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Review Article

A VITAL ROLE OF AQUASOME'S ON CONTROLLED AND NOVEL DRUG DELIVERY

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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.The delivery system has been successfully utilized for the delivery of insulin, hemoglobin, and enzymes like serratiopeptidase etc. This reviews the principles of self assembly, the challenges of maintaining the conformational integrity and biochemical activity of immobilized surface pairs, the convergence of these principles into a single functional composition and its application in various fields of pharmacy.

Keywords: Aquasomes; Method of Preparation; Characterization; Applications.

Introduction

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 bioactive molecules like peptide and protein hormones, enzymes, antigens and genes to specific sites [1]. Aquasomes were first developed by Nir Kossovsky and these carbohydrate stabilize nanoparticles of ceramic are known as "aquasomes". The pharmacologically active molecule incorporated by co-polymerization, diffusion or adsorption to carbohydrate surface of pre formed nanoparticles [2]. Aquasomes are three layered structure having a self assembled by non covalent and ionic 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 [3]. 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. 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, vander Waal need to be buffered. In aquasomes, sugars help in molecular plasticization. [4-6].

OBJECTIVES OF AQUASOMES

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 dena-turing interaction between drug and solid carriers [7].
2. Aquasomes maintains molecular confirmation and optimum pharmacological activity. Normally, active molecules possess following qualities i.e. 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 [8]. 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 [9].

PROPERTIES OF AQUASOMES [10-12]

- 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. Mechanism of action of Aquasomes 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 after formation of heterophagosome).
- Aquasomes are mainly characterized for structural analyses, morphology these are evaluated by X-ray powder diffractometry, transmission electron microscopy, scanning electron microscopy. X-ray analysis of samples and drug loading efficiency and in vivo performance.

FORMULATION OF AQUASOMES

Principles of self assembly [5, 6]

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 nano structured 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.

METHOD OF PREPARATION OF AQUASOMES

The general procedure consists of an inorganic core formation, which will be coated with lactose forming the polyhydroeylated core that finally will be loaded by model drug. The core is coated with a polyhydroxyl oligomeric film, and the coated particles are then allowed to adsorb a drug or antigen. The final product consists of three layers: drug (or antigen), polybydroxyl oligomeric film, and the nanocrystalline ceramic core. The aquasomes are prepared using the principle of self preparation of core, coating of core, and immbolization of drug molecule.

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 [14].

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 [15].

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 [16]. 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 [15]. 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 makes 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 [14]. 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

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 [17-19].

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}. The characteristic peaks observed are then matched with reference peaks. Identification of sugar and drug loaded over the ceramic core can also be confirmed by FT-IR analysis of the sample [17, 20-21].

Crystallinity: The prepared ceramic core can be analyzed for its crystalline or amorphous behavior using xray 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 [17, 21-22].

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 [17, 20-21].

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 [17].

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 [18].

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 samples are withdrawn and centrifuge at high speed for certain length of time which is later on analysed [17].

APPLICATIONS OF AQUASOMES

1. Insulin delivery: Cherian et al prepared aquasomes using a calcium phosphate ceramic core for the parenteral delivery of insulin. The core was coated with various disaccharides such as cellobiose, trehalose, and pyridoxal-5-phosphate. Subsequently the drug was loaded to these particles by adsorption method. The in vivo performance of various aquasome formulations of insulin was evaluated using albino rats. Prolonged reduction of blood glucose was observed with all formulations except cellobiose-coated particles. Pyridoxal-5-phosphate coated particles were found to be more effective in reducing blood glucose levels than aquasomes coated with trehalose or cellobiose. This could be attributed to the high degree of molecular preservation by pyridoxal-5-phosphate. The prolonged activity was attributed to slow release of drug from the carrier and structural integrity of the peptide [22].

