determine the factors affecting drug the solubility and bioavailability of the drug. In the solubility studies changes in solubility of the drug is plotted against the concentration of cyclodextrin, and observe complex formation in solution when the solubility of a drug increases with increasing cyclodextrin concentration.[17]

2. **Microscopic studies:** Microscopic studies of nanosponges can be studied by using scanning electron microscope (SEM) and transmission electron microscope (TEM). Formation of inclusion complex is indicated by the difference in the crystallization state and the product seen under an electron microscope.[17]

3. **Zeta potential determination:** Zeta potential is the major key indicator for the stability of the nanosponge measured by adding extra electrode on particle size equipment or zeta seizer. It is the difference of potential between two layers (dispersion medium and immobile layer) of fluid locked up with dispersed particles. Ideal range for high degree of stability is ± 25 mV[129].

4. **Thermodynamical method:** Thermal degradation of nanosponges by melting, evaporation, oxidation and decomposition and polymeric changes can be determined by the thermo-chemical method. The changes in the drug molecules indicate the formation of a good complex.[18]

5. **Particle size analysis and polydispersity index determination**

Particle size is determined by laser light diffractometry, dynamic light scattering (DLS) using 90Plus particle size determining software or zeta seizer. To study the effect of particle size on drug release, a graph is plotted against the cumulative percentage drug release from nanosponges of different particle size versus time. Particle size range from 10- 30µm can be preferred for topical drug delivery.

Polydispersity index (PDI) can be measured from dynamic light scattering instruments. It is an index of width or variation with particle size distributes. Monodisperse samples have a lower PDI value, whereas polydisperse have higher value of PDI. PDI can be calculated by the following equation,

PDI = Δd/davg

where, **Δd** is the width of distribution, and **davg** is the average particle size **(nm).**[7,22,23]

Table 2: Range of polydispersity index.

PDI	Type of dispersion
0-0.05	Monodisperse standard
0.05-0.08	Nearly monodisperse
0.08-0.7	Mid-range polydispersity
> 0.7	Very polydisperse

6. **Determination of production yield of nanosponges:** It is determined by calculating ratio the initial weight of the raw materials and the final weight of the nanosponges obtained. Normal values of production yield of nanosponges are in the range of 85%-98%.[20]

Production yield = practical mass of nanosponges ×100 / Theoretical mass

7. **%Drug entrapment efficiency:** It is determined by centrifugation method, in which drug loaded nanosponges taken in centrifuge tube and centrifuged in cooling centrifuge tube at 1300 rpm for 20 min. After centrifugation, the supernatant layer was removed and dilute with appropriate solvent, and calculate % Drug entrapment efficiency by using the formula,

$$\% \text{ drug Entrapment Eficiency} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

8. **Loading efficiency:** It is determined by subtracting the un-entrapped drug from the total amount of drug. The drug entrapment efficiency will be determined by separating un-entrapped drug estimated by any suitable UV spectrophotometer or high-performance liquid chromatography method. The method used for separation of un-entrapped drug by gel filtration, dialysis and ultra centrifugation.[12,14]

Loading efficiency = Actual drug content in nanosponge / Theoretical drug × 100.

9. **Porosity:** It is performed to check the extent of nanochannels and nanocavities formed by using helium pycnometer. Due to their porous nature, they exhibit higher porosity compared to the parent polymer.

% Porosity = Bulk volume - True volume / Bulk volume × 100

10. **Swelling and water uptake:** For swellable polymers like polyamidoamine, water uptake can be determined by soaking the prepared nanosponges in aqueous solvent.[19,24]

% Swelling = Marking of cylinder at a specified time point / Initial marking before soaking × 100.

% Water uptake = Mass of hydrogel after 72 hrs / Initial mass of dry polymer × 100.

11. **Compatibility Studies:** The drug should be compatible with the polymers which are used for the preparation of nanosponges. The compatibility of drug with adjuvants can be determined by Thin Layer Chromatography (TLC) and Fourier Transform Infra-red Spectroscopy (FT-IR). Crystalline characteristics can be studied by powder X-ray diffraction (XRD) and Differential Scanning Colorimetry (DSC).[20]

12. **Resiliency (Viscoelastic properties):** Resiliency of sponges can be modified to produce beadlets that is softer or firmer according to the needs of the final formulation. Increased crosslinking tends to slow down the rate of release. Hence resiliency of sponges will be studied and optimized as per the requirement by considering the release as a function of cross- linking with time.

