



Patent review on cyclodextrin based nanosponges prepared by different methods: physicochemical characterization, factors influencing formation and applications

Pritesh Patel, Ashwini Deshpande*

SVKM's NMIMS, School of Pharmacy and Technology Management, Shirpur, Dist-Dhulia, Maharashtra-425405, India

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ABSTRACT

Cyclodextrin nanosponges are solid, porous, biocompatible nanoparticulate three dimensional structures and have been used as delivery system of different drugs. Nanosponges are diminutive sponges with a size of about a virus. Specific sites are being targeted by tiny nanosponges in the body during circulation and release the drug in controlled expected manner. Porous insoluble nanosponges with a crystalline or amorphous structure and spherical shape can be formed by using different types of cyclodextrins. Different types of cyclodextrins, cross-linkers and degree of cross-linking between them can alter the dimension and polarity of the polymer mesh. Cyclodextrin-based nanosponges can enclose different types of lipophilic or hydrophilic drug molecules by forming inclusion complex. Inclusion Complexes formed between drug and nanosponges can be characterized by different methods like microscopy, solubility studies, zeta potential, differential scanning calorimetry, fourier transform-infrared spectroscopy etc. Different factors like type of polymer, type of drug, temperature and method of preparation can affect formation as well as performance of nanosponges. Benefits of cyclodextrin based nanosponges are aqueous solubility improvement, increment in stability period, masking unpleasant flavours, preventing gastrointestinal irritation and obtaining sustained delivery systems or designing innovative drug carriers for nanomedicine.

Key Words: cyclodextrin, nanosponges, cross-linker, inclusion complex, solubility, stability, drug carrier.



INTRODUCTION

Cyclodextrin-based nanosponges are biocompatible nanoparticles used for improvement in dissolution rate, solubility and stability of drugs, masking unpleasant flavors, converting liquid substances to solids and prolonging the release of drug. Mostly two methods used for the preparation of nanosponges are cross-linking reaction by condensation polymerization and cross-linking reaction by interfacial phenomenon. Nanosponges prepared by polymerization reaction showed the promising results in anticancer drug delivery system, proteins delivery system, anti-inflammatory drugs and antifungal drug delivery system^(1,2,3).

By using organic carbonates as cross-linkers, cyclodextrin based nanosponges are prepared for eliminating the chlorinated aromatic compounds present in water in traces^(1,2,3). For solubility enhancement, various formulation approaches like

use of co-solvents, surfactants, inclusion complex formation, solid dispersion, hot melt extrusion, supercritical fluid technology etc. are adopted in which most interesting approach is inclusion complex formation of active ingredients and cyclodextrins by using cross-linker⁽³⁾. Cyclodextrins (CD) are shaped like truncated cone structure in which arrangement of the functional groups of the glucose molecules is such that glucose molecule's upper surface is polar and inner cavity is lipophilic which facilitates CD's to form inclusion complexes that are stable in organic solutions of suitable size and polarity⁽³⁾. Inclusion complexes are generally prepared for solubility improvement of poorly water-soluble drug molecules, increment in stability period of drug molecules and masking unpleasant flavors⁽³⁾. These inclusion complexes carry lipophilic drug inside the hydrophobic cavity of CD's^(1,2,3). Only because of this reason cyclodextrin is having numerous applications in various fields like pharmaceutical, catalysis, analytical, cosmetics etc.

*Corresponding Author Address: Ashwini Deshpande, Department of Pharmaceutics, School of Pharmacy & Technology Management, NMIMS, Shirpur, India; Email ID: ashwinideshpande4@gmail.com

wherein the properties of the inclusion compounds are exploited⁽³⁾. Cross-linkers like Diisocyanates, epichlorohydrin or organic carbonates were used to get polymerization⁽³⁾. Nanosponges are covalently bound functionalised cyclodextrin systems which can be used as carriers of active ingredients. Because of these features, cyclodextrin based nanosponges can be used to solve the intrinsic problems of the active ingredient such as the protection, poor hydro-solubility, instability, gastric irritation and toxicity^(1, 2, 3).

