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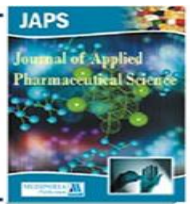

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AQUASOMES: A NOVEL DRUG CARRIER

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ABSTRACT

Aquasomes are one of the most recently developed delivery system for bioactive molecules like peptide, protein, hormones, antigens and genes to specific sites. Aquasomes are spherical in shape with 60–300 nm particles size. These are nanoparticulate carrier systems but instead of being simple nanoparticles these are three layered self assembled structures, comprised of a solid phase nanocrystalline core coated with oligomeric film to which biochemically active molecules are adsorbed with or without modification. These structures are self assembled by non covalent and ionic bonds. The solid core provides the structural stability, while the carbohydrate coating protects against dehydration and stabilizes the biochemically active molecules. The delivery system has been successfully utilized for the delivery of insulin, hemoglobin, and enzymes like serratiopeptidase etc. This reviews the principles of self assembly, the challenges of maintaining the conformational integrity and biochemical activity of immobilized surface pairs, the convergence of these principles into a single functional composition and its application in various fields of pharmacy.

Keywords: Bioactive molecules, Nanoparticulate carrier system, Oligomeric film, Carbohydrate, Self assembly, Conformational integrity

INTRODUCTION

Within the last decade diverse technological strategies have been proposed in order to obtain nanoparticles of a distinct nature, charged with drugs which in turn have revolutionized the systems of drug administration, particularly those of controlled release and the ones oriented at the vectoring of the active principle for release at target tissue or organs. Various methods used for the preparation of nanoparticles use polymers and encounter difficulties such as the compatibility of solvents and other constituents and the polymers and co-polymers with the active principle and biological fluids and factors of the collection system (Oviedo *et al.*, 2007). Kossovsky proposed a system to prepare nanoparticles transporting the so-called aquasomes (Kossovsky *et al.*, 1995), whose particle size (lower than 1000 nm), is appropriate to parenteral administration because it prevents the obstruction into the bloodstream capillaries (Banker and Rhodes, 1990).

Aquasomes are nanoparticulate carrier system but instead of being simple nanoparticle these are three layered self assembled structures, comprised of a solid phase nanocrystalline core coated with oligomeric film on which biochemically active molecules are adsorbed with or without modification.

Aquasomes are like “bodies of water" and their water like properties protect and preserve fragile biological molecules, and this property of maintaining conformational integrity as well as high degree of surface exposure is exploited in targeting of bio-active molecules like peptide and protein hormones, enzymes, antigens and genes to specific sites. These three layered structures are self-assembled by non covalent and ionic bonds. These carbohydrate stabilize nanoparticles of ceramic are known as “aquasomes”. The pharmacologically active molecule incorporated by co-polymerization, diffusion or adsorption to carbohydrate surface of pre formed nanoparticles. Aquasomes discovery comprises a principle from microbiology, food chemistry, biophysics and many discoveries including solid phase synthesis, supramolecular chemistry, molecular shape change and self assembly (Jain *et al.*, 2001).

Principle of self assembly

Self assembly implies that the constituent parts of some final product assume spontaneously prescribed structural orientations in two or three dimensional space. The self assembly of macromolecules in the aqueous environment, either for the purpose of creating smart nanostructure materials or in the course of naturally occurring biochemistry, is governed basically by three physicochemical processes: the interactions of charged groups, dehydration effects and structural stability.

I- Interaction between charged groups

The interaction of charged groups, such as amino, carboxyl, sulphate, phosphate groups facilitates long range approach of self assembly sub units. Charged group also plays a role in stabilizing tertiary structures of folded proteins.

II- Hydrogen bonding and dehydration effect

Hydrogen bond helps in base pair matching and stabilization of secondary protein structure such as alpha helices and beta sheets. Molecules forming hydrogen bonds are hydrophilic and this confers a significant degree of organization to surrounding water molecules. In case of hydrophobic molecules, which are incapable of forming hydrogen bond. However, their tendency to repel water helps to organize the moiety to surrounding environment. The organized water decreases the overall level of disorder/ entropy of the surrounding medium. Since, organized water is thermodynamically unfavorable, the molecule loose water/dehydrate and get self assembled (Kossovsky *et al*., 1994).