13. ***In-vitro* drug permeation studies:** The diffusion studies of the prepared nanosponges can be carryied out in Franz diffusion cell using semipermeable membrane. Nanosponge sample can take on membrane and the diffusion studies carried out at 37 ± 1°C using dissolution medium. Samples can be withdrawn periodically and analyzed spectrophotometrically.[23]

14. **Drug release kinetics:** The mechanism of drug release from the nanosponges can be analysed by using Zero order, First order, Higuchi, Korsemeyer-Peppas, Hixon Crowell, Kopcha and Makoid-Banakar models, using graph pad prism software.

Applications of nanosponges

Nanosponges have a wide range of applications due to its biocompatibility and versatility. β–CD nanosponges deliver the drug to the target site 3-5 times more effective than direct injection.[4]

1. **Solubility enhancement:** Nanosponges can improve the wetting and intrinsic solubility of poorly water soluble drugs by molecularly dispersed within the cavities and then released as molecules, avoiding the dissolution step. Eg.: β-Cyclodextrin-based nanosponges of itraconazole, simvastatin, and rilpivirine etc.[6]

2. **Topical drug delivery:** Local anaesthetics, antifungal and antibiotics formulated as gel, lotion, cream, ointment, liquid, or powder nanosponges provide controlled release and retention of drug form on skin, reduces the occurrence of rashes or

more serious side effects. Eg., Econazole nitrate nanosponge hydrogel fabricated by emulsion solvent diffusion method diffused more and provide therapeutic action and sustained drug release at low doses when compared to cream, ointment, lotion and solution.[2,24]

3. **Nanosponges as a carrier for biocatalysts and in the delivery of enzymes, proteins, vaccines and antibodies:** The major disadvantage in formulation of proteins is to maintain its structure during processing and long term storage. Nanosponges loaded proteins encapsulated in swellable cyclodextrin-based poly (amidoamine) increase the stability of proteins.[18]

4. **Nanosponges in anti-viral therapy:** Delivery of antiviral drugs or small interfering RNA (siRNA) to the nasal epithelia and lungs can be targeted by nanocarriers such as nanosponges used for the treatment of human immunodeficiency virus (HIV), Hepatitis-B virus (HBV) and herpes simplex virus (HSV). They can deliver the drugs through ocular, nasal and pulmonary administration routes. Examples of drugs delivery includes zidovudine, saquinavir, interferon-α, acyclovir, nelfinavir, etc.[12,14]

5. **Nanosponges in chemotherapy:** Anticancer drugs formulated as nanosponges increases bioavailability, activity and minimizes side effects and exhibit three to five times more effective at reducing tumor growth than direct injection of the drugs. The tiny drug loaded nanosponges encounter tumor cells they attach to the surface and are triggered to release their drug.[17,19]