Nanosponges based on cyclodextrins can be obtained using organic carbonates as cross-linkers, operating in particular with the aid of ultrasounds without a solvent⁽³⁾. Under sonication, cyclodextrin based nanosponges can be obtained by a reaction between natural cyclodextrin (α , β and γ) and the diphenyl carbonate (DPC) or other organic carbonates without a solvent addition which can be operated between the ambient temperature and 90°C⁽³⁾. Cyclodextrin based nanosponges can also synthesized by using organic carbonates which involves the use of solvents such as DMF or DMSO⁽³⁾. In which reaction is carried out at temperatures higher than 130-140°C and leads to the formation of a reticulate transparent block which has proved completion of polymerization reaction resulting inclusion complex formation⁽³⁾. The product, which has suitable characteristics for its use as carrier of active ingredients, shows under the optical microscope a peculiar morphology: it is made up of spheroidal particles with regular dimensions smaller than 5 microns^(1,2,3). **Table 1** shows list of different drug molecules complexed by nanosponges and **Table 2** shows list of chemicals (polymers and cross-linkers) used for synthesis of nanosponges.

Methods for Preparation of nanosponges:

1) *Different methods for preparation of β -CD based nanosponges using Diphenyl Carbonate.*

Method 1^(1,2,3)

In a flask mix 0.001 mols of anhydrous β -CD and 0.004 mols of diphenyl carbonate (DPC) in the molar ratio of CD:DPC=1:4. Subject this mixture for sonication in an ultrasound bath filled with water at 90°C for 5 hrs. The reaction mixture is allowed to cool down. The resultant cross-linked transparent block is to be broken down roughly. Phenol crystals of fine needle shape get deposited on the flask's neck and part of the phenol developed helps the product to be agglomerated. Wash the product (roughly ground) with water for the removal of non-reacted cyclodextrin, and additionally wash in Soxhlet's extraction apparatus with ethanol to ensure removal of unknown by

products formed during reaction. A fine white powder, insoluble in water and other organic solvents is obtained as final product at the end of reaction. This can be further confirmed by the supporting studies.

Method 2^(1,2,3)

Same procedure, as mentioned in Method 1 up to sonication is to be employed. The reaction mixture is allowed to cool and then rotavapor is used to concentrate mixture in a small volume. Excess water is added in the end, followed by filtering and washing with water for an extensive period. A fine white powder, insoluble in water and other organic solvents is obtained as final product at the end of reaction which is to be dried.

The same procedure can be repeated with molar ratio of CD: DPC 1:2

Method 3^(1,2,3)

Mixture in the molar ratio of CD:DPC = 1:2 in a beaker is placed in an oil bath bain-marie under sonication and heated at 90°C for 4 h at 19kHz using an ultrasound probe capable of supplying a maximum power of 250W. The reaction mixture is allowed to cool down and break-down of the obtained product is roughly facilitated. For removal of the non-reacted cyclodextrin, the product is washed with water followed by washing in Soxhlet apparatus with acetone to eliminate the phenol developed and the residual DPC. A fine white powder which is insoluble in water and other organic solvents is obtained as final product at the end of reaction. This is the same method can be repeated with molar ratio of CD: DPC=1:3 and CD:DPC=2:1.

2) *Different methods for preparation of α , β and γ -CD based nanosponges using different cross-linkers according to Table 3*

Factors influencing Nanosponge Formation:

Type of polymer: Type of polymer used has direct impact on the formation as well as the performance of Nanosponges. Hydroxy propyl β -cyclodextrin possess good affinity to form inclusion complex as compared to α , β and γ -cyclodextrin. Suitability of cavity size in inclusion complex confirm the accommodation of a drug molecule of particular size in nanosponge⁽⁵⁾.

Type of drugs: Complex of drug molecules with nanosponges should possess certain characteristics which are enlisted below⁽⁷⁾.

- Molecular weight should be in between 100 and 400 gm/mole.

- Drug molecule consists of less than five condensed rings.
- Solubility of drug molecule in water should be less than 10mg/mL.
- Melting point of the substance should be below 250°C.

Temperature: Temperature change can play vital role in affecting Drug/Nanosponge complexation. In general, the magnitude of stability constant of the Drug/Nanosponge complex decreases with increase in the temperature which may be due to reduction in drug/nanosponge interaction forces⁽⁸⁾.

Method of preparation: Nanosponges can be prepared by different methods like polymerization condensation, interfacial polymerization, emulsion solvent diffusion, ultrasound etc. Drug/Nanosponge complexation can be affected by method of loading the drug into the nanosponge. Nature of the drug, polymer and temperature can affect the effectiveness of a method of preparation and in most cases of nanosponge formation, drug complexation was effectively obtained by freeze drying.⁽⁹⁾

Degree of substitution: The nanosponge complex forming ability may be greatly affected by type, number and position of the substituent on the parent polymeric molecule. Number of substitutions present is directly correlated to the degree of crosslinking. As the number of substituents increases, the probability of crosslinking increases correspondingly. Highly porous nanosponges can be obtained due to higher degree of cross-linking resulting in formation of a mesh type network⁽⁹⁾.

