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Review Paper

Nano sponge as versatile carrier systems - an updated review

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Abstract

Nano sponge is a recent advancement in the nanotechnology based drug delivery system. These are 3- dimensional scaffolds formed by extensive cross linking of polymers to form small nano size cavities capable of incorporating both hydrophilic and hydrophobic drugs. This inclusion complexation behaviour enhances the aqueous solubility of drugs with low aqueous solubility. The use of biodegradable polymers can release the drug in a controlled and predictable fashion to maintain constant drug levels. Further advancement in the nanosponge drug delivery is the use of peptide linkers to specifically target a receptor, usually in case of tumors. This will minimize the adverse effects caused mainly due to unspecific release of drug other than tumor cells. In this review, an attempt is made to summarize the methods of development, evaluation techniques, molecular environment and possible areas of applications and future of nanosponge drug delivery systems.

Keywords: Targeted drug delivery technology, β cyclodextrins, Waste water treatment, Sustained release, Drug encapsulation.

Introduction

Around $65 billion go in vain every year in the drug development process due to poor bioavailability[1,2]. Many potent drugs which showed promising results in the preclinical trials fail to make it into the market due to potential side effects associated with unspecified drug delivery. Developing a sustained and targeted drug delivery system pose many challenges in the drug development process due to the complex chemistry involved in it. Nanoparticulate drug delivery showed some promising results in improving the bioavailability and targeted therapy. Nanosponge (NS) is an offshoot of nanoparticulate drug deliverywith a great potential for sustained and specific release of drug with enhanced bioavailability[3]. NS are innovative nanoparticulate drug carrier system with spherical shape and a size range of 150-400 nm (Figure-1)[4]. They consist of small cavities resulted from the cross linking of polymers with cross linker[5]. They are synthesized in neutral or acidic forms depending on the agent used as cross linker [6]. The brilliance of NS is their capacity to encapsulate both hydrophilic and lipophilic drugs within their structure by forming inclusion and non-inclusion complexes with them[7,8]. The cross linker gets bind to certain portions of the polyester strand and form a scaffold structure. The pore size is controlled by using different polymer and cross linkers in different proportions. As shown in Figure-2, when a peptide linker is attached to its structure, they get localized at the target tissue. The polymeric strand is biodegradable which release the drug in a predictable fashion[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, this peptide bound NS circulate within the blood stream until they get attached to the target tissue. The possibility of being detected by the reticulo endothelial system (RES) is limited by using hydrophilic polymers. Different drugs with varying structure and solubility profile are used to evaluate the incorporation efficiency of NS[9]. NS are used to increase aqueous solubility of poorly water-soluble drugs, in purification of water, and nanocarriers for biomedical applications[10]. The simple chemistry involved in NS formulation should take them onto commercial production levels without requiring unusual equipment. This review will focus on the molecular environment, fabrication techniques and applications of NS.

Advantages: It offers predictable release of drug which is the major advantage when compared to other nanoparticle delivery systems under development[3]: i. The pore size of these NS is very less that bacteria cannot be penetrated into its cavity hence self-sterilized[11]. ii. It can be used for both hydrophilic (e.g., dexamethasone and fulrbiprofen) or lipophilic (e.g., doxorubicin) drugs to protect degradable molecules and to formulate various dosage forms[7,12]. iii. Method of preparation requires simple chemistry of polymers and cross linker which does not pose many problems in the technology and can easily scale up the commercial production[6]. iv. Drug is incorporated into the NS cavity forming the inclusion complex; it can be used to mask unpleasant taste and odour[10]. v. NS can be easily reusable by different treatments like stripping with moderately inert hot gases, washing with eco-compatible solvents, mild heating, or changing pH or ionic strength[4]. For this reason NS are employed in cosmetics and many pharmaceuticals[13]. vi. It is

a useful technology to enhance the stability of photodegradable drugs and drugs which are highly hygroscopic, thus increase the shelf life of the formulation[14,15].

Disadvantages: i. NS has the disadvantage of incorporating only small molecules of molecular weight below 500 Daltons only. ii. The crystalline form is also a disadvantage of the NS as the loading capacity of the NS depends on the degree of crystallization. The NS obtained can be crystalline or Para crystalline form. The loading capacity is more for Para crystalline form[16].

