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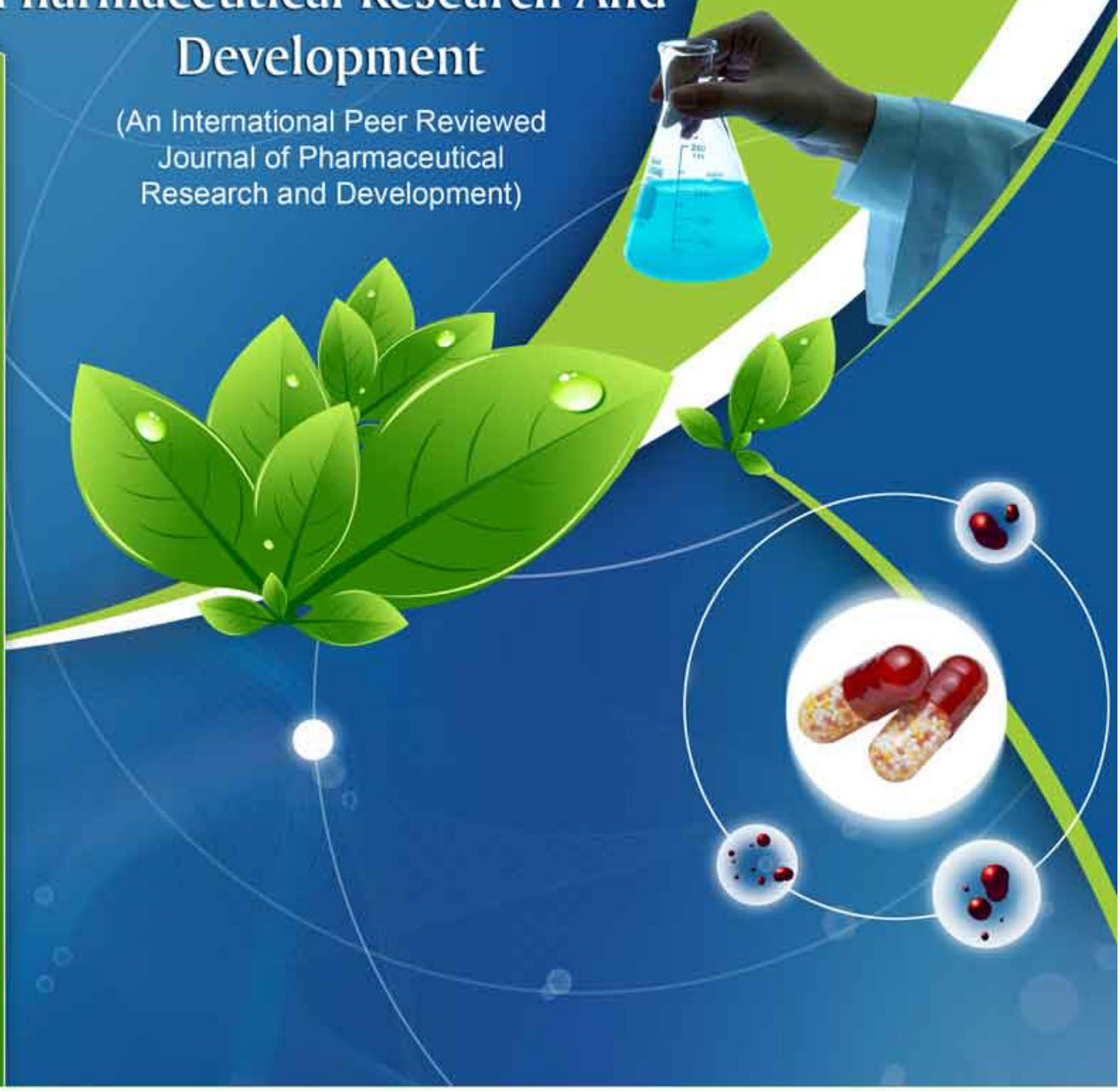

