Review Article

An Overview on Aquasomes

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ABSTRACT

Aquasomes are spherical particles composed of calcium phosphate or ceramic diamond covered with a polyhydroxyloligomeric film and act as nanoparticulate carrier system but instead of being simple nanoparticle these are three layered self-assembled structures, comprised of a solid phase nanocrystalline core coated with oligomeric film on which biochemically active molecules are adsorbed with or without modification. The solid core provides the structural stability, while the carbohydrate coating protects against dehydration and stabilizes the biochemically active molecules. After synthesis of core of ceramic and polyhydroxyoligomeric material coating like cellulobiose and trehalose the final stage were drug loading during which the aquasomes act as host particles to non-covalently interact with bio-active molecule via hydrogen and cationic bonding. It provides delivery of various protein molecules such as liable enzymes and insulin via non covalently adsorption over polysaccharide layer and conforms the stability and remain orally active.

Keywords: Disaccharide, Vesicles, Prodrug, Nanoparticles.

INTRODUCTION[1-6]

Aquasomes are termed as “bodies of water”, since they have water like properties which protect and preserve fragile biological molecules and this property of maintaining conformational integrity as well as high degree of surface exposure is used in targeting and delivering of bio-active molecules like peptide and protein hormones, antigens and genes to specific sites where action is required. Aquasomes were first developed by Nir Kossovsky and these carbohydrate stabilize nanoparticles of ceramic are known as “aquasomes”. Preparation of aquasomes is shown in Figure 1. The pharmacologically active molecule incorporated by co-polymerization, diffusion or adsorption to carbohydrate surface of pre formed nanoparticles. Aquasomes are three layered structure having a self assembled by non-covalent bonds. Principal of “self assembly of macromolecule” is covered by three physiochemical process that are 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 protein. 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 unfavourable, the molecule dehydrate and get self assembled. Self assembly leads to altered biological activity, vander waals needs to be buffered. In aquasomes, sugars help in molecular plasticization .

COMPOSITION OF AQUASOMES[7-9]

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.

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.

Bioactive

They have the property of interacting with film via non covalent and ionic interactions.

ROLE OF DISACCHARIDES IN AQUASOMES

Disaccharides like trehalose are seen to have stress tolerance in fungi, bacteria, insects, yeast and some plants. Trehalose have mechanism of action by protecting proteins and membranes within plant cell during the desiccation process and thus preserves cell structures, inherent flavors, colors and textures. 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. These disaccharides contain a large quantity of hydroxyl group and help to replace the water around polar residues in proteins, thus 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.

PROPERTIES OF AQUASOMES[6]

Aquasomes can be efficiently loaded with substantial amounts of agents through ionic, non co-valent bonds, van der waals forces and entropic forces since they possess large size and active surface. As solid particles dispersed in aqueous environment, exhibit physical properties of colloids. Aquasomes mechanism of action is controlled by their surface chemistry. Aquasomes allow delivery of drug contents through combination of specific targeting, molecular shielding, and slow and sustained release process. Aquasomes have water like properties which provides a great advantage for preserving the conformational integrity and biochemical stability of bio-actives. Aquasomes avoid clearance by reticuloendothelial system or degradation by other environmental challenges due to their size and structure stability. 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. The chemical composition and the crystalline structure ofsamples can be obtained by X-ray powder diffractometry.

OBJECTIVES OF PREPARING AQUASOMES[10-12]

The main objective of preparing aquasomes is to protect bio-actives. Various other carrier system are there like prodrugs and liposomes but they have disadvantage that they are prone to undergo destructive interactions between drug and carrier, so in that condition in aquasomes can be termed as a important carrier. In aquasomes carbohydrate coating prevents destructive denaturing interaction between drug and solid carriers. Aquasomes maintains molecular confirmation and optimum pharmacological activity. An active molecule possess qualities like unique three-dimensional conformation, a freedom of internal molecular rearrangement which is induced by molecular interactions, freedom of bulk movement but protein undergo irreversible denaturation when desiccated, even unstable in aqueous state. In the aqueous state pH, temperature, solvents, salts cause denaturation thus bio-activity face many biophysical constrain and hurdles. In such case, aquasomes with natural stabilizers like various polyhydroxy sugars act as dehydroprotectant aiding in maintaining water like state thereby preserves molecules in dry solid state.

MATERIAL USED AND ITS IMPORTANCE[13-16]

Polymers and ceramic can be used for preparation of nanoparticles core. Albumin, gelatin or acrylates are polymers used and diamond particles, brushite, and tin oxide core are ceramics used in preparation of aquasomes.

