Recent Advances in Nanosponges as Drug Delivery System

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ABSTRACT

Major problem of many newly developed chemical entities is their poor solubility in water and pharmacokinetic issues. These poorly-water soluble drugs show many problems in formulating them in conventional dosage forms and the critical problem associated is its very low bio-availability. Nanotechnology has attracted increasing attention during recent years and it can resolve the problems associated with solubility and bio-availability. Nanosponges are a part of nanotechnology. Nanosponges delivery system, which was originally developed for topical delivery of drugs, can also be used for controlled oral delivery of drugs using water soluble and bioerodible polymers.

Nanosponges are tiny sponges with a size of about a virus, which can be filled with wide variety of drugs. These tiny sponges can circulate around the body until they encounter the specific target site and stick on the surface and begin to release the drug in a controlled and predictable manner. Because the drug can be released at the specific target site instead of circulating throughout the body it will be more effective for specific disease targeted treatment. Another important character of these sponges is their aqueous solubility; this allows the use of these systems effectively for drugs with poor solubility.

Key Words: Nanosponges; Topical drug delivery; Controlled release; Poor solubility; Biodegradable polymers.

Introduction

An ideal drug therapy achieves effective concentration of drug at the target site for a specified period of time in order to minimize general and local side effects. To obtain a desirable therapeutic response, the correct amount of drug should be transported and delivered to the site of action with subsequent control of drug input rate. The distribution of drug to other tissues therefore seems unnecessary, wasteful and a potential cause of toxicity. Targeted drug delivery is the delivery of drug to receptor, organ or any part of the body to which one wishes to deliver the drug exclusively.

Effective targeted drug delivery systems have been a dream for a long time now but it has been largely frustrated by the complex chemistry that is involved - how to get them to the right place in the body and how to control the release of the drug to prevent overdoses. The developments of new and complex molecules called nanosponges have the potential to solve these problems.

Nanosponges are a new class of tiny sponges that are about the size of a virus, filling them with a drug and attaching -special chemical “linkers” that bond preferentially to a feature found only on the surface of tumour cells and then injecting them into the body. These tiny sponges circulate around the body until they encounter the surface of a tumour cell where they stick on the surface (or are sucked into the cell) and begin releasing their potent drug in a controllable and predictable fashion.

Nanosponges (NS) are like a three-dimensional network or scaffold, whose backbone is a long length of polyester. It is mixed in solution with small molecules called cross-linkers that act like tiny grappling hooks to fasten different parts of the polymer together. The net effect is to form spherically shaped particles filled with cavities where drug molecules can be stored. The polyester is biodegradable, so it breaks down gradually in the body (David, 2011).

It is also possible to control the size of nanosponge particles. By varying the proportion of cross-linker to polymer, the nanosponge particles can be made larger or smaller. This is important because research has shown that drug delivery systems work best when they are smaller than 100nm, about the depth of the pits on the surface of a compact disc. The nanosponge particles used in the current study were 50 nm in size (David, 2011).

The simple chemistry of polymers and cross linkers does not pose many problems in the preparation and this technology can be easily ramp up to commercial production levels. Nanosponges are water soluble but does not breakup chemically in water. They mix with water and use as a transport fluid. They can be used to mask unpleasant flavours, to convert liquid substances to solids. The chemical linkers enable the nanosponges to bind preferentially to the target site.

Nanosponges are a new class materials made of microscopic particles with few nanometres in range where a large variety of substances can be encapsulated.
These particles are capable of carrying both lipophilic and hydrophilic substances and of improving the solubility of poorly water soluble molecules (Trotta et al., 2007). Nanosponges can be administered by different routes besides oral route which protect the degradable molecules like proteins and enzymes. Nanosponges are tiny mesh-like structures with nanoparticle sized system to deliver the drug payload, that may revolutionise the treatment of many diseases and early trials suggest this technology is up to five times more effective at delivering drugs for breast cancer than conventional methods (David F, 2011).

Predictable release is one of the major advantages of this system compared to other nanoparticle delivery systems under development. When they reach their target, many other nanoparticle delivery systems unload most of their drug in a rapid and uncontrollable fashion. This is called the burst effect and makes it difficult to determine effective dosage levels, whereas nanosponges when they reach their target site the drug is released in a predictable and controlled manner which helps to determine the effective dosage levels.

The nanosponges are encapsulating type of nanoparticles which encapsulates the drug molecules within its core. By the method of associating with drugs, the nanoparticles can be classified into:

1. **Encapsulating nanoparticles** These are represented by nanosponges and nanocapsules. Nanosponges such as alginate nanosponge, which are spongelike nanoparticles containing many holes that carry the drug molecules. Nanocapsules such as poly(isobutyl-cyanoacrylate) (IBCA) are also encapsulating nanoparticles. They can entrap drug molecules in their aqueous core

2. **Complexing nanoparticles** These nanoparticles attract the molecule by electrostatic charges.