Characterization of Nanosponges:

Solubility Studies: Phase solubility study is most widely used approach for inclusion complexation which scrutinizes the effect of a nanosponge on the solubility of the drug. Nanosponges are confirmed by insolubility in water and organic solvents like DMF (Dimethyl Formamide) and DMSO (Dimethyl Sulfoxide)⁽⁵⁾.

Loading efficiency: Disperse the drug loaded nanosponges in the solvent in which drug is soluble, sonicate for 5 min to break the complex, diluted with suitable solvent and analyze applying suitable method⁽⁵⁾.

FT-IR(Infrared) spectroscopy: FTIR spectra of pure drug, β -cyclodextrin, nanosponge formulation can be compared to understand interaction between pure drug and nanosponge complex by KBr pellet

method using a FT-IR spectrometer, at specific wavelength^(5,10,11).

Zeta potential: Zeta potential is widely used for quantification of the magnitude of the electrical surface charge at the double layer. The significance of zeta potential is that its value can be related to the stability of formulation. More than 30 mV zeta potential value in water indicates good stability of Nanosponge. It can be measured by using zetasizer or particle size equipment⁽¹⁰⁾.

Microscopy studies: For morphological study (particle size, shape) of drug, nanosponges and product (drug/nanosponge complex), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) can be used. Formation of the inclusion complexes can be confirmed by observing difference in crystalline state of pure drug, cyclodextrin and product under electron microscope^(10, 11).

Thin Layer Chromatography (TLC): TLC can also denote the complex formation. On running the sample of pure drug and nanosponges complex on TLC plate, the retention factor(R_f) was changed as compared to pure drug which confirms the complex formation⁽¹¹⁾.

Differential scanning calorimetry (DSC): The thermal behaviour of drug-CD complex can be studied using DSC in order to confirm the formation of complex. DSC thermogram of pure drug shows an endothermic peak at its melting point. This peak for drug will be absent in DSC thermogram of nanosponge⁽¹²⁾.

In vitro release studies: In vitro release of drug from nanosponge formulations confers the release behaviour of the drug from nanosponges. Suitable dissolution and analysis method can be employed based on drug and type of formulation as per the aim of the study⁽¹²⁾.

Accelerated Stability Studies: As stability is the main issue related to drug product, these studies are mandatory to be performed. Charge freshly prepared formulation in stability chamber as per the ICH guidelines and study is to be performed⁽¹²⁾.

APPLICATIONS OF NANOSPONGES^(4,5):

Solubility Enhancement: One of the key hindrance to the formulation and development in pharmaceuticals is poor solubility of drugs in water. To solve the problem of solubility, numerous approaches have been investigated. Inclusion complex of cyclodextrin approach is widely used in pharmaceutical field for

improvement of solubility and bioavailability of lipophilic drugs. Nanosponges can enhance the wetting and solubility of poorly soluble molecules⁽⁴⁾. Swaminathan *et al.* studied a nanosponge formulation of **itraconazole** having solubility of 1 ng/mL at physiological pH. Nanosponges improved the solubility of the itraconazole by over 27-fold; and on adding PVP solubility was increased by 55-fold. Thus Nanosponge formulation could increase solubility and the bioavailability of itraconazole⁽¹³⁾.

Nanosponges for Drug Delivery: The nanosponges can be formulated as Oral, Parenteral and Topical dosage forms. For the oral administration, capsules or tablets can be prepared by dispersion of nanosponge complex in a matrix of excipients, diluents and lubricants. For the parenteral administration, sterile water, saline or other aqueous solutions are used as carrier for nanosponge complex. For topical administration, nanosponge complex is incorporated in topical hydrogel⁽⁴⁾.

Vavia prepared nanosponges of **nelfinavir mesylate** to improve dissolution rate, solubility and ultimately bioavailability of the drug⁽¹⁷⁾. Nelfinavir is a HIV-1 and HIV-2 protease inhibitor which used for the treatment of HIV infections. From nanosponges, found drug release rate was less than from a β -CD complex which shows that nanosponges were proposed as a sustained drug delivery system for oral administration⁽¹⁷⁾.

Acyclovir is a drug with poor bioavailability, medium polarity and solubility of 1.5 mg/mL in water. Special carboxylated nanosponges, enclosing dissociable carboxylic groups in their structure were developed for its encapsulation⁽⁹⁾. In addition to the cyclodextrin cavities, they also represent additional electrostatic contribution for encapsulation of drug. Carboxylic groups present in the nanosponge structure and the amino group of acyclovir may show some electrostatic interactions.

