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An overview on nanocarrier technology- Aquasomes

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ABSTRACT

Aquasomes are the nanobiopharmaceutical carrier system contains the particle core composed of nanocrystalline calcium phosphate or ceramic diamond, and is covered by a polyhydroxyl oligomeric film. Aquasomes are spherical 60–300 nm particles used for drug and antigen delivery. Properties like protection and preservation of fragile biological molecules, conformational integrity, and surface exposure made it as a successful carrier system for bioactive molecules like peptide, protein, hormones, antigens and genes to specific sites. Three types of core materials are mainly used for producing aquasomes: tin oxide, nanocrystalline carbon ceramics (diamonds) and brushite (calcium phosphate dihydrate). Calcium phosphate is the core of interest, owing to its natural presence in the body. The brushite is unstable and converts to hydroxyapatite upon prolong storage. Hydroxyapatite seems, therefore, a better core for the preparation of aquasomes. It is widely used for the preparation of implants for drug delivery. It has been reported haemoglobin loaded aquasomes using hydroxyapatite core as potential artificial oxygen carrying system. Conformational integrity of aquasomes exploited as a red blood cell substitutes, vaccines for delivery of viral antigen (Epstein-Barr and Immune deficiency virus) to evoke correct antibody and as targeted system for intracellular gene therapy. Enzyme activity and sensitivity towards molecular conformation made aquasome as a novel carrier for enzymes like DNAses and pigment/ dyes.This report reviews the principles of self assembly, the challenges of maintaining both the conformational integrity and biochemical activity of immobilized surface pairs, and the convergence of these principles into a single functional composition.

Keywords: Aquasomes, self assembling carrier system, nanoparticles.

INTRODUCTION

Aquasomes are nanoparticulate carrier system 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. Alternatively aquasomes are called as "bodies of water", 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 are exploited in targeting of bio-active molecules like peptide and protein hormones, antigens and genes to specific sites. These carbohydrate stabilize nanoparticles of ceramic are known as "aquasomes" which was first developed by Nir Kossovsky. The pharmacologically active molecule incorporated by co-polymerization, diffusion or adsorption to carbohydrate surface of pre formed nanoparticles.

These three layered structure are self assembled by non-covalent bonds. Principle of "self assembly of macromolecule" is governed by three physiochemical process [1, 2] i.e.

1. Interaction between charged group
2. Hydrogen bonding and dehydration effect
3. Structural stability

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Self-assembly, broadly defined as the spontaneous fabrication of multi-component molecular structures, is the elegant mechanism through which the most complex biological molecules achieve their ultimate form.

As an approach to macromolecular synthesis, self-assembly is appealing because biomimetic processes imply more biochemically functional products. This report reviews the principles of self assembly, the challenges of maintaining both the conformational integrity and biochemical activity of immobilized surface pairs, and the convergence of these principles into a single functional composition.

OBJECTIVES

1. Aquasomes protect bio-actives. Many other carriers like prodrugs and liposomes utilized but these are prone to destructive interactions between drug and carrier in such case aquasomes proof to be worthy carrier, carbohydrate coating prevents destructive denaturing interaction between drug and solid carriers.
2. 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 but proteins undergo irreversible denaturation when desiccated, even unstable in aqueous state. In the aqueous state pH, temperature, solvents, salts cause denaturation [3]

hence bio-active faces many biophysical constrain. In such case, aquasomes with natural stabilizers like various polyhydroxy sugars act as dehydroprotectant maintains water like state thereby preserves molecules in dry solid state.

FORMULATION OF AQUASOMES

I.Principles of Self Assembly [1, 2]

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 nanostructured 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.

1. Interactions between Charged Groups: 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. The intrinsic chemical groups or adsorbed ions from the biological milieu lend to most biological and synthetic surfaces a charge polarity. Most biochemically relevant molecules, in fact are amphoteric. The interactions of charged groups such as amino-, carboxyl-, sulfate-, and phosphate-groups, facilitate the long range approach of self assembling subunits. The long range interaction of constituent subunits beginning at an intermolecular distance of around 15 nm, is the necessary first phase of self assembly. With hydrophobic structures, long range forces may extend up to 25 nm. Charged groups also play a role in stabilizing tertiary structures of folded proteins.
2. Hydrogen Bonding and Dehydration Effects: Hydrogen bond helps in base pair matching and stabilization 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, their tendency to repel water helps to organize the moiety to surrounding environment, organized water decreases level of entropy and is thermodynamically unfavorable, the molecule dehydrate and get self assembled.
3. Structural Stability: 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 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 assembly leads to altered biological activity, van der Waals need to be buffered. In aquasomes, sugars help in molecular plasticization. Van der Waals forces, most often experienced by the relatively hydrophobic molecular regions that are shielded from water, play a subtle but critical role in maintaining molecular conformation during self assembly. Van der Waals forces largely internal to the molecule also play a small but measurable role in the interaction of polypeptides with carbohydrates and related polyhydroxyloligomers. When molecules change their shape substantially following an interaction, the energy minima assumed upon conformational denaturation tend to preclude reversal.