III- Structural stability

Molecules that carry less charge than formally charged groups exhibit a dipole moment. The forces associated with dipoles are known as van der waals forces. Structural stability of protein in biological environment determined by interaction between charged group and hydrogen bonds largely external to molecule and by van der waals forces largely internal to molecule. The Vander Waals forces, most often experienced by hydrophobic molecular regions that are shielded from water play a subtle but critical role in maintaining molecular shape or conformation during self-assembly. The van der waals forces are largely responsible for hardness or softness of molecules. The van der waals interaction among hydrophobic side chain promotes stability of compact helical structures which are thermodynamically unfavorable for expanded random coils. It is the maintenance of internal secondary structures, such as helices which provides sufficient softness, and allows maintenance of conformation during self assembly, small changes are necessary for successful antigen- antibody interactions. In biotechnological self-assembly, this can lead to altered molecular function and biological activity. Thus, the van der waals need to be buffered for maintaining the optimal biological activity. In case of aquasomes, sugars help in molecular plasticization (Jain *et al.*, 2001).

Strategies used in chemical synthesis of nanostructure

Aquasomes are self-assembled three layered nanostructures. Therefore the strategies involved in chemical synthesis of nanostructure need elaboration. The strategies normally used in the chemical synthesis of nanostructures are discussed below.

I- Sequential covalent synthesis

This can be used to generate arrays of co-valently linked atoms generated with well defined composition, connectivity and shape i.e. vitamin B12. It can generate the structures that are far from the thermodynamic minimum for that collection of atoms (Frankel *et al*., 1989).

II- Covalent polymerization

This strategy is used for preparing molecules with high molecular weight. Here a relatively simple low weight substance is allowed to react with itself to produce molecule comprising many covalently linked monomers. For example: Formation of polyethylene from ethylene. The molecular weight of polyethylene can be high (>106 Daltons), and it is easily prepared, but the molecular structure is simple and repetitive and the process by which it is formed offers only limited opportunity for controlled variation in the structure or for control of its three dimensional shape. Polymerization indirectly provides synthetic routes to stable nanostructures e.g. phase separated polymers (Crowe *et al*., 1983).

III- Self –organizing synthesis

This strategy abandons the covalent bond as required connection between atoms and relies instead on weaker and less directional bonds such as ionic, hydrogen and van der waals interactions to organize atoms, ions or molecules into structures. The different type of structures prepared by this stratergy includes molecular crystals, ligand crystals, colloids, micelles, emulsions, phase separated polymers and self assembled monolayer. Self organization is the peculiar feature of these methods. The molecules or ions adjust their own position to reach thermodynamic minimum. By self-organization, true nanostructures can be prepared (Haberland *et al.*, 1992).

IV- Molecular self assembly

It is the spontaneous assembly of molecules into structured, stable, non-covalently joined aggregates. Molecular self-assembly combines features of each preceding strategies to make large structurally well defined assemblies of atoms:

Formation of well defined molecules of intermediate structural complexity through sequential covalent synthesis.

Formation of large, stable structurally defined aggregates of these molecules through ionic, hydrogen and van der waals interactions or other non covalent links.

Use of multiple copies of one or several of the constituent molecules or of a polymer, to simplify the synthetic task. The key to this type of synthesis is to understand and overcome intrinsically unfavorable entropy together in a single aggregate.

For final assembly to be stable and to have well defined shape, the non covalent connection between molecules must be stable. The strength of the individual Vander waals interactions and hydrogen bonds are weak (0.1 to 5 Kcal/mole) relative to typical covalent bonds (40 to 100 Kcal/mole) and comparable to thermal energies. Thus to achieve acceptable stability, molecules in self assembled aggregates must be joined by many of these weak non-covalent interaction or by multiple hydrogen bonds or both (Jain *et al*., 2001).

Objective behind development of aquasomes:

Firstly, aquasomes protect bio-actives. Many other carriers like prodrugs and liposomes utilized but these are prone to destructive interactions between drug and carrier. The drugs are often inevitable and these always bring limitation to drug delivery system. In such case aquasomes proof to be worthy carrier, which are comprised of solid carriers whose film has been treated with a film of carbohydrate to prevent destructive denaturing interaction between drug and solid carriers (Bryan *et al.*, 1994). Secondly aquasomes maintains molecular confirmation and optimum pharmacological activity. Normally, active molecules possess following qualities i.e. a unique three-dimensional conformation, a freedom of internal molecular rearrangement induced by molecular interactions and a freedom of bulk movement. This is to be maintained for optimal pharmacological activity. Dehydration, degradation and decomposition can change these spatial qualities. Many of the biological molecules like proteins undergo irreversible denaturation and become non functional when desiccated, at the same time, they are not resistant to denaturation for a long time in aqueous state. In the aqueous state pH, temperature, solvents, salts etc can cause denaturation. So the challenge is to maintain water like circumstance otherwise it may lead to dehydration and conformational changes, which in turn lead to degradation and alteration of chemical composition. The intrinsic biophysical constraints, dehydration and conformational changes caused by the drug delivery system can lead to adverse or allergic reaction with suboptimal pharmacological activity. By incorporating such biological molecules on aquasomes with natural stabilizers one can preserve the molecular conformation since these natural sugar acts as dehydroprotectant. Sugars and polyols stabilize protein against heat denaturation and stabilization is due to the effect of sugars and polyols on hydrophobic interactions. The extent of stabilization by different sugars and polyols is explained by different influences on structure of water. The hydroxyl group on carbohydrate interacts with polar and charged groups of biological molecules in a manner similar to water molecules alone and preserves the aqueous structure of biological molecules like protein on dehydration. Since these disaccharides are rich in hydroxyl groups and help to replace water around the polar residues in proteins, thus maintaining their integrity in the absence of water. The free mobility associated with rich hydroxyl component creates a unique hydrogen binding subsrate that produces glassy aqueous state (Dunitz J D *et al*., 1994, Franks F *et al*., 1994). There are many systemic biophysical and intrinsic biophysical constraints, which tend to destabilize the drug.

