International Journal for Pharmaceutical Research Scholars (IJPRS)
ISSN (Online): 2277 - 7873
REVIEW ARTICLE **V-1, I-1, 2012**

Aquasomes: A Self Assembling Nanobiopharmaceutical Carrier System for Bio-Active Molecules: A Review

Patel JK[1], Patel KN[1], Patel HK[1], Patel BA[1], Patel PA[1]
[1]*Shree Swaminarayan Sanskar Pharmacy College, Zundal, Gujarat-382421, India.*
Manuscript No: IJPRS/V1/I1/00012, Received On: 12/02/2012, Accepted On: 19/02/2012

ABSTRACT

Aquasomes are the self-assembling nanobiopharmaceutical carrier system, contains nanocrystalline calcium phosphate or ceramic diamond, is covered by a glassy polyhydroxyl oligomeric film. Aquasomes are spherical (5–925nm) particles used for drug and antigen delivery. Aquasomes are called as “bodies of water" their water like properties protect and preserve fragile biological molecules. Its high degree of surface exposure is used in targeting of bio-active molecules to specific sites. Three types of core materials are mainly used for producing aquasomes: Tin oxide, Nanocrystalline carbon ceramics and Brushite. Calcium phosphate is the core of interest, due to its natural presence in the body. The brushite is unstable and converts to hydroxyapatite upon prolong storage and seems a better core for the preparation of aquasomes. It is widely used for the preparation of implants. Aquasomes exploited as a RBC substitutes, vaccines for delivery of viral antigen and as targeted system for intracellular gene therapy. Enzyme activity and sensitivity towards molecular conformation made aquasome as a novel carrier for enzymes like DNAses and pigment/dyes. This report reviews the principles of self assembly, the challenges of maintaining both the conformational integrity and biochemical activity of immobilized surface pairs.

KEYWORDS

Aquasomes, Self assembling carrier system, Carbon ceramics (diamonds) and Brushite (calcium phosphate dihydrate)

INTRODUCTION

Recent advances in he fields of biotechnology and genetic research have resulted in promotion of proteins and peptides as a major class of therapeutic agents. Administration of bioactive molecules in their active state has been a formidable challenge to the pharmaceutical as well as biotechnological industries. Drug associated challenges such as suitable route of drug delivery, physical and chemical instability, poor bioavailability, and potentially serious side effects of these bioengineered molecules are some potential limitations on their successful formulation.

***Address for Correspondence:**
Jayeshkumar K. Patel,
M.Pharm, Department of Pharmaceutics,
Shree Swaminarayan Sanskar Pharmacy College, Zundal,
Gandhinagar, Gujarat, India.
E-Mail Id: jay_patel2008143@yahoo.com

The combination of biotechnology and nanotechnology (i.e., nanobiotechnology) has proposed a new approach as a solution to their formulation problem in the form of aquasomes.[1,2] Various new delivery systems used as carriers for various pharmaceutical applications are listed in Table 1.

They consist of a ceramic core whose surface is noncovalently modified with carbohydrates to obtain a sugar ball, which is then exposed to adsorption of a therapeutic agent. The core provides structural stability to a largely immutable solid.[3,4] Aquasomes offer an attractive mode of delivery for therapeutic agents belonging to the class of proteins and peptides, since they are able to overcome some inherent problems associated with these molecules.