3. **Conjugating nanoparticles** These nanoparticles linked to drug molecules through a strong covalent bond (Trotta et al., 2009).

Nanosponges represent a novel class of nanoparticles usually obtained by natural derivatives. As compared to the other nanoparticles, they are soluble both in water and organic solvents, porous, non-toxic and stable at high temperatures up to 300°C. Due to its 3D structure containing cavities of nanomeric size, tunable polarity and high solubility they are able to capture, transport and selectively release a wide variety of substances.

Furthermore, nanosponges show a remarkable advantage in comparison with the common nanoparticles: indeed, they can be easily regenerated by different treatments, such as washing with eco-compatible solvents, stripping with moderately inert hot gases, mild heating, or changing pH or ionic strength. For all these characteristics, nanosponges have been already employed in different applied fields, such as cosmetic and pharmaceutical sectors (Liang L et al., 2002).

The main disadvantage of these nanosponges is their ability to include only small molecules. The nanosponges could be either paracrystalline or in crystalline form. The loading capacity of nanosponges depends mainly on degree of crystallisation. Paracrystalline nanosponges can show different loading capacities.

These nanosponges can be magnetized when they are prepared in the presence of compounds having magnetic properties (Jenny A et al., 2011). The tiny shape of nanosponges enables the pulmonary and venous delivery of nanosponges (Trotta et al., 2007).

The list of polymers and cross linking agents used for the synthesis of nanosponges are presented in Table-1 (Selvamuthukumar et al., 2012).

**TABLE 1**

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Crosslinkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyper cross linked Polystyrenes,</td>
<td>Diphenyl Carbonate, Diarylcarbonyl-</td>
</tr>
<tr>
<td>Cycloexetrins and its derivatives</td>
<td>Carbonates, Di-isocyanates,</td>
</tr>
<tr>
<td>like Methyl β- Cycloexetrin,</td>
<td>Pyromellitic anhydride,</td>
</tr>
<tr>
<td>Alkoxycarbonyl-Cycloexetrins,</td>
<td>Carbonyl-di-Imidazoles,</td>
</tr>
<tr>
<td>2-Hydroxy Propyl β-Cycloexetrins</td>
<td>Epichlorhydrin, Glutaraldehyde,</td>
</tr>
<tr>
<td>and Copolymer like Poly</td>
<td>Carboxylic acid, Acid</td>
</tr>
<tr>
<td>(valerolactone -allylvalerolactone)</td>
<td>dihydrides, 2,2-bis(acrylamido)</td>
</tr>
<tr>
<td>(valerolactone -allylvalerolactone)</td>
<td>Acetic acid and Dichloromethane</td>
</tr>
</tbody>
</table>

**Advantages of Nanosponges drug delivery system**
- This technology offers entrapment of wide variety of ingredients and reduced side effects (Patel et al., 2012).
- Improved stability, increased elegance, and enhanced formulation flexibility.
- Nanosponges systems are non-irritating, non-mutagenic, non-allergenic, and non-toxic (Patel et al., 2012).
- Controlled release - continuous action for more than 12 hours.
- Reduces dosing interval and increases the patient compliance.
- Allows incorporation of immiscible liquids which improves material processing - liquid can be converted to powders (Khopade et al 1996; Patel et al., 2008)
- These formulations are stable over wide range of pH (1-11) and temperature (up to 130°C)
- These are self-sterilizing as their average pore size is 0.25μm where bacteria cannot penetrate.
- These are free flowing, highly compatible with wide variety of ingredients and cost effective.

**Preparation of Nanosponges**

1. **Emulsion solvent diffusion method**

Nanosponges can be prepared by using different proportions of ethyl cellulose and polyvinyl alcohol. The dispersed phase containing ethyl cellulose and drug was dissolved in 20ml dichloromethane and slowly added to a definite amount of polyvinyl
alcohol in 150ml of aqueous continuous phase. The reaction mixture was stirred at 1000 rpm for 2 hrs. The nanosponges formed were collected by filtration and dried in oven at 40°C for 24 hrs. The dried nanosponges were stored in vacuum desiccators to ensure the removal of residual solvent (Sharma et al., 2011).

2. Nanosponges prepared from hypercross-linked β-cyclodextrins
Nanosponges can be obtained by cross linking with different types of cyclodextrins (CD's) with a carbonyl or a dicarboxylate compound as a cross linker (Trotta et al., 2009). The ratio of CD's can be varied during preparation to improve the drug loading and to obtain a tailored release profile (Cavalli et al., 2006, Swaminathan et al., 2007; Vavia et al., 2006).