Designing a NS drug delivery system: considerations

Physio chemical characteristics of the drug: The drug molecules used for NS drug delivery should possess some characteristics like small molecular weight, less solubility, a lower melting point and the number of condensed rings present in its structure. All the drugs using NS as carriers should meet the primary requirement of small molecular weight. The drugs with molecular weight in the range of 100-400 Daltons can be easily incorporated into the tiny pores of the NS and the loading efficiency achieved is also high. The aqueous solubility should be less than 10mg/ml[17]. The number of condensed rings present in the drug should be less than five so that the formulation will be more stable.

Polymer and cross linker characteristics: The polymer used for the formulation of NS determines its pore size [18]. Different types of polymers and cross linkers used in the formulation of NS are given in Table-1. The polymer and the cross linkers ratio should be optimized to achieve the optimum pore size [19]. The cross linkers will bind to some particular sites on the polymer chains thus forming a mesh like structure with a particular cavity size. For complexation the cavity size of NS should be suitable enough to accommodate drug molecule[20,21]. β-CD and its derivatives has been most commonly employed in the preparation of NS due to their high complexation and encapsulation capacity[22].

Synthesis method: The particle size and shape of NS depends on the synthesis method employed. Spherical and uniform sized NS are formed by using ultra sonication. When the drug or the polymer is highly reactive with the solvent i.e., DMSO or DMFA, then ultra-sonication is used where cross linking occurs directly in the absence of solvent. The method used for drug loading also affect NS complexation. Generally freeze drying is found to be more effective for drug complexation than other loading techniques[23].

Fabrication techniques

Emulsion solvent diffusion technique: The polymer is cross linked with a suitable crosslinking agent in the presence of a polar aprotic solvent like DMSO or DMFA. The dispersed phase containing polymer and drug is mixed in a definite quantity of cross linking agent and then slowly added to a solution of copolymer in aqueous continuous phase. The reaction mixture was stirred at 1000rpm for 2hrs. The NS formed are collected by filtration and dried in oven at 400°C for 24 hrs. The dried NS are stored in vacuum desiccator to ensure the removal of residual solvent[24].

Ultra sound assisted synthesis: Ultrasound is being employed in the laboratory for various purposes which have numerous effects both physical and chemical. An ultrasound probe or a bath called sonicator is used to produce ultrasound. In nanotechnology sonication is usedfor evenly dispersing nanoparticles in a liquid system. NS are prepared by direct ultra-sonication of β-CD and it's by products.Organic carbonates are used as cross linkers. Solvent is not required in this method. The reaction time usually ranges from 4-5hrs. Then the mixture is allowed to cool and the product is roughly broken. Then the product is washed with excess water to remove unreacted solvent and soxhlet extraction is performed with ethanol. The NS obtained by this method are spherical with uniform particle size[12,25].

Solvent method: A polar aprotic solvent like dimethylsulfoxide (DMSO) or dimethylformamide (DMF) is placed in a round bottom flask followed by the addition of desired polymer. After complete dissolution of the polymer in the solvent a required quantity ofcross linkers is added. This is allowed to react for 4 h at 100°C [5]. After the completion of polymerization reaction, theproduct is roughly ground and washed with excess of deionized water to remove unreacted solvent. Further purification is doneby Soxhlet extraction with ethanol. The resulted white powder thus obtained was dried overnight in an oven at 60°C and size reduced. The fine powder obtained is dispersed in water and the colloidal fraction in water was recovered and lyophilized. The NS obtained by this method are sub-micron in dimension and with a spherical shape[12].

Molecular environment at 3D network of CDNS: The chemical environment of NS is reported recently using molecular mechanics and molecular dynamics simulation studies[26]. CDNS have swelling behaviour and can absorb or form inclusion complexes with chemicals and release of active compounds. Use of different cross linking agent will dramatically modulate important parameters such as the swelling capability and hydrophilicity / hydrophobicity of the final polymer[27,28]. Raman spectroscopy and x-ray crystallography are employed to study the molecular structure of NS. Hydration studies are employed to gain information on the state of water anda model solute dissolved in water inside the nanoporous network of swollen CDNS. This will be useful to understand the diffusion phenomenon in gel like state.

