REVIEW PAPER

Pharmaceutical Science

AN OVERVIEW ON AQUASOMES : A NOVEL APPROACH FOR DELIVERING MACROMOLECULES

KEY WORDS: Aquasomes, carrier, self-assembled structure, nanocrystalline ceramic core

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ABSTRACT

In recent blossoming era of research in nanobiotechnology, aquasomes are viewed as an effective and efficient carrier system for drug delivery or biochemically active long chain macromolecules like protein and peptide, different hormones, antigens, enzymes and gene delivery. Aquatones are spherical in shape and having particle size within 60-300nm. Self-assembled structure of aquasome (due to its natural property) it generates a focus on nanobiotechnology research as a carrier system. Mainly, aquasomes contain three-layers, that is core material, coating material, drug layer attached by ionic or non-covalent bond. Aquasomes include mainly the core materials like , hydroxyapatite, solid phase nanocrystalline ceramic diamond (carbon) and calcium phosphate dihydrate(brushite) .The coating of core material is done with glassy polyhydroxyl oligomeric film such as cellobiose and trehalose, on which modification of biochemically active molecules are attached. Whereas, calcium phosphate used as a core material, because of it is naturally present in the body. Whether calcium phosphate is unstable in nature, due to prolong storage it converts into hydroxyapatite which is a better core than calcium phosphate to develop the aquasomes. The solid core material renders stability of structures, while the coating material stabilizes the biochemically active molecules and protects against dehydration. In this review, we tried to an overview of aquasomes, and about its advantages over conventional drug delivery system, shielding effect of aquasomes on desired drug and how its self-assembled structures, makes them an attractive carrier to deliver biochemically active molecules.

INTRODUCTION :

Now a day, nanotechnology involves the delivery of biologically active pharmaceutical products through different types of bio material like nanoparticles, aquasome, liposome, niosome, quantum dots, magnetic nanoparticles and dendrimers. In the pharmaceutical industries, nanobiotechnology is an ideal choice for delivering the drugs. Nir Kossovsky in 1995 developed first Aquasomes. Aquasome drug delivery systems are recent and rapidly developing science where materials in the nanoscale range are used to aid as a diagnostic tool or to deliver therapeutic agents to specific targeted sites in a pre-determined controlled manner (Patra et al,2018) The developers of nanoparticle have to face many challenges, namely, choosing suitable polymer, solvents compatibility with the components and affinity of polymers and copolymers with the drug and biological fluids (Shaji and Patole,2008)These tri layered self assembled structures, based drug delivery can overcome all the challenges mentioned above that's why it can be an ideal choice for delivering the drugs like long chain molecules, proteins and peptides (Patel et.al.2016)

(Kossovsky et al.) formed innovative nanovesicular drug career which ranges from 60 to 300 nanometer in size. These drug delivery system are used for parenteral drug delivery because it can easily pass through the blood capillaries (Chaudhary, 2018).. Aquasomes are spherical shaped having particles size ranges from 60–300 nm and looks like "bodies of water" used for delivery of drug and antigen (Patel et al., 2012). In muscles and liver, the aquasomes are heavily concentrated because of biodegradable nature and without any change in the surface of the system the drug is absorbed. As aquasomes are covered by oligosaccharides, drug molecules are easily remaining stable at water like environment. Whereas, calcium phosphate used as a core material, because of it is naturally present in the body.. Aquasomes have some enthralling properties like protection and maintenance of fragile biological molecules, surface exposure and conformational integrity. bioactive molecules like peptide, protein, hormones, genes and antigens to its specific sites (Patel et al., 2012

Hence, carbohydrate stabilizes the ceramic nanoparticles also known as aquasomes and it act as a natural stabilizer. The biochemically active molecules present in the aquasomes are incorporated to carbohydrate surface by adsorption or diffusion, co-polymerization method of preformed aquasomes.

Objectives for development of Aquasomes

The main objectives are :

1) Protect Bioactives
2) Maintain Molecular Confirmation
3) Maintains Optimum Pharmacological activity.

Properties :

1) Aquasome act as the preservation of bio-active conformation integrity and biochemical stability.
2) Due to certain environmental challenges ,aquasomes for their size and structure stability ,undergo deterioration
3) Aquasomes possess massive size and active surface area and therefore be filled efficiently with significant quantities of agents by ionic, non-covalent bonds, van der waals and entropic forces.