Ceramic materials are mostly used because ceramics are structurally the most regular materials known for core, being crystalline high degree of order ensures-

Bulk properties of ceramic will be preserved because any surface modification will have only limited effect on nature of atoms below surface layer. The surface will exhibit high level of surface energy that will favor the binding of polyhydroxy oligomer surface film. Within a very less time the freshly prepared particles possess good property of adsorbing molecules. Second step followed by coating of carbohydrate epitaxially over nanocrystalline ceramic core. The commonly used coating materials 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 bioactives molecules adsorbed which possess property of interacting with film via non-covalent and ionic interactions.

METHOD OF PREPARATION OF AQUASOMES[17-19]

The method of preparation of aquasomes involves three steps.

FORMATION OF AN INORGANIC CORE

Calcium phosphate and diamond are the two most commonly used ceramic cores. This method involves the fabrication of a ceramic core, and the procedure depends upon the materials selected.

Synthesis of nanocrystalline tin oxide core ceramic

It can be synthesized by direct current reactive magnetron sputtering. In a high pressure gas mixture of orgon and oxygen, a 3 inches diameter target of high purity tin is sputtered. The ultrafine particles formed in the gas phase are then collected on copper tubes cooled to 770K with flowing nitrogen.

Self assembled nanocrystalline brushite (calcium phosphate dihydrate)

These can be prepared by colloidal precipitation and sonication by reacting solution of disodium hydrogen phosphate and calcium chloride.

Nanocrystalline carbon ceramic, diamond particles

After ultra cleansing and sonication, nanocrystalline carbon ceramic, diamond particles can also be used for the core synthesis. The main feature of various cores is that they are crystalline. When they are introduced into the synthetic processes they measures between 50-150 nm and exhibit extremely clean spectrum and are therefore reactive species. Ceramic materials are structurally highly regular thus they are mostly used for core fabrication. The high degree of order in crystalline ceramics ensures only a limited effect on the nature of atoms below the surface layer when any surface modification is being done, thus preserving the bulk properties of ceramics. This high degree of order also offers a high level of surface energy that favors the binding of pohyhydroxyl oligomeric surface film. During the reaction the precipitated cores are centrifuged and then washed with enough distilled water to remove sodium chloride formed. To collect the particles of desired size the precipitates are resuspended in distilled water and passed through a fine membrane filter. The equation for the reaction is as follows:

$2\ Na_2HPO_4 + 3\ CaCl_2 + H_2O \rightarrow Ca_3(PO_4)_2 + 4\ NaCl + 2\ H_2 + Cl_2 + (O)$

COATING OF THE CORE WITH POLYHYDROXY OLIGOMER

The commonly used coating materials are cellobiose, citrate, pyridoxal-5- phosphate, trehalose and sucrose. It is the second step in which ceramic cores are coated with carbohydrate. The carbohydrate which we mainly use can be polyhydroxyl oligomer. By addition of carbohydrate into an aqueous dispersion of the cores under sonication the coating is carried out. These are then subjected to lyophilization which make an irreversible adsorption of carbohydrate onto the ceramic surface. By centrifugation the unadsorbed carbohydrate are removed.

LOADING OF THE DRUG OF CHOICE TO THIS ASSEMBLY

The loading of drug to the coated particles by adsorption is the last and final stage for the preparation of aquasomes. In this stage a solution of known concentration of drug is prepared in suitable pH buffer and coated particles are dispersed into it. The dispersion is then kept overnight at low temperature which governs drug loading or lyophilized after some time so as to obtain the drug-loaded formulation. The preparation thus obtained is then characterized using various techniques .

CHARACTERIZATION OF AQUASOMES[20-21]

They are characterized for the structural and morphological properties, particle size distribution and drug loading capacity.

Size distribution

Morphological properties and particle size distribution can be characterized by scanning electron microscopy and transmission electron microscopy. For the measurement of man particle size and zeta potential of the particle photon correlation spectroscopy is used.

Structural analysis

For structural analysis FT-IR spectroscopy is used. In FT-IR Potassium bromide sample disk method is used, core as well as coated core is analysed by recording their IR spectra in wave number range 4000-400 cm^{-1}.

Crystallinity

X –ray diffraction is used to determine crystalline or amorphous behavior of ceramic core.

CHARACTERIZATION OF COATED CORE

Carbohydrate coating

For coating of sugar over ceramic core Concanavalin A-induced aggregation method or anthrone method is used. By the help of zeta potential measurement, absorption of sugar over the core is recorded.

Glass transition temperature

The transition from glass to rubber state as a change in temperature upon melting of glass DSC analyser can be used to analyse.

CHARACTERIZATION OF DRUG -LOADED AQUASOMES

Drug payload

It is determined by measuring the drug in the supernatant liquid after loading which can be estimated by analysis method.

