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Sphingosomes: A Novel Approach For Vesicular Drug Delivery System

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ABSTRACT

Sphingosomes is bilayer vesicles in which an aqueous volume is entirely enclosed by membrane lipid bilayer mainly composed of natural or synthetic sphingolipid. Sphingosomes solve the major drawback of vesicle system(liposomes, niosomes) like less stability, less in vivo circulation time, low tumor loading efficacy in case of cancer therapy. The outcome of this review is that Sphingosomes represents a promising vesicular drug delivery system to delivers therapeutic compounds for a range of possible application. Sphingosomes are clinically used delivery system for chemotherapeutic agent, biological macromolecule and diagnostics. Due to flexibility in size and composition, different types of Sphingosomes have been developed.

Keywords: Sphingosomes, Lipid bilayer, Vesicular drug delivery, sphingolipid, cholesterol.

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INTRODUCTION

In the past few decades, considerable attention has been focused on the development of new drug delivery system (NDDS). The NDDS should ideally fulfill two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of treatment. Secondly, it should channel the active entity to the site of action. Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery [1]. Recently different carrier systems and technologies have been extensively studied with the aim of controlling the drug release and Improving the efficacy and selectivity of formulation. Now a day's vesicles as a carrier system have become the vehicle of choice in drug deliver and lipid vesicles were found to be of value in immunology, membrane biology and diagnostic technique and most recently in genetic engineering [2]. Vesicular delivery system provides an efficient method for delivery to the site of infection, leading to reduce of drug toxicity with no adverse effects. Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both by hydrophilic and lipophilic drugs. Different novel approaches used for delivering the drug by vesicular system include liposomes, niosomes, Sphingosomes, transferosomes and pharmacosomes. Thus a main aim to design this review article is to introduce different vesicular drug delivery system with their marketed formulation and limitation for a student, guide or researcher or who might keen to know about vesicular drug delivery system [3].

Novel drug delivery system aims at providing some control, whether this is of temporal or spatial nature, or both, of drug release in the body. Novel drug delivery attempts to either sustain drug action at a predetermined rate, or by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects. It can also localize drug action by spatial placement of controlled release systems adjacent

to, or in the diseased tissue or organ; or target drug action by using carriers or chemical derivatization to deliver drug to particular target cell type.

Sphingosome may be defined as "concentric, bilayered vesicle in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphingolipid". Liposomal formulation based on sphingomyelin based cholesterol has several advantages when compared to other formulation [4]. The sphingosomes are much more stable to acid hydrolysis, have better drug retention characteristics. Sphingosomes are administered in many

ways these include parental route of administration such as intravenous, intramuscular, subcutaneous, and intra-arterial. Generally it will be administered intravenous or some cases by inhalation. Sphingosomes may be administered orally or transdermally (Webb et al., 1996). Sphingosome are comprised of sphingolipid (sphingomyelin) and cholesterol and have an acidic intra liposomal pH ratio of sphingomyelin and cholesterol varies in the range of 75/25 mol%/mol% (55/45mol%/mol% most preferably present at a percentage molar ratio from 75:25 to 30:50 and most preferable associate and interact with those of the neighboring molecules, and the polar headgroups orient themselves to the exterior of the assembly. Sphingosomes are forming ordered membranes as the sphingolipids in general show a preference for partitioning into ordered domains. The head group structures and acyl chain compositions of naturally occurring sphingolipids vary greatly. The ceramide moieties with the long- chain base and long saturated N-acyl chains promote the partitioning of sphingolipids into ordered membrane domains. The polar head group, which varies from the single hydroxyl of ceramide and the phosphocholine group of sphingomyelin, to large assemblies of carbohydrates for the complex glycol sphingolipid, will undoubtedly also affect the partitioning of these lipids [5, 6].