Systemic biophysical constraints

There are physical and chemical degradative agents, which cause compositional changes and loss of spatial activity by breaking chemical bonds in the drug candidate. Such agents include UV radiation, heat, ozone, peroxide and other free radicals. Likewise mammalian body also contains certain agents viz. inflammatory, peroxides, free radicals and degradative enzymes related to serine proteases. Other than these physical and chemical degradative agents, those agents that promote dehydration also cause molecular inactivation. Since water is critical structural component of most biochemically reactive molecules, its loss leads to change in energies and results in altered molecular conformation and impaired spatial qualities. Exposure and surface immobilization often promotes dehydration. Degradative agents present in mammals can destroy rapidly complex and expensive polypeptide biopharmaceuticals, while denaturation during dehydration can impair polypeptides on long term storage (Norde and Lyklema *et al.*, 1992).

Intrinsic biophysical constraints

The intrinsic biophysical constraint is normally posed by drug delivery system. When drug candidates are immobilized to nanoparticulate substrate, it can cause surface induced dehydration and, in turn molecular conformation. The altered molecular conformation can produce adverse or allergic reaction with suboptimal pharmacological activity. In short, biochemically active molecules lose their functional properties in either case, means in a ‘dry’ or ‘wet’ state. At the same time, a water environment is vital for molecular activity. Therefore, the challenge is to store and transport promising and useful biomolecules in the dry state without causing them to lose too much of their potential activity. In such case, aquasomes with natural stabilizers like various polyhydroxy sugars act as dehydroprotectant, maintains water like state and thereby helps to preserves the molecular conformation of bioactive molecules in dry solid state. Fungal spores producing ergot alkaloids were stabilized by sucrose rich solution. Desiccation induced molecular denaturation is reported to be prevented by certain disaccharides (Crowe *et al*., 1988).

Composition of aquasomes

I- Core material

Ceramic and polymers are most widely used core materials. Polymers such as albumin, gelatin or acrylate are used. Ceramic such as diamond particles, brushite (calcium phosphate) and tin oxide are used.

II- Coating material

Coating materials commonly used are cellobiose, pyridoxal 5 phosphate, sucrose, trehalose, chitosan, citrate etc. Carbohydrate plays important role act as natural stabilizer, its stabilization efficiency has been reported. Beginning with preformed carbon ceramic nanoparticle and self assembled calcium phosphate dihydrate particles (colloidal precipitation) to which glassy carbohydrate are then allowed to adsorb as a nanometer thick surface coating a molecular carrier is formed.

III- Bioactive

They have the property of interacting with film via non covalent and ionic interactions (Cherian *et al*., 2000).

Role of disaccharides

The hydroxyl group on carbohydrate interacts with polar and charged groups on the proteins, in a similar manner to water molecules alone and preserve the aqueous structure of proteins on dehydration. Disaccharides such as trehalose are reported to have stress tolerance in fungi, bacteria, insects, yeast and some plants. Trehalose works by protecting proteins and membranes within plant cell during the desiccation process and thereby preserves cell structures, inherent flavors, colors and textures. These disaccharides rich in hydroxyl group and help to replace the water around polar residues in proteins, thereby maintaining their integrity in the absence of water.

The studies indicated that the structure and function of cellular components could be protected by sugar during lyophilization, were conducted with Ca-transporting microsomes isolated from rabbit muscles and lobster muscles. When Ca-transporting microsomes were lyophilized without stabilizing sugar, the rehydrated vesicles shows greatly reduced Ca-uptake and uncoupling of ATPase activity. Vesicles lyophilized in presence of as little as 0.3 g. Of trehalose per g. membrane upon rehydration are morphologically distinguishable from freshly prepared vesicles (Crowe *et al.*, 1983, 1984).

