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Aquasome: A Novel Drug Delivery Approach Using Nanocrystaline Biomaterial

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ABSTRACT

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.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..

Keywords: Aquasomes, nanoparticles, biochemically active, lipid systems.

1. INTRODUCTION

The field of biomaterials has been grown and evolved in its capacity to study the molecular biology and cell biology of the implant tissue interface, used as vehicles to deliver nano and large bioactive molecules to specific tissues in order to restore normal physiological function. The strategy of using materials as delivery agents provides many opportunities related to the design of micro-material and eventually nanomaterial, which are miniaturized constructs designed to encapsulate, target, and deliver drugs to a specific site [1]. Nanotechnology can be defined as the design, characterization, production, and application of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale (atomic, molecular, and macromolecular scale) that produces structures, devices, and systems with at least one novel/superior characteristic or property [2].

Biomaterials are natural or synthetic nonviable materials introduced in a medical device, intended to interact with biological systems in order to evaluate, treat, deliver, augment, or replace any tissue, or function of body [3]. Biomaterials when reduced to the size scale of nano, properties like surface-area, quantum, and optical effects, electrical and magnetic behaviors are unassailable for diagnosis and treatment of diseases.

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues.

From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology.

To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers one can name soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles.

Controlled drug release and subsequent biodegradation are important for developing successful formulations. Potential release mechanisms involve: (i) desorption of surface-bound /adsorbed drugs; (ii) diffusion through the carrier matrix; (iii) diffusion (in the case of nanocapsules) through the carrier wall; (iv) carrier matrix erosion; and (v) a combined erosion /diffusion process. The mode of delivery can be the difference between a drug's success and failure, as the choice of a drug is often influenced by the way the medicine is administered. Sustained (or continuous) release of a drug involves polymers that release the drug at a controlled rate due to diffusion out of the polymer or by degradation of the polymer over time. Pulsatile release is often the preferred method of drug delivery, as it closely mimics the way by which the body naturally produces hormones such as insulin. It is achieved by using drug-carrying polymers that respond to specific stimuli (e.g., exposure to light, changes in pH or temperature).

Targeting is the ability to direct the drug-loaded system to the site of interest. Two major mechanisms can be distinguished for addressing the desired sites for drug release: (i) passive and (ii) active targeting.

The main objective behind the development of controlled drug delivery careers is to maintain the release of a drug in a constant manner to get its optimum bio-availability.

Bioavailability is defined as the rate and the extent to which the ingredients or active moiety is absorbed from the drug product and becomes available at the site of action. As per the definition of bioavailability, a drug with poor bioavailability is one with poor aqueous solubility, slow dissolution rate in biological fluids, poor stability of dissolved drug at physiological pH, poor permeation through biomembrane, extensive presystemetic metabolism. Bioavailability of poorly water soluble drugs is a major problem. There are three major approaches to overcome the bioavailability problems.

A) Pharmaceutics approach: Modification of formulation, manufacturing processes or physiochemical properties of the drug is done.

B) Pharmacokinetic approach: Pharmacokinetics of drug is altered by modifying its chemical structure.

C) Biological approach: In this, route of drug administration may be changed such as parenteral form instead of oral form. Rate dissolution and its solubility are very important factors in third approach. The second approach of chemical modification has number of drawbacks such as being very expensive, time consuming, requires repetition of chemical studies, risk of precipitation and adverse effects. So generally only pharmaceutics approach is considered there. The technology which has the potential to solubilise varying quantities of poorly water soluble drugs with the help of lipids or lipid systems is known as lipid technology. [4]

Various lipid systems are as follows:

a) Oil based formulation
b) Triglycerides
c) Liposomes and proliposomes
d) Niosomes
e) Lipid Emulsions
f) Multiple emulsions: o/w/o emulsion, w/o/w emulsion
g) Hydrogel
h) nanoparticles
i) Aquasomes
j) Solid lipid nanoparticles
k) Nanostructure lipid carriers (NLC)
l) L-OROS Technology

SEDDS (Self emulsifying drug delivery systems) and SMEDDS (Self micro emulsifying drug delivery systems)

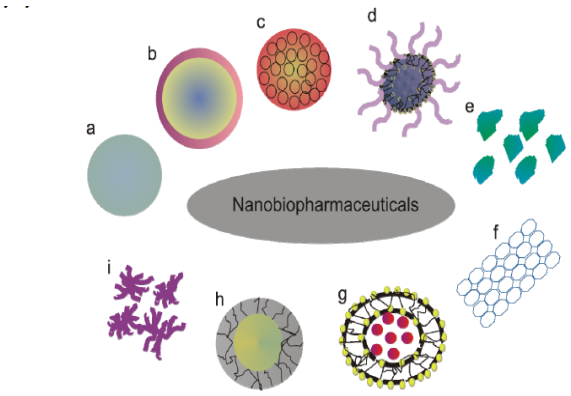

Fig.1. illustrates the different biomaterials as nanobiopharmaceuticals. (a) multifunctional nanoparticle (b) quantum dot (c) aquasomes (d) polyplexes/lipopolyplexes (e) superparamagnetic iron oxide crystals (f) carbon nanomaterial (g) liposomes (h) polymeric micelles and (i) dendrimers

Aquasome Are Nano-Decoy

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. [5]
Aquasomes are the nanobiopharmaceutical carrier system contains the particle core composed of nanocrystalline calcium phosphate or ceramic diamond, and is covered by a polyhydroxyl ligomeric 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. [6]

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 [7] and desiccation induced molecular denaturation prevented by certain disaccharides [8]. These three layered structure are self assembled by non-covalent bonds.