2. Oral Delivery of Enzyme: Rawat et al proposed the use of a nanosized ceramic core based system for oral administration of the acid-labile enzyme serratiopeptidase. The nanocore was prepared by colloidal precipitation under sonication at room temperature. The core was then coated with chitosan under constant stirring, after which the enzyme was adsorbed over it. The enzyme was protected by further encapsulating the enzyme-loaded core into alginate gel. The TEM images of particles showed them to be spherical in shape, with an average diameter o 925 nm. The enzyme-loading efficiency of the particles was found to be approximately 46%. The in vitro drug release data followed the Higuchi model in acidic medium (pH 1.2) for period of up to 2 to 6 hours, while the alkaline medium (pH 7.4) showed sustained and nearly complete first-order release of enzyme for up to 6 hours. These aquasomes were found to beprotecting the structural integrity of enzymes so as to obtain a better therapeutic effect [23].

3. As Oxygen Carrier: Khopade et al prepared hydroxyapatite core by using carboxylic acid–terminated half generation poly(amidoamine) dendrimers as templates or crystal modifiers. These cores were further coated with trehalose followed by adsorption of hemoglobin. The size of the particles was found to be in the nanometer range, and the loading capacity was found to be approximately 13.7 mg of hemoglobin per gram of the core. The oxygen-binding properties of the aquasomes were studied and compared to those of fresh blood and hemoglobin solution. Hill coefficient values determined for fresh blood, for hemoglobin solution, as well as for the aquasome formulation indicated that the properties of hemoglobin including its oxygencarrying capacity were retained by the aquasomes. Studies carried out in rats showed that aquasomes possess good potential for use as an oxygen carrier. Moreover, the formulation was found to retain its oxygen-binding characteristics over a period of 30 days [21].

4. Antigen Delivery: The adjuvants generally used to enhance the immunity to antigens have a tendency either to alter the conformation of the antigen through surface adsorption or to shield the functional groups. So Kossovsky et al demonstrated the efficacy of a new organically modified ceramic antigen delivery vehicle. These particles consisted of diamond substrate coated with a glassy carbohydrate (cellobiose) film and an immunologically active surface molecule in an aqueous dispersion. These aquasomes

(5-300 nm) provided conformational stabilization as well as a high degree of surface exposure to protein antigen. Diamond, being a material with high surface energy, was the first choice for adsorption and adhesion of cellobiose. It provided a colloidal surface capable of hydrogen bonding to the proteinaceous antigen. The disaccharide, being a dehydro-protectant, helps to minimize the surface-induced denaturation of adsorbed antigens (muscle adhesive protein, MAP). For MAP, conventional adjuvants had proven only marginally successful in evoking an immune response. However, with the help of these aquasomes a strong and specific immune response could be elicited by enhancing the availability and in vivo activity of antigen [24].

5. For delivery of gene: Aquasomes can be studied for the delivery of genes. It illustrates the attractive delivery system loaded with genetic material. Studies reveal that aquasomes protect and maintain structural integrity of the gene segment. A five layered composition comprised of the ceramic nanocrystalline core, the polyhydroxyl oligomeric film coating, the non covalently bound layer of therapeutic gene segment, an additional carbohydrate film and a targeting layer of conformationally conserved viral membrane proteins, have been proposed for gene therapy. The aquasome vehicle would afford all of the potential advantages of viral vectors and simultaneous overwhelming the risk of irrelevant gene integration [15].

Recent development

1. Development of hemoglobin aquasomes from spherical hydroxyapatite cores precipitated in the presence of half-generation poly(amidoamine) dendrimer .
2. AQUASOMES: A Novel Nanocarrier for Drug Delivery
3. An overview on nanocarrier technology
4. Aquasomes: a promising carrier for peptides and protein delivery.Nanomedicine.

CONCLUSION

Aquasomes, the self-assembling surface-modified nanocrystalline ceramic cores, seem to have potential and promising carriers capable of preserving the structural integrity of protein pharmaceuticals and carrier for delivery of broad range of molecules including viral antigens, heamoglobin and insulin, thus promoting a better therapeutic effect. Also, these formulations have been found to evoke a better immunological response and could be used as immunoadjuvants for proteinaceous antigens. This strategy may be beneficially extended to the novel delivery of other bioactive molecules. However, the roles of molecular plasticizers and core crystallinity need further extensive investigations

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