Among three layers of aquasomes, carbohydrate fulfills the objective of aquasomes. The hydroxyl groups on oligomer interact with polar and charged groups of proteins, in a same way as with water thus preserve the aqueous structure of proteins on dehydration. The most commonly used carbohydrates are cellobiose, pyridoxal-5-phosphate, trehalose, sucrose, citrates etc (Jain *et al*., 2001).

Properties of aquasomes

Aquasome possess large size and active surface hence can be efficiently loaded with substantial amounts of agents through ionic, non co-valent bonds, van der waals forces and entropic forces. As solid particles dispersed in aqueous environment, exhibit physical properties of colloids.

Aquasome mechanism of action is controlled by their surface chemistry. Aquasomes deliver contents through combination of specific targeting, molecular shielding, and slow and sustained release process.

Aquasome water like properties provides a platform for preserving the conformational integrity and bio chemical stability of bio-actives.

Aquasomes due to their size and structural stability, avoid clearance by reticuloendothelial system or degradation by other environmental challenges.

Aquasome is colloidal range biodegradable nanoparticles, so that they will be more concentrated in liver and muscles. Since the drug is absorbed on to the surface of the system without further surface modification as in case of insulin and antigen delivery, they may not find any difficulty in receptor recognition on the active site so that the pharmocological or biological activity can be achieved immediately

In normal system, calcium phosphate is biodegradable. Biodegradation in vivo achieved by monocytes and multicellular cells called osteoclast. Two types of phagocytosis reported, either crystals taken up alone and then dissolved in cytoplasm after disappearance of phagosome membrane or dissolution after formation of heterophagosome.

Aquasomes are mainly characterized for structural analysis, particle size, and morphology. These are evaluated by X-ray powder diffractometry, transmission electron microscopy, and scanning electron microscopy. (Vays *et al.*, 2004)

Method of preparation of aquasomes

The method of preparation of aquasomes involves three steps. The general procedure consists of Formation of an inorganic core, followed by Coating of the core with polyhydroxy oligomer, and finally loading of the drug of choice to this assembly (Kossovsky *et al.*, 1990, Kossovsky *et al.*, 1991, Kossovsky *et al.*, 1994a).

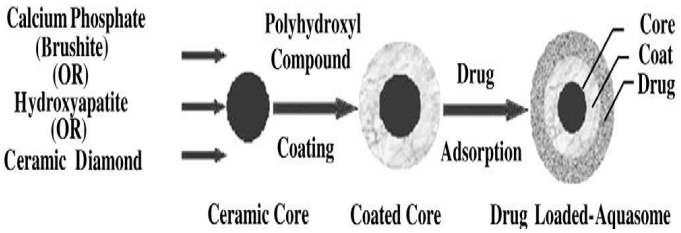

Fig. 1: Method of Preparation of Aquasomes.

I- Formation of an inorganic core

It involves the fabrication of a ceramic core, and the procedure depends upon the materials selected. The two most commonly used ceramic cores are calcium phosphate and diamond.

a) Synthesis of nanocrystalline tin oxide core ceramic

It can be synthesized by direct current reactive magnetron sputtering. Here, a 3 inches diameter target of high purity tin is

sputtered in a high pressure gas mixture of orgon and oxygen. The ultrafine particles formed in the gas phase are then collected on copper tubes cooled to 77 ^{0}K with flowing nitrogen.

b) Self assembled nanocrystalline brushite (calcium phosphate dihydrate)

These can be prepared by colloidal precipitation and sonication by reacting solution of disodium hydrogen phosphate and calcium chloride.

c) Nanocrystalline carbon ceramic, diamond particles

These can also be used for the core synthesis after ultra cleansing and sonication.

The common feature of various cores is that they are crystalline and that when they are introduced into the synthetic processes, they measures between 50-150 nm and exhibit extremely clean and therefore reactive species. Ceramic materials, being structurally highly regular, are most widely used for core fabrication. The high degree of order in crystalline ceramics ensures only a limited effect on the nature of atoms below the surface layer when any surface modification is being done, thus preserving the bulk properties of ceramics. This high degree of order also offers a high level of surface energy that favors the binding of pohyhydroxyl oligomeric surface film. The precipitated cores are centrifuged and then washed with enough distilled water to remove sodium chloride formed during the reaction. The precipitates are resuspended in distilled water and passed through a fine membrane filter to collect the particles of desired size. The equation for the reaction is as follows:

$$2\ Na_2HPO_4 + 3\ CaCl_2 + H_2O \rightarrow Ca_3(PO_4)_2 + 4\ NaCl + 2\ H_2 + Cl_2 + (O)$$