Properties Of Aquasome

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 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.
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. As solid particles dispersed in aqueous environment, exhibit physical properties of colloids.
4. 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.
5. Aquasomes water like properties provides a platform for preserving the conformational integrity and bio chemical stability of bio-actives.
6. Aquasomes due to their size and structure stability, avoid clearance by reticuloendothelial system or degradation by other environmental challenges.
7. 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 dsissolution after formation of heterophagosome. [9, 10]

Rational Behind Development Of Aquasomes

There are several reasons behind the development of this noval drug delivery system comprising natural material: some of them are described here as;

1. The careers like prodrug, macromolecules and liposomes have served to attain the intended purpose, but they all are prone to have biological constraints. The destructive interaction between the drug career and the drug are often present several limitations for the development of the newer carrier system.
2. The intrinsic biophysical constraints, dehydration and conformational changes caused by the drug delivery system can lead to adverse or allergic reactions with some advers pharmacological activities (heberland et al. 1992). By incorporation such molecules in aquasomes with natural stabilizers , one can preserve the molecular conformation since these natural sugars act as dehydroprotactants.
3. There are several systemic biophysical and intrinsic biophysical constraints, which tend to destabilize the drug. These can be overcome by using an natural stabilizer like sugar. [11]

Formulation Of Aquasomes

1. Principles 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 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.
(i) 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. [9-11]

2. METHOD OF PREPARATION OF AQUASOMES [12-16]

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.

a. ***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.

b. ***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.

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.

The carbohydrates used for this purpose are: [17]

(a) *Cellobiose*- It is 4-0-beta-D glucopyranosil –D-Glucose [$C_{12}H_{22}O_{11}$ m.w. 342.30]. It does not acure in free in nature or as gluycoside. It is prepared from cell-free enzymatic hydrolysis of cellulose. Its 1 gm is soluble in 8 ml. of water and 1.5 ml. of boiling water and almost insilube in alcohol.

(b) *Pyridoxil -5-phosphate*- it is 3- hydrpoxy 2 mthyle -5-[(phosphonoxy) methyl 1]- 4 ester. It is prepared by the action of phosphorus oxychloride on pyridoxyl in aqueous solution by the phosphorylation of pyridoxamine with 100% hypoclorus acid.

(c) *Trehalose* – It is alpha-D glucopuyranosyl alpha D-glucopyranoside [$C_{12}H_{22}O_4$ m.w. 342]. It is fund on parasite beetle, Larinus species and in fubgi *Amanita muscaria*. It can be isolated from bakers yeast. It is solubel is water and hot alcohol and insoluble in ether.

c. ***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.

3. CHARACTERIZATION OF AQUASOMES

Aquasomes are mainly characterized for

- Structural analyses, particle size, and morphology evaluated by X-ray powder diffractometry, transmission electron microscopy, and scanning. electron microscopy.
- Morphology and the size distribution were evaluated through images of scanning electron microscopy.
- Chemical composition and the crystalline structure of all samples were obtained through X-ray powder diffractometry.
- Dissolution and drug release profiles are evaluated by invitro studies using semi permeable membrane.

4. APPLICATIONS OF AQUASOMES

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.

Aquasome has resolved the problems associated with other systems, as-

1. Aquasomes can effectively deliver the large complex labile molecules and hemoglobin. This solves the problem stability of such biomolecules. 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 [18]

2. The development of compound that enhances immune responses to recombinant or synthetic epitopes is of considerable importance in vaccine research. Of the many different types of immunopotentiating compounds that have been researched, aquasomes are of considerable promise, because of their potency and adjuvanticity. Aquasomes were prepared by self-assembling of hydroxyapatite by co-precipitation method and thereafter preliminary coated with polyhydroxyl oligomers (cellobiose and trehalose) and subsequently adsorbed with bovine serum albumin (BSA) as a model antigen. 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 [19]. The clean ceramic was coated with the disaccharide cellobiose and mixed with the emulsified viral protein and then dialyzed into the final delivery vehicle to elicite both cellular and humoral response.

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 [20].

4. 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. 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 [21]

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. [22]

6. The insulin-bearing aquasomes were fabricated by first preparing the nanosize calcium phosphate dihydrate core. The calcium phosphate dihydrate core was prepared by colloidal precipitation and sonication of disodium hydrogen phosphate solution and calcium chloride solution at low temperature. This core was coated with cellobiose, pyridoxal-5-phosphate, or trehalose under sonication and was further loaded with the drug at low temperature by a partial adsorption mechanism. The prepared systems were characterized for size, shape, size distribution, drug loading efficiency, and in vivo performance. The in vivo performance of the formulated aquasome was compared with standard porcine insulin solution, and better results were observed compared to insulin solution.[23]

Fate Of Aquasome

Aquasomes are colloidal biodegradable carrier system and they specifically target the liver and muscles. The ceramic core was built of calcium phosphate and the biodegradation of such core was achieved by monocytes and multi-cellular cells called osteoclasts because they intervene first at the biomaterial implantation site during inflammation reactions.

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