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AQUASOMES: A NOVEL DRUG CARRIER SYSTEM FOR BIOACTIVE MOLECULES

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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. Aquasomes contains the particle core composed of nanocrystalline calcium phosphate or ceramic diamond, and is covered by a poly hydroxyl oligomeric film used for drug and antigen delivery. Three types of core material are mainly used for producing aquasomes: tin oxide, nanocrystalline carbon ceramics (diamonds) and brushite (calcium phosphate dehydrates). Aquasomes is one of the part of novel drug delivery carrier which deal with principle of self assembly. We can see better biological activity even in case of conformationally sensitive drug candidates because of the presence of the unique carbohydrate coating the ceramic. This strategy may be beneficially extended to the novel delivery of other bioactive molecules. The molecular plasticizer, carbohydrate prevent the destructive drug-carrier interaction and helps to preserve the spatial qualities. The structural stability and overall integrity is controlled by crystalline nature of the core. We can say aquasomes can be used as a potential carrier for the delivery of a broad range of molecules including viral antigens, hemoglobin and insulin.

***Keywords*:** Aquasomes, Nanoparticles, Polyhy droxyl oligomeric, Nano crystal-line Carbon ceramics

INTRODUCTION

Aquasomes are called as “bodies of water" their water like properties protect and preserve fragile biological molecules. Aquasomes are the combination of biotechnology and nanotechnology.

Aquasomes was first developed by Nir Kossovsky. These carbohydrates stabilize nanoparticles of ceramic. The pharmacologically active molecule incorporated by co-polymerization, diffusion or adsorption to carbohydrate surface of pre formed nanoparticles. Carbohydrate plays important role act as natural stabilizer, its stabilization efficiency has been reported i.e. fungal spores producing alkaloid stabilized by sucrose rich solution [1] and desiccation induced molecular denaturation prevented by certain disaccharides [2].These three layered structure are self assembled by non-covalent bonds. Principal of “self assembly of macromolecule” is governed by three physiochemical process i.e.

1) Interaction between charged groups: [3, 4]

The interaction of charged group facilitates long range approach of self assembly sub units charge group also plays a role in stabilizing tertiary structures of folded proteins.

1) **Hydrogen bonding and dehydration effect**: **[5, 6]**

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.

2) **Structural stability of protein in biological environment**: **[7, 8]**

Determined by interaction between charged group and Hydrogen bonds largely external to molecule and by van der waals forces largely internal to molecule experienced by hydrophobic molecules, responsible for hardness and softness of molecule and maintenance of internal secondary structures, provides sufficient softness, allows maintenance of conformation during self assembly. Self

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assembly leads to altered biological activity, van der waals need to be buffered. In aquasomes, sugars help in molecular plasticization.

Advantages of aquasomes: [9, 10]

1) Mechanism of action of aquasomes is controlled by their surface chemistry. They deliver contents through combination of molecular shielding, specific targeting, and slow and sustained release process.
2) Aquasomes provides a platform for preserving the conformational integrity and bio chemical stability of bio-actives due to its water like properties.
3) Aquasomes 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.
4) 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 after formation of heterophagosome [11].
5) Aquasomes due to their size and structure stability, avoid clearance by reticuloendothelial system or degradation by other environmental challenges.
6) Aquasomes are mainly characterized for structural analysis, morphology these are evaluated by X-ray powder diffractometry, transmission electron microscopy, scanning electron microscopy. X-ray analysis of samples and drug loading efficiency and in vivo performance [12].
7) Aquasomes-based vaccines offer many advantages as a vaccine delivery system. Both cellular and humoral immune responses can be elicited to antigens adsorbed onto the surface of aquasomes [13, 14].