β-Cyclodextrin nanosponges were prepared as reported in the patent by Trotta and Tumiai (Trotta et al., 2003). 100 ml of Dimethyl Formamide (DMF) was placed in a round bottomed flask and 17.42g of anhydrous β-CD was added to achieve complete dissolution. Then 9.96 g of carbonyl di-imidazole (61.42mmol) was added and the solution allowed reacting for 4 hrs at 100°C. Once condensation polymerization was completed, the transparent block of hyper cross linked cyclodextrin was roughly ground and an excess of de-ionised water added to remove DMF. Finally residual by-products or unreacted reagents were completely removed by Soxhlet extraction with ethanol.

The white powder thus obtained was dried overnight in an oven at 60°C and subsequently ground in a mortar. The fine powder obtained was dispersed in water. The colloidal part that remained suspended in water was recovered and lyophilised. The obtained nanosponges are sub-micron in dimension and with a spherical shape.

3. Solvent method
Mix the polymer with a suitable solvent, in particular in a polar aprotic solvent such as dimethylformamide (DMF), dimethylsulfoxide (DMSO). Then add this mixture to excess quantity of the cross-linker, preferably in cross linker/polymer molar ratio of 4 to 16. Carry out the reaction at temperature ranging from 10°C to the reflux temperature of the solvent, for time ranging from 1 to 48hrs. Preferred cross linkers are carbonyl compounds (dimethyl carbonate and carbonyl diimidazole) (Jenny A et al., 2011).

After completion of the reaction, allow the solution to cool at room temperature, then add the product to large excess of distilled water and recover the product by filtration under vacuum and subsequently purify by prolonged Soxhlet extraction with ethanol. Dry the product under vacuum and grind in a mechanical mill to obtain homogeneous powder (Lala et al., 2011).

4. Ultrasound-Assisted synthesis
Nanosponges can be obtained by reacting polymers with cross-linkers in the absence of solvent and under sonication. The obtained nanosponges will be spherical, uniform in size and smaller than 5 microns (Trotta et al., 2007).

In this method di-phenyl carbonate (or)pyromellitic anhydride is used as cross-linker. An amount of anhydrous CD was put to react in melted di-phenyl carbonate at 90°C for at least 5 hrs. Then, the solid was ground in a mortar and soxhlet extracted with ethanol to remove either impurities (or) unreacted diphenyl carbonate. After purification nanosponges were stored at 25°C until further use (Trotta et al., 2007; Lala et al., 2011).

Loading of Drug into Nanosponge
Nanosponges for drug delivery should be pre-treated to obtain a mean particle size below 500nm. Suspend the nanosponges in water and sonicate to avoid the presence of aggregates and then centrifuge the suspension to obtain the colloidal fraction. Separate the supernatant and dry the sample by freeze drying (Lala et al., 2011).

Prepare the aqueous suspension of Nanosponge and disperse the excess amount of the drug and maintain the suspension under constant stirring for specific time required for complexation. After complexation, separate the uncomplexed (undissolved) drug from complexed drug by centrifugation. Then obtain the solid crystals of nanosponges by solvent evaporation or by freeze drying (Lala et al., 2011; Jenny et al., 2011).

Crystal structure of nanosponges plays a very important role in complexation with drug. A study revealed that paracrystalline nanosponges showed different loading capacities when compared to crystalline nanosponges. The drug loading is greater in crystalline nanosponges than paracrystalline one. In poorly crystalline nanosponges, the drug loading occurs as a mechanical mixture rather than inclusion complex (Shankar et al., 2010).

FACTORS INFLUENCING NANOSPONGES FORMULATION

Type of polymer
Type of polymer used can influence the formation as well as the performance of Nanosphges. For complexation, the cavity size of nanosponges should be suitable to accommodate a drug molecule of particular size (Amber et al., 2008).

Type of drug
Drug molecules to be complexed with nanosponges should have certain characteristics mentioned below (Amber et al., 2008).

- Molecular weight of drug should be in between 100 to 400 Daltons.
The structure of the drug molecule should contain no more than five condensed rings.
- Solubility in water should be less than 10mg/ml.
- Melting point of the substance should be less than 250°C.

**Temperature**

Temperature changes can affect drug/nanosponge complexation. In general, increase in the temperature decreases the magnitude of the apparent stability constant of the drug/nanosponge complex which may be due to a result of possible reduction of drug/nanosponge interaction forces, such as van-der Waal forces and hydrophobic forces with rise of temperature (Rajeswari et al., 2005).

**Method of preparation**

Method of preparation. The method of loading the drug into the nanosponges can affect drug/nanosponge complexation. However, the effectiveness of a method depends on the nature of the drug and polymer, in many cases freeze drying was found to be most effective method for drug complexation (Rajeswari et al., 2005).

