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# Aquasomes: A Nanoparticulate Drug Carrier System

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## ABSTRACT

Nanotechnology has emerged fields of biomedical research in the last few decades the presents context is an attempt to present the brief information about nanobiotechnological applications. 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. Aquasomes are nanoparticulate carrier system but instead of being simple nanoparticles these are three layered self assembled structures, comprised of a solid 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. This article reviews the principles of self assembly, strategies used in chemical synthesis, methods of preparation, characterization and application of Aquasomes.

**Keywords:** Aquasomes, Nanoparticulate Carrier System, Oligomers and Self assembly

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# INTRODUCTION

The "Some" is the cell like formulations of novel drug delivery system. There are different types of 'somes' like Aquasomes (Carbohydrates-ceramic nanoparticles) are the nano-biopharmaceutical carrier system contains the particle core composed of nanocrystalline calcium phosphate or ceramic diamond, and is covered by a polyhydroxyl oligomeric film. Alternatively aquasomes are called as "bodies of water". 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[1]. These carbohydrate stabilize the 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[2] and desiccation induced molecular denaturation prevented by certain disaccharides[3]. 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.

**Interaction between charged group:**

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[4].

**Hydrogen bonding and dehydration effect :**

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[5].

**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 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, vander waals need to be buffered. In aquasomes, sugars help in molecular plasticization[6].

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 DNA and pigment/dyes[7]. Aquasomes deliver their contents through a combination of specific targeting, molecular shielding and a slow sustained release processes. Their large sized and active surfaces enable them to be loaded with water insoluble drugs through non covalent processes.

## 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[8].

**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[9]. 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 vander 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[10].

The molecules or ions adjust their own position to reach thermodynamic minimum. By self-organization, true nanostructures can be prepared.

## OBJECTIVES

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[11].

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. Hence, bio-active faces many biophysical constrain[12]. In such case, aquasomes with natural stabilizers like various polyhydroxy sugars act as dehydro protectant maintains water like state thereby preserves molecules in dry solid state.

## RATIONALE

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[13].

## PROPERTIES

Aquasomes performs following properties

1. Aquasomes possess large size and active surface hence can be efficiently loaded with substantial amounts of agents through ionic, non co-valent bonds, vander waals forces and entropic forces. Solid particles dispersed in aqueous environment, exhibit physical properties of colloids[14].
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[15].
4. Aquasomes due to their size and structure stability, avoid clearance by reticuloendothelial

system or degradation by other environmental challenges.

## METHOD OF PREPARATION OF AQUASOMES

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.

**Preparation of the core:**

The first step of aquasome preparation is the fabrication of the ceramic core[16]. 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. The precipitated cores are centrifuged and then washed with enough distilled water to remove sodium chloride formed during the action. The precipitates are resuspended in distilled water and passed through a fine membrane, filter to collect the particles of desired size. Two ceramic cores that are most often used are diamond and calcium phosphate.

**Carbohydrate coatings:**

The second step involves coating 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 carbohydrates on to the surfaces. Excess and readily desorbing carbohydrate is removed by by stir cell ultra-filtration[17]. The commonly used coating materials are cellobiose, citrate, pyridoxal-5-phosphate, sucrose and trehalose.

**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[18].

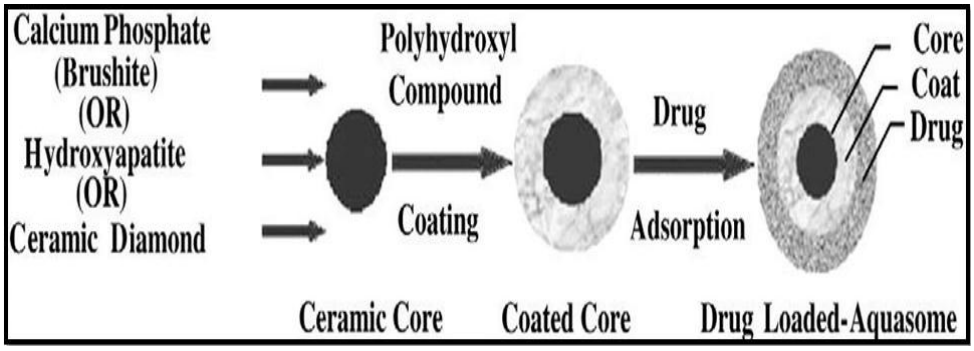


**Figure 1: Preparation of Aquasomes**

## CHARACTERIZATION OF AQUASOMES

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

**I. 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[19]. 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 electron photon correlation spectroscopy.