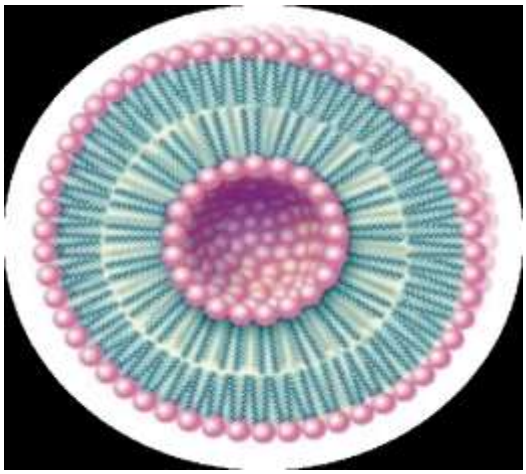

Figure I: Cross Section View Of Sphingosome

There are different types of pharmaceutical carriers are present. They are - particulate, polymeric, macromolecular, and cellular carrier. Particulate type carrier also known as a colloidal carrier system, includes lipid particles (low and high density lipoprotein-LDL and HDL, respectively), microspheres, nanoparticles, polymeric micelles and vesicular like liposomes, sphingosome, niosomes pharmacosomes, virosomes.

General advantages of sphingosomes:

- ❖ Provide selective passive targeting to tumor tissue.
- ❖ Increase efficacy and therapeutic index.

- Increase stability via encapsulation.
- Reduction in toxicity of the encapsulated agent.
- Improve pharmacokinetic effect (increase circulation time).
- Flexibility to couple with site specific ligands to achieve active targeting**:**
- Vesicular drug delivery systems are emerging with the diverse application in Pharmaceutics, Cosmetics and food industries. Their delivery of drug directly to the site of infection, leading to reduction of drug toxicity with no adverse effects. It also reduces the cost of therapy by imparting better biopharmaceutical properties to the drug, resulting in improved bioavailability, especially in case of poorly soluble drugs.
- Now a day's various non-steroidal anti inflammatory drugs, proteins, cardiovascular, anti neoplastic, anti glucoma, anti diabetic drugs that are incorporated with vesicular system are available in a commercial market that are playing a vital role to cure from a disease, hence improving the health of human kinds [7, 8].

Advantages over the Phospholipid Liposomes:

It is more stable than the phospholipid liposome because [9]

a. Sphingolipid built up by only amide and ether linkage. They are more resistant to hydrolysis then ester linkage of lecithin.

b. They also contain less double bond then lecithin and thus less subject to rancidity.

c. They also absorb less oil then lecithin which in consequence change in geometry and diameter.

Disadvantages:

1. Higher cost of sphingolipid hinders the preparation and use of these vesicular systems.
2. Low entrapment efficacy.

Classification of sphingosomes:

Sphingosomes can be classified based on structural parameter like number of bilayer formed and diameter of their resultant vesicles [10, 11]. The Sphingosomes are unilamellar or multilamellar and will typically have mean diameter of about 0.05 µ to 0.45 µ. More preferably diameter range is 0.05 to 0.2 µ.

i. Small unilamellar vesicles (SUV): It consists of single lipid bilayer and having diameter in size range 10nm-100nm.

ii. Large unilamellar vesicles (LUV): It consists of single lipid bilayer. Having greater diameter than SUV. Having size range 100nm-1µm.

iii. Multilamellar vesicles (MLV): it consists of several bilayers of lipid and having size range 100nm-20µm.

iv. Oligolamellar vesicles (OLV): bilayer is more than one but not as many as MLV's. Having size range 0.1-1μ.

v. Multivesicular vesicles (MVV): size range 100nm-20μm.

vi. Vesicles above 1 μm are known as Giant vesicles (GV).

Composition of sphingosomes:

Sphingosome are comprised of sphingolipid (sphingomyelin) and cholesterol and have an acidic intraliposomal pH ratio of sphingomyelin and cholesterol varies in the range of 75/25 mol%/mol% (55/45 mol%/mol% most preferably). Liposomal formulation based on sphingomyelin and cholesterol has several advantages when compared to other formulation. The Sphingosomes are much more stable to acid hydrolysis, have better drug retention characteristics [12].

Sphingolipid:

Sphingolipid have been known as cell component. Their name was given by J.L.W. Thudichum in 1884, because of their enigmatic nature [9]. Sphingolipid contain a polar head attached to hydrophobic body. The sphingolipid being polar lipid is related to the composition and structure of human skin lipid, specifically in the epidermis layer. The sphingolipid obtained from natural source like mammals milk, preferably bovine milk, brain, egg yolk, erythrocytes from animal blood, preferably sheep. The sphingolipid may be synthetic or semi synthetic. The simplest sphingolipids are sphingosine and Ceramide which are scaffold and complex sphingolipid such as sphingomyelin(SM) and glycosphingolipid. Different types of sphingolipid can be used in Sphingosomes and are described in table 1.