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Review Article

AQUASOMES -THE BEST CARRIERS FOR PROTEIN AND PEPTIDE DELIVERY

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ABSTRACT

Nanoparticulate carrier system constitute one of the self assembling approaches for development of pharmaceutical agents. Aquasomes are the nano biopharmaceutical carrier systems containing particle core composed of nano crystalline calcium phosphate or ceramic diamond, and is covered by a poly hydroxyl oligomeric film. The solid core provides the structural stability, while the carbohydrate coating protects against dehydration and stabilizes the biochemically active molecules. The delivery system has been successfully utilized for the delivery of insulin, haemoglobin, and enzymes like serratiopeptidase etc. Aquasomes technology represents a platform system for conformation integrity and biochemical stability of bioactives. Three types of core materials are mainly used for producing aquasomes: tin oxide, nano crystalline carbon ceramics (diamonds) and brushite (calcium phosphate dihydrate). Calcium phosphate is the core of interest, owing to its natural presence in the body. Aquasomes discovery comprises a principle from microbiology, food chemistry, biophysics and many discoveries including solid phase synthesis, supra molecular chemistry, molecular shape change and self assembly. This review mainly deals with the advantages, properties, method of preparation, fate and characterization of aquasomes.

Key Words: *Aquasomes, Nanoparticulate carrier system, Sonication, Core, Glass transition temperature, Self assembling carrier system.*

INTRODUCTION

Aquasomes means "water bodies" are the combination of biotechnology and nanotechnology. These drug delivery systems were first discovered by Nirkossovsky. Aquasomes are the nano biopharmaceutical carrier systems containing the particle core composed of nano crystalline calcium phosphate or ceramic diamond, and are covered by polyhydroxyl oligomeric film. These three layered structures are self assembled by non-covalent bonds.

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The pharmacologically active molecule can be incorporated by following methods like co-polymerization, diffusion or adsorption to carbohydrate surface of pre-formed nanoparticles [1]. Aquasomes are spherical 60-300 nm particles used for drug and antigen delivery. As these are solid or glossy particles dispersed in aqueous environment, they exhibit the physical properties of colloids and their mechanism of action is controlled by their surface chemistry. Aquasomes delivers their contents through a combination of special targeting molecular shielding and slow & sustained release process. Aquasomes technology represents a platform system for conformation integrity and biochemical stability of bioactives. Their intended route of administration is parenteral. Some researchers have extended the route of administration from parenteral to oral.

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 [2]. 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 6M is unstable and converts to hydroxyapatite upon prolonged storage. Hydroxyapatite seems, therefore, a better core for the preparation of aquasomes. It is widely used for the preparation of implants for drug delivery [3]. Aquasomes discovery comprises a principle from microbiology, food chemistry, biophysics and many discoveries including solid phase synthesis, supra molecular chemistry, molecular shape change and self assembly.

PRINCIPLE OF SELF ASSEMBLY:

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 is governed basically by three physicochemical processes:

- ***Interaction between charged groups***

The interaction of charged groups, such as amino, carboxyl, sulphate, phosphate groups facilitates long range approach of self assembly sub units. Charged group also plays a role in stabilizing tertiary structures of folded proteins.

- ***Hydrogen bonding and dehydration effect***

Hydrogen bond helps in base pair matching and stabilization of 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, however, their tendency to repel water helps to organize the moiety to surrounding

environment. The organized water decreases the overall level of disorder/ entropy of the surrounding medium. Since, organized water is thermodynamically unfavorable, the molecule loose water/dehydrate and get self assembled.

- ***Structural stability***

Structural stability of protein in biological environment was determined by interaction between charged group and hydrogen bonds largely external to molecule and by vanderwaals forces largely internal to molecule experienced by hydrophobic molecules. These are responsible for hardness and softness of molecule and maintenance of internal secondary structures, provides sufficient softness and allows maintenance of conformation during self assembly. Self assembly leads to altered biological activity, vanderwaals need to be buffered. In aquasomes, sugars help in molecular plasticization. Vander Waals forces, most often experienced by the relatively hydrophobic molecular regions that are shielded from water, play a suitable but critical role in maintaining molecular conformation during self assembly. Vander Waals forces largely internal to the molecule also play a small but measurable role in the interaction of polypeptides with carbohydrates and related polyhydroxyoligomers .When molecules change their shape substantially following an interaction, the energy minima assumed upon conformational denaturation tend to preclude reversal.

