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REVIEW: AQUASOMES A POTENTIAL DRUG DELIVERY CARRIER

Bhairav Bhushan A .[1]* ,Wagh Yogesh B.[2], Saudagar Ravindra B.[3]

1. Department of Quality Assurance Techniques, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.
2. Department of Pharmaceutical Chemistry, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.

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For Correspondence:

Bhairav Bhushan A .

Department of Quality Assurance Techniques, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India

E-mail:

yogeshwagh68@gmail.com

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. Aquasomes 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. 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.

INTRODUCTION [2, 4, 22, 24]

Aquasomes are nanoparticulate carrier system but instead of being simple nanoparticles these are three layered self assembled structures, comprised of a solid phase nano crystalline 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 site.These carbohydrate stabilize nanoparticles of ceramic are known as "aquasomes" which was first developed by Nir Kossovsky. The pharmacologically active molecule incorporated by copolymerization, 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 and desiccation induced molecular denaturation prevented by certain disaccharides .These three layered structure are self assembled by non-covalent bonds.

Principal "self assembly of macromolecule" is governed by three physiochemical process i.e.

1) 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.

2) 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.

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 , 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, sugarshelp in molecular plasticization.

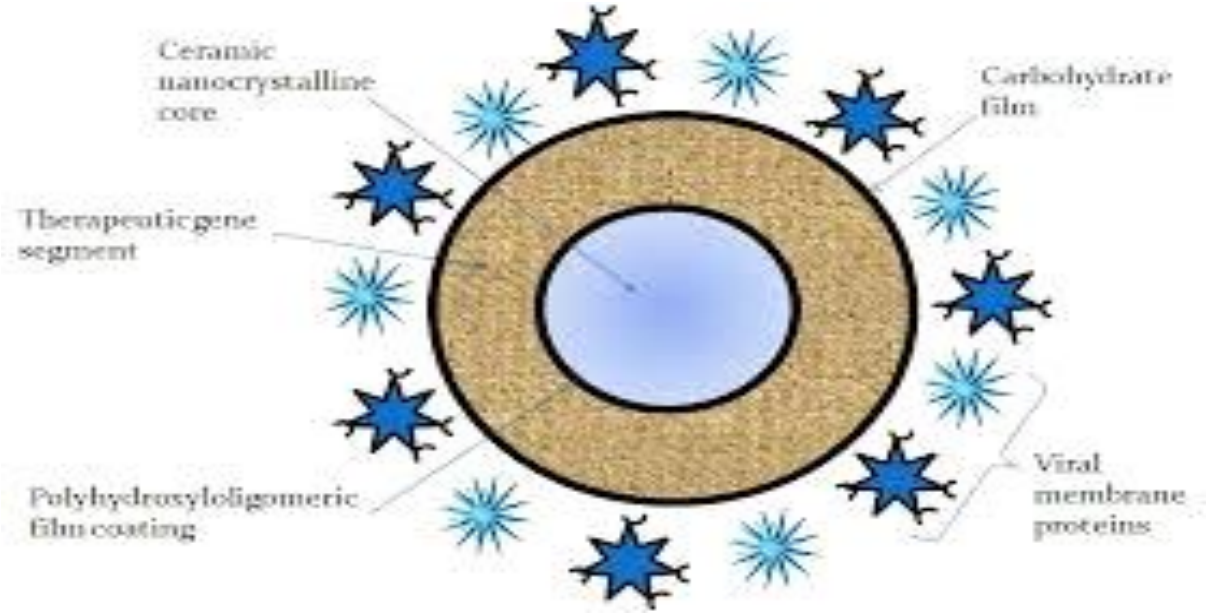


Fig: Aquasomes

STRATEGIES USED IN CHEMICAL SYNTHESIS OF NANOSTRUCTURES [6, 9, 13]

1) Arrays of co-valently linked atoms generated with well defined composition, connectivity and shape.

2) Covalent polymerization, 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.

4) Molecular self assembly , 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 [7,11, 16]

1. 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.

2. Secondly aquasomes maintains molecular confirmation and optimum pharmacological activity.

3. 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 dehydro protectant maintains water like state thereby preserves molecules in dry solid state.

PROPERTIES OF AQUASOMES [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. 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 biochemical 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.

ROLE OF DISACCHARIDES [14,15, 22].

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.

MATERIAL USE AND ITS IMPORTANCE [17,18]

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 tinoxide core. 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.

(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 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.

AQUASOMES[3]

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.

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 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 waal 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]

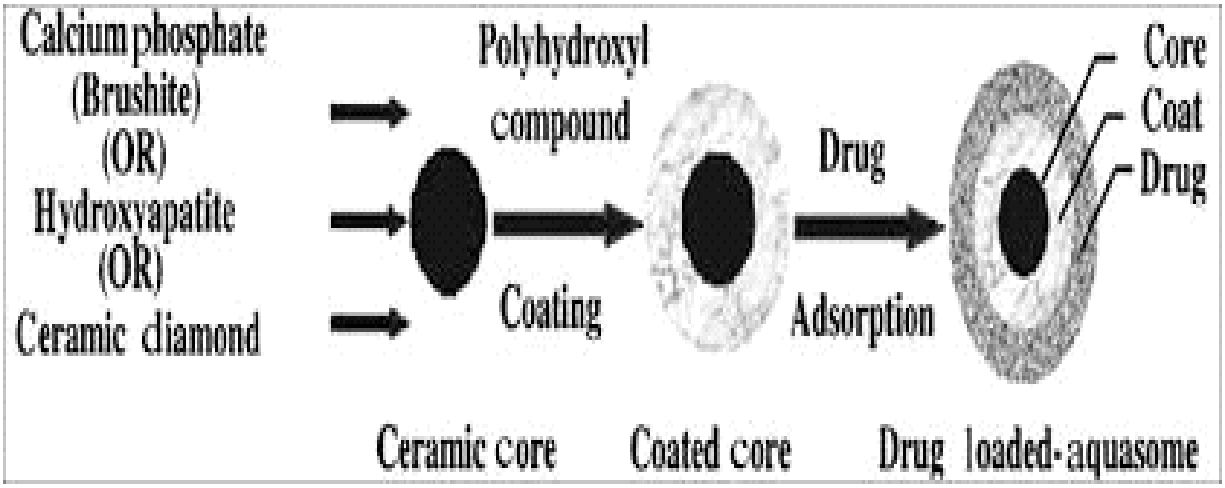
The general procedure consists of an inorganic core formation, which will be coated with Lactose forming the polyhydroxylated core that finally will be loaded by model drug By using the principle of self-assembly, the aquasomes are prepared in three steps i.e., preparation of core, coating of core, and 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 magnetron 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 favour 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.



Preparation method of Aquasomes

CHARACTERIZATION OF AQUASOMES [29]

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 wavenumber 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 load : 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 370^0C with continuous stirring. The sample are withdrawn and centrifuge at high speed.

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

Table 1: FDA Approved 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

APPLICATIONS OF AQUASOMES

1. Insulin delivery [22]: 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 .

2. Oral Delivery of Enzyme [23] : 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 upto 6 hours. These aquasomes were found to beprotecting the structural integrity of enzymes so as to obtain a better therapeutic effect.

3. As Oxygen Carrier [21]: 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 oxygen carrying 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.

4. Antigen Delivery [24]: 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 Kossovskyet 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.

5. For delivery of gene [15] : 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.

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 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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