Cholesterol:

Incorporation of sterol in Sphingosomes bilayer can bring about major changes in the preparation of this membrane. Cholesterol does not by itself from bilayer structure, but can be incorporated in to sphingolipid membranes in very high concentration up to 1:1 or even 2:1 molar ratio cholesterol to sphingolipid. Cholesterol incorporation increase the separation between the choline head group and eliminate the normal electrostatic and hydrogen bonding interaction. The stability of Sphingosomes can be increased by addition of stearylamine (SA) a positive charge inducing agent. Additional components may be added to the Sphingosomes to target them to specific cell types. For example, the Sphingosomes can be conjugated to monoclonal antibodies or binding fragments thereof that bind to epitopes present only on specific cell types, such as cancer-related antigens, providing a means for targeting the Sphingosomes following systemic administration. Alternatively, ligands that bind surface receptors of the target cell types may also be bound to the liposomes [12].

Table 1: Classification of Sphingolipid Based On Source

Natural Sphingolipids: Sources	**Name of sphingolipid**
Egg,brain,milk	**1.Sphingosine derivatives:** D-erythrosphingosine, Sphingomyelin,Ceremides, brain sufatides **2.gangliosides:** ovine braingangliosides, Porcine brain gangliosides
Soy-bean	Glucosylceramides
Plant (yeast)	Phytosphingosine, D-ribo-Phytosphingosine-1-Phosphate, N-Acyl Phytosphingosine
Natural Sphingolipids:	
Sphingosine derivatives	D-erythro Sphingosine (synthetic) Sphingosine -1-Phosphate N,N-Dimethyl sphingosine , Sphingomyelin , Glycosylated Sphingosine
OmegaLabeled Sphingosine	Omega-Biotinyl Sphingosine , Omega-Biotinyl D-erythro-Sphingosine-1-Phosphate
Ceramide Derivatives	Ceramides , D-erythro Ceramide-1Phosphate Glycosulated Ceramides , Fluorescent Ceramide
Sulfated Ceramide derivatives	3-O-Sulfo-ß-D-C12-galactosylceramide (ammonium salt) 3,6-di-O-Sulfo-ß-D-C12-galactosylceramide (diammonium salt)
Sphinganine (Dihydrosphingosine)	Sphinganine-1-Phosphate ,Sphinganine (C20), D-erythro Sphinganine , N-Acyl-Sphinganine

METHOD OF PREPARATION OF SPINGOSOMES

Lipid film formation (hand shaking method):

Surfactant/cholesterol mixture was dissolved in diethyl ether in a round bottom flask and ether was removed at room temperature under reduced pressure, in a rotary evaporator. The dried surfactant film was hydrated with aqueous phase at 50- 60°C , Ether angham, in which a lipid solution in diethyl ether was slowly introduced into warm water typically the lipid mixture was injected into an aqueous solution of the material to be encapsulated (using syringe type infusion pump) at 55-65oC and under reduced pressure. Vaporization of ether leads to the formation of single layered vesicles (SLVs) depending upon the conditions used, the diameter of vesicles varies with gentle agitation; this method produces multilamellar vesicles (MLVs) with large diameter [13, 14].

Micro Fluidization:

This is a recent technique to prepare small MLVS. A Micro fluidizer is used to pump the fluid at a very high pressure (10,000psi) through a screen. Thereafter; it is forced along defined micro

channels, which direct two streams of fluid to collide together at right angles, thereby affecting a very efficient transfer of energy. The lipids can be introduced into the fluidizer. The fluid collected can be recycled through the pump until vesicles of spherical dimensions are obtained. This results in greater uniformity, small size and better reproducible niosomes [15, 16].

Reverse phase evaporation:

The novel key in this method is the removal of solvent from an emulsion by evaporation. Water in oil emulsion is formed by bath sonication of a mixture of two phases, and then the emulsion is dried to a semi-solid gel in a rotary evaporator under reduced pressure. The next step is to bring about the collapse of certain portion of water droplets by vigorous mechanical shaking with a vortex mixture. In these circumstances, the lipid monolayer, which encloses the collapse vesicles, is contributed to adjacent intact vesicles to form the outer leaflet of the bilayer of large unilamellar niosomes. The vesicles formed are unilamellar and have a diameter of 0.5 μm [17, 18].