ADVANTAGES

- Aquasomes based vaccines offer many advantages as a vaccine delivery system. Cellular and humoral immune responses can be elicted to antigens adsorbed on to aquasomes [4].
- Multilayered aquasomes conjugate with biorecognition molecules such as antibodies, nucleic acids, peptides which are known as

biological labels can be used for various imaging tests.

- They increase the therapeutic efficacy of pharmaceutically active agents and protects the drug from phagocytosis and degradation.
- These nanoparticles offer favourable environment for proteins thereby avoiding their denaturalization.
- These systems act as a reservoirs to release the molecules either in a continuous or a pulsatile manner, avoiding a multiple injection schedule.

PROPERTIES [5, 6]

- Aquasomes possess large size and active surface hence can be efficiently loaded with substantial amounts of agents.
- Aquasomes mechanism of action is controlled by their surface chemistry.
- They deliver contents through combination of specific targeting, molecular shielding, and slow and sustained release process.
- water like properties provides a platform for preserving 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.
- Calcium phosphate is biodegradable and its degradation can be achieved by monocytes and osteoclasts.

METHOD OF PREPARTION [7, 8, 9]

Aquasomes preparation is considered to be a relatively simple and staright forward approach with minimum solvent usage and no homogenization steps. The general procedure consists of an inorganic core formation, which will be coated with carbohydrate forming the polyhydroxylated core that finally will be loaded by protein/antigen/drug. By using the principle of self-assembly aquasomes are prepared in 3 steps.

Formation of an inorganic core:

It involves the fabrication of a ceramic core and the procedure depends upon the materials elected. The two most commonly used ceramic cores are calcium phosphate and diamond.

Synthesis of nanocrystalline tin oxide core ceramic

It can be synthesized by direct current reactive magnetron sputtering. Here, a 3 inches diameter target of high purity tin is sputtered in a high pressure gas mixture of argon and oxygen. The ultrafine particles formed in the gas phase are collected on copper tubes cooled to77 k with flowing nitrogen.

Self assembled nanocrystalline brushite

These can be prepared by colloidal precipitation and sonication by reacting solution of Na_2HPO_4 and $CaCl_2$.

Nanocrystalline carbon ceramic

These can also be used for the core synthesis after ultra cleansing and sonication. The common feature of various cores is that they are crystalline and they measure between 50-150 nm and exhibit extremely clean and therefore reactive species. 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 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 surface film. The precipitated cores are centrifuged and then washed with enough distilled water to remove NaCl 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:

$2\ Na_2HPO_4 + 3\ CaCl_2 + H_2O \rightarrow Ca_3(PO4)_2 + 4\ NaCl + 2\ H_2 + Cl_2 + (O)$

Coating of the core with polyhydroxy oligomer :

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 ceramic cores. These processes generally essential to 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.

Immobilization of Drugs:

The surface modified nano-crystalline cores provide the solid phase for the subsequent non-denaturing self assembly for broad range of biochemically active molecules. The drug can be loaded by partial adsorption.

AQUASOMES FATE [10]:

The drug delivery vehicle of 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 $Ca3(PO_4)_2$ is a biodegradable ceramic. Biodegradation of ceramic in vivo is achieved essentially by monocytes and multicellular cells called osteoclasts. Two types of phagocytosis were reported when cells come in contact with biomaterial a) $Ca3(PO_4)_2$ crystals were taken up alone and then dissolved in the cytoplasm after disappearance of the phagosome membrane b) dissolution after formation of heterophagosomes.

CHARECTERIZATION OF AQUASOMES

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

Characterization of ceramic core

Size distribution:

For morphological characterization and size distribution analysis, scanning electron microscopy and transmission electron microscopy 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 photon correlation spectroscopy.

Structural analysis:

FT-IR spectroscopy can be used for structural analysis. Using KBr sample disk method, the core as well as the coated core can be analyzed by recording their IR spectra in the range of 4000–400 cm–1; the characteristic peaks observed are 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 [11].

Crystallinity:

The ceramic core can be analyzed for its crystalline or amorphous behavior using X-ray diffraction by comparing the diffraction patterns of the sample and standard and the interpretations are made.

Characterization of coated core

- ***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.

- ***Glass transition temperature***

DSC can be used to analyze the effect of carbohydrate on the drug loaded 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 [12].

CHARACTERIZATION OF DRUG-LOADED AQUASOMES [13]

- ***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 suitable method of analysis.

- ***In vitro drug release studies***

It was determined to study the release pattern of drug from the aquasomes by incubating a known quantity of drug-loaded 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 suitable method.

- ***In-process stability studies***

SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) can be performed to determine the stability and integrity of protein during the formulation of the aquasomes.

APPLICATIONS

- Aquasomes used as vaccines for delivery of viral antigen i.e., Epstein-Barr and Immune deficiency virus [14] to evoke correct antibody, objective of vaccine therapy must be triggered by conformationally specific target molecules.
- Aquasomes have been used for successful targeted intracellular gene therapy, a five layered composition comprised of ceramic core, polyoxyoligomeric film, therapeutic gene segment, additional carbohydrate film and a targeting layer of conformationally conserved viral membrane protein [15].
- Aquasomes for pharmaceuticals delivery i.e. insulin, developed because drug activity is conformationally specific. Bioactivity preserved and activity increased to 60% as compared to i.v. administration and toxicity was not reported [16].
- Aquasomes also used for delivery of enzymes like DNAase and pigments/dyes because enzymes activity fluctuates with molecular conformation and cosmetic properties of pigments are sensitive to molecular conformation.
- Aquasomes as red blood cell substitutes, haemoglobin immobilized on oligomer surface because release of oxygen by haemoglobin is conformationally sensitive. By this toxicity is reduced, haemoglobin concentration of 80% was achieved and reported to deliver blood in non linear manner like natural blood cells [17].
- Various recombinant products used for the delivery through aquasomes thus preventing degradation in stomach pH and increases the drug targeting and availability.

Table: 1 *Fda Approved (Recombinant Genes) Proteins Which Can Be Transferred Through Aquasomes* **[18]**

Trade Name	*Recombinant Product*	*Year of Aproval*
Activase	Tissue plasminogen activator	1987 US
Avonex	IFN_1a	1996 US
Aldurazyme	Laronidase	2003 US
Amevive	LFA-3-IgG fragment fusion protein	2003 US
Aranesp	Darbepoetin	2001 US
Advate	Clotting factor VIII	2003 US
Benefix	Clotting factor VIII	1993 US
Cerezyme	Glucocerebrosidase	1994 US
Epogen/procrit	Erythropoietin	1989/1990 US
Enbrel	TNF alpha receptor _ receptor- IgG fusion protein	1998 US
Fabrazyme	Galactosidase a	2003 US
Gonal-f	Follicle stimulating hormone	1995 EU
Herceptin	Anti-HER 2 humanized mAb	1998 US
Helixate FS	(sucrose formulation)	2000 US
Humira	Anti-TNF human mAb	2002 US
Luveris	Luteinizing hormone	2004 US
Myozyme	Acid –glucosidase	2005 US
Mircera	Methoxy polyethylene glycol-epoetin	2007 US
Novoseven	Clotting factor VII a	1999 US
Naglazyme	N-acetylgalactosamine 4 sulfate	2006 EU
Ovidrel	Human chronic gonadotropin	2000 US
Orencia	Ig-CTLA4 fusion	2005 US
Optaflu	Cell based seasonal influenza virus	2007 US
Osigraft	Osteogenic protein-1 bone morphogenetic protein-7	2001 US
Raptiva	Anti-CD11a humanized mAb	2003 US
Refib	INF-1	2002 US
Rebif New formulation	Rh IFN-1a	2007 EU
Recothrom	Topical human thrombin	2008 US
Refacto	B domain-delected clotting factor VIII	1998 US
Remicade	Anti- TNF _ chimeric mAb	1998 US
Recombinate	Clotting factor VIII	1992 US
Simulect	Anti-IL2 receptor- chimeric mAb	1988 US
Thyrogen	Thyrotropin	1998 US
TNKase	Tissue plasminogen activator	2000 US