Self -Assembly – A Concept of Aquasome :

Self-assembly means assumption of the component parts of some finished products spontaneously prescribed their structural orientations in two or three-dimensional layer (Kaushik and Bhatt, 2003; Kossovsky et al., 1996). The three physicochemical processes that governs the concept of Self - Assembly of macromolecules include

- The charged groups interactions
- Effects due to dehydration
- Structural stability.

1.Interaction among charged groups-

The interaction of charged groups, such as amino, carboxyl,

sulphate, and phosphate groups can smooth the self-assembling of macromolecules in aqueous environment and they also facilitate long range approach of self-assembling sub units (Jagdale and Karekar, 2020). The natural chemical groups or absorbed ions from nature provide a charge polarity on biological and synthetic surfaces. (Shahabade Gururaj et al., 2009).

2. Hydrogen bonding and dehydration effect-

In the self assembly of aquasome, the effect of hydrogen bonding plays an important role to prepare its appropriate structure. Mainly the hydrogen bonding happens in between carbohydrate and drug molecules (Jagdale and Karekar, 2020).Generally, hydrogen bonds are helps in base pair matching and also give a modification effect of alpha helices and beta sheets that are considered as secondary proteins.(Shahabade Gururaj et al., 2009.). In carbohydrates, only trehalose form intramolecular hydrogen bond therefore trehalose has many sites available to form hydrogen bonding with drugs. In that way, trehalose act as a substrate for drugs or proteins (Jagdale and Karekar, 2020).

3. Structural stability-.

Structural stability of protein in biological environment is resolved by van der waals forces largely internal to molecule experienced by hydrophobic molecules, accountable for hardness and softness of molecule and supportive of internal secondary structures, allows sufficient softness, and maintenance of conformation during self assembly. These conditions may leads to irreversible denaturation of proteins (Jagdale and Karekar, 2020

4.Most of the times the Van der Waals forces, are shielded from water which play a minor role but also play a critical role in interactivity of protein with carbohydrate (Snehal et al., 2016).. As we know, carbohydrates act as a natural stabilizer and also dehydroprotectant so that they can be maintain structural stability of protein and can be prevent denaturation (Jagdale and Karekar, 2020).

Method Of Preparation :

From the above, we know aquasomes have self-assembled structure, which consist of 3 layers that is core material which is coated by polyhydroxyl oligomeric film, after this finally drug is loaded. So, using these self-assemble principle, aquasomes are prepared into three steps. Those are following:

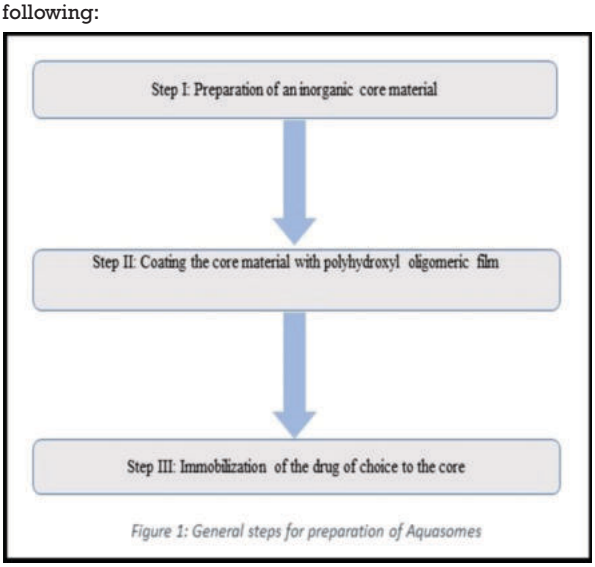


Figure 1: General steps for preparation of Aquasomes

Step I: Inorganic core material Preparation

This is the first step for preparation of aquasomes, which include construction of ceramic core. This process depends upon the choice of core materials. Three types of core material are generally used that is calcium phosphate, hydroxyapatite, carbon ceramic. Inorganic materials are widely used to prepare the core because they have high degree of order in their crystalline structure. They also provides a high level of surface energy that will be help to bind polyhydroxyl oligomeric surface film (Shahabade Gururaj et al. 2009).. A fine membrane filter is used to obtain desired particle size. The equation of this reaction is follows. (Sutariya and patel, 2018):

$2Na2HPO4 + 3CaCl2 + H2O \rightarrow Ca3[PO4]2 + 4NaCl + 2H2 + Cl2 + [O]$

Step II: Polyhydroxyl Oligomeric film for coating the core material

This is the second step, where the surface of ceramic cores is covered by carbohydrate that is polyhydroxyl oligomers. Commonly, cellobiose, trehalose, citrate, pyridoxal-5-phosphate and sucrose are used as covering material. Various types of processes are used to cover the surface of the nano-crystalline ceramic cores in an ordered manner by carbohydrate (polyhydroxyl oligomers) (Vengala et al., 2012). Firstly, carbohydrates are added into the aqueous dispersion of the cores under sonication. Others methods like non-solvent addition, adsorption, and by direct incubation are also used to coated the core. Ultra-centrifugation is done to remove unabsorbed carbohydrate (Jagdale and Karekar, 2020).