Rao *et al.* studied that **telmisartan** affected by carbonate nanosponges. Telmisartan is an angiotensin II receptor antagonist, antihypertensive BCS class II drug and having solubility of 9.9 μ g/ml in water which ultimately lowers the bioavailability. The formation of complex of telmisartan with nanosponges and NaHCO_3 was observed synergistically to enhance the dissolution rate as well as bioavailability of telmisartan⁽²³⁾.

Nanosponges as carriers to protect molecules from light or degradation: Alternative use for

nanosponges is as carriers to safeguard encapsulated molecules from light or from degradation (chemical or enzymatic). Light-sensitive model drug **5-fluorouracil** was used for evaluation of the potential protection application. Up to 30% of 5-fluorouracil can be incorporated by β -CD nanosponges formation⁽¹⁸⁾. Furthermore, encapsulation of 5-fluorouracil in nanosponges protected the drug and maintained its cytotoxicity against MCF-7 cells.

Another example was the cyclodextrin based nanosponges of **camptothecin**⁽¹⁰⁾. The anti-tumour activity of camptothecin has been widely studied in both hematological and solid malignancies. Due to its poor solubility and high chemical instability, the lactone ring of the molecule is very susceptible to hydrolysis at physiological pH. The encapsulation of camptothecin in nanosponges was used to extend the shelf life as well as release of the drug. The nanosponges improved solubility of large amounts of the drug and protected the lactone ring from opening due to its high inclusion abilities and thereby increasing stability⁽⁴⁾.

Nanosponges as carrier for biocatalysts and in release of enzymes, proteins, vaccines, antibodies:

For carrying enzymes and proteins a number of systems have been developed, for example, nanoparticles, microparticles, liposomes and hydrogels. It has been found that Cyclodextrin based nanosponges are predominantly appropriate carrier to adsorb enzymes, antibodies, proteins, and macromolecules. Specifically, when enzymes are used, nanosponge formation can maintain their activity, efficiency, extend their operation, pH and temperature range of activity and allows the conduct of continuous flow processes. Furthermore, proteins and other macromolecules can be carried by adsorbing or encapsulating them in cyclodextrin nanosponge. Swellable cyclodextrin-based nanosponges were purposely prepared for protein delivery by using a different synthetic routes⁽⁵⁾.

New swellable cyclodextrin-based **poly (amidoamine)** nanosponges (PAA-NS) were synthesised by cross linking β -cyclodextrins with either short polyamido-amine chain or 2,2-bis (acrylamidoacetic acid)⁽⁹⁾. Swellable nanosponges were discovered to be sensitive to the pH of the encompassing media. By using the high pressure-homogenisation technique, PAA-NS were abridged in nanosuspensions. For study of encapsulation capacity of these new nanosponges, bovine serum albumin (BSA) was used as a model protein. For achievement of high protein complexation capacity, nanosponges with BSA-encapsulation

efficiency greater than 90% were used. By forming carbonate nanosponges, encapsulation of albumin and its prolonged release was obtained⁽⁹⁾.

Other applications of nanosponges: Even at very low concentrations nanosponges based on cyclodextrins can strongly bind organic molecules and remove them from water⁽¹⁹⁾. The identical notion can be suitable for eradication of bitter components from grape fruit juice by careful combination of polymer and cross-linker. The microporous hyper cross linked nanosponges have been used in selective separation of inorganic electrolytes by size exclusion chromatography. Vital role would be played by three dimensional nanosponges in the fractionalization of peptides for proteomic applications⁽²⁰⁾. Nanosponges can be used as **carrier for gases** like oxygen and carbon dioxide. Many biomedical applications can also take advantage of these nanosponges. Specifically, oxygen to the hypoxic tissues which are present in various diseases could be supplied by oxygen-filled nanosponges⁽²¹⁾. For the diagnosis purpose,

nanosponges can soak up biomarkers selectively.⁽¹¹⁾

CONCLUSION

Cyclodextrin based nanosponges possess different properties like encapsulation ability, unpleasant flavors masking, biocompatibility, solubilisation capacity and stabilization capacity which give benefits to poorly soluble BCS class II drugs. By controlling the ratio of polymer to cross-linker and drug to nanosponge complex, particle size and drug release can be modified. Different dosage forms like oral, parenteral and topical can be developed for both types of drug molecules like lipophilic as well as hydrophilic because of spherical shape and tiny size of nanosponges. Nanosponges can broaden the range of applications like solubility enhancement, for drug delivery, as carrier for biocatalyst and in release of enzymes, proteins, vaccines, antibodies etc. For the delivery of poorly soluble active drug molecules, cyclodextrin based nanosponges can be considered as multifunctional nanoscale drug delivery system in nanoscience.