CONCLUSION

Aquasomes have given a new hope for the pharmaceutical scientists to deliver bioactive molecules. Aquasomes appear to be promising carriers for the delivery of broad range of conformational sensitive molecules with better activity due to presence of unique carbohydrate coating over the ceramic core. The drug candidates delivered through the aquasomes show better biological activity even in case of conformationally sensitive ones. Molecular plasticizers, carbohydrates prevent the destructive drug carrier interactions and help to prevent spatial qualities. The crystalline nature of core gives structural stability and overall integrity. Furthermore coating on carbohydrates prevents destructive interactions between drug and carrier which occur in prodrug and liposomes system and then it helps to prevent the special qualities. Finally aquasomes are the best carriers for the delivery of vaccines, haemoglobin, protein and peptides.

REFERENCES

1. *Vyas SP, Khar RK,Targeted and controlled drug delivery, CBC publisher and distributors, New delhi, 2004; 28-30*
2. *Kossovsky N, Gelman A, Sponsler EE, Hnatyszyn HJ, Rajguru S, Torres M. Surface modified nanocrystalline ceramics for drug delivery applications, Biomaterials 1994; 15: 1201-1207*

3. *Barroug A, Lernoux E Lemaitre , J, Rouxhet PG. Adsorption of catalase on hydroxyapatite, J. Colloid Interf. Sci. 1998; 208: 147-152.*
4. *Rege K, Huang HC, Barua S, Sharma G, Dey SK. Inorganic nanoparticle for cancer imaging and theraphy.J Control Release 2011;155:344-57.*
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. *Jain S, Jain NK, Liposomes As Drug Carriers, In Jain NK, Controlled and Novel Drug Delivery, CBS Publishers & Distributors, New Delhi, 1997; 1: 304-352.*
7. *Cherian AK,"Self-Assembled Carbohydrate-Stabilized Ceramic Nanoparticles for the Parenteral Delivery of Insulin", Drug Development and Industrial Pharmacy, 2000; 26: 459-463.*
8. *Kossovsky N, Millett D. "Materials biotechnology and blood substitutes." Matr. Res. Soc. Bull., 1991; 78-81.*
9. *Kossovsky N, Gelman A, Sponsler EE, Hnatyszyn AJ, Rajguro S, Torres M, Pham M, Crowder J, Zemanovich J, Chung A, Shah R. "Surface modified nanocrystalline ceranlic for drug delivery applications." Biomaterials, 1994; 15: 1201-1207.*
10. *Shahabade GS, Bhosale AV, Mutha SS, Bhosale NR, Khade PH, Bhadane NP. An overview on nannocarrier technology-Aquasomes.J Pharm Res 2009; 2:1174-1177.*
11. *Khopade AJ, Khopade S, Jain NK. Development of haemoglobin aquasomes from spherical hydroxyapatite cores precipitated in the presence of poly(amidoamine) dendrimer. Drug Dev Ind Pharm. 2002; 241:145-154.*
12. *Vyas SP, Goyal AK, Vaidya B, "Aquasomes-A Nanoparticulate Approach for the Delivery of Antigen", Drug Development and Industrial Pharmacy, 2008; 34: 1297-1305.*
13. *Vyas SP, Goyal AK. "Nanodecoy system: A Novel Approach to Design Hepatitis B Vaccine for Immunopotentiation", International Journal of Pharmaceutics, 2006; 309: 227-233.*
14. *Cherian A, Jain SK. "Self assembled carbohydrate stabilized ceramic nanoparticles for the parenteral drug delivery of insulin". 2000; 459-463.*
15. *Kossovsky GA, 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.*
16. *Vays SP, Khar RK. Targeted & Controlled Drug Delivery, CBC Publisher & distributors, New Delhi, 2004; 28-30.*
17. *Kossovsky N, Gelman A, Sponsler EE. Cross linking encapsulated haemoglobin solid phase supports: lipid enveloped haemoglobin adsorbed to surface modifies ceramic particles exhibit physiological oxygen lability artif.cells blood sub. Biotech 1994; 223:479-485.*
18. *Rathore P, Duggal S, Swami G. A review on aquasomes a promising nanobiopharmaceutical drug delivery system for proteins and peptides IJPT, 2012; 4(1): 1875-1888.*