**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 400-400 $cm^{-}1$; the characteristic peaks observed are then matched with references peaks[20]. Identification of sugar and drug loaded over the ceramic core can also be confirmed by FT-IR analysis of the sample.

**Crystallinity:**

The prepared ceramic core can be analyzed for its crystalline or amorphous behaviour 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[21].

**II. 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)[22]. Furthermore, the adsorption of over the core can also be confirmed by measurement of zeta potential.

**Glass transition temperature:**

DSC can be used analyze the effect of carbohydrate on the drug loaded to aquasomes[23]. 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.

**III. Characterization of drug-loaded aquasomes:**

**Drug Loading:**

The drug loading can be determined by measuring the drug remaining in the supernatant liquid after loading which can be estimated by any suitable method of analysis[24].

**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 by incubating a known quantity of drug-loaded aquasomes in a buffer of suitable pH at 37° C with continuous stirring[25]. Samples are withdrawn periodically and centrifuged at high speed for certain lengths of time. Equal volumes of medium must be replaced after each with drawl. The supernatants are then analyzed for the amount of drug released by any suitable method[26].

## APPLICATIONS

Aquasomes used in following applications

1. 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[27].
2. Aquasomes as **red blood cell substitutes**, hemoglobin immobilized on oligomer surface because release of oxygen by hemoglobin is conformationally sensitive[28]. By this toxicity is reduced, hemoglobin concentration of 80% achieved and reported to deliver blood in non linear manner like natural blood cells.
3. Aquasomes have been used for successful targeted **intracellular gene therapy**[29,30], 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**[31], developed because drug activity is conformationally specific. Bio activity preserved and 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[32].

## CONCLUSION

Aquasomes, the self assembling surface-modified nanocrystalline ceramic cores, represent one of the simplest yet a carrier. The drug candidates delivered through the aquasomes show better biological activity even in case of conformationally sensitive ones. The crystalline nature of the core

gives structural stability and overall integrity. The molecular plasticizer, carbohydrate prevents the destructive drug carrier interaction and helps to preserve the spatial qualities. Hence these appear to be promising carriers for the delivery of broad range of molecules including viral antigens, heamoglobin and insulin, and other bioactive molecules, thus promoting a better therapeutic effect. This approach thus provides pharmaceutical scientists with new hope for the delivery of bioactive molecules.

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## REFERENCES

1. N. Kossovsky, A. Gelman, E.E. Sponsler, H.J. Hnatyszyn, S. Rajguru, M. Torres. Biomaterials. 1994; 15: 1201.
2. Arakawa, T. and Timasheff, S. N. Stabilization of protein structure by sugars, Biochemistry. 1982: 21: 6536-6544.
3. 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. 1947; 367-384.
4. Kossovsky, Gelman. A. and Sponsler, E.E. Cross linking encapsulated haemoglobin solid phase supports. 1994; 223: 479-485.
5. Cherian A.K., Rana A.C., Jain S.K. Self-assembled carbohydrate-stabilized ceramic nanoparticles for the parenteral delivery of insulin. Drug Dev Ind Pharm. 2000; 26:459-463.
6. Goyal A.K., Khatri K., Mishra N., Mehta A., Vaidya B., Tiwari S., *et al.* Development of self-assembled nanoceramic carrier construct(s) for vaccine delivery. J Biomater Appl. 2009; 24:65-84.
7. Mesariya S, Joshi K, Jain H, Upadhyay U. Aquasomes—A Self-Assembled Nanotechnology System. Chem Inform. 2012; 43.
8. Frankel D.A., Lamparski H. Liman U, O' Brien d.f. Photoinduced destabilization of bilayered vesicles. J Am. Chem.Soc. 1989; 111: 9262
9. Crowe J.H., Crowe L.M. and Jackson S.A. Preservation ofStructural and functional activity in lyophilized sarcoplasmin reticulum. 1983; 2: 477-484.
10. Haberland M.E., Fless G.M., Scannu A. M., and Fogelman A.M. Malondiaalde hydede modification of lipoprotein produces avid uptake by human monocytes macrophages. J.boil.

chem, 1992; 267: 4143-4159.