Table: 1 Some emerging novel carriers for drug delivery

Carrier	Description	Application
Aquasomes	Three layered self-assembly compositions with ceramic nanocrystalline particulate core loaded with glassy layer of polyhydroxy compounds	Molecular shielding, specific targeting
Archaeosomes	vesicles composed of glycerolipids of Archaea with potent adjuvant activity	Potent adjuvant activity
Cryptosomes	Lipid vesicles with a surface coat composed of PC and of suitable polyoxyethylene derivative	Ligand-mediated drug targeting
Discomes	Niosomes solubilized with nonionic surfactant solution	Ligand-mediated drug targeting
Emulsomes	Nanosized lipid particles consisting of microscopic lipid assembly with a polar core	Parenteral delivery of poorly water soluble drugs
Enzymosomes	Liposomes designed to provide a mini bioenvironment in which enzymes are covalently immobilized or coupled to the surface of liposomes	Targeted delivery to tumor cells
Erythrosomes	Human erythrocyte cytoskeletons used as a support to which lipid bilayer is coated.	Effective targeting of macromolecular drugs
Ethosomes	Lipid-based soft, malleable vesicles containing a permeation enhancer and composed of phospholipids, ethanol and water	Targeted delivery to deep skin layers
Genosomes	Artificial macromolecular complexes for functional gene transfer. Cationic lipids are most suitable because they possess high biodegradability and stability in the blood stream	Cell-specific gene transfer
Novasomes	Consist of glyceryl dialurate, cholesterol and polyoxyethylene 10-stearyl ether at a weight percent ration of 57:15:28 respectively	Drug delivery to pilosebaceous compartment
Photosomes	Photolyase encapsulated in liposomes that release the contents by phototriggered changes in membrance permeability characteristics	Photodynamic therapy
Proteosomes	High-molecular-weight multi-subunit enzyme complexes with catalytic activity that is specifically due to assembly pattern of enzymes	Better catalytic activity turnover than nonassociated enzymes, may serve as adjuvant as well as protein carrier
Transferosomes (elastic liposomes)	Modified lipid-based soft, malleable carriers tailored for enhanced systemic delivery of drugs	Noninvasive delivery of drugs into or across the deeper skin layers and/or the systemic circulation
Vesosomes	Nested-bilayer compartments with “interdigitated” bilayer phase formed by adding ethanol to a variety of saturated phospholipids	Multiple compartments of the vesosomes give better protection to the interior contents in serum
Virosomes	Liposomes spiked with virus glycoprotein, incorporated into the liposome bilayers based on retrovirus-derived lipids	Immunological adjuvants

These problems include suitable route of delivery, physical as well as chemical instability, poor bioavailability, and potent side effects. The surface modification with carbohydrates creates a glassy molecular stabilization film that adsorbs therapeutic proteins with minimal structural denaturation.

Thus, these particles provide complete protection of an aqueous nature to the adsorbed drugs against the denaturing effects of external pH and temperature, because there are no swelling and porosity changes with change in pH or temperature.[5]

Aquasomes are nanoparticulate carrier system but instead of being simple nanoparticles these are three layered self assembled structures. They 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 also known as "bodies of water" as 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, 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 preformed nanoparticles.[6-7]

As an approach to macromolecular synthesis, self-assembly is appealing because biomimetic processes imply more biochemically functional products. This review article focuses on the principles of self assembly, the challenges of maintaining both the conformational integrity and biochemical activity of immobilized surface pairs, and the convergence of these principles into a single functional composition.

OBJECTIVES

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[8] 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.

FORMULATION OF AQUASOMES

1. Principles of Self Assembly[6,7]

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.

1.1 Interactions between Charged Groups:

The interaction of charged group facilitates long range approach of self assembly subunits 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.

1.2 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.

1.3 Structural Stability:

Structural stability of protein in biological environment determined by interaction between charged group and hydrogen bonds largely external to molecule and by 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 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.

2. Method of Preparation[9,10,11,12]

The method of preparation of aquasomes involves three steps. The general procedure consists of (1) formation of an inorganic core, followed by (2) coating of the core with polyhydroxy oligomer, and finally (3) loading of the drug of choice to this assembly.