Step III: Immobilization of the drug of option to the core

This is the final stage of these preparation method, where drug is loaded onto the core. Primarily a suitable pH buffer solution is used to preparation of known concentration of drug Henceforth dispersion of coated particles are done into this solution. Then, to obtain drug loaded formulation (aquasomes), this dispersion is either lyophilized or kept at low temperature for overnight. After this, the collection of the the aquasomes of formulation are collected and then characterized by several techniques (Jagdale and Karekar, 2020).The synthesis of an aquasome is represented in Fig 2.

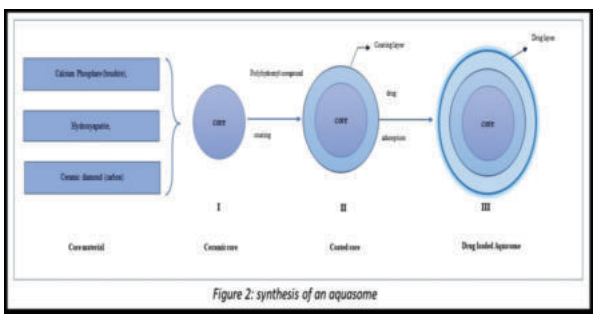


Figure 2: synthesis of an aquasome

Aquasomes characterisation :

Usually aquasomes are characterized to determine their structural as well as morphological properties, particle size distribution, and drug loading capacity.

Depiction of ceramic core

Size Grouping : -

Aquasomes should possess particle size in a range of 60-300nm. Particle size distribution is a well known parameter to assess the physical and chemical properties of aquasome and also control the colloidal properties like surface area, rheology, and density (Jagdale and Karekar, 2020). SEM and TEM are used as techniques to analyse core, coated core of as well as drug-loaded aquasomes

Analysis Of Structure:

Aquasomes are determined for structural analysis using Potassium Bromide pellets sample disk method through FTIR-spectroscopy, where the analysis of basic core as well as the coated core of aquasomes are performed by recording their IR spectra and the wave number range of 4000–400

cm–1.Reference peaks are compared with characteristic peaks..(Khopade et al. 2002; Vyas et al. 2006a, 2008a; Monica et. al. 2015).

Crystallinity:
Evaluation the degree of crystallinity of aquasome is very important characterization method because degree of crystallinity may affect the mechanical and thermal properties of aquasome. Therefore, X-ray diffraction study are generally used to know the crystalline or amorphous behaviour of the prepared ceramic core of aquasomes and to understand the kinetics of formulations (Jagdale and Karekar, 2020 et. al., 2015).

Coated Core Characterisation :
Coating-of Carbohydrate:
The two methods involved in the coating of sugar over the ceramic core can be verified by a) concanavalin A - induced aggregation method and b) by anthrone method (determines the residual sugar unbound or residual sugar remaining after coating). In addition , the adsorption of sugar over the core can also be established by measurement of zeta potential.

Glass transition temperature-
To analyse the effect of carbohydrate over the drug loaded aquasomes, DSC are widely used . To know the study of glass transition temperature of carbohydrates and proteins, The reversible transition from glassy state to rubbery state with increasing the temperature, can be measured by using a DSC analyzer. (Vyas et al. 2008a; Monica et. al., 2015).

Drug-loaded aquasomes characterisation :
Drug load-
Reduces the amount of matrix material, higher the drug loading capacity of aquasome is showed. Also smaller the size of core, larger the surface area so that drug loading capacity increases in aquasome (Jagdale and Karekar, 2020). To know the drug loading of aquasomes, firstly basic formulation of aquasome devoid of drug is incubated in a known concentration of the drug solution at a temperature of 4°C for 24hrs.. Estimation of the amount of drug in the supernatant liquid after loading is done by any suitable method of analysis (Cherian et al. 2000; Monica et. al., 2015).

***In-vitro* drug release studies-**
It is necessary to know the delivery of drug and to understand the bioavailability of drug molecules in the formulation by In-vitro drug release study. The mechanism of drug release is also understand by this method (Jagdale and Karekar, 2020). In this method, an incubation of investigated quantity of drug-loaded aquasomes is done with continuous stirring utilizing buffer of suitable pH at temperatue about 37°C. Then samples are removed periodically and high speed centrifugation is used for a period of times and equal volumes of medium are restored after each withdrawal. Then the obtained supernatants are analysed to note down the amount of drug released (Vyas et al. 2008a; Monica et. al. 2015).

Benefits of Aquasomes :
Aquasomes are reservoir like system which entraps the loaded drug and slowly releases the drug in perpetual or in a pulsatile manner and avoiding multiple parenteral administration. The protective property is due to the presence of inorganic core which is covered by poly-hydroxyl compounds which is having hydrophilic properties (Sahoo et al., 2018). Drug delivery via aquasomes are preferred over other delivery system because both humoral and cellular immune responses can absorb antigen on the surface of aquasomes (Jain et al., 2012). Aquasomes are also used in diagnostic purpose. The multi-layered aquasomes are loaded with biologically identifiable molecule such as peptides, nucleic acid, antibodies called biological labels used for various imaging testing purposes. Due to following properties of aquasomes like sensitivity and enzyme activity towards specific molecular integrity makes it an absolute carrier for enzymes like DNAase and proteins (Saurabh et al., 2013

Applications :
Insulin Delivery:
The water like bodies also known as aquasomes formulated with calcium phosphate are used for insulin delivery via parenteral route. The ceramic core is coated with numerous carbohydrates such as cellabios and trehalose etc. Parallelly by absorption phenomena the drug is loaded into the particles. These insulins loaded aquasomes are assessed in albino rat by in vivo study. All the insulin loaded aquasomes showing its action for long period of time except cellabios coated particles. The Pyridoxal-5-phosphate coated aquasomes reduces the blood glucose level up to (94.40% ± 0.90%) in 2-3 hrs which is higher than plain insulin (74.92% ± 0.88%). The prolonged action is due to the structural arrangement of peptide and the drug release slowly from the carrier. (Cherian et al., 2000; Beherei et al., 2008)

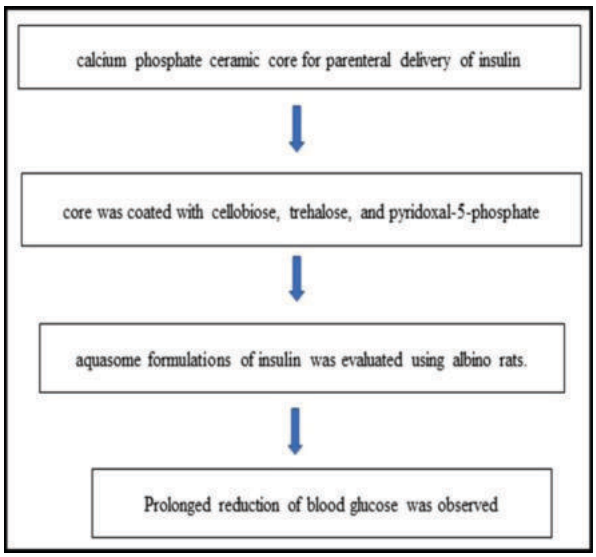


Figure 3: Insulin Delivery Via Aquasome

1. Delivery Of Acid Labile Enzyme:
The acid labile enzymes like DNAase and cyclosporine are very much sensitive in terms of their molecular integrity and their activity changes with its molecular integrity. For effective treatment of various diseases like cystic fibrosis the therapeutic enzymes are incorporated into aquasomes and targeted to the desired sites for effective therapeutic action (Vays and Khar, 2004).

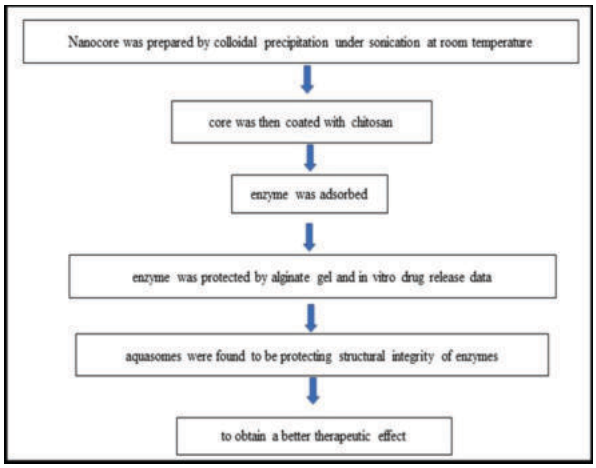


Figure 4: Delivery Of Acid Labile Enzyme Via Aquasomes

2. Anti-thrombotic Activity:

(Leclerc et al., 2004) formulated nanoparticles of heparin-polymer aquasomes are copolymers with isobutyl cyanoacrylate to carry haemoglobins in which haemoglobin is loaded on the surface of the aquasome instead of being encapsulated. The anti-thrombotic activity of heparin coated nanoparticle was assessed by anti-Xa factor activity using a coagulometer.

As Oxygen Carrier:

(Khopade et al., 2002) formulated aquasomes using hydroxyapatite core as template or modifier by using carboxylic acid-terminated half generation poly (amidoamine) dendrimer. The aquasomes are coated with trehalose by adsorption of haemoglobin. Aquasome act as a red blood cell substitute and due to release of oxygen by haemoglobin, it is immobilized on the oligomer surface. By this 80 % of haemoglobin is achieved and toxicity is reduced and deliver the blood in non-linear manner like natural blood cells.

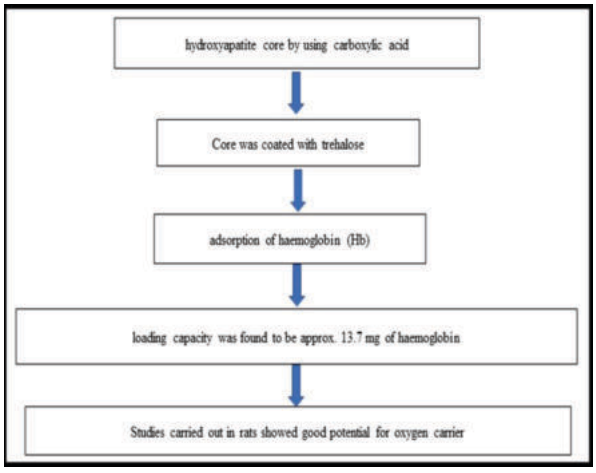


Figure 5: Aquasome As Oxygen Carrier

3. Antigen Delivery:

To enhance the immunity of antigens the adjuvant plays a major role through shielding the functional groups or to modify the integrity by surface absorption. The disaccharides help in reducing the surface-induced denaturation of absorbed antigen called muscle antigen protein. In case of muscle adhesive protein by this adjuvant successfully achieved effective immune response. Aquasome helps in elicit strong immunity response by facilitate the availability of antigen and improving the in vivo activity (kossovsky et al., 1994). Aquasomes used for delivery of viral antigen as a vaccine i.e., Immune deficiency virus and Epstein-Barr to recorrect antibody, conformationally triggering the specific target molecules is the main objective of vaccine therapy.

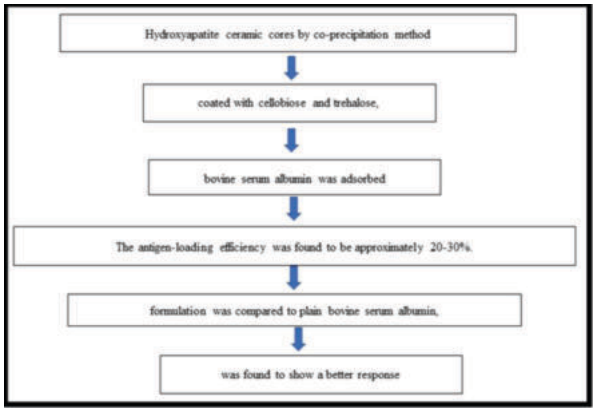
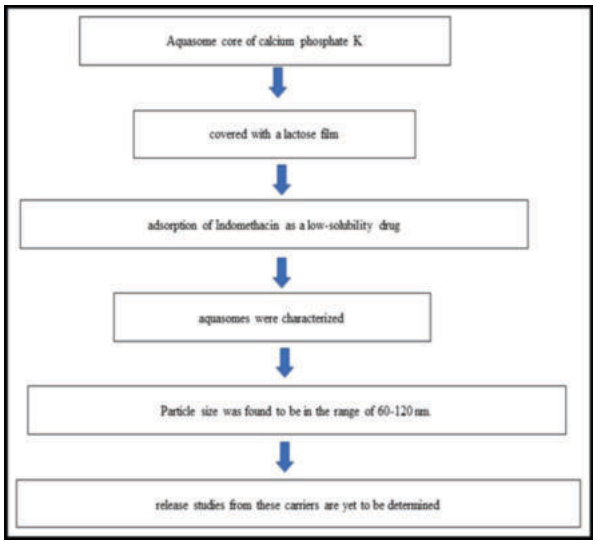


Figure 6: Antigen Delivery Via Aquasome

4. Delivery of drug (60-120 nm):

The poor soluble drugs like lornoxicam is loaded in aquasomes using co-precipitation method followed by sonication method. The cellabiose film is coated on the calcium phosphate core in which lornoxicam was absorbed. Aquasomes showed better release rate in compare to pure drug because of uniform and spherical shape of the aquasomes (Vengala et al., 2017).

Figure 7: Delivery Of Drug Via Aquasomes



5. Delivery of gene:

Aquasomes are five layered structure consists of ceramic core, therapeutic gene segment, polyhydroxyl oligomeric film, supplementary carbohydrate film, targeting layer of conformationally preserve viral membrane protein and have been used for successful targeted intracellular gene therapy. It was observed that the structural conformity of gene segment is protected and shielded by aquasomes. On using aquasomes as a vehicle its advantageous in providing all possible capabilities of viral vector (Kossovsky et al., 1994a).

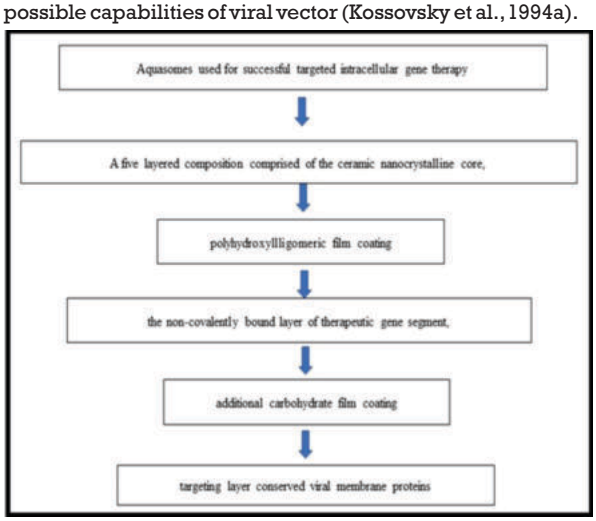


Figure 8: Delivery of gene via aquasomes

6. Vaccine Delivery:

The vaccines are prone to immunogenicity and stability problems due to these limitations (Goyal et al., 2006) formulated hepatitis B surface antigen (HBsAg) which are loaded into aquasomes. The cellabios hydroxy apatite core was loaded with HBsAg and the spherical shape and size was observed in nanometric range.

7. The water like bodies also termed as aquasomes are mainly considered for proteins, peptides and related

molecules but they are versatile compounds that can have great reproducibility and robustness to be used in different ways e.g. solubility enhancing, targeting approach and many others.

Table 1: Applications Of Aquasome

Active ingredient	Therapeutic application	Reference
Dithranol	Antipsoriasis	Tiwari *et al.,* 2012
Etoposide	Anticancer	
Haemoglobin	Blood component, oxygen carrier	Khopade *et al.,* 2002
Hepatitis B vaccine	Antigen for prevention of Jaundice	Vyas *et al.,* 2006
MSP 119	Antimalarial	Goyal *et al.,* 2009
INF á	Hairy cells	Mizushima *et al.,* 2006
Insulin	Glucose regulation	Cherian *et al.,* 2000
Polypeptide-k	Glucose regulation	Gulati *et al.,* 2014
Testosterone Enanthate	Growth hormone	Mizushima *et al.,* 2006
Serum Albumin	Organizing the colloid osmotic pressure required for distribution of body fluids between intravascular compartments and body tissues	Vyas *et al.,* 2008
Serratiopeptidase	Antispasmodic	Rawat *et al.,* 2008

Future Prospectus

We conclude that, aquasomes in novel drug delivery will provide glorious future prospectus as a self-assembled carrier system for biochemically active drug delivery, where the sensitive large size drugs such as protein and peptide drug, DNA-based drug, genes, enzymes, hormones are delivered by showing better biological activity. The presence of carbohydrate coated ceramic core in aquasome is leading part to enhance biological activity of these drugs. This is the new expectation for pharmaceutical researchers to deliver large size drugs into the system without showing any toxicity. Aquasomes or water like bodies are providing a ray of hope for the pharmaceutical scientists to overcome various drawbacks and challenges related to conventional delivery of pharmaceutical drugs .

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