Raman spectroscopy: It is a useful tool to study the molecular structure of the compound. The width and the intensity, as well as the wave number of the Raman peaks are sensitive to the

environmental and conformational changes of the molecules and intermolecular interactions. A bump at the lower wave number (15-30 cm^{-1}) is observed in the Raman spectrum of NS, which is a characteristic in disordered systems. Change in the lower energy vibrational dynamics is connected to the increasing density of cross linking of the whole system[27].

X-ray diffraction: The crystallinity of the NS depends on the type of cross linker and the molar ration of polymer and cross linker used. In a study, NS prepared by using diphenyl carbonate as cross linker, yield crystalline and para crystalline forms, whereas NS prepared by using pyrometallic dianhyride showed predominantly amorphous. However, increasing the molar ratio of polymer to cross linker to 1:8, presents several crystalline peaks, indicating that a different spacing position between the polymers[27].

Table-1: List of polymers, cross linkers and solvents used for fabrication of Nanosponge.

Polymers	Hypercross linked polystyrenes, cyclodextrines and its derivatives like methyl β-cyclodextrin, alkyloxycarbonyl cyclodextrins, 2-hydroxy propyl β-cyclodextins and copolymers like poly (valerolactone-allylvalerolactone) and poly (valerolactone-allylvalerolactone-oxepanedione) and ethylcellulose & PVA
Cross linkers	Diphenyl carbonate, pyromellitic anhydride Diarylcarbonates, Diisocyanates, carbonyldiimidazoles, dichloromethane, epichloridrine, glutarldehyde, carboxylic acid anhydrides, 2,2-bis(acrylamido) acetic acid
Solvents	dimethyl sulfoxide (DMSO), dimethyl formamide {DMF)

Therapies which could benefit from NS technology

Biological and future drugs: With the advancement in biotechnology and molecular biology many novel drugs are developed for various diseases. These novel drugs include nucleic acids, antibodies and proteins, monoclonal antibodies. These drugs fail to show the desirable effects due to their poor stability in-vivo. The commercial production is also hindered by their poor stability at normal environmental conditions. Till date these drugs are administered by parental route for chronic conditions. Encapsulation of these drugs into NS will certainly improve their stability both in-vitro and in-vivo[29].

Gene therapy: Detection and correction of the defective gene by gene targeting have attracted increasing attention in recent times. Chimeric RNA- DNA oligonucleotide (RDO) is one of them[30]. Several novel delivery systems have been used successfully for plasmid DNA transfer or oligonucleotide transfer including Nanoparticles which can be divided as encapsulating, complexing and conjugating nanoparticles based on the method of associating with oligonucleotides. Alginate NS is an example of encapsulating type which is sponge like nanoparticles containing many cavities that carry the oligonucleotides[31].

Proteins and Peptide delivery: Proteins and peptides are the evolving class of therapeutics for the treatment of cancer or type 1 mucopolysaccharidosis[29]. The efficiency of these drugs is limited due to their large molecular size, hydrophilic nature, degree of ionization, high surface charge, chemical and enzymatic instability and low permeability through mucous membrane. Oral administration of proteins will result in the degradation in the GI tract. Intravenous administration results in rapid clearance from blood, plasma protein binding and enzymatic digestion by proteolytic enzymes. A number of carrier systems are developed for carrying these proteins and peptides which increase its pharmacokinetics and in-vivo stability. β-CD based NS are effective carrier system for these drugs. These proteins and peptides can be incorporated into the NS by adsorption or encapsulation. This technology is still in infantile stage and the NS should be modified to incorporate large size proteins and peptides.

Delivery of enzymes: In the process of fermentation on industrial scale, many steps are involved which are catalysed by enzymes[32]. The use of enzymes as biocatalysts will reduce the consumption of energy and water for the downstream process. Enzymes are specific in their nature which results in high yields at lower temperatures and pressure. Immobilization of enzymes on NS will increase their effectiveness. α- amylase, trypsin, cellulose and pectinase are used for the clarification of fruit juice along with NS.

Tissue engineering: Silicon NS prepared by wet etching of Ag-NP's followed by vapor desorption of various organosilane chemicals[33,34]. These NS are nano topographic oxidized. These are employed in tissue engineering to gain insight into the fundamental biology of cell environment interactions in-vitro. They influence the cell development process and are applied in tissue engineering and basic biology[35].

