AQUASOMES: A POTENTIAL DRUG DELIVERY CARRIER

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Summary

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. 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.

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 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 [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 molecule [24], 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 polymerization [13], 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 vander waals. Molecules adjust their own position to reach thermodynamic minimum, true nanostructures prepared. [9]
4) Molecular self assembly [24], it combines features of preceding strategies, involves

- Formation of intermediate structural complexity through co valent synthesis.
- Formation of stable structure through ionic, hydrogen and vander 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 proof to be worthy carrier, carbohydrate coating prevents destructive denaturing interaction between drug and solid carriers. [16] 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 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 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.

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 heterophagosome [3].
- 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 performance [12]

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]

Formulation of Aquasomes

I. Principles of Self Assembly [22, 24]
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 [22, 23, 26, 27, 28]
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 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 nondenaturing self assembly for broad range of biochemically active molecules. The drug can be loaded by partial adsorption 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.

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

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.[29]

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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References

1. Arakawa, T., Timasheff, S. N. Stabilization of protein structure by sugars 1982 Biochemistry 21:6536- 6544.
2. Batz, H.G.; ringsford, H. and Ritter, H. Pharmacologically active polymers"., Macromol. Chem., 1974.175(8):2229-2239.
3. Bauman. and Gauldie, J.The acute phase response Immunol. Today.1994. 15:74-78.
4. Bhave, S.; Sewak, P and Saxena, j., Nanoparticles, A new colloidal drug delivery system" the Eastern pharmacist.1994. 17-21.
5. Cherian, A. and Jain S.K. "Self assembled carbohydrate stabilized ceramic nanoparticles for the parentral drug delivery of insulin" Drug development and industrial pharmacy 2000, vol. 26, 459-463
6. Bovey, F.A and Winslow, F.H. Macro molecules academic press. New York 1998.
7. Bryan, W.P.Science, 1994 26:1726.
8. Cherian, A. and Jain S.K. "Self assembled carbohydrate stabilized ceramic nanoparticles for the parentral drug delivery of insulin"2000. 459-463.
9. Crowe, J.H Crowe, L.M and Jackson S.A."Preservation of structural and functional activity in lyophilized sarcoplasmic reticulum" 1983. 220(2):477-484.
10. Crowe, J.H.; Crowe, L.M.; Carpenter, J.F.; Rudolph, A.S.; Wistrom. C.A.; Spargo, B.J. and

Acnhordoguy.T.J "Interaction of sugars with membrane"Biochem biophys acta 1988. 1947:367-384.
11. Dunitz, J.D. "The entropic cost of bound water in crystals and biomolecules"science. 1994. 264-670.
12. Frankel, D.A Elaboration and structural analysis of aquasomes loaded with Indomethacin 2007 Nov; 32(3):223-30.
13. Frankel, D.A.; Lamparski, H.Liman, U; O'Brien, d.f."Photoinduced destabilization of bilayer vesicles"J.Am.chem.soc.1989. 111:9262.
14. Franks, F.,"Long term stabilization of biologicals" bio technology, 1994. 12:253
15. Green, J.L. and Angel, C.A."Phase relations and vitrification in sacchride Solutions and trehalose anomaly."J. Phys. Chem. 1989. 93:2880-2882.
16. Haberland, M.E,;Fless,G.M.;Scannu,A.M.andFogelman,A.M. "Malondiaalde hyde de modification of lipoprotein produces avid uptake by human monocytes macrophages " J. boil.chem, 1992. 267:4143-4159.
17. Horbett, T.A.; Brash, J.L"proteins at interface; current issues and future prospects"in ; Brash. J.L. and Horbett, T.A,"Proteins at interfaces physiochemical and biological studies" ACS Symposium Series, 343; Washington: Acs, 1987. pp 1-33.
18. Israelachvilli, J. N.;"Intermolecular and surface force" New York .Academic press.1985
19. Johnson, L.N; Cheetham, J: Mclaunglin, P.J.; Acharya, k. R.: Barford, D and Philips. D. C. "Proteinoligosacchride interactions: lysozymephosphorylase amylase."curr top. 1985. 139:81-134.
20. Kossovsky, N."Perfecting delivery"chemistry in Britain. 43-45. 1996.
21. Kossovsky, N..; Millet, d; Gelman, L.A.; Sponsler.E.Dand Hnatyszyn.H.J"Self assembling nano structures" matr. Res. Soc bull. Seot. 1993. 78-81.
22. Kossovsky, N..; Gelman.A. and Sponsler, E.E."Cross linking encapsulated haemoglobin solid phase supports: lipid enveloped haemoglobin adsorbed to surface modified ceramic particles exhibit physiological oxygen lability artif.cells blood sub"biotech 1993. 223: 479-485.
23. Kossovsky, N.; Gelman, A; Sponsler, E.E.; Hnatyszyn, AJ.; Rajguro, S.; Torres, M.; Pham, M.; Crowder, J.; Zemanovich, J.; Chung, A and Shah, R "Surface modified nanocrystalline ceranlic for drug delivery applications." Biomaterials, 1994a 15: 1201-1207.
24. Jain. N. K. "Advances in controlled drug delivery system"; 317-328.
25. Vyas S P., Khar R k., Targeted & controlled Drug Delivery, 2004, CBC Publisher & distributors, New Delhi 28-30.
26. Kossovsky N. and Millett D. "Materials biotechnology and blood substitutes." Matr. Res. Soc. Bull., Sept.: 1991 78-81.
27. Kossovsky, N.; Bunshah, R F.; Gelmm, A; Sponsler, E.D.; Dmarjee, D.M.; Suh; T.G.; Pralash, S.; Doel; H. J. and Deshpandey, Cv. "A non-denaturing solid phase pharmaceutical carrier comprised of surface modified nanocrystalline materials." 1. Appl. Biomater. 1990 1: 289 294.
28. Kossovsky, N.; Gelman, A; Sponsler, E.D.; Millett, D. "Nano-crystalline Epstein-Bar Vims decoys." 1. Appl. Biomater. 1991 2: 251-259.
29. Irma Rojas-Oviedo, Rodrigo A. Salazar-L ´opez, "Elaboration and structural analysis of aquasomes loaded with Indomethacin" european journal of pharmaceutical sciences Nov; 32(3):223-30.