II.Method of Preparation of Aquasomes [4, 5, 6, 7, 8]

The general procedure consists of an inorganic core formation, which will be coated with Lactose forming the polyhydroxylated core that finally will be loaded by model drug [Fig. No.1].

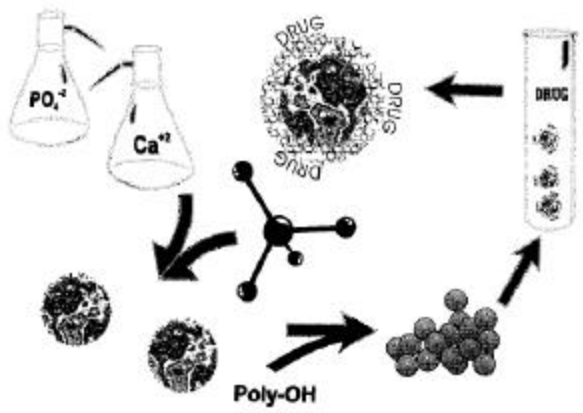

Fig No.: 1 Synthesis of aquasomes consists of fabricating a nanocrystalline core of a calcium phosphate (brushite) colloidal precipitate or ceramic diamond. The core is coated with a polyhydroxyl oligomeric film, and the coated particles are then allowed to adsorb a drug or antigen. The final product consists of three layers: drug (or antigen), polybydroxyl oligomeric film, and the nanocrystalline ceramic core.

By using the principle of self-assembly, the aquasomes are prepared in three steps i.e., preparation of core, coating of core, and immobilization of drug molecule.

1. **Preparation of the core:** The first step of aquasome preparation is the fabrication of the ceramic core. The process of ceramic core preparation depends on the selection of the materials for core. These ceramic cores can be fabricated by 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. Being crystalline, the high degree of order in ceramics ensures that any surface modification will have only a limited effect on the nature of the atoms below the surface layer and thus the bulk properties of the ceramic will be preserved. The high degree of order also ensures that the surfaces will exhibit high level of surface energy that will favor the binding of polyhydroxy oligomeric surface film. Two ceramic cores that are most often used are diamond and calcium phosphate.
2. **Carbohydrate coatings:** The second step involves coating by carbohydrate on the surface of ceramic cores. There are number of processes to enable the carbohydrate (polyhy-

droxy 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.

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

FATE OF AQUASOME [9]

The drug delivery vehicle aquasome is colloidal range 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 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 the 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.

PROPERTIES OF AQUASOMES [10]

1. 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. As solid particles dispersed in aqueous environment, exhibit physical properties of colloids.
2. Aquasomes 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.
3. Aquasomes water like properties provides a platform for preserving the conformational integrity and bio chemical stability of bio-actives.
4. Aquasomes due to their size and structure stability, avoid clearance by reticuloendothelial system or degradation by other environmental challenges.

CHARACTERIZATION OF AQUASOMES [10]

Aquasomes are mainly characterized for structural analyses, particle size, and morphology these are evaluated by X-ray powder diffractometry, transmission electron microscopy, and scanning electron microscopy.

The morphology and the size distribution were obtained through images of scanning electron microscopy. The chemical composition and the crystalline structure of all samples were obtained through X-ray powder diffractometry.

Role of disaccharides [11, 12]

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. 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 hydroxyl component creates unique hydrogen binding substrate that produces a glassy aqueous state.

APPLICATIONS OF AQUASOMES

1. Aquasomes as red blood cell substitutes, haemoglobin immobilized on oligomer surface because release of oxygen by haemoglobin is conformationally sensitive. By this toxicity is reduced, haemoglobin concentration of 80% achieved and reported to deliver blood in non linear manner like natural blood cells [11].
2. 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 conformationally specific target molecules [13].
3. Aquasomes have been used for successful targeted intracellular gene therapy, a five layered composition comprised of ceramic core, polyoxyoligomeric film, therapeutic gene segment, additional carbohydrate film and a targeting layer of conformationally conserved viral membrane protein [11].
4. Aquasomes for pharmaceuticals delivery i.e. insulin, developed because drug activity is conformationally specific. Bioactivity preserved and activity increased to 60% as compared to i.v. administration and toxicity not reported [14].
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

Aquasomes represent one of the simplest yet a novel drug carrier based on the fundamental principle of self assembly. The drug candidates delivered through the aquasomes show better biological activity even in case of conformationally sensitive ones. This is probably due to the presence of the unique carbohydrate coating the ceramic. This molecular plasticizer, carbohydrate prevents the destructive drug-carrier interaction and helps to preserve the spatial qualities. Moreover, the crystalline nature of the core gives structural stability and overall integrity. In conclusion, aquasomes appear to be promising carriers for the delivery of a broad range of molecules including viral antigens, hemoglobin and insulin. This strategy may be beneficially extended to the novel delivery of other bioactive molecules. However, the roles of molecular plasticizers and core crystallinity need further extensive investigations.

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