Drug targeting by NS: Drug loaded NS are targeted to particular sites in the body like tumour cells or vasculature by binding the nano network with some special chemical linkers[35]. Peptide linkers are used to target the NS loaded paclitaxel to breast cancer cells. Eva Harth *et. al*.,[3] linked Gly-lle-Arg-Leu-Arg-Gly (GlRLRG) a peptide that selectively recognizes GPR78 receptor on certain tumour cells. The conjugation of this peptide to NS has increased the concentration of drug at the tumour site thereby suppress the tumour growth by five folds. These peptide linked NS circulate in the blood stream until they bind to the targeting site there by reduces the side effects associated with burst release of drugs by other drug delivery systems. The polyester chain is biodegradable which breakdown gradually in the body releasing the drug in a predictable fashion.

Table 2: Examples of Various drugs successfully encapsulated in to nanosponge.

Drug	Mol. Wt (Daltons)	Polymer	Cross linker	Category	Study	References
Paclitaxel	853.90	β- CD	Carbonyldiimidazole	Anti-tumour	Bio availability and cytotoxicity	3,36
Tamoxifen	371.51	β- CD	Carbonyldiimidazole	Anti-tumour	cytotoxicity	37
Campothecin	348.35	β- CD	Diphenylcarbonate	Anti-tumour	Haemolytic activity and cyto toxicity	38
Curcumin	368.37	β- CD	Dimethyl carbonate	Anti-tumour	Bio availability and Cyto toxicity	39
Itraconazole	705.63	β- CD	Copolyvidonum	Anti-fungal	Saturation solubility study	40
Econazole	444.69	Ethyl cellulose	Poly vinyl alcohol	Anti-fungal	Irritation study	4
Orizanol	602.98	β- CD	Diphenylcarbonate	Anti-oxidant	Anti-oxidant study	14
BSA	66,463	β-CD	2,2-bisacrylamido acetic acid or polyamidoamine	Protein	Stability study	41
Resveratrol	228.24	β- CD	Carbonyldiimidazole	Anti-inflammatory	Cytotoxicity Ex-Vivo Study Permeation study	44
Cefpodoxime proxetil	427.45	β- CD	Diphenyl carbonate	Anti-bacterial	Saturation solubility study	45
Anti-sense oligo nucleotide	7000	Sodium alginate	Poly L-lysine	Gene therapy	Pharmacokinetic study	31