Film method:

Film method described by Bangham et.al 1965. In this method the mixture of appropriate amount of lipid are casted as stack of film form this organic solution using flash rotary evaporator under reduced pressure (or by hand shaking) and then the casted film is dispersed in aqueous medium. Upon hydration the lipid swell and peel off from the wall of round bottom flask and vasiculate forming multi lamellar sphingosomal vesicles (MLSV's). the mechanical energy required for swelling of lipid in dispersion casted lipid film is imparted by manual agitation(hand shaking technique) or exposing the film to the stream of nitrogen for 15 minutes followed by swelling in aqueous medium without shaking (non shaking methods). The hand shaking method produce MLSV's, but the vesicles produced by non shaking method are large unilamellar sphingosomal vesicles. MLSV's formed on hydration of lipid could be further modified for their size and other characteristics [19].

Extrusion technique is generally applied to reduce the size of Sphingosomes. In this technique all the dispersion are extruded through polycarbonate membrane/ an asymmetric ceramic membrane, filter with core of 0.6μm (once) and 0.2μm (ten times). The dispersion subsequently freeze thaw ten times to increase the encapsulation efficiency of the Sphingosomes. The non entrapped drug removed by ultracentrifugation for thirty minute at 55,000 rpm and 4ºC. The pellets subsequently redisperse in buffer. Other method for size reduction of Sphingosomes:

Sonication:

At high energy level the average size of sphingosome is further reduced. This was first achieved on exposure of MLSV's to ultrasonic irradiation and still remains the method most widely used for

producing small vesicles. There are two method of sonication based on the use of either probe or bath. Ultrasonic disintegrator bath sonicators are most widely used for preparation of small unilamellar vesicles.

French pressure cells:

This is very useful method. In this extrusion of preformed sphingosome in French press under very high pressure. This technique yields rather uni or oligo lamellar Sphingosomes. These Sphingosomes are more stable as compared to sonicated vesicles.

APPLICATIONS OF SPHINGOSOMES:

A wide variety of therapeutic compound may be delivered by the sphingosome. "Therapeutic compound" is meant to include example- nucleic acid, proteins, peptides, oncolytics, anti-infective, anxiolytics, psycho tropics, ionotrops, toxins such as gelonin and inhibitors of eukaryotic protein synthesis and the like.. Particularly preferred among the therapeutic compounds for entrapment in the Sphingosomes are "lipophilic cations". Among these are therapeutic agents of the class of lipophilic molecules which are able to partition into the lipid bilayer phase of Sphingosomes and which therefore are able to associate with the Sphingosomes in a membrane form [12]. Sphingosomes may prove to be efficient carrier for targeting the drug to the site of action, because of being biodegradable, innocuous nature and being identical to biological membrane.

Sphingosomes in tumor therapy:

Most of the medical applications that have reached the pre-clinical and clinical stages are in cancer. Ex. Vinorelbine (semi synthetic vinca alkaloid) sphingosomal product has reached in phase I clinical trials [20]. Sphingosomes increased drug concentration at the tumor site is associated with increased clinical activity. The link between drug exposure and anti-tumor efficacy is especially pronounced for cell cycle-specific agents such as vincristine, vinorelbine and topotecan, which kill tumor cells by interfering with mitosis at a precise step during the cancer cell cycle. Thus, this proprietary sphingosomal drug delivery platform encapsulates approved anticancer agents within the aqueous interior of small liposomes to potentially enhance the therapeutic index of these existing anticancer treatments.

Sphingosomal products such as Marqibo(TM) (sphingosomal vincristine) are loaded with active, cell cycle-specific anticancer agents that may benefit from increased targeting and long duration of drug exposure at the tumor site. Vincristine, vinorelbine and topotecan are approved cancer therapies which have been selected for sphingosomal formulation specifically for their ability to benefit from this novel encapsulation. Vincristine (Oncovin(R); Eli Lilly and Company), a

microtubule inhibitor, is approved for acute lymphoblastic leukemia (ALL) and is widely used as a single agent and in combination regimens for treatment for hematologic malignancies such as lymphomas and leukemias. Vinorelbine (Navelbine(R) GlaxoSmithKline), a microtubule inhibitor, is approved for use as a single agent or in combination with cisplatin for the first-line treatment of unrespectable, advanced non-small cell lung cancer.