II- Coating of the core with polyhydroxy oligomer

In the second step, ceramic cores are coated with carbohydrate (polyhydroxyl oligomer). The coating is carried out by addition of carbohydrate into an aqueous dispersion of the cores under sonication. These are then subjected to lyophilization to promote an irreversible adsorption of carbohydrate onto the ceramic surface. The unadsorbed carbohydrate is removed by centrifugation. The commonly used coating materials are cellobiose, citrate, pyridoxal-5- phosphate, trehalose and sucrose.

III- Loading of the drug of choice to this assembly

The final stage involves the loading of drug to the coated particles by adsorption. For that, a solution of known concentration of drug is prepared in suitable pH buffer, and coated particles are dispersed into it. The dispersion is then either kept overnight at low temperature for drug loading or lyophilized after some time so as to obtain the drug-loaded formulation (i.e., aquasomes). The preparation thus obtained is then characterized using various techniques. The procedure for preparation of aquasomes is depicted in above figure (Jain *et al*., 2006).

Fate of aquasomes

Since aquasomes are biodegradable nanoparticles, so that they will be more concentrated in liver and muscles. Since the drug is adsorbed on to the surface of the system without further surface modification as in case of insulin and antigen delivery, they may not find any difficulty in receptor recognition on the active site so that the pharmacological or biological activity can be achieved immediately, in normal system, the calcium phosphate is a biodegradable ceramic.

Biodegradation of ceramic in vivo is achieved essentially by monocytes and multicellular cells called osteoclasts because they intervene first at the biomaterial implantation site during inflammatory reaction. Two types of phagocytosis were reported when cells come in contact with biomaterial; either calcium phosphate crystals were taken up alone and then dissolved in cytoplasm after disappearance of the phagosome membrane or dissolution after formation of heterophagosomes. Phagocytosis of calcium phosphate coincided with autophagy and the accumulation of residual bodies in the cell (Israelachvili *et al*., 1985).

Monocytic activities can be modulated by many soluble factors and are increased by IFN-g (interferon gamma) or 1, 25 dihydroxy cholecalciferol. Other cytokines can also contribute to inflammatory mechanism and may be involved in the biodegradation process (Bauman and Gauldie *et al*., 1994).

Characterization of aquasomes

Aquasomes are characterized chiefly for their structural and morphological properties, particle size distribution, and drug-loading capacity.

Characterization of ceramic core

Size distribution

For morphological characterization and size distribution analysis, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are generally used. Core, coated core, as well as drug-loaded aquasomes are analyzed by these techniques. Mean particle size and zeta potential of the particles can also be determined by using photon correlation spectroscopy (Cherian *et al*., 2000 and Oviedo *et al*., 2007).

Structural analysis

FT-IR spectroscopy can be used for structural analysis. Using the potassium bromide sample disk method, the core as well as the coated core can be analyzed by recording their IR spectra in the wave number range 4000–400 cm^{-1}; the characteristic peaks observed are then matched with reference peaks. Identification of sugar and drug loaded over the ceramic core can also be confirmed by FT-IR analysis of the sample (Vyas *et al*., 2006 and Khopade *et al*., 2002).

Crystallinity

The prepared ceramic core can be analyzed for its crystalline or amorphous behavior using X-ray diffraction. In this technique, the X-ray diffraction pattern of the sample is compared with the standard diffractogram, based on which the interpretations are made (Khopade et at., 2002 and Vyas *et al*., 2008).

Characterization of coated core

Carbohydrate coating

Coating of sugar over the ceramic core can be confirmed by concanavalin A–induced aggregation method (determines the amount of sugar coated over core) or by anthrone method (determines the residual sugar unbound or residual sugar remaining after coating). Furthermore, the adsorption of sugar over the core can also be confirmed by measurement of zeta potential (Vyas *et al*., 2006, Khopade *et al.*, 2002, Khopade et at., 2002 and Vyas *et al*., 2008).

Glass transition temperature

DSC can be used to analyze the effect of carbohydrate on the drug loaded to aquasomes. DSC studies have been extensively used to study glass transition temperature of carbohydrates and proteins. The transition from glass to rubber state can be measured using a DSC analyzer as a change in temperature upon melting of glass (Vyas et al 2008).