11. Khopade A.J., Khopade S., Jain N.K. Development of haemoglobin aquasomes from spherical hydroxyapatite cores precipitated in the presence of poly(amidoamine) dendrimer. Drug Dev Ind Pharm. 2002; 241:145-154.
12. Shahabade Gururaj S, Bhosale Ashok V, Mutha Swati S, Bhosale Nilesh R, Khade Prashant H. An overview on nanocarrier technology-Aquasomes. Journal of Pharmacy Research. 2009; 2; 7.
13. Wani S, Yerawar A. Aquasomes (2011), A novel nanocarrier for drug delivery. Chem Inform, 42-46.
14. Gholap AD, Borude SS, Mahajan AM, Gholap MAD. Aquasomes: A potential drug delivery carrier. Pharmacol Online. 2011; 3: 230-37.
15. Jain NK, Umamaheshwari RB. Control and novel drug delivery systems. In: Jain NK, editor. Pharmaceutical product development. CBS Publishers & Distributors, New Delhi. 2006; 419-455.
16. N. Kossovsky, A. Gelman, E.E. Sponsler, Artif. Cells Blood Sub. Biotech. 1994; 223, 479.
17. Vyas S.P., Goyal A.K., Khatri K., Mishra N., Mehta A., Vaidya, B., *et al.* Aquasomes a nanoparticulate approach for the delivery of antigen. Drug Dev Ind Pharm. 2008; 34:1297-1305.
18. Vays SP, Khar RK. Targeted & controlled Drug Delivery. CBC Publisher & distributors, New Delhi. 2004; 28-30.
19. Irma Rojas-oviedo, Rodrigo A, Salazar-L openz. Elaboration and structural analysis of aquasomes loaded with inomethacin. European journal of pharmaceutical sciences, 1994; (3): 223-30.
20. Cherian AK, Rana AC, Jain SK. Self-assembled carbohydrate-stabilized ceramic nanoparticles for the parentral delivery of insulin. Drug Dev Ind Pharm. 2000; 26: 459-463.
21. Vysa SP, Goyal AK, Rawat A, Manohar S, Gupta PN, KhatriK. Nanodecoy system: a novel approach to design hepatitis B vaccine for immune potentiation. Int J Pharm. 2006; 309: 227-233.
22. Vyas SP, Goyal AK, Khatri K, Mishra N, Mehta A, Vaidya B, et al. Aquasomes-a nanoparticulate approach for the delivery of antigen. Drug Dev Ind Pharm. 2008; 34:1297-1305.
23. Sutariya V, Patel P. Aquasomes: a novel carrier for drug delivery. International Journal of Pharmaceutical Sciences and Research. 2012; 3: 688

24. Sirikonda AK, Kumar BV, Soma Sekhar A, Gopi M, Babu HS, Rao GS. Aquasomes: A Review. Chem Inform. 2014; 45 - 60.
25. Shahabade Gururaj S, Bhosale Ashok V, Mutha Swati S, Bhosale Nilesh R, Khade Prashant H. Thakkar. An overview on nanocarrier technology-Aquasomes. Journal of Pharmacy Research, 2009; 2 - 7.
26. Oviedo R.I., Lopez S.A.D., Gasga R.J., Barreda C.T.Q. Elaboration and structural analysis of aquasomes loaded with indomethecin. Eur J Pharm Sci. 2007; 32: 223-230.
27. Vyas S.P., Goyal A.K., Rawat A., Mahor S., Gupta P.N., Khatri K. Nanodecoy system: a novel approach to design hepatitis B vaccine for immune potentiation. Int J Pharm. 2006; 309: 227-233.
28. Patil S., Pancholli S.S., Agrawal S., Agrawal G.P. Surface modified meso porous ceramics as delivery vehicle for haemoglobin. Drug Deliv. 2004; 11:193-199.
29. Pavani V, Dintakurthi S, Aslam S, Gollagudem R, Pabbapi K. Aquasomes: A Novel Drug Carrier System. ChemInform. 2013; 22 - 44.
30. Rathore P, Duggal S, Swami G. Aquasomes: a promising nanobiopharmaceutical drug delivery system for proteins and peptides. Chem Inform. 2013; 44 - 61.
31. Chaudhari M, Pandya D, Thakkar P, Soni A, Modi D. Aquasomes: a novel drug delivery system. Chem Inform. 2013; 38 - 44.
32. Girotra L, Bajaj R. Emerging Trends And Recent Advances In Aquasomes: A Review. Inventi Rapid: Pharm Tech. 2012; 24 - 29