Topotecan (Hycamtin(R); GlaxoSmithKline), a topoisomerase I inhibitor, is approved for use in relapsed small-cell lung cancer and in relapsed ovarian cancer. Sphingosomes generally refers to uni- or multilamellar lipid structures enclosing an aqueous interior, depending on the number of lipid membranes formed. Typically liposomes can be loaded with drugs, i.e. the drug is encapsulated in the interior of the vesicle, and/or drugs can be attached to the sphingosome or incorporated into the lipid bilayer. Such drug comprising liposomal formulations have been shown to have an increased efficacy in comparison to the free drug. For example, it has been shown that a liposomal formulation including the vinca alkaloid vincristine has a greater efficacy if compared to free vincristine and it shows less overall toxicity.

Sphingosome act as vehicle useful for the treatment of proliferative disease, immune disease, infectious disease, vascular disease, rheumatoid disease and inflammatory disease. The representative drugs include prostaglandins, amphotericin B, methotrexate, cisplatin, vincristine, vinblastine, doxorubicin, camphothecin, ciprofloxacin, progesterone, testosterone, estradiol, beclometasone and esters vitamin-E, dexamethasone and other steroids [21]. Sphingosomes are also used in the cosmetic industry and drug delivered through transdermal route. The skin compatibility of topically applied sphingolipid is very high. Because of the membrane

lipid of sphingosome belong to same class of chemical compound as do epidermal lipid, they have characteristic that enhance their penetration.

Sphingosomes in cosmetic industry:

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Sphingosomes in ophthalmic drug delivery:

A major problem in ocular therapeutics is the delivery of an optimal drug concentration at the site of action. The ocular drug bioavailability is often modified by the physical and chemical properties of a drug as well as by physical properties of the vehicle in which the drug is placed. Thus, the selection of vehicles has been limited and semisolid varieties, principally because of the

anatomical construct of the conjunctival sac and the sensitivity of the cornea to foreign object. Amongst various vehicles and carrier, vesicles have gained considerable attention for ocular drug delivery [22]. Ex. In the treatment of acute and chronic herpetic keratitis, iodoxuridine entrapped in Sphingosomes is more effective than a comparable therapeutic regimen of unentraped drug.

Sphingosomes used for enzyme delivery:

Many enzymes including streptokinase, urokinase esterase encapsulated in Sphingosomes. Enzyme catalysis in Sphingosomes has been used for variety of reaction such as synthesis of esters, peptides and sugar acetal transformation [19, 23].

Other therapeutic application of sphingosomes:

I. Sphingosomes in antimicrobial, antifungal and antiviral (anti-HIV) therapy [17, 24].

ii. Ex. ciprofloxacin, oflaxacin, vancomycin, amoxicillin, amphotericin B, iodoxuridine.

iii. Sphingosomes may be used in gene delivery [17].

iv. Sphingosomes may be used in enzyme immobilization [17].

v. Sphingosomes may be used in immunology [17]. The concepts of Sphingosomes as drug or bioactive carrier still need further optimization. Researchers all world continue to put in their in improving vesicular system by making them steady in nature to prevent leaching of content, oxidation and their uptake by natural defence mechanism. Genetic engineering aspect can be coupled to give newer dimension to the existing cellular drug carrier concept. Their potential pharmaceutical application include immobilization of enzyme, masking the taste of drug, enhancement of gastrointestinal absorption and as carrier for sustained release and transdermal drug delivery, treatment of drug overdosing. With the evolution of various newer techniques of preparation, stabilization, characterization of these system, they can serve as potential carrier for drug cosmetic and pharmaceutical agents. these are therapeutic agents of the class of lipophilic molecules which are able to partition into the lipid bilayer phase of sphingosomes and which therefore are able to associate with the Sphingosomes in a membrane form [14].

Sphingosomes may prove to be efficient carrier for targeting the drug to the site of action, because of being biodegradable, innocuous nature and being identical to biological membrane.

CONCLUSION

Over the years, vesicular systems have been investigated as a major drug delivery system, due to their flexibility to be tailored for varied desirable purposes. Sphingosomes is bilayered vesicles in which an aqueous volume is entirely enclosed by membrane lipid bilayer mainly composed of natural or synthetic sphingolipid. Lipophilic cations are the preferred category which is to be

encapsulated. Application of sphingosomes clinically used for delivery of chemotherapeutic compound, diagnostic purpose and in cosmetic industry. Sphingosomes is made up of lipid which is similar class of skin lipid so it is more compatible and safe to host cell and there are no restrictions concerning their use neither in the EU nor for regulations of the US Food and Drug Administration; Sphingosomes are generally accepted as safe (GRAS status).

REFERENCES:

1. Robinson JR, Lee VHL. Controlled drug delivery: Fundamentals and Applications. 2nd ed., Informa healthcare; Vol 20: 56-57.
2. Molema G, Meijer DKF. Drug Targeting Organ-Specific Strategies. Wiley-VCH Verlag GmbH, 2001.
3. Shah Adarsh A *et al.* Organelle Specific Targeted Drug Delivery- A Review. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011; 2(3):895-912.
4. Shailza Singh *et al.* Molecular drug targets and structure based drug design: A holistic approach. Bio information. 2006; 1(8): 314-320.
5. Schreier H. Drug Targeting Technology: Physical, Chemical, Biological Methods. Marcel Dekker, 2001.
6. Gupta Manish, Sharma Vimukta. Targeted drug delivery system: A Review. Research Journal of Chemical Sciences. 2011; 1(2): 135-138.
7. Biju SS, Sushama Talegaonkar, Mishra PR, Khar RK. Vesicular systems: An Overview. Indian Journal of Pharmaceutical Sciences. 2012, Jun 28; IP: 117. 211.123.49
8. Ravi Kumar, Shivjee Kumar, Shyam Shankar Jha, Amit Kumar Jha. Vesicular System-Carrier for Drug Delivery. Der Pharmacia Sinica. 2011; 2(4):192-202.
9. Muller *et al.* Drug delivery vehicles and uses thereof. European Patent. 1 537 858 A1, 2005. 10 Vyas SP, Khar RK. Targeted and controlled drug delivery. Ist edition New Delhi: CBS publisher; 2002
10. Vyas SP, Khar RK. Targeted and controlled drug delivery. Ist edition New Delhi: CBS publisher; 2002
11. Biju SS, Talegaonkar S, Mishra PR. et al. Vesicular system: An overview. Indian J Pharm Sci 2006; 68:141-153.
12. Webb MS, Bally MB, Mayor LD; Inex Pharmaceutical Corporation. Sphingosome for enhanced drug delivery.US Patent 5543152. 1996 June 8.

13. Stuti Gupta, Ravindra Pal Singh, Priyanka Lokwani, Sudhir Yadav, Shivjee KG. Vesicular system as targeted drug delivery system: An overview. International journal of pharmacy and technology. 2011; 3: 987-1021.
14. Webb MS, Bally MB, Mayor LD; Inex Pharmaceutical Corporation. Sphingosomes for enhanced drug delivery. World Patent 035094. 1995Dec. 28.
15. Mayank Gangwar, Ragini Singh, Goel RK, Gopalnath. Recent Advances in various emerging vesicular system: An Overview. Asian Pacific Journal of Tropical Biomedicine. 2012:1-4.
16. Jain NK. Controlled and Novel Drug Delivery. 1st ed. CBS Publishers and Distributors, New Delhi, 2001.
17. Vyas SP, Khar RK. Targeted and controlled drug delivery. CBS publisher and Distributor, New Delhi, 2001.
18. Swarnalatha Saraf, Paliwal S, Shailendra Saraf. Sphingosomes A novel approach to vesicular drug delivery. International Journal of current scientific research, 2011; 1(1):63-68.
19. Erdogan S, Ozer Y, Bilgili H. et al. In vivo behavior of vesicular urokinase. Int J Pharm 2005; 295:1-6.
20. http://findarticles.com/p/articles/mi_m0EIN/is_/ai_n26798401
21. Mueller R, Hilka T, Nahde T; Vectron therapeutic AG. Drug delivery vehicles and uses their of European Patent 1537858. 2005 June 6.
22. Jain NK. Controlled and novel drug delivery. 1st ed. New Delhi: CBS publishers and distributors; 2001.
23. Erdogan S, Ozer Y, Bilgili H. et al Thrombus Localization by Using Streptokinase Containing Vesicular Systems. Drug Delivery 2006; 13(4):303-309.
24. Webb MS, Bally MB, Mayor LD; Inex Pharmaceutical Corporation. Sphingosome for enhanced drug delivery.US Patent 5741516. 1998 April21