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Pharmacosomes: A novel drug delivery system

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Abstract
Pharmacosomes are colloidal dispersions of drugs covalently bound to lipids, and may exist as ultrafine vesicular, micellar, or hexagonal aggregates, depending on the chemical structure of drug-lipid complex. It is based on the principle that the drug binds covalently to a lipid where the resulting compound is the carrier and the active compound at the same time. The physicochemical properties depend on drug as well as the lipid. This system shows low entrapment efficiency and drug leakage during storage for hydrophilic drugs. Pharmacosomes have advantages over liposomal, transferosomal, and niosomal drug delivery systems. They are mainly prepared by hand-shaking and ether injection method. The Pharmacosomes were evaluated for different parameters such as size, NMR, surface morphology and In vitro release rate. They minimize drug degradation and increase bioavailability of poorly soluble drugs. Pharmacosomes can only encapsulate the water insoluble drugs in relatively small hydrophobic regions within membrane bilayer rather than relatively large surface. Pharmacosomes are used in drug targeting in cancer and also brain targeting by using 5-flouro-2-deoxyuridine Pharmacosomes. Marketed preparations of include veterinary iron dextrans and other dextrans.

Keywords: Pharmacosomes, Hydrophobic, Bioavailability, Micellar.

1. Introduction
From the very beginning of the human race; the quest is going on for newer and better alternatives, and in case of drugs it will continue; continue till we find a drug with maximum efficacy and no side effects. Many drugs, particularly chemotherapeutic agents, have narrow therapeutic window, and their clinical use is limited and compromised by dose limiting toxic effect. Thus, the therapeutic effectiveness of the existing drugs is improved by formulating them in an advantageous way.
In the past few decades, considerable attention has been focused on the development of Novel Drug Delivery System (NDDS).

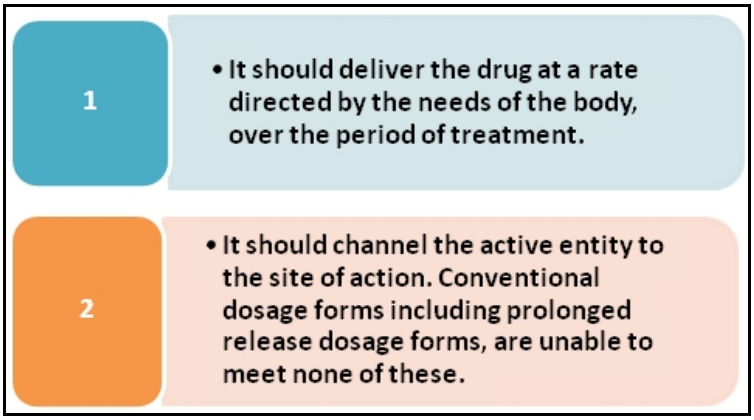

Fig 1: Two prerequisites of NDDS

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]. Approaches are being adapted to achieve this goal, by paying considerable attention either to control the distribution of drug by incorporating it in a carrier system, or by altering the structure of the drug at the molecular level, or to control the input of the drug into the bio-environment to ensure an appropriate profile of distribution. 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.

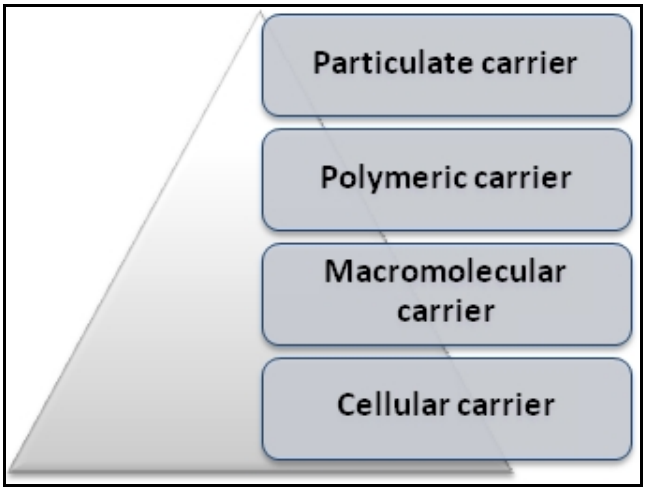

Fig 2: Different types of Pharmaceutical Carriers

Particulate type carrier also known as a colloidal carrier system. Which includes

- Lipid Particles (Low and High Density Lipoprotein-LDL and HDL, Respectively),
- Microspheres,
- Nanoparticles,
- Polymeric Micelles and vesicular Like Liposomes, Niosomes Pharmacosomes, Virosomes etc [2].

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayers formed, when certain amphiphilic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphilic building blocks. The terms such as synthetic bilayers allude to the non-biological origin of such vesiculogenes. Biologic origin of these vesicles was first reported in 1965 by Bingham and was given the name Bingham bodies. Much water has flown since then.

The novel drug delivery system is ever growing. In recent years, vesicles have become the vehicle of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques, and most recently, genetic engineering. Vesicles can play a major role in modeling biological membranes, and in the transport and targeting of active agents. Vesicular systems that have evolved are Liposomes, Niosomes, Transfersomes, and Pharmacosomes.

2. Liposomes

Liposomes are simple microscopic vesicles in which lipid bilayer structures are present with an aqueous volume entirely enclosed by a membrane, composed of lipid molecule. There are a number of components present in liposomes, with phospholipid and cholesterol being the main ingredients. The type of phospholipids includes phosphoglycerides and sphingolipids, and together with their hydrolysis products.