The first step involves the fabrication of a ceramic core, and the procedure depends upon the materials selected. The two most commonly used ceramic cores are calcium phosphate and diamond. These can be fabricated by colloidal precipitation and sonication, inverted magnetron sputtering, or plasma condensation, among other methods. Ceramic materials, being structurally highly regular, are most widely 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. The precipitated cores are centrifuged and then washed with enough distilled water to remove sodium chloride formed during the reaction. The precipitates are resuspended in distilled water and passed through a fine membrane filter to collect the particles of desired size. The equation for the reaction is as follows:

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

In the second step, ceramic cores are coated with carbohydrate (polyhydroxyl oligomer). The coating is carried out by addition of carbohydrate into an aqueous dispersion of the cores under sonication. These are then subjected to lyophilization to promote an irreversible adsorption of carbohydrate onto the ceramic surface. The unadsorbed carbohydrate is

removed by centrifugation. Finally, the drug is loaded to the coated particles by adsorption. For that, a solution of known concentration of drug is prepared in suitable pH buffer, and coated particles are dispersed into it. The dispersion is then either kept overnight at low temperature for drug loading or lyophilized after some time so as to obtain the drug-loaded formulation (i.e., aquasomes). The preparation thus obtained is then characterized using various techniques. The procedure for preparation of aquasomes is depicted in Fig 1.

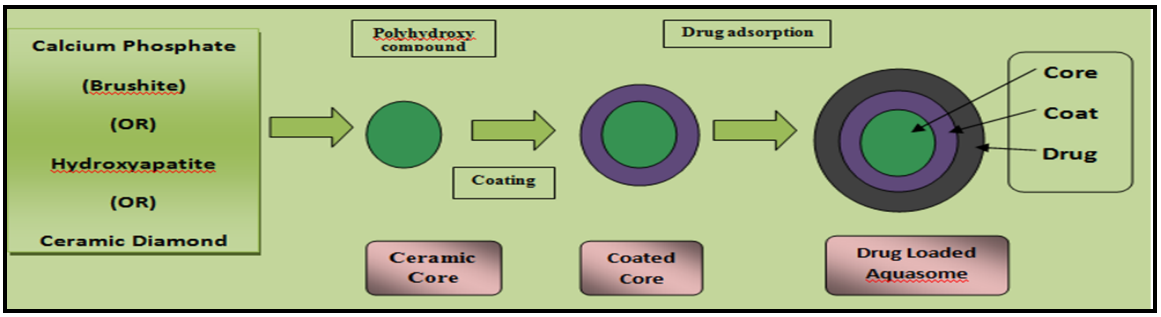

Figure: 1 Preparation of aquasomes.

PROPERTIES OF AQUASOMES[13]

- 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.
- Aquasomes mechanism of action is controlled by their surface chemistry. These 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 reticulo-endothelial system or degradation by other environmental challenges.

CHARACTERIZATION

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

1. Characterization of ceramic core

A. Size distribution

For morphological characterization and size distribution analysis, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are generally used. 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 photo correlation spectroscopy.[14-16]

B. 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 wavenumber 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.[16-18]

C. Crystallinity

The prepared ceramic core can be analyzed for its crystalline or amorphous behavior 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.[13,16,17]

2. Characterization of coated core

A. 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). Furthermore, the adsorption of sugar over the core can also be confirmed by measurement of zeta potential.[16-18]

B. Glass transition temperature

DSC can be used to analyze the effect of carbohydrate on the drug loaded to aquasomes. 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.[16]

3. Characterization of drug-loaded aquasomes

A. Drug payload

The drug loading can be determined by incubating the basic aquasome formulation (i.e., without drug) in a known concentration of the drug solution for 24 hours at 4°C. The supernatant is then separated by high-speed centrifugation for 1 hour at low temperature in a refrigerated centrifuge. The drug remaining in the supernatant liquid after loading can be estimated by any suitable method of analysis.[14]

B. *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 drugloaded aquasomes in a buffer of suitable pH at 37°C with continuous stirring. Samples are withdrawn periodically and centrifuged at high speed for certain lengths of time. Equal volumes of medium must be replaced after each withdrawal. The supernatants are then analyzed for the amount of drug released by any suitable method.[16]

C. In-process stability studies

SDS-PAGE can be performed todetermine the stability and integrity of protein during the formulation of the aquasomes.[16,18]

D. Role of disaccharides[19,20]

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.

APPLICATIONS

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. The authors therefore proposed aquasomes as a promising carrier for therapeutic protein and peptide drug delivery.[14] The utility of nanocarriers for effective delivery of insulin was also proved by Paul and Sharma. They prepared porous hydroxyapatite nanoparticles entrapped in alginate matrix containing insulin for oral administration. The optimum controlled release of insulin was also achieved in this study.[21]

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 of 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 a 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 be protecting the structural integrity of enzymes so as to obtain a better therapeutic effect.[22]

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 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.[17]

In another study Patil and co-workers prepared hydroxyapatite ceramic cores by co-precipitation and self-precipitation. These cores were coated with various sugars including cellobiose, trehalose, maltose, and sucrose. Subsequently, hemoglobin was adsorbed over the coated ceramic core, and the percentage drug loading was estimated by the benzidine method. The oxygen carrying capacity of aquasome formulation was found to be similar to that of fresh blood. Also, the Hill coefficients were found to be good for its use as an oxygen carrier. The aquasome formulations neither induced hemolysis of the red blood cells nor altered the blood coagulation time. The hemoglobin loading to various sugar-coated particles was found to be approximately 7.4%. The formulation was able to retain the hemoglobin over a period of 30 days. No significant increase in arterial blood pressure and heart rate was observed in rats transfused with aquasome suspension on 50% exchange transfusion.[23]

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]

Vyas et al prepared aquasomes by self-assembling of hydroxyapatite using the co-precipitation method. The core was coated with

cellobiose and trehalose, and finally bovine serum albumin was adsorbed as model antigen onto the coated core. The aquasomes were found to be spherical in shape with diameter around 200 nm. The coating of carbohydrate over the surface of the core was confirmed by concanavalin A–induced aggregation assay method as well as IR spectroscopy. The antigen-loading efficiency was found to be approximately 20–30%. When the immunological activity of the prepared formulation was compared to plain bovine serum albumin, the former was found to exhibit a better response. In view of these results, aquasomes were proposed to have superior surface immutability, in that they protect the conformation of protein structure and present it in such a way to immune cells that it triggers a better immunological response.[16]

The use of ceramic core–based nanodecoy systems was proposed by Vyas et al. as an adjuvant and delivery vehicle for hepatitis B vaccine for effective immunization. Self-assembling hydroxyapatite core was coated with cellobiose, and finally hepatitis B surface antigen was adsorbed over the coated core. The drug-loaded particles were in the nanometer range and almost spherical in shape. The antigen-loading efficiency of plain hydroxyapatite core (without cellobiose coating) was found to be approximately 50%, whereas the coated core was observed to load approximately 21% antigen. The preparation was found to be better than the conventional adjuvant alum followed by subcutaneous immunization in Balb/c mice. The nanodecoy systems were also found to be able to elicit a combined Th1 and Th2 immune response.[18]

Vyas et al demonstrated the immunoadjuvant properties of hydroxyapatite by administering it with malarial merozoite surface protein-119 (MSP-119). Hydroxyapatite nanoceramic carrier was prepared by co-precipitation. Prepared systems were characterized for crystallinity, size, shape, and antigen-loading efficiency. Small size and large surface area of prepared hydroxyapatite demonstrated good adsorption efficiency of immunogens. Prepared nanoceramic formulations also showed slower in vitro antigen release and slower biodegradability behavior, which may lead to a prolonged exposure to antigenpresenting cells and lymphocytes. Furthermore, addition of mannose in nanoceramic formulation may additionally lead to increased stability and immunological reactions. Immunization with MSP-119 in nanoceramic-based adjuvant systems induced a vigorous IgG response, with higher IgG2a than IgG1 titers. In addition, a considerable amount of interferon γ (IFNγ) and interleukin2 was observed in spleen cells of mice immunized with nanoceramic-based vaccines. In contrast, mice immunized with MSP-119 alone or with alum did not show a significant cytotoxic response. The antibody responses to vaccine co-administered with hydroxyapatite was a mixed Th1-Th2 compared to the Th2-biased response obtained with alum. The prepared hydroxyapatite nanoparticles exhibit physicochemical properties that point toward their potential as a suitable immunoadjuvant for used as antigen carriers for immunopotentiation.[25]

He et al. compared a new nanoparticulate adjuvant composed of calcium phosphate with commonly used aluminum (alum) adjuvants for its ability to induce immunity to herpes simplex virus type 2 and Epstein-Barr virus infections. Calcium phosphate was observed to cause little or no inflammation at the site of administration, induced high titers of immunoglobulin G2a (IgG2a) antibody and neutralizing antibody, and facilitated a high percentage of protection against herpes simplex virus type 2 infections. Thus, calcium phosphate proved to be a more potent adjuvant than alum. Moreover, being a natural constituent of the body, it was found to be very well tolerated and absorbed in the animal studies. These studies, by virtue of potency and relative absence of any side effects of calcium phosphate, recommended it as an adjuvant for use in human beings.[26]

The uses of drug-delivery systems in allergen specific immunotherapy appear to be a promising approach due to their ability to act as adjuvants, transport the allergens to immune-

competent cells and tissues and reduce the number of administrations. The aim of this work was to evaluate the carbohydrate modified ultrafine ceramic core based nanoparticles (aquasomes) as adjuvant/delivery vehicle in specific immunotherapy using ovalbumin (OVA) as an allergen model. Prepared nanoparticles were characterized for size, shape, zetapotential, antigen integrity, surface adsorption efficiency and in vitro release. The humoral and cellularinduced immune responses generated by OVA adsorbed aquasomes were studied by two intradermal immunizations in BALB/c mice. OVA sensitized mice were treated with OVA adsorbed aquasomes and OVA adsorbed aluminum hydroxide following established protocol. Fifteen days after therapy, animals were challenged with OVA and different signs of anaphylactic shock were evaluated. Developed aquasomes possessed a negative zeta potential (−11.3 mV) and an average size of 47 nm with OVA adsorption efficiency of ~60.2 μg mg−1 of hydroxyapatite core. In vivo immune response after two intradermal injections with OVA adsorbed aquasomes resulted in a mixed Th1/Th2-type immune response. OVA-sensitized mice model, treatment with OVA adsorbed aquasomes elicited lower levels of IgE (pb0.05), serum histamine and higher survival rate in comparison with alum adsorbed OVA. Symptoms of anaphylactic shock in OVA aquasometreated mice were weaker than the one induced in the alum adsorbed OVA group. Results from this study demonstrate the valuable use of aquasomes in allergen immunotherapy.[27]

5. Miscellaneous

Mizushima and co-workers prepared spherical porous hydroxyapatite particles by spray-drying. These particles were tried as a carrier for the delivery of drugs such as IFNα, testosterone enanthate, and cyclosporin A. Spherical porous hydroxyapatite was found to have an average diameter of 5 μm with approximately 58% porosity. These particles could be injected subcutaneously through a 27-gauge needle. IFNα was adsorbed well to spherical hydroxyapatite particles. Addition of HAS and zinc (for reinforcement) to IFNα-adsorbed hydroxyapatite particles caused marked prolongation of release in vivo.

The in vivo release of testosterone enanthate and cyclosporin A was also prolonged from oil preparation. Thus, the spherical porous hydroxyapatite particles were shown to be useful as a biodegradable and subcutaneously injectable drug carrier. The reinforcement of spherical porous hydroxyapatite particles was suggested to be very effective for sustained release of drugs.[28]

Oviedo and co-workers prepared aquasomes loaded with indomethacin through the formation of an inorganic core of calcium phosphate covered with a lactose film and further adsorption of indomethacin as a low-solubility drug. The aquasomes were characterized for their structural analysis, particle size, and morphology by using x-ray powder diffractometry, TEM, and SEM. Particle size of drug-loaded aquasomes was found to be in the range of 60–120 nm. SEM and TEM techniques confirmed the spherical shape of aquasomes. However, results of drug (indomethacin) release studies from these carriers are yet to be determined.[13]

CONCLUSION

Aquasome is colloidal range biodegradable novel drug delivery carrier, which is based on the fundamental principle of self assembly. The drug candidates delivered through the aquasomes show better biological activity even in case of conformationally sensitive ones. This is probably due to the presence of the unique carbohydrate coating the ceramic. Furthermore, carbohydrate coating on aquasomes prevent destructive interaction between drug and carrier and thus it helps to preserve the spatial qualities. In conclusion, aquasomes appear to be promising carriers for the delivery of a broad range of molecules including viral antigens, hemoglobin and insulin. This approach thus provides pharmaceutical scientists with new hope for the delivery of bioactive molecules.

REFERENCES

1. Juliano RL, Microparticulate Drug Carriers: Liposomes, Microspheres and Cells. In Robinson JR, Lee VHL, editors, Controlled Drug Delivery, 2nd Edn, Marcel Dekker, New York, 2005, 555-580.
2. Rawat M, Singh D, Saraf S, "Nanocarriers: Promising Vehicles for Bioactive Drugs", Biological & Pharmaceutical Bulletin, 2006, 29, 1790-1798.
3. Vyas SP, Khar RK, Introduction to Parenteral Drug Delivery, In: Vyas SP, Khar RK, editors, Targeted And Controlled Drug Delivery, CBS Publishers & Distributors, New Delhi, 2002, 3-37.
4. Kossovsky N, Gelman A, Rajguru S, Nguyan R, Sponsler E, Hnatyszyn CK, "Control of Molecular Polymorphism by a Structured Carbohydrate/Ceramic Delivery Vehicle-Aquasomes", Journal of Controlled Release, 1996, 39, 383-388.
5. Luo D, Han E, Belcheva N, Saltzman WM, "A Self-Assembled, Modular Delivery System Mediated by Silica Nanoparticles", Journal of Controlled Release, 2004, 95, 333-341.
6. Kossovsky N, Gelman A and Sponsler EE. "Cross Linking Encapsulated Haemoglobin Solid Phase Supports: Lipid Enveloped Haemoglobin Adsorbed to Surface Modified Ceramic Particles Exhibit Physiological Oxygen Lability Artif. Cells Blood Sub", Biotech, 1994, 223, 479-485.
7. Jain S, Jain NK, Liposomes As Drug Carriers, In Jain NK, Controlled and Novel Drug Delivery, 1st Edn, CBS Publishers & Distributors, New Delhi, 1997, 304-352.
8. Dunitz JD, "The Entropic Cost of Bound Water in Crystals and Biomolecules", Science 1994, 264, 5159, 670.
9. Kossovsky N, Gelman A, Sponsler EE, Hnatyszyn AJ, Rajguro S, "Surface Modified Nanocrystalline Ceranlic for Drug Delivery Applications", Biomaterials, 1994a, 15, 1201-1207.
10. Kossovsky N, Millett D, Gelman LA, Sponsler ED and Huatyszyn HJ, "Self Assembling Nanostructures", Biotechnology, 1993, 11, 1534.
11. Kossovsky N and Millett D, "Materials Biotechnology and Blood Substitutes". Matr. Res. Soc. Bull., 1991, 78-81.
12. Jain NK, Umamaheshwari RB, Control and Novel Drug Delivery Systems, In: Jain NK, editor, Pharmaceutical Product Development, 2nd Edn., CBS Publishers & Distributors, New Delhi, 2011, 521-564.
13. Oviedo RI, Lopez SAD, Gasga RJ, Barreda CTQ, "Elaboration and Structural Analysis of Aquasomes Loaded with Indomethecin", European Journal of Pharmaceutical Sciences 2007, 32, 223-230.
14. Cherian AK, Rana AC, Jain SK, "Self-Assembled Carbohydrate-Stabilized Ceramic Nanoparticles for the Parenteral Delivery of Insulin", Drug Development and Industrial Pharmacy, 2000, 26, 459-463.
15. Frankel DA, Lamparski H, Liman U, O'Brien DF, "Photoinduced Destabilization of Bilayer Vesicles", Journal of the American Chemical Society, 1989, 111, 26, 9262-9263.
16. Vyas SP, Goyal AK, Khatri K, Mishra N, Mehta A, Vaidya B, "Aquasomes-A Nanoparticulate Approach for the Delivery of Antigen", Drug Development and Industrial Pharmacy, 2008, 34, 1297-1305.
17. Khopade AJ, Khopade S, Jain NK, "Development of Haemoglobin Aquasomes from Spherical Hydroxyapatite Cores Precipitated in the Presence of Poly(amidoamine) Dendrimer" Drug Development and Industrial Pharmacy, 2002, 241, 145-154.
18. Vyas SP, Goyal AK, Rawat A, Mahor S, Gupta PN, Khatri K, "Nanodecoy system: A Novel Approach to Design Hepatitis B Vaccine for Immunopotentiation", International Journal of Pharmaceutics, 2006, 309, 227-233.