Degree of substitution. The complexation ability of the nanosponges may be greatly affected by type, number and position of the substituent on the parent molecule (Rajeswari et al., 2005).

**Characterization of Nanosponges**

Inclusion complexes formed between the drug and nanosponges can be characterized by following methods:

Solubility studies. The method used to study inclusion complexation is the phase solubility method described by Higuchi and Connors, which examines the effect of a nanosponge on the solubility of drug. Phase solubility diagrams indicate the degree of complexation (Rajeswari et al., 2005; Jenny et al., 2011). In the solubility studies (Aithal et al., 1995; Wang et al., 2007) changes in solubility of the guest are plotted as a function of the cyclodextrin concentration, if the solubility of a potential guest increases with increasing cyclodextrin concentration; complex formation in solution is indicated. Solubility studies were performed to evaluate the effect of multicomponent complexation on drug solubility (Fouda et al., 2006; Ribeiro et al., 2007; Ramnik et al., 2010).

**Particle size and polydispersity**

The particle size can be determined by dynamic light scattering using 90 Plus particle sizer equipped with MAS OPTION particle sizing software. From this the mean diameter and polydispersity index can be determined (Shankar et al., 2010).

The polydispersity index (PDI) can also be measured from Dynamic light scattering Instruments. PDI is an index of width or spread or variation within the particle size distribution. Monodisperse samples have a lower PDI value, whereas higher value of PDI indicates a wider particle size distribution and the polydisperse nature of the sample. PDI can be calculated by the following equation:

$$\text{PDI} = \frac{\Delta d}{d_{avg}}$$

Where, $\Delta d$ is the width of distribution denoted as $SD$ and $d_{avg}$ is the average particle size denoted as $MV$ (nm) in particle size data sheet. (Wolfgang et al., 2007)

**Polydispersity index.**

<table>
<thead>
<tr>
<th>Polydispersity index</th>
<th>Type of dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 0.7</td>
<td>very polydisperse</td>
</tr>
<tr>
<td>0.08-0.7</td>
<td>mid range polydispersity</td>
</tr>
<tr>
<td>0.05-0.08</td>
<td>nearly monodisperse</td>
</tr>
<tr>
<td>0-0.05</td>
<td>monodisperse standard</td>
</tr>
</tbody>
</table>

**Zeta potential**

Zeta potential is a measure of surface charge. It can be measured by using additional electrode in the particle size equipment (Shankar et al., 2010).

**Microscopy studies**

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) can be used to study the microscopic aspects of the drug, nanosponges and the product (drug/nanosponge complex). The difference in crystallization state of the raw materials and the product seen under electron microscope indicates the formation of the inclusion complexes, even if there is a clear difference in crystallization state of the raw material and the product obtained by co-precipitation (Shankar et al., 2010; Ramnik et al., 2010; Sinha et al., 2005; Glomot et al., 1988).

**Thin Layer Chromatography**

In Thin Layer Chromatography, the Rf values of a drug molecule diminishes to considerable extent and this helps in identifying the complex formation between the drug and nanosponges (Ramnik et al., 2010 and Bekers et al 1991). Inclusion complexation between the guest and host molecules is a reversible process. Consequently, the complex may separate completely in guest and host molecules during the chromatographic process and only the spots of the guest and host molecules are found on the TLC-plate (Aithal et al., 1995).

**Infra-Red spectroscopy**

Infra-Red spectroscopy is used to estimate the interaction between nanosponges and the drug molecules in the solid state.

Nanosponge bands often change only slightly upon complex formation and if the fraction of the guest molecules encapsulated in the complex is less than 25%, bands which could be assigned to the included part of the guest molecules are easily masked by the bands of the spectrum of nanosponges (Uekama et al., 1982; Uekama et al., 1983). The technique is not generally suitable to detect the inclusion complexes and is less clarifying than other methods (Ramnik et al., 2010).
The application of the Infra-red spectroscopy is limited to the drugs having some characteristic bands, such as carbonyl or sulfonyl groups. Infrared spectral studies give information regarding the involvement of hydrogen in various functional groups. This generally shifts the absorbance bands to the lower frequency, increases the intensity and widens the band caused by stretching vibration of the group involved in the formation of the hydrogen bonds. Hydrogen bond at the hydroxyl group causes the largest shift of the stretching vibration band (Ramnik et al., 2010).

Thermo-analytical methods

Thermo-analytical methods (Duchene, 1988; Erdem, 1988) determine whether the drug substance undergoes some change before the thermal degradation of the nanosponges. The change of the drug substance may be melting, evaporation, decomposition, oxidation or polymorphic transition. The change of the drug substance indicates the complex formation. The thermo gram obtained by DTA and DSC can be observed for broadening, shifting and appearance of new peaks or disappearance of certain peaks. Changes in the weight loss also can provide supporting evidence for the formation of inclusion complexes (Ramnik et al., 2010). The nature of the drug and cyclodextrin used and method of preparation of complex have been found to influence the above finding considerably. If the interaction between the drug and the excipient is weak, the shift in the endothermic peak is very small.