Methods of preparation of Aquasomes: [15, 16, 17]

There are three steps of preparation of Aquasomes:

1) Preparation of core
2) Carbohydrate coating
3) Immobilization of drug

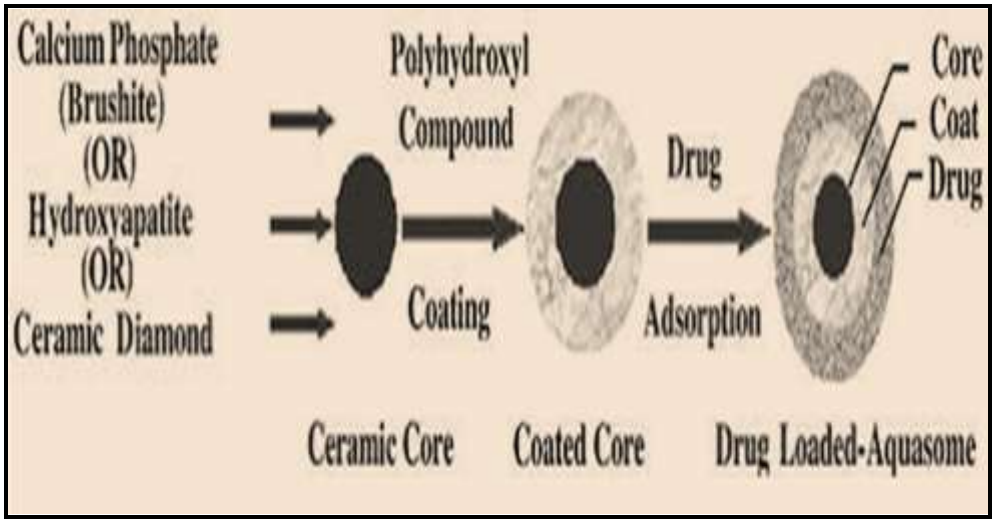

Figure 1: Method of Preparation of Aquasomes.

Preparation of core: The first step of aquasome preparation is the fabrication of the ceramic core. This process of depends on the selection of the materials for core. Cores can be fabricated by one of the method colloidal precipitation and sonication, inverted rnagnetron sputtering, plasma condensation and other processes.For the core, ceramic materials were widely used because ceramics are structurally the most regular materials known. Ceramics materialsensure that any surface modification will have only a limited effect on the nature of the atoms below the surfacelayer and thus the bulk properties of the ceramic will be preserved. The high surface will exhibit high level of surface energy that will favour the binding of poly hydroxy oligomeric surfacefilm. Two ceramic cores that are most often used are diamond and calcium phosphate.

The equation for the reaction is as follows;

$$2Na_2HPO_4 + 3CaCl_2 + H_2O \rightarrow Ca_3(PO4)_2 + 4NaCl + 2H_2 + Cl_2 + (O)$$

Carbohydrate coating: It is the second step in preparation of an aquasomes. It involves coating by carbohydrate on the surface of ceramic cores. There are number of processes to enable the carbohydrate (polyhydroxy oligomers) coating to adsorb epitaxially on to the surface of the nano-crystalline ceramic cores. The processes generally entail the addition of polyhydroxy oligomer to a dispersion of meticulously cleaned ceramics in ultra pure water, sonication and then lyophilization to promote the largely irreversible adsorption of carbohydrate on to the ceramic surfaces. Excess and readily desorbing carbohydrate is removed by stir cell ultra-filtration. The commonly used coating materials are cellobiose, citrate, pyridoxal-5-phosphate, sucrose and trehalose.

Immobilization of drug: The drug in aquasomes can be loaded by partial adsorption. The surface modified nano-crystalline cores provide the solid phase for the subsequent non denaturing self assembly for broad range of biochemically active molecules.

Role of disaccharides: [18]

Carbohydrate fulfills the objective of aquasomes among three layers of aquasomes. As cyclodextrins which arethe complex cyclic oligosaccharides which recently have been noticed as useful pharmaceutical ingredient and identified as and cyclodextrin composed of various glucose molecules forming truncated cone which can enclose

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various complex structure form of proteins and peptides. 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. These disaccharides rich in hydroxyl group help to replace the water around polar residues in protein, maintaining integrity in absence of water. The free bound mobility associated with a rich hydroxylcomponent creates unique hydrogen binding substrate that produces a glassy aqueous state.

Characterization of Aquasomes:

Aquasomes [19, 20] are characterized chiefly for their ceramic core, coated core, and drug-loaded capacity.