Characterization of drug-loaded aquasomes

Drug payload

The drug loading can be determined by incubating the basic aquasome formulation (i.e., without drug) in a known concentration of the drug solution for 24 hours at 4°C. The supernatant is then separated by high-speed centrifugation for 1 hour at low temperature in a refrigerated centrifuge. The drug remaining in the supernatant liquid after loading can be estimated by any suitable method of analysis (Oviedo *et al.*, 2007).

In vitro drug release studies

The in vitro release kinetics of the loaded drug is determined to study the release pattern of drug from the aquasomes by incubating a known quantity of drug-loaded aquasomes in a buffer of suitable pH at 37°C with continuous stirring. Samples are withdrawn periodically and centrifuged at high speed for certain lengths of time. Equal volumes of medium must be replaced after each withdrawal. The supernatants are then analyzed for the amount of drug released by any suitable method (Vyas *et al.*, 2008).

In-process stability studies

SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) can be performed to determine the stability and integrity of protein during the formulation of the aquasomes (Vyas *et al.*, 2006, Khopade et at., 2002 and Vyas *et al.*, 2008).

Applications of aquasomes

I- Insulin delivery

Cherian et al prepared aquasomes using a calcium phosphate ceramic core for the parenteral delivery of insulin. The core was coated with various disaccharides such as cellobiose, trehalose, and pyridoxal-5-phosphate. Subsequently the drug was loaded to these particles by adsorption method. The in vivo performance of various aquasome formulations of insulin was evaluated using albino rats. Prolonged reduction of blood glucose was observed with all formulations except cellobiose-coated particles. Pyridoxal-5-phosphate coated particles were found to be more effective in reducing blood glucose levels than aquasomes coated with trehalose or cellobiose. This could be attributed to the high degree of molecular preservation by pyridoxal-5-phosphate. The prolonged activity was attributed to slow release of drug from the carrier and structural integrity of the peptide (Oviedo *et al.*, 2007). The utility of nanocarriers for effective delivery of insulin was also proved by Paul and Sharma. They prepared porous hydroxyapatite nanoparticles entrapped in alginate matrix containing insulin for oral administration. The optimum controlled release of insulin was also achieved in this study (Paul *et al.*, 2001).

II- Oral delivery of acid labile enzyme

Rawat et al proposed the use of a nanosized ceramic core–based system for oral administration of the acid-labile enzyme serratiopeptidase. The nanocore was prepared by colloidal precipitation under sonication at room temperature. The core was then coated with chitosan under constant stirring, after which the enzyme was adsorbed over it. The enzyme was protected by further encapsulating the enzyme-loaded core into alginate gel. The TEM images of particles showed them to be spherical in shape, with an average diameter of 925 nm. The enzyme-loading efficiency of the particles was found to be approximately 46%. The in vitro drug release data followed the Higuchi model in acidic medium (pH 1.2) for a period of up to 2 to 6 hours, while the alkaline medium (pH 7.4) showed sustained and nearly complete first-order release of enzyme for up to 6 hours. These aquasomes were found to be protecting the structural integrity of enzymes so as to obtain a better therapeutic effect (Rawat *et al.*, 2008).

III- As oxygen carrier

Khopade et al prepared hydroxyapatite core by using carboxylic acid–terminated half-generation poly(amidoamine) dendrimers as templates or crystal modifiers. These cores were further coated with trehalose followed by adsorption of hemoglobin. The size of the particles was found to be in the nanometer range, and the loading capacity was found to be approximately 13.7 mg of hemoglobin per gram of the core. The oxygen-binding properties of the aquasomes were studied and compared to those of fresh blood and hemoglobin solution. Hill coefficient values determined for fresh blood, for hemoglobin solution, as well as for the aquasome formulation indicated that the properties of hemoglobin including its oxygen-carrying capacity were retained by the aquasomes. Studies carried out in rats showed that aquasomes possess good potential for use as an oxygen carrier. Moreover, the formulation was found to retain its oxygen-binding characteristics over a period of 30 days (Khopade et at., 2002). In another study Patil and co-workers prepared hydroxyapatite ceramic cores by co-precipitation and self-precipitation. These cores were coated with various sugars including cellobiose,

trehalose, maltose, and sucrose. Subsequently, hemoglobin was adsorbed over the coated ceramic core, and the percentage drug loading was estimated by the benzidine method. The oxygen-carrying capacity of aquasome formulation was found to be similar to that of fresh blood. Also, the Hill coefficients were found to be good for its use as an oxygen carrier. The aquasome formulations neither induced hemolysis of the red blood cells nor altered the blood coagulation time. The hemoglobin loading to various sugar-coated particles was found to be approximately 7.4%. The formulation was able to retain the hemoglobin over a period of 30 days. No significant increase in arterial blood pressure and heart rate was observed in rats transfused with aquasome suspension on 50% exchange transfusion (Patil *et al.*, 2004).