X-ray diffractionometry and single crystal X-ray structure analysis

Powder X-ray diffractionometry can be used to detect inclusion complexation in the solid state. When the drug molecule is liquid since liquid have no diffraction pattern of their own, then the diffraction pattern of a newly formed substance clearly differs from that of uncomplexed nanosponges. This difference of diffraction pattern indicates the complex formation. When the drug compound is a solid substance, a comparison has to be made between the diffractogram of the assumed complex and that of the mechanical mixture of the drug and polymer molecules (Ramnik et al., 2010).

A diffraction pattern of a physical mixture is often the sum of those of each component, while the diffraction pattern of complexes are apparently different from each constituent and lead to a “new” solid phase with different diffractograms. Diffraction peaks for a mixture of compounds are useful in determining the chemical decomposition and complex formation (Ramnik et al., 2010).

The complex formation of drug with nanosponges alters the diffraction patterns and also changes the crystalline nature of the drug. The complex formation leads to the sharpening of the existing peaks, appearance of a few new peaks and shifting of certain peaks (Ramnik et al., 2010).

Single crystal X-ray structure analysis

This method used to determine the detailed inclusion structure and mode of interaction. The interaction between the host and guest molecules can be identified and the precise geometrical relationship can be established. This information obtained during the analysis lead to know about the formation of inclusion complexes (Ramnik et al., 2010; Tayade et al., 2006; Jadhav et al., 2008).

Loading efficiency

The loading efficiency of nanosponges can be determined by the quantitative estimation of drug loaded into nanosponges by UV spectrophotometer & HPLC methods.

In-vitro drug release study

Drug release from the Nanosponges can be measured across the dialysis membrane using Franz Diffusion cell with a diffusional area of 2.26 cm² and receptor volume of 11ml. The dialysis membrane soaked in receptor medium for 8 hrs is used as a barrier between the donor and receptor compartment. A one gram nanosponge was placed on the membrane surface in the donor compartment that was sealed from the atmosphere with aluminium foil. The receptor compartment was filled with 11ml of phosphate buffer of pH 6.8 (skin pH). During the experiment, the solution of receptor side compartment was kept at 37±0.5°C and stirred at 100 rpm with Teflon-coated magnetic stirring bars. Aliquots were collected from the receptor compartment at designated time intervals and replaced by the same volume of fresh receptor solution to maintain sink condition and constant volume. The sample was analysed using UV spectrophotometer (Renuka et al., 2010).

Drug release kinetics

To investigate the mechanism of drug release from the Nanosponge the release data was analysed using Zero order, First order, Higuchi, Peppas, Hixon Crowell, Kopcha and Makoid-Banakar models. The data can be analysed using graph pad prism software. The software estimates the parameters of a non-linear function that provides the closest fit between experimental observations and non-linear function (Renuka et al., 2010). The mathematical expressions that describe the dissolution curves are summarized in table3 (Renuka et al., 2010).

| zero order | Qt = Q0 + K0 t |
| first order | Qt = Q0 + K1 t |
| higuchi | Qt = K0 t 1/2 |
| Korsemeyerpeppas model | Qt = K0 t^0.4 |
| Kopcha model | Qt = At^0.5 + Bt |
| Makoid-bankar model | Qt = Kuit^0.5 e^-at |

TABLE 3

The mathematical expressions of dissolution curves.
Applications of Nanosponges

Nanosponges for drug delivery

Because of their nanoporous structure, nanosponges can advantageously carry water insoluble drugs (Biopharmaceutical Classification System class-II drugs). These complexes can be used to increase the dissolution rate, solubility and stability of drugs, to mask unpleasant flavours and to convert liquid substances to solids. β-Cyclodextrin based nanosponges are reported to deliver the drug to the target site three to five times more effectively than direct injection (David F, 2011).

Drugs which are particularly critical for formulation in terms of their solubility can be successfully delivered by loading into the nanosponges. List of some BCS Class II drugs that can be developed as nanosponges are given in Table 4 (Leslie et al., 2007).

The nanosponges are solid in nature and can be formulated as Oral, Parenteral, Topical or Inhalation dosage forms. For the oral administration, the complexes may be dispersed in a matrix of excipients, diluents, lubricants and anticaking agents suitable for the preparation of capsules or tablets (Jenny et al., 2011). For the parenteral administration the complex may be simply carried in sterile water, saline or other aqueous solutions. For topical administration they can be effectively incorporated into topical hydrogel (Renuka et al., 2011; Renuka et al., 2011). The nanosponges used in the formulation of some drugs are provided in the Table 5.