Table 1: DRUG MOLECULES COMPLEXED BY NANOSPONGES⁽¹⁴⁾

Drug	Therapeutic activity	Administration Route
Dexamethasone	anti-inflammatory	Oral, Parenteral
Flurbiprofen	anti-inflammatory	Oral
Doxorubicin	Antineoplastic	Parenteral
Progesterone	Hormonal	Oral
Itraconazole	Antifungal	Oral, Topical
5-fluorouracile	Antineoplastic	Parenteral Topical
Tamoxifen	Antiestrogen	Oral
Resveratrol	Antioxidant	Topical
Paclitaxel	Antineoplastic	Parenteral
Camptothecin	Antineoplastic	Parenteral
Omeprazole	Antiulcerative	Oral
Nelfinavirmesylate	Antiviral	Oral
Acetylsalicylic acid	Analgesic	Oral
Acyclovir	Antiviral	Topical, Parenteral
Gamma-oryizanol	Antioxidant	Topical
Telmisartan	Antihypertensive	Oral

Table 2: CHEMICALS USED FOR SYNTHESIS OF NANOSPONGES⁽¹⁵⁾

Polymers	Crosslinkers
Hyper cross linked Polystyrenes	Carbonyldiimidazoles
Cyclodextrins and its derivatives like Methyl β -Cyclodextrin	Diphenyl Carbonate
Alkyloxycarbonyl Cyclodextrins	Diarylcyanates
2-Hydroxy Propyl β -Cyclodextrins	Diisocyanates
Copolymers like Poly(valerolactone-allylvalerolactone)	Pyromellitic anhydride, Epichloridrine, , Glutaraldehyde
Poly(valerolactone-allylvalerolactoneoxepanedione)	Carboxylic acid dianhydrides
Ethyl Cellulose	2,2-bis(acrylamido) Acetic acid
PVA	Dichloromethane

Table 3 Different formula for preparation of nanosponges⁽⁶⁾

Aqueous Phase	Organic Phase	Dextrin Type	Cross-linking agent	Drug: Cross-linker Molar Ratio
0.1 M KOH	CH ₂ Cl ₂	β-CD	CDI	1:8
0.1 M KOH	Methyl Isobutyl Ketone	β-CD	CDI	1:8
0.1 M NaOH	CH ₂ Cl ₂	β-CD	CDI	1:8
0.1 M KOH	CH ₂ Cl ₂	β-CD	CDI	1:8
0.1 M KOH	Hexane	β-CD	PMDA	1:8
0.1 M KOH	CH ₂ Cl ₂	β-CD	TPh	1:2
0.1 M KOH	CH ₂ Cl ₂	β-CD	TPh	1:4
0.1 M KOH	DMC	β-CD	DMC	1:8
0.1 M KOH	CH ₂ Cl ₂	α-CD	CDI	1:8
0.1 M KOH	CH ₂ Cl ₂	γ-CD	CDI	1:8
0.1 M LiOH	CH ₂ Cl ₂	β-CD	CDI	1:8
0.1 M KOH	CH ₂ Cl ₂	β-CD	DPC	1:8
0.1 M KOH	CH ₂ Cl ₂	α-CD	CDI	1:8
0.1 M KOH	CH ₂ Cl ₂	γ-CD	CDI	1:8
0.1 M KOH	Hexane	β-CD	PMDA	1:8
0.1 M KOH	CH ₂ Cl ₂	β-CD	TPh	1:2
0.1 M KOH	CH ₂ Cl ₂	β-CD	TDI	1:8
0.1 M KOH	CH ₂ Cl ₂	β-CD	HDI	1:8
0.1 M KOH	CH ₂ Cl ₂	β-CD	TDI	1:8
0.1 M KOH	CH ₂ Cl ₂	β-CD	CDI	1:8
0.1 M KOH	CH ₂ Cl ₂	β-CD	Epichlorohydrin	1:8
0.1 M KOH	CH ₂ Cl ₂	β-CD	Epichlorohydrin	1:8

PMDA-Pyromellitic anhydride, *TPh*- Triphosgene, *CDI*-Carbonyl Di-Imidazole, *SSC*-SebacoylChloride, *DCM*-Dicholomethane, *DPC*- Diphenylcarbonate, *DMC*-Dimethyl carbonate, *HDI*-Hexamethyl-diisocyanate

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