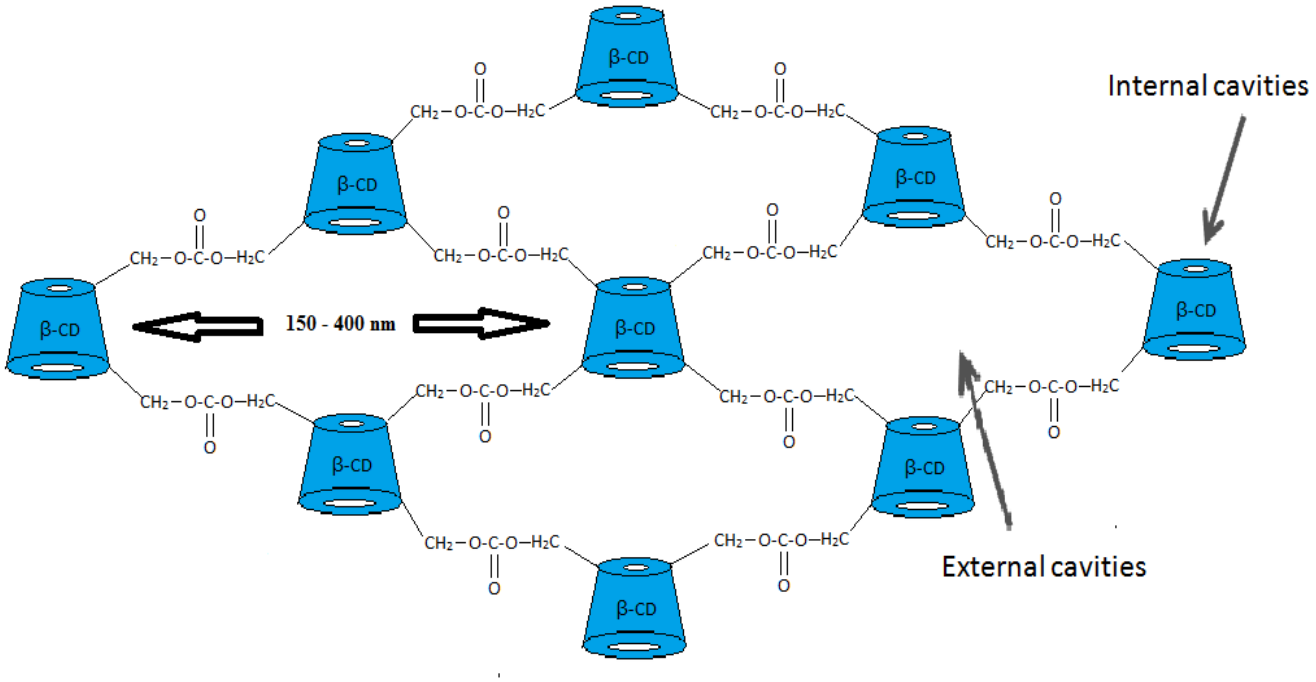

Figure-1: Illustration representing the internal and external cavities of nanosponge carrier system.

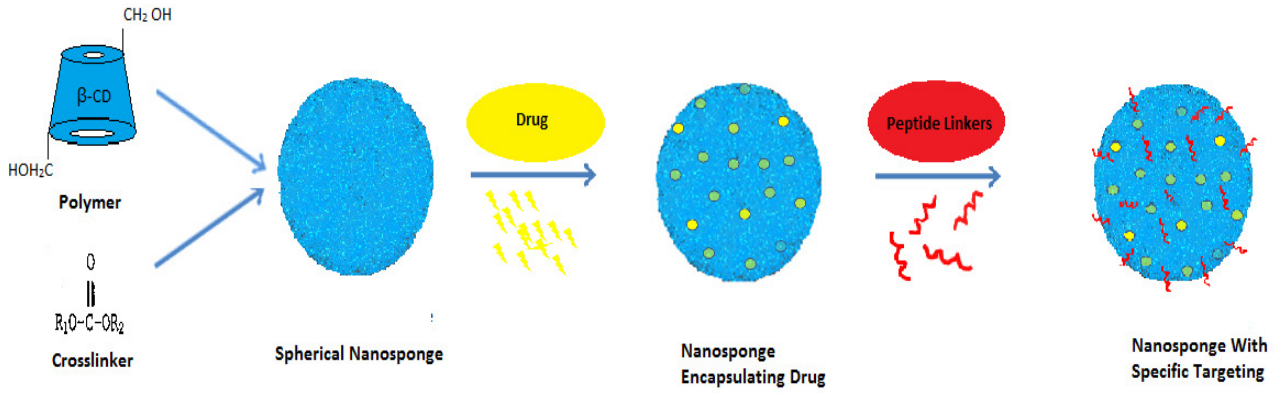

Figure-2: Fabrication of nanosponge carriers using β-CD, crosslinking agent and peptide linkers.

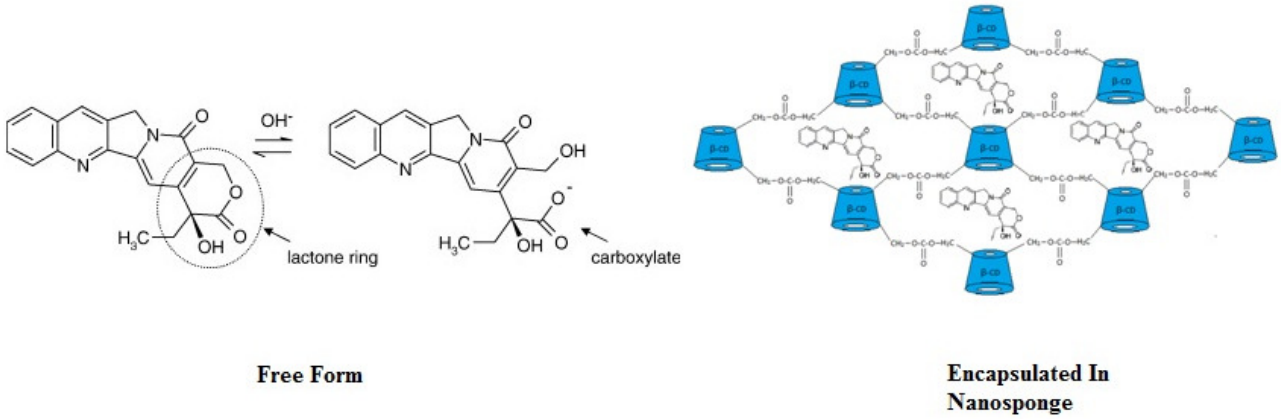

Figure-3: Internalization of campothecin in nanosponge carriers increases its stability.