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Examples of nanosponges.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Nanosponge vehicle</td>
</tr>
<tr>
<td>Antisense Oligonucleotides (Isabelle et al., 1999)</td>
<td>Sodium alginate Poly L-lysine</td>
</tr>
<tr>
<td>Bovine serum albumin (Swaminathan et al., 2010)</td>
<td>Cycloextrin based poly (amidoamine)</td>
</tr>
<tr>
<td>Camptothecin (Shankar et al., 2010; Rosalba et al., 2011)</td>
<td>β-Cyclodextrin</td>
</tr>
<tr>
<td>Dexamethasone (Lala et al., 2011)</td>
<td>β-Cyclodextrin</td>
</tr>
<tr>
<td>Econazole nitrate (Renuka et al., 2011)</td>
<td>Ethyl cellulose Polyvinyl alcohol</td>
</tr>
<tr>
<td>Itraconazole (Shankar et al., 2007)</td>
<td>β-Cyclodextrin &amp; copolyvidonum</td>
</tr>
<tr>
<td>Paclitaxel (Torne et al., 2010; Ansari et al., 2011)</td>
<td>β-Cyclodextrin</td>
</tr>
</tbody>
</table>

Table 5 Contd...
Nanosponges as a carrier for biocatalysts

Nanosponges act as carriers in the delivery of enzymes, proteins, vaccines and antibodies. Many industrial processes involving chemical transformation are associated with operational disadvantages. Non-specific reactions lead to low yields, and the frequent need to operate at high temperatures and pressures requires consumption of large amounts of energy, and very large amounts of cooling water in the down-stream process. All these drawbacks can be eliminated or significantly reduced by using enzymes as biocatalysts. These enzymes operate under mild reaction conditions, have high reaction speed, and are highly specific. They have a beneficial effect on the environment because they reduce energy consumption and reduce production of pollutants. Developments in genetic engineering have increased the stability, economy, specificity of enzymes and number of their industrial applications is continually increasing.

**Examples.** Examples of industrially useful enzymes include alpha amylase, trypsin, cellulase and pectinase for fruit juice clarification processes, ligninase to breakdown lignin, lipase in the detergent industry and biodiesel production, etc. The catalytic activity of enzyme depends mainly on the correct orientation of the active site (Amber et al., 2008).

Proteins, peptides, enzymes and derivatives thereof also can be used in the biomedical and therapeutic field. Proteolytic enzymes can be used to treat cancer or type I mucopolysaccharidosis, while DNA and oligonucleotides are used in gene therapy. The administration of these molecules presents various problems and limitations. Most protein drugs are poorly absorbed through the biological membranes due to the some factors such as large molecular size, hydrophilic nature, degree of ionization, high surface charge, chemical and enzymatic instability and low permeability through mucous membranes. Following intravenous administration, protein molecules may be rapidly cleared from blood, bind to plasma proteins, and sensitive towards Proteolytic enzymes. With oral administration bioavailability is the problem. Various approaches exist for therapeutic use, such as increasing the dose or using absorption promoters, which can cause toxicity problems (Amber et al., 2008).

A number of systems for carrying enzymes and proteins have been developed, such as nano and micro particles, liposomes and hydrogels. Carriage in a particular system can protect proteins from breakdown, modify their pharmacokinetics and improve their stability in vivo. Now, it has been found that cyclodextrin based nanosponges are particularly suitable carrier to adsorb proteins, enzymes, antibodies and macromolecules. In particular when enzymes are used, it is possible to maintain their activity, efficiency, prolong their operation and extends the pH and temperature range of activity and allows the conduct of continuous flow processes. Moreover, proteins and other macromolecules can be carried by adsorbing or encapsulating them in cyclodextrin nanosponges (Amber et al., 2008).

**Cancer Therapy**

**Nanosponges Carrying Anticancer Drugs Effectively Slow Tumour Growth**

Researchers at Vanderbilt University have developed “nanosponges” which can be used as a delivery system for anticancer drugs to tumors. They claim that the method is three to five times more effective at reducing tumor growth than direct injection of the drugs. The tiny nanosponges are filled with a drug load and expose a targeting peptide that binds to radiation-induced cell surface receptors on the tumor. When the sponges encounter tumor cells they stick to the surface and are triggered to release their cargo. Benefits of targeted drug delivery include more effective treatment at the same dose and fewer side-effects. Studies so far have been carried out in animals with paclitaxel as the sponge load. Camptothecin, a plant alkaloid and a potent antitumor agent, has a limited therapeutic utility because of its poor aqueous solubility, lactone ring instability and serious side effects. Cyclodextrin-based nanosponges are a novel class of cross-linked derivatives of cyclodextrin. They have been used to increase the solubility of poorly soluble actives, to protect the labile groups and control the release. This study aimed at formulating complexes of Camptothecin with βcyclodextrin based nanosponges (Swaminathan et al., 2010).
**Treatment for Fungal Infections**

Econazole nitrate, an antifungal used topically to relieve the symptoms of superficial candidiasis, dermatophytosis, versicolor and skin infections available in cream, ointment, lotion and solution. Adsorption is not significant when econazole nitrate is applied to skin and required high concentration of active agents to be incorporated for effective therapy. Thus Econazole nitrate nanosponges were fabricated by emulsion solvent diffusion method and these nanosponges were loaded in hydrogel as a local depot for sustained drug release (Sharma et al., 2011).