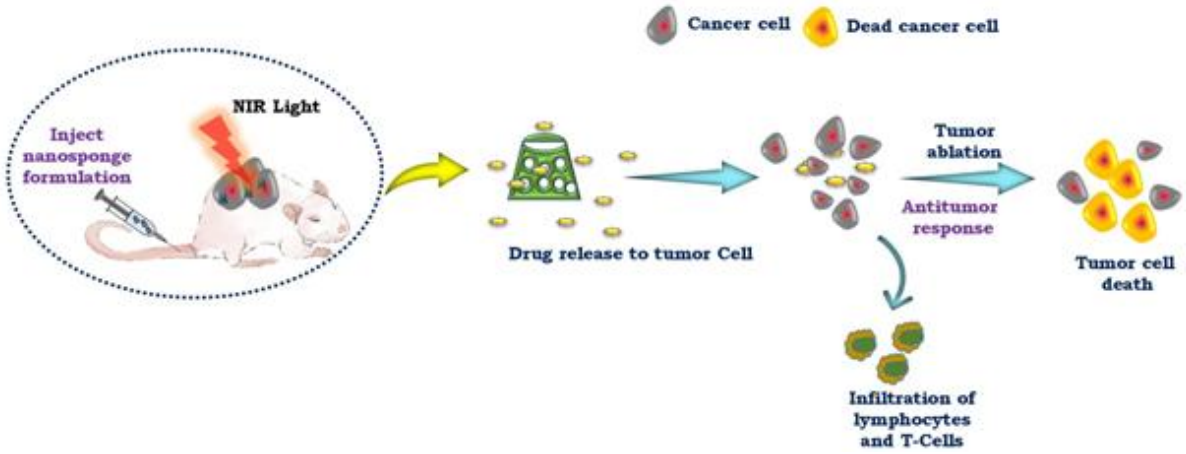

Fig. 11: Mechanism of drug release through nanosponges in chemotherapy.

6. **Nano-carriers for biomedical applications:** Nanosponge has been used for the removal of organic impurities in water.[36]

7. **Analytical applications:** The microporous hyper cross-linked nanosponges have been used in selective preparation of inorganic electrolytes by size exclusion chromatography. The three-dimensional nanosponges will play an important role in the fractionalization of peptides for proteomic applications.[21]

8. **Nanosponges as protective agent against photodegradation:** Gammaoryzanol loaded nanosponges gel and an O/W emulsion exhibit good protection from photodegradation.[14]

9. **Removal of organic pollutants from water:** Betacyclodextrin nanosponges are completely insoluble in water, and encapsulate organic pollutants from water.[12,16]

10. **Nanosponges as absorbent in treating poison in blood:** Instead of using antidotes, if we injected nanosponges, they look like red blood cells, tricks toxins into attacking it, and then absorb it. The number of toxin molecules each nanosponge can absorb depends upon the amount of toxin.[22,24]

11. **Nanosponges as a carrier for delivery of gases:** Cavalli developed topical nanosponges formulations as oxygen delivery systems which have the ability to store and to release oxygen slowly over time.[15]

Table 3: List of research studies done on formulation of nanosponges of various drugs.

Drug	Nanosponge vehicle	Category of drug	Study	Reference
Cephalexin	Ethyl cellulose, PVA	Antibiotic	Drug release	[27]
Econazole Nitrate	β-CD, poly vinyl alcohol (PVA)	Antifungal	Drug release	[28]
Ibuprofen	Ethyl cellulose, PVA	NSAID	Drug release	[29]
Isoniazid	Ethyl cellulose, PVA	Anti-tubercular	Drug release	[30]
Itraconazole	β-CD, copolyvidonum	Antifungal	Solubility	[31]
Miconazole Nitrate	β-CD, Di-phenyl carbonate	Antifungal	Drug release	[32]
Nifedipine	β-CD	Calcium channel blocker	Solubility	[33]
Paclitaxel	β-CD	Antineoplastic	Bioavailability	[34]
Voriconazole	Ethyl cellulose, Polymethyl methacrylate (PMMA), Pluronic F-68.	Antifungal	Drug release	[35]

Table 4: Patents reported on nanosponges[36]

S No	Patent/App No. Year of Issue	Title
1.	WO/2012/147069 (2012)	Method for preparing dextrin nanosponges
2.	PCT/EP2009/004098 (2009)	Cyclodextrin nanosponges as a carrier for biocatalysts, and in the delivery enzymes, proteins, vaccines, and antibodies.
3.	W02003085002A1 (2003)	Cross-linked polymers based on cyclodextrin for removing polluting agents.

CONCLUSION

Nanosponges are innovative drug carriers which encapsulate both lipophilic and hydrophilic drugs, and can be developed as different dosage forms like parenteral, aerosol, topical, oral tablets and capsules. They improve the bioavailability of poorly water soluble drugs, prevent drug and protein physiochemical degradation, prolong drug release in a controlled manner at the target site of action, improve stability, drug dosing, and patient compliance. Due to their small size, they possess 4–5 times more valuable at delivering drugs than the conventional method. They also used in various fields of cosmetics, biomedicine, chemistry, and catalysis. Thus, nanosponges are gaining interest for targeted drug delivery system among the other nanotechnologies.

Authors' contributions

Ms. Prasanthi G has been compiled the data and summarized all the information.

Ms. Sravani B has supervised the manuscript and revised it critically for important intellectual content.

Conflicts of interest

There are no conflicts of interest.

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