19. Prausnitz MR, "Microneedles for Transdermal Drug Delivery" Advanced Drug Delivery Review, 2004, 56, 581-587.
20. Pillai O, Nair V, Panchagnula R, "Transdermal Iontophoresis of Insulin: Influence of Chemical Enhancers", International Journal of Pharmaceutics, 2004, 269, 109-120.
21. Paul W, Sharma CP, "Porous Hydroxyapatite Nanoparticles for Intestinal Delivery of Insulin", Trends in Biomaterials and Artificial Organs, 2001, 14, 37-38.
22. Rawat M, Singh D, Saraf S, Saraf S, "Development and In vitro Evaluation of Alginate Gel-Encapsulated, Chitosan-Coated Ceramic Nanocores for Oral Delivery of Enzyme", Drug Development and Industrial Pharmacy, 2008, 34, 181-188.
23. Patil S, Pancholli SS, Agrawal S, Agrawal GP, "Surface-modified Mesoporous Ceramics as Delivery Vehicle for Haemoglobin", Drug Delivery, 2004, 11, 193-199.
24. Kossovsky N, Gelman A, Hnatyszyn HJ, Rajguru S, Garrell LR, Torbati S, "Surface-Modified Diamond Nanoparticles as Antigen Delivery Vehicles" Bioconjugate Chemistry, 1995, 6, 507-510.
25. Goyal AK, Khatri K, Mishra N, Mehta A, Vaidya B, Tiwari S, "Development of Self-assembled Nanoceramic Carrier Construct(s) for Vaccine Delivery" Journal of Biomaterals Applications, 2009, 24, 65-84.
26. He Q, Mitchell AR, Johnson ST, Wagner-Bartak C, Morcol T, Bell SJD, "Calcium Phosphate Nanoparticle Adjuvant", Clinical and Diagnostic Laboratory Immunology, 2000, 7, 899-903.
27. Pandey RS, Sahu S, Sudheesh MS, Madan J, Manoj Kumar, Dixit V, "Carbohydrate Modified Ultrafine Ceramic Nanoparticles for Allergen Immunotherapy", International Immunopharmacology, 2011, 11, 925–931.
28. Mizushima Y, Ikoma T, Tanaka J, Hoshi K, Ishihara T, Ogawa Y, "Injectable Porous Hydroxyapatite Microparticles as a New Carrier for Protein and Lipophilic Drugs" Journal of Controlled Release, 2006, 110, 260-265.