In vitro drug release studies

In this the release pattern of drug from the aquasomes is determined by incubating a known quantity of drug loaded aquasomes in Ph at 37^0C with continuous stirring. The sample are withdrawn and centrifuge at high speed for certain length of time which is later on analysed .

APPLICATIONS OF AQUASOMES[22-24]

Since they act as red cell substitute haemoglobin is immobilized on oligomer surface because the oxygen released by haemoglobin is sensitive thus toxicity is reduced, concentration of haemoglobin reaches to 80% and blood is delivered as natural blood cells in non linear manner. For the delivery of viral antigen like Epstein –Barr and immune deficiency viruses produce correct antibody which is done by triggering with conformationally specific target molecules. The five layer composition consisting of ceramic core, polyoxyoligomeric film, therapeutic gene segment, additional carbohydrate film and a targeting layer of conformationally conserved viral membrane protein is used for successful targeted intracellular gene therapy. Aquasomes are used for the delivery of pharmaceutical product like insulin in which activity is conformationally specific. Activity is increased up to 60% and bio activity is preserved ,toxicity is not reported in comparision to intravenous administration of drugs. Enzymes like DNAase and pigment, dyes are also delivered by aquasomes,this is due to reason thatenzymes activity fluctuates with molecular conformation and cosmetic properties of pigments are sensitive to molecular conformation.

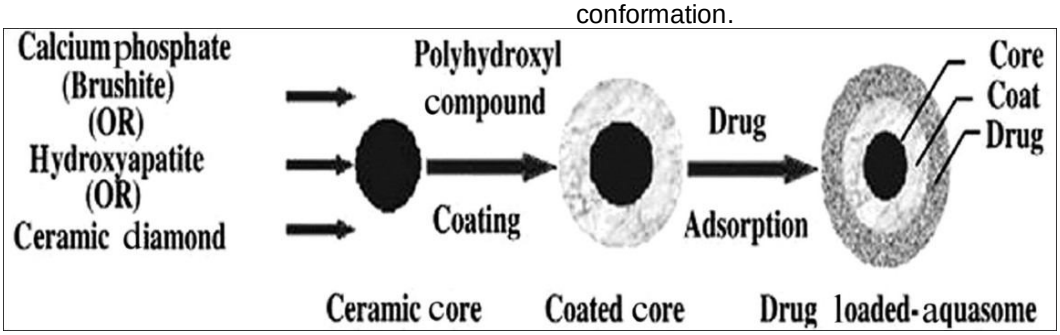

Fig. 1: Aquasome Preparation

FDA APPROVED (RECOMBINANT GENES) PROTEINS WHICH CAN BE TRANSFERRED THROUGH AQUASOMES (Table 1)

Table 1: FDA Approved (Recombinant Genes) Proteins Which Can Be Transferred Through Aquasomes

TRADE NAME	RECOMBINANT PRODUCT	YEAR OF APPROVAL
Activase	Tissue plasminogen activator	1987 US
Epogen/procrit	Erythropoietin (epoetinα)	1989/1990 US
Recombinate	Clotting factor VIII	1992 US
Kogenate; Helixate	Clotting factor VIII	1993 US
Kogenate FS;	Clotting factor VIII	1993 US
Helixate FS	(sucrose formulation)	2000 US
Cerezyme	β –glucocerebrosidase	1994 US
Avonex	IFN-β-1a	1996 US
Benefix	Clotting factor IX	1997 US
Rituxan (US)/ Mabthera(EU)	Anti-CD20 chimeric mAb	1997 US
Gonal-f	Follicle stimulating hormone	1997 US
	(follitropin α)	1995 EU
Simulect	Anti-IL2 receptor- α chimeric mAb	1998 US
Remicade	Anti- TNF α chimeric mAb	1998 US
Herceptin	Anti-HER 2 humanized mAb	1998 US
Enbrel	TNF α receptor- IgG fusion protein	1998 US
Thyrogen	Thyrotropin α	1998 US
Novoseven	Clotting factor VII a	1999 US
Ovidrel or Ovitrelle	Human chorionic gonadotropin α	2000 US
Refacto	B domain-delected clotting factor VIII	2000 US

CONCLUSION

Aquasomes is one of the part of novel drug delivery carrier which deal with principle of self assembly. We can see better biological activity even in case of conformationally sensitive drug candidates because of the presence of the unique carbohydrate coating the ceramic. This strategy may be beneficially extended to the novel delivery of other bioactive molecules.The molecular plasticizer , carbohydrate prevent the destructive drug-carrier interaction and helps to preserve the spatial qualities. The structural stability and overall integrity is controlled by crystalline nature of the core. We can say aquasomes can be used as a potential carrier for the delivery of a broad range of molecules including viral antigens, hemoglobin and insulin.

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