IV- Antigen delivery

The adjuvants generally used to enhance the immunity to antigens have a tendency either to alter the conformation of the antigen through surface adsorption or to shield the functional groups. So Kossovsky et al demonstrated the efficacy of a new organically modified ceramic antigen delivery vehicle. These particles consisted of diamond substrate coated with a glassy carbohydrate (cellobiose) film and an immunologically active surface molecule in an aqueous dispersion. These aquasomes (5–300 nm) provided conformational stabilization as well as a high degree of surface exposure to protein antigen. Diamond, being a material with high surface energy, was the first choice for adsorption and adhesion of cellobiose. It provided a colloidal surface capable of hydrogen bonding to the proteinaceous antigen. The disaccharide, being a dehydroprotectant, helps to minimize the surface-induced denaturation of adsorbed antigens (muscle adhesive protein, MAP). For MAP, conventional adjuvants had proven only marginally successful in evoking an immune response. However, with the help of these aquasomes a strong and specific immune response could be elicited by enhancing the availability and in vivo activity of antigen (Kossovsky *et al.*, 1995). Vyas et al prepared aquasomes by self-assembling of hydroxyapatite using the co-precipitation method. The core was coated with cellobiose and trehalose, and finally bovine serum albumin was adsorbed as model antigen onto the coated core. The aquasomes were found to be spherical in shape with diameter around 200 nm. The coating of carbohydrate over the surface of the core was confirmed by concanavalin A–induced aggregation assay method as well as IR spectroscopy. The antigen-loading efficiency was found to be approximately 20–30%. When the immunological activity of the prepared formulation was compared to plain bovine serum albumin, the former was found to exhibit a better response. In view of these results, aquasomes were proposed to have superior surface immutability, in that they protect the conformation of protein structure and present it in such a way to immune cells that it triggers a better immunological response (Vyas *et al.*, 2008).

The use of ceramic core–based nanodecoy systems was proposed by Vyas et al as an adjuvant and delivery vehicle for hepatitis B vaccine for effective immunization. Self-assembling hydroxyapatite core was coated with cellobiose, and finally hepatitis B surface antigen was adsorbed over the coated core. The drug-loaded particles were in the nanometer range and almost spherical in shape. The antigen-loading efficiency of plain hydroxyapatite core (without cellobiose coating) was found to be approximately 50%, whereas the coated core was observed to load approximately 21% antigen. The preparation was found to be better than the conventional adjuvant alum followed by subcutaneous immunization in mice. The nanodecoy systems were also found to be able to elicit a combined Th1 and Th2 immune response (Vyas *et al.*, 2006).

Vyas *et al.*, demonstrated the immunoadjuvant properties of hydroxyapatite by administering it with malarial merozoite surface protein-119 (MSP-119). Hydroxyapatite nanoceramic carrier was prepared by co-precipitation. Prepared systems were characterized for crystallinity, size, shape, and antigen-loading efficiency. Small size and large surface area of prepared hydroxyapatite demonstrated good adsorption efficiency of immunogens. Prepared nanoceramic formulations also showed slower in vitro antigen release and slower biodegradability behavior, which may lead to a prolonged exposure to antigen-presenting cells and lymphocytes. Furthermore, addition of mannose in nanoceramic formulation may additionally lead to increased stability and immunological reactions. Immunization with MSP-119 in nanoceramic-based adjuvant systems induced a vigorous IgG response, with higher IgG2a than IgG1 titers. In addition, a considerable amount of interferon γ (IFNγ) and interleukin 2 was observed in spleen cells of mice immunized with nanoceramic-based vaccines. In contrast, mice immunized with MSP-119 alone or with alum did not show a significant cytotoxic response. The antibody responses to vaccine co-administered with hydroxyapatite was a mixed Th1-Th2 compared to the Th2-biased response obtained with alum. The prepared hydroxyapatite nanoparticles exhibit physicochemical properties that point toward their potential as a suitable immunoadjuvant for use as antigen carriers for immunopotentiation (Goyal *et al.*, 2009). He et al compared a new nanoparticulate adjuvant composed of calcium phosphate with commonly used aluminum (alum) adjuvant for its ability to induce immunity to herpes simplex virus type 2 and Epstein-Barr virus infections. Calcium phosphate was observed to cause little or no inflammation at the site of administration, induced high titers of immunoglobulin G 2a (IgG 2a) antibody and neutralizing antibody, and facilitated a high percentage of protection against herpes simplex virus type 2 infections. Thus, calcium phosphate proved to be a more potent adjuvant than alum. Moreover, being a natural constituent of the body, it was found to be very well tolerated and absorbed in the animal studies.