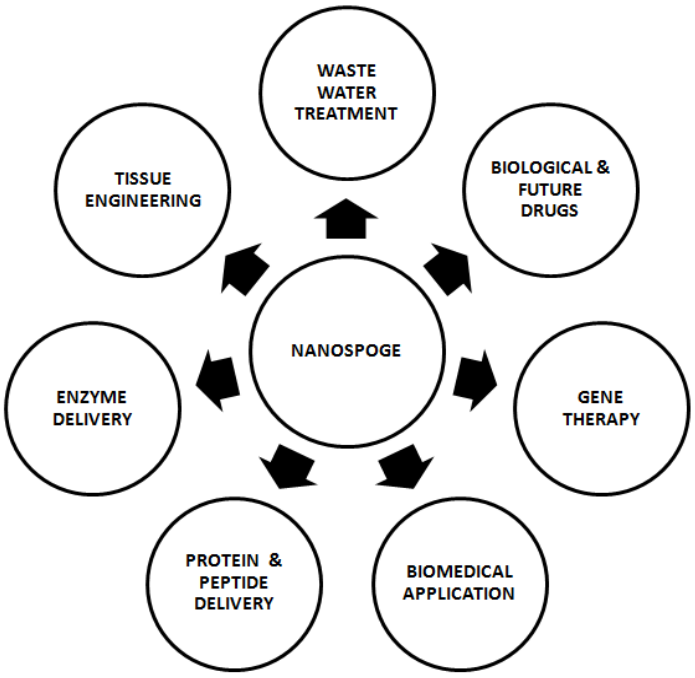

Figure-4: Application of nanosponge in various fields.

Applications

Anti-cancer agents: *Paclitaxel* loaded NS are prepared by using Beta cyclodextrin (β-CD) as nanosponge vehicle using the technique of inclusion complex formation, to improve its bioavailability and cytotoxic efficacy. It is a potent anti-mitotic drug used in cancer therapy but has poor bioavailability of 6.5%. Incorporation of paclitaxel into NS complex eventually enhanced bioavailability when tested on Sprague drawly rats and also the cytotoxic efficacy when tested on MCF-7 cell lines[36].

Tamoxifen is currently employed in the treatment of both early and advanced estrogen receptor positive breast cancer. Tamoxifen loaded β-CD NS for oral drug delivery with particle size of 400–600 nm was developed by freeze drying method using carbonyldiimidazole as a cross linker and tested for bioavailability and cytotoxic activity. They showed an increase in AUC and C_{max} of 1.44 fold and 1.38 fold higher than plain drug and increased cytotoxic activity when tested on MCF-7 celllines[37].

Camptothecin is a potent antitumor agent which acts as topoisomerase 1 inhibitor is used in cancer therapy. But its activity is limited due to poor aqueous solubility and also lactone ring instability which further lead to adverse effects. β-CD based NS loaded with camptothecin are prepared by using β-CD and varying concentrations of cross linkers to protect the lactone ring from hydrolysis and sustain the release of CAM (Figure-3). The *in vitro* studies indicated a slow and prolonged CAM release over aperiod of 24 hr. The haemolytic activity performed on diluted blood showed enhanced action. The cytotoxicity studies areperformed on HT-29 cell lines showed promising tumour inhibition[38].

Curcumin has some potent antitumor properties, but its therapeutics utility is limited due to poor aqueous solubility. Cyclodextrin based NS are used to enhance the solubility and to control the release of curcumin. Dimethyl carbonate is used as a cross linker. Curcumin loaded NS showed 50 times more solubility compared to plain drug. The *invitro* cyctotoxicity studies on MCF-7 cell lines showed significant inhibition of cell lines compared to plain drug[39].

Antifungal Agents: *Itraconazole*, a novel antifungal agent is a BCS Class II drug that has a dissolution rate limited poor bioavailability. To enhance the bioavailability, β-CD based NS of itraconazole are prepared by Solid dispersion technique using copolyvidonumas cross linking agent. When saturation solubility studies are performed by taking Higuchi model as mathematical reference, the solubility of itraconazole was enhanced more than 50-folds with a ternary solid dispersion system[16,40].