All methods of preparation of liposomes involve dissolution of cholesterol, lecithin, and charge in organic solvent, followed by drying it to a thin film, and then dispersion of film in an aqueous medium to obtain liposome suspension at a critical hydrating temperature. The methods of preparation have been classified to the two basic modes of dispersions:

- Physical dispersion
- Solvent dispersion [3]

The liposomes are characterized for their physical attributes i.e. size, shape, and size distribution [4] surface charge percent capture entrapped volume lamellarity through freeze fracture microscopy and P-NMR [5].

3. Application of Liposomes

1. Topical ocular drug delivery.
2. Oral administration of insulin.
3. Photodynamic therapy.

4. Niosomes

Vesicular delivery system consisting of unilamellar or multilamellar vesicles called Niosomes. In this case, an aqueous solution is enclosed in a highly ordered bilayer made up of non- ionic surfactant, with or without cholesterol and dicetyl phosphate, and exhibit a behaviour similar to liposomes *in vivo.* Niosomes have unique advantages over liposomes. Noisomes are quite stable structures, even in the emulsified form [6]. They require no special conditions such as low temperature or inert atmosphere for protection or storage, and are chemically stable. Relatively low cost of materials makes it suitable for industrial manufacture.

Methods of preparation of Niosomes:

- Lipid layer hydration method
- Reverse phase evaporation method
- Transmembrane pH gradient uptake process (Remote loading)
- Hand Shaking method
- Ether Injection
- Sonication.

Types of Niosomes:

- Multilamellar Vesicles (MLV, Size >0.05 µm),
- Small Unilamellar Vesicles (SUV, Size -0.025-0.05 µm),
- Large Unilamellar Vesicles (LUV, Size >0.10 µm).

Niosomes are characterized for different attributes such as vesicle diameter using light microscope, photon correlation microscopy, freeze capture microscopy, entrapment efficiency, and *in vitro* release rate. Other aspects studied are drug stability, drug leakage in saline and plasma on storage, pharmacokinetic aspect, toxicity, etc.

Applications of Niosomes:

1. Highest protection of insulin against proteolytic enzymes.
2. Stable carriers for tretinoin.
3. Enhanced anti-inflammatory activity of Nimesulide drug.
4. An Antileishmanial property of Bacopasaponin C was maximal without any side effects.
5. Carriers for iobitridol, a diagnostic agent used for X-ray imaging [7].

5. Transfersomes

Liposomal as well as niosomal systems, are not suitable for transdermal delivery, because of their poor skin permeability, breaking of vesicles, leakage of drug, aggregation, and fusion of vesicles.

To overcome these problems, a new type of carrier system called "Transfersome", has recently been introduced, which is capable of transdermal delivery of low as well as high molecular weight drugs.

Transfersomes are specially optimized, ultradeformable (ultraflexible) lipid supramolecular aggregates, which are able to penetrate the mammalian skin intact.

Each transfersome consists of at least one inner aqueous compartment, which is surrounded by a lipid bilayer with specially tailored properties, due to the incorporation of "edge activators" into the vesicular membrane [8]. Due to their deformability, transfersomes are good candidates for the non-invasive delivery of small, medium, and large sized drug.

Transfersomes are prepared in two steps

1. Thin film, comprising phospholipid and surfactant is prepared, hydrated with buffer (pH 6.5) by rotation, and then brought to the desired size by sonication. The concentration of surfactant is very crucial in the formulation of transfersomes, because at sublytic concentration, these agents provide flexibility to vesicles membrane, and at higher concentration, cause a destruction of vesicles.
2. Sonicated vesicles are homogenized by extrusion through a polycarbonate membrane.

Transfersomes are characterized for different physical properties such as vesicle diameter, entrapment efficiency, vesicle diameter degree of deformability or permeability, *in vitro* drug release, confocal scanning laser microscopy (CSLM) study, *in vivo* pharmacokinetic aspects [9] such as toxicity studies etc.

Applications of Transfersomes

1. Carrier for protein and peptides like insulin, bovine serum albumin, vaccines, etc.
2. Transfersomes provides a very successful means for the noninvasive therapeutic use of such large molecular weight drugs on the skin.
3. It improve the site specificity, overall drug safety, and lower the doses Transfersomes.
4. Enhanced passive estradiol penetration
5. Improve the therapeutic efficacy of cyclosporine, and the site specificity and safety of corticosteroids.

Limitations of Transfersomes

1. Chemically unstable because of their predisposition to oxidative degradation.
2. Lack of purity of the natural phospholipids.
3. Expensive.

6. Pharmacosomes

Pharmacosomes are defined as “the colloidal dispersion of drugs covalently bound to lipids, and may exist as an ultrafine vesicular, micellae or hexagonal aggregates depending upon the chemical structure of drug-lipid complex.” The term “Pharmacosomes” is derived from pharmacon, the active principle and soma, the carrier. The idea for the development of vesicular pharmacosomes is based on surface and bulk interaction of lipids with water. Any drug possessing an active hydrogem atom (-COOH, -OH, -NH2, etc.) can be esterified to the lipid with or without spacer chain. Synthesis of such compound may be guided in such a way that strongly amphiphilic compound results, which will facilitate membrane, tissue or cell wall transfer in the organism.

7. Principle

It is based on the principle that the drug binds covalently to a lipid where the resulting compound is the carrier and the active compound at the same time. The physicochemical properties depend on drug as well as the lipid.

This system shows low entrapment efficiency and drug leakage during storage for hydrophilic drugs. Pharmacosomes have some importance in escaping the tedious steps of removing the free from entrapped drug. Similar to other vesicular system pharmacosomes provide an efficient method for delivery of drug directly to the site of infection, leading to reduction of drug toxicity with no adverse effects also reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. