



Review Article

A REVIEW ON AQUASOMES: A POTENTIAL DRUG DELIVERY CARRIER

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Abstract: Aquasomes are one of the most recently developed delivery systems that are finding a niche as peptide and protein carriers. These are nanoparticulate carrier systems with three-layered self-assembled structures. They comprise a central solid nanocrystalline core coated with polyhydroxy oligomers onto which biochemically active molecules are adsorbed. The solid core provides the structural stability, while the carbohydrate coating protects against dehydration and stabilizes the biochemically active molecules. This property of maintaining the conformational integrity of bioactive molecules has led to the proposal that aquasomes have potential as a carrier system for delivery of peptide-based pharmaceuticals. The delivery system has been successfully utilized for the delivery of insulin, hemoglobin, and various antigens. Oral delivery of enzymes like serratiopeptidase has also been achieved. This article discusses the problems faced in the delivery of clinically important peptides and presents aquasomes as a reliable approach to troubleshoot them. Aquasomes are nanoparticulate carrier systems with three layered self-assembled structures enabling the delivery peptide/protein based pharmaceuticals including enzymes, structural proteins or even antigens.

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.^{22, 24} These carbohydrate stabilize nanoparticles of ceramic are known as “aquasomes” which was first developed by Nir Kossovsky. The pharmacologically active molecule incorporated by copolymerization, 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 solution¹ and desiccation induced molecular denaturation prevented by certain disaccharides.¹⁰ 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 group^{2, 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.
2. Hydrogen bonding and dehydration effect^{22, 24},

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 of protein in biological environment determined by interaction between charged group and Hydrogen bonds largely external to molecule and by vander waals forces largely internal to molecule²⁴, 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 needs to be buffered. In aquasomes, sugars help in molecular plasticization.

Strategies used in chemical synthesis of nanostructures -

1. Arrays of co-valently linked atoms generated with well defined composition, connectivity and shape.⁶
2. Covalent polymerization¹³, used for preparing molecules with high molecular weight, low weight substance allowed to react with itself to produce

molecule comprising many covalently linked monomers.

3. Self-organizing synthesis relies on weaker and less directional bonds as ionic, hydrogen and van der Waals. Molecules adjust their own position to reach thermodynamic minimum, true nanostructures prepared.⁹
4. Molecular self assembly²⁴ it combines features of preceding strategies, involves
 - Formation of intermediate structural complexity through covalent synthesis.
 - Formation of stable structure through ionic, hydrogen and van der Waals links
 - Use of multiple copies. For final assembly, non-covalent connection between molecules must be stable.

Objectives -

Firstly, 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 prove to be worthy carrier, carbohydrate coating prevents destructive denaturing interaction between drug and solid carriers.¹⁶ Secondly, aquasomes maintain molecular conformation 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^{7, 11} hence bio-active faces many biophysical constraints. In such case, aquasomes with natural stabilizers like various polyhydroxy sugars act as dehydroprotectant maintaining water-like state thereby preserving molecules in dry solid state.

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 loses water/dehydrates and gets self assembled.

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 Van der 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 chains 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.

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 covalently 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.

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.

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 strategy 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.

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 noncovalent interaction or by multiple hydrogen bonds or both.

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.

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. 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.

Role of Disaccharides -

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.^{14, 15 & 22}

Material used and its importance -

Initially for preparation of nanoparticles core both polymers and ceramic can be used. Polymers used are albumin, gelatin or acrylates and ceramics used are diamond particles, brushite, and tin oxide core.^{17,18} For core, ceramic materials were widely used because ceramics are structurally the most regular materials known, being crystalline high degree of order ensures

- (a) Any surface modification will have only limited effect on nature of atoms below surface layer and thus bulk properties of ceramic will be preserved.¹⁹
- (b) The surface will exhibit high level of surface energy that will favor the binding of polyhydroxy oligomer surface film. The freshly prepared

particles possess good property of adsorbing molecules within fraction of seconds. Second step followed by coating of carbohydrate epitaxially over nanocrystalline ceramic core. The commonly used coating materials 3, 5 are cellobiose, pyridoxal-5-phosphate, sucrose and trehalose, presence of carbohydrate film prevents soft drug from changing shape and being damage when surface bound. Thirdly bioactive molecules adsorbed which possess property of interacting with film via non-covalent and ionic interactions.

Properties^{20, 21}

- 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.
- 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.
- Aquasomes water like properties provides a platform for preserving the conformational integrity and bio chemical stability of bio-actives.
- Aquasomes due to their size and structure stability, avoid clearance by reticuloendothelial system or degradation by other environmental challenges.
- 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 heterophagosome³.
- 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 X-ray analysis of the samples and drug loading efficiency and in vivo performance¹²

Method of preparation of aquasomes -

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 .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 magnetron 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 (polyhydroxy oligomers) coating to adsorb epitaxially on to the surface of the nanocrystalline 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 nanocrystalline cores provide the solid phase for the subsequent nondenaturing self assembly for broad range of biochemically active molecules. The drug can be loaded by partial adsorption.

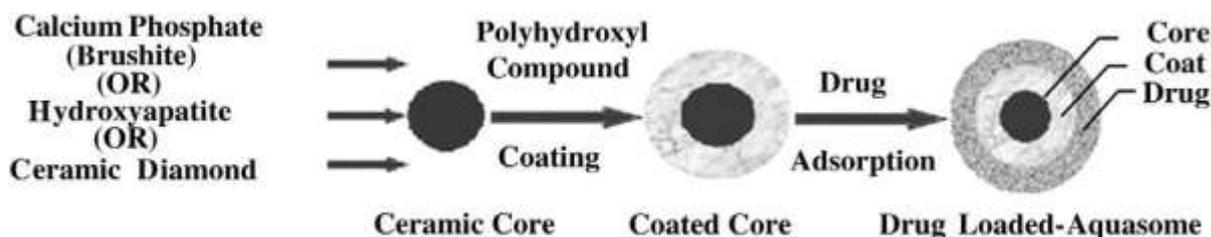


Fig. 1: Method of Preparation of Aquasomes.

Characterization of aquasomes -

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. In this technique, the x-ray diffraction pattern of the sample is compared with the standard diffractogram, based on which the interpretations are made .

Applications -

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.²²

2) Aquasomes used as vaccines for delivery of viral antigen i.e. Epstein-Barr and Immune deficiency virus.²³ to evoke correct antibody, objective of vaccine therapy must be triggered by conformationally specific target molecules.

3) Aquasomes have been used for successful targeted intracellular gene therapy ²², 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.

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 ⁸ 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.²⁵

Fate of Aquasome -

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.³

Characterization of Aquasomes -

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Conclusion -

Aquasomes appear to be promising carriers for the delivery of a board range of conformational sensitive molecules with better biological activity due to presence of unique carbohydrate coating over the ceramic core. Molecular plasticizer, carbohydrates prevent the destructive drug carrier interaction and helps to preserve the spatial qualities and the crystalline nature of core, gives structural stability and overall integrity. This strategy may be beneficially extended to the novel delivery of other bioactive molecules

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