Econazole nitrate, a topically used antifungal agent has poor adsorption properties when applied directly to the skin. A higher concentration of drug has to be applied at the infected site for effective therapy. For this reason econazole NS are prepared by using ethyl cellulose and poly vinyl alcohol as polymers by emulsion solvent diffusion technique. These NS are further loaded into hydrogel and applied as local depot for sustained release. This eventually increased the concentration of econazole at the infected site[4].

Anti-oxidants: *γ-Orizanol* (GO), which is popularly used as anti-oxidant in cosmetic, food and medicinal product is a powerful inhibitor of hydroxyl radical formation. It is highly light sensitive ingredient, so it is advantageous to incorporate it in a carrier system to increase its stability and ensure its efficacy. β-CD based NS of GO are prepared by mixing GO and NS in the ratio of 1:1. The solid dispersions were freeze dried and stored. The effect of encapsulation in β-CD NS on anti-lipoperoxidative activity and in-vitro skin permeability were investigated[14].

Protein Delivery: *Bovine Serum Albumin* (BSA) a protein obtained from cow serum, has numerous biochemical applications including ELISA. Being a protein solution it is highly unstable and need to be stored in a lyophilized state. To enhance the stability of BSA, swellable NS of BSA are prepared by using β-CD and 2,2-bisacrylamidoacetic acid or polyamidoamine as a cross linking agent. The in vitro BSA release studies reported a prolonged release of albumin from the swollen BSA loaded β-CD PAA-NS for 24 hr[41].

Anti-inflammatory: *Resveratrol* is a potent anti-inflammatory agent used from decades in medicine[42]. The drug is highly hydrophobic which limits the dissolution rate of the drug and results in poor bioavailability[43]. To overcome this problem resveratrol loaded β-CDNS were prepared by solvent method by using β-CD as carrier and carbonyldiimidazole as cross linker in the presence of DMF. The ex-vivo study on rabbit buccal mucosa reported the accumulation of drug in the buccal mucosa[44].

Antibiotic: *Cefpodoxime proxetil* (CP) belongs to cephalosporin class of antibiotic poses limited bioavailability due to poor aqueous solubility. To enhance the solubility profile of CP, NS are prepared by cross linking β-CD with diphenyl carbonate. These colloidal nanocarriers showed a marginal increase in the saturation solubility of CP, but the in vitro studies showed an increase in drug release by 11 folds as compared with plain drug[45].

Non Pharmaceutical Applications

Waste water treatment: Ether activated carbon and zeolites are the conventional method of water purification which is proved ineffective at very lowconcentration of contaminants in water[46,47]. β-CD based NS can strongly bind to the organic pollutants present in the water and this polymer is 10000 times more effective in removal of organic pollutants from water than

the conventionalmethods. This reduces the clean-up cost[48]. Cyclodextrin -based NS are used to improve the vase life of Dianthuscaryophyllus cut flowers[49].

Biomedical Application: Polyionic NS is applied in the fractionalization of peptides by MALDI-MS analysis for the proteomic application[50]. Theyare used as biomarkers for cancer research[51]. Hyper cross linked NS are used as carrier for gases like oxygen and carbondioxide. NS carrying oxygen are employed in the supply of oxygen to the hypoxic tissue associated with various diseases likeCOPD (chronic obstructive pulmonary disease)[52].

Conclusion

The real challenge is in the development of modified NS for the delivery of proteins and peptides, gene therapy and antibodies. These agents should be engineered in a way that they are stable trough other routes of administration other than parental route. Further research should be done to know the solubilisation efficiency of tertiary complexes of NS. There is a lot of scope in this area for patient filling of more advanced techniques for NS preparation. If this can be transferred from strong results in laboratory to safe and effective clinical treatment then it can definitely revolutionize the drug delivery pattern. It can be concluded that NS drug delivery system opens new approaches for delivery of many existing drugs as well as older drugs which showed poor bioavailability, adverse effects and poor in-vitro and in-vivo stability. Innovative NS delivery systems are expected to have meaningful effects in the treatment of several lethal diseases. Further it is an opt platform not only to deliver small molecule drugs in combinational therapies but also peptides, proteins and biological. The day is not far ahead when insulin can also be delivered using nanosponge technology.

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