These studies, by virtue of potency and relative absence of any side effects of calcium phosphate, recommended it as an adjuvant for use in human beings (He *et al.*, 2000).

V- Delivery of drug

Oviedo and co-workers prepared aquasomes loaded with indomethacin through the formation of an inorganic core of calcium phosphate covered with a lactose film and further

adsorption of indomethacin as a low-solubility drug. The aquasomes were characterized for their structural analysis, particle size, and morphology by using X-ray powder diffractometry, TEM, and SEM. Particle size of drug-loaded aquasomes was found to be in the range of 60–120 nm. SEM and TEM techniques confirmed the spherical shape of aquasomes. However, results of drug (indomethacin) release studies from these carriers are yet to be determined (Oviedo *et al.*, 2007).

VI- For delivery of gene

Aquasomes can be studied for the delivery of genes. It illustrates the attractive delivery system loaded with genetic material. Studies reveal that aquasomes protect and maintain structural integrity of the gene segment. A five layered composition comprised of the ceramic nanocrystalline core, the polyhydroxyl oligomeric film coating, the non covalently bound layer of therapeutic gene segment, an additional carbohydrate film and a targeting layer of conformationally conserved viral membrane proteins, have been proposed for gene therapy. The aquasome vehicle would afford all of the potential advantages of viral vectors and simultaneous overwhelming the risk of irrelevant gene integration (Kossovsky *et al.*, 1994a).

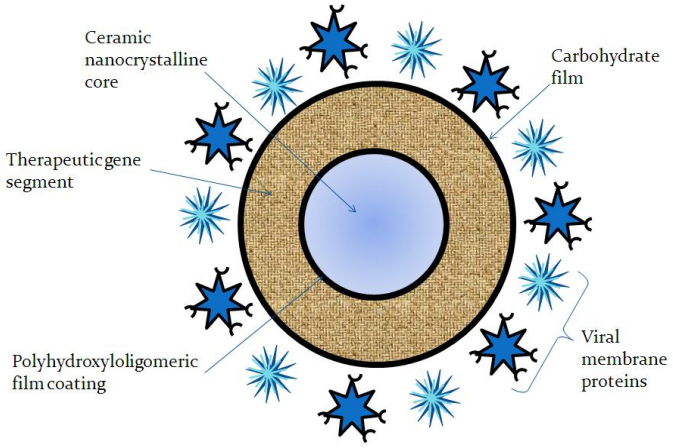

Fig. 2: Gene Delivery Through Aquasomes.

VII- For delivery of enzymes

Aquasomes also used for delivery of enzymes like DNAase and pigment/dyes because enzymes activity fluctuates with molecular conformation and cosmetic properties of pigment are sensitive to molecular conformation. DNAase a therapeutic enzyme used in the treatment of cystic fibrosis was successfully immobilized on aquasomes and targeted to the specific site and elicited significant therapeutic effect as desirable. A marked retention of biological activity was observed with surface immobilized DNAase on the solid phase of a colloidal calcium phosphate nanoparticle coated with polyhydroxyl oligomeric films (Vays *et al.*, 2004).

VIII- Miscellaneous

Mizushima and co-workers prepared spherical porous hydroxyapatite particles by spray-drying. These particles were tried as a carrier for the delivery of drugs such as interferon α (IFN α), testosterone enanthate, and cyclosporine A. Spherical porous hydroxyapatite was found to have an average diameter of 5 µm with approximately 58% porosity. These particles could be injected subcutaneously through a 27-gauge needle. IFN α was adsorbed well to spherical hydroxyapatite particles. Addition of HAS and zinc (for reinforcement) to IFN α-adsorbed hydroxyapatite particles caused marked prolongation of release in vivo. The in vivo release of testosterone enanthate and cyclosporine A was also prolonged from oil preparation. Thus, the spherical porous hydroxyapatite particles were shown to be useful as a biodegradable and subcutaneously injectable drug carrier. The reinforcement of spherical porous hydroxyapatite particles was suggested to be very effective for sustained release of drugs (Mizushima *et al.*, 2006).

CONCLUSION

Aquasomes, the self-assembling surface-modified nanocrystalline ceramic cores, seem to have potential and promising carriers capable of preserving the structural integrity of protein pharmaceuticals and carrier for delivery of broad range of molecules including viral antigens, heamoglobin and insulin, thus promoting a better therapeutic effect. Also, these formulations have been found to evoke a better immunological response and could be used as immunoadjuvants for proteinaceous antigens. This approach thus provides pharmaceutical scientists with new hope for the delivery of bioactive molecules.

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