Itraconazole is a BCS class-II drug that has a dissolution rate limited poor bioavailability. Rationale of the work was to enhance the solubility of itraconazole so that the bioavailability problem was solved. In this nanosponges of β-cyclodextrin cross linked with carbonate bonds were prepared and loaded with itraconazole so that its solubility was increased. (Swaminathan et al., 2007)

**Nanosponges in Proteins Delivery**

Bovine serum albumin (BSA) protein in solution is not stable; it is stored in lyophilized state. However proteins can reversibly denature on lyophilisation and adopts conformation markedly different from native structure. Major drawback in protein formulation and development is to maintain its native structure during processing and long term storage. In the nanosponges based approach protein like BSA are encapsulated in swell able cyclodextrin based poly (amidoamine) nanosponges to increase the stability of proteins (Swaminathan et al., 2010).

**Other applications of Nanosponges**

**Purification of water**

Cyclodextrin “Nanosponges” can be used for the removal of organic pollutants from water. β-Cyclodextrin nanosponges are completely insoluble in water, have the property of encapsulating organic pollutants from water. Ceramic porous filters can be impregnated with these Nanosponges resulting in hybrid organic/inorganic filter modules. These hybrid filter modules were tested for the effective purification of water, employing a variety of water pollutants. It has been established that polycyclic aromatic hydrocarbons (PAHs) can be removed very efficiently (more than 95%). Representatives of the pollutant group of trihalogenmethanes (THMs), monoaromatic hydrocarbons (BTX), and pesticides (simazine) can also be removed (>80%) (Arkas et al., 2006).

Nanosponges based on cyclodextrin can strongly bind organic molecules and remove them from water even at very low concentrations (Trotta et al., 2003).

**Encapsulation of gases**

Cyclodextrin based carbonate nanosponges were used to form inclusion complexes with three different gases i.e. 1-methylcyclop propane, oxygen and carbon dioxide. The complexation of oxygen or carbon dioxide could be useful for many biomedical applications. In particular the oxygen-filled nanosponges could supply oxygen to the hypoxic tissues which are present in various diseases (Trotta et al., 2011).

**Bio medical Applications**

The oxygen-filled Nanosponges could supply oxygen to the hypoxic tissues which are present in various diseases (Cavalli et al., 2010).

Nanosponges can selectively soak up biomarkers for the diagnosis. One study concluded that Nanosponges can harvest rare cancer marker from blood (Longo et al., 2011).

Nanosponges can be used as carrier for gases like oxygen and carbon dioxide.

**Analytical Applications**

The Micro porous hyper cross linked Nanosponges have been used in selective separation of inorganic electrolytes by size exclusion chromatography. The three dimensional nanosponges will play important role in the fractionalization of peptides for proteomic applications (Wong et al., 2009).

**Food Industry**

Nanosponges are useful for elimination of bitter components from grape fruit juice by selective combination of polymer and cross linker.

**Nanosponges for Storage of Hydrogen**

Hydrogen is considered as an alternative energy for the future, but one of the problems to be solved before it achieves the versatility of other fuel sources as oil is how to store it. Recent studies claim to find materials that could act as sponges that absorb hydrogen and store it until ready to use. But until now had not found a material with the capability to store hydrogen under the necessary pressure and temperature.

A team of scientists from the Universities of Newcastle and Liverpool have discovered a new class of materials which composed of long carbon chains linked by metal atoms. To crystallize, these molecules form cavities that are less than a nanometer, which are connected by “windows” that are even smaller than a molecule of hydrogen.

While these cavities are filled, hydrogen fits through the windows, because the carbon chains are flexible. But once filled the cavities, the chains lose their flexibility, thus closing the windows. Consequently, it can be loading the high-pressure hydrogen gas, and when pressure levels drop, forming a sort of molecular size seal.

Although so far the materials created by this team of scientists do not have enough capacity for most applications that use fuel cells, their work represents a new approach to the problem, and nanosponges could potentially have a key role in the hydrogen storage system.
Nanosponges for Intelligent Agriculture

Plants that grow more have a better appearance. What counts is not just the climate, but technology. This is so for functionalized nanosponges (FNS), an agricultural invention that allows plants to grow more and improve their appearance by feeding them with an optimal dosage of micro-nutrients and active ingredients that are necessary for healthy growth. Another notable advantage is that nanosponges allow a significant reduction in the use of herbicides and fertilizers, thereby increasing productivity and improving both the environmental and cultivation quality levels.

Nano-sponges are porous macro-molecules with nano-cavities (hence the name) synthesised using a completely natural product: starch. Nutritive substances (such as iron and zinc), or active ingredients, are encapsulated in the nano-cavities during the synthesis process. One of the big advantages of this innovative product is the possibility of making ad-hoc formulations for different types of applications.

The nutritive substances incorporated in the nanosponges are dosed and fed to the plants in a very precise manner, “drop by drop”, thereby optimising photosynthesis. The significant reduction in the use of fertilisers makes their cultivation similar to that of organic products, although production levels are much higher. This means lower production costs and access to healthier food for many more people.

For example, FNSs with iron solve one of the most common problems for plants: iron chlorosis (yellowing of the leaves), thereby allowing a more efficient photosynthesis conversion and higher plant growth rate (with a production yield up to 20% or more of the dry weight), compared to plants treated with products that are currently on the market. Also, flowers that are cut off have a significantly longer duration in terms of their appearance compared to those treated with products that are currently on the market.

Nanosponge in Oil Cleaning

Nanotech allows for the creation of new materials with unique and enhanced properties, and has specific implications for the electronics and biomedical industries.

One of the latest nanotech discoveries came through researchers at Rice University and Penn State. They found that adding boron to carbon during nanotube construction creates spongy blocks that have amazing oil absorbing properties.

The nanosponges are extremely hydrophobic, giving it the natural tendency to float on water and not absorb it even when submerged. It is also ferromagnetic, meaning it can be controlled or retrieved using a magnet. The density of the material is extremely low, making the available volume for oil uptake very high. Not only can it soak up over 100 times its weight in oil as it floats on the water, but it can store the oil for later retrieval. The oil can then be squeezed out or burned off, allowing the sponge to be reused. The researchers also tested the sponge’s robustness and reusability in the lab - it maintained elasticity even after 10,000 compressions. Safe to say, this material has tremendous power as an agent for surface oil clean-up.

Conclusions

From the above study it is concluded that the Nanosponges have the capability to include either lipophilic or hydrophilic drugs and release them in a controlled and predictable manner at the target site. Nanosponges can be incorporated into a formulated product such as a gel, lotions, cream, ointments, liquid or powder. This technology offers entrapment of ingredients and thus reduced side effects, improved stability, increases elegance and enhanced formulation flexibility. Nanosponges can be effectively incorporated into topical drug delivery system for retention of dosage form on skin, and also use for oral delivery of drugs using bioerodiblepolymers, especially for colon specific delivery and controlled release drug delivery system thus improving patient compliance by providing site specific drug delivery system and prolonging dosage intervals.

Future Opportunities and Challenges

Nanoparticles and nanoformulations have already been applied as drug delivery systems with great success; and nanoparticulate drug delivery systems have still greater potential for many applications, including anti-tumour, gene, AIDS and radiotherapy in the delivery of proteins, antibiotics, virostatics, and vaccines as vesicles to pass the blood - brain barrier.

Nanoparticles provide massive advantages regarding drug targeting, delivery, and release along with their additional potential to combine diagnosis and therapy, emerged as one of the major tools in nanomedicine. The main goals are to improve their stability in the biological environment, to mediate the bio-distribution of active compounds, improve drug loading, targeting, transport, release, and interaction with biological barriers. The cytotoxicity of nanoparticles or their degradation products remains a major problem, and improvements in biocompatibility obviously are a main concern of future research.

There are many technological challenges to be met, in developing the following techniques:

- Nano-drug delivery systems that deliver large but highly localized quantities of drugs to specific areas to be released in controlled ways;
- Controllable release profiles, especially for sensitive drugs;
- Materials for nanoparticles that are biocompatible and biodegradable;
- Architectures / structures, such as bio mimetic polymers, nanotubes;
- Technologies for self-assembly;
- Functions (active drug targeting, on-command delivery, intelligent drug release devices/ bioresponsive triggered systems, self-regulated
delivery systems, systems interacting with the body, smart delivery;

- Virus-like systems for intracellular delivery;
- Nanoparticles to improve devices such as implantable devices/nanochips for nanoparticle release, or multi reservoir drug delivery-chips;
- Nanoparticles for tissue engineering e.g. for the delivery of cytokines to control cellular growth and differentiation, and stimulate regeneration or for coating implants with nanoparticles in biodegradable polymer layers for sustained release;

References


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