Characterization of ceramic core:

1) Size distribution:

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are generally used for size distribution analysis. Core, coated core, as well as drug-loaded aquasomes are analyzed by these techniques. Photon correlation spectroscopy are used to determine Mean particle size and zeta potential of the particles.

2) Structural analysis: For structural analysis FT-IR spectroscopy can be used.

The core and the coated core can be analyzed by using the potassium bromide sample disk method, 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.

3) Crystallinity: The prepared ceramic core can be analyzed for its or amorphous behavior using X-ray diffraction. In this technique, the X-ray diffraction pattern of the sample is compaired with the standard diffractogram, based on which the interpretations are made.

Characterization of coated core

1) Carbohydrate coating

Concanavalin A induced aggregation method (determines the amount of sugar coated over core) or anthrone method (determines the residual sugar unbound or residual sugar remaining after coating) are used to confirm the coating of sugar over the ceramic core. Furthermore, the adsorption of sugar over the core can also be confirmed by measurement of zeta potential.

2) 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.

Characterization of drug-loaded Aquasomes [21, 22]

1) 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.

2) In vitro drug release studies

The in vitro release kinetics of the drug loaded is determined to study the release pattern of drug from the aquasomes. This can be achieved 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.

3) 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.

Applications of Aquasomes: [23, 24]

1. Aquasomes as red blood cell substitutes, by heamoglobin is confomationally sensitive. By this toxicity is reduced, haemoglobin concentration of 80% achieved and reported to deliver blood in non linear manner like natural blood cells.
2. Aquasomes have been used for successful targeted intracellular gene therapy, a five layered composition comprised of ceramic core, polyoxyoligomeric film, therapeutic gene segment, additional carbohydarte film and a targeting layer of conformationally conserved viral membrane protein.

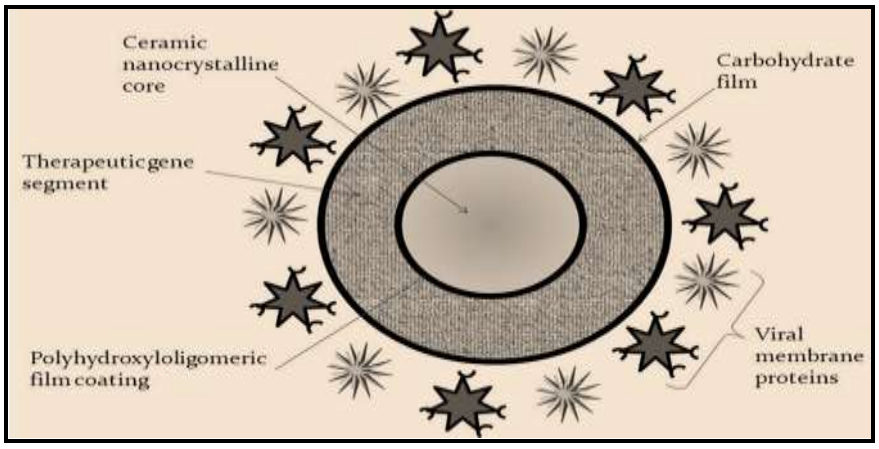

Figure 2: Gene Delivery through Aquasomes.

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3. Aquasomes used as vaccines for delivery of viral antigen i.e. Epstein-Barr and Immune deficiency virus to evoke correct antibody, objective of vaccine therapy must be triggered by cnformationally specific target molecules.
4. Aquasomes for pharmaceuticals delivery i.e insulin, developed because drug activity increased to 60% as compared to i.v. administration and toxicity not reported.
5. Aquasomes also used for delivery of enzymes like DNAase and pigments/dyes because enzymes activity fluctuates with molecular conformation and cosmetic properties of pigments are sensitive to molecular conformation.

CONCLUSION

Due to presence of unique carbohydrate coating over the ceramic core aquasomes appear to be promising carriers for the delivery of a board range of conformational sensitive molecules with better biological activity. The drug candidates which are delivered through aquasomes are peptide and protein hormones, antigens and genes and show better biological activity even in case of conformationally sensitive ones. Various other ranges of broad molecules delivered through aquasomes include viral antigens, heamoglobin and insulin. This strategy may be beneficially extended to the novel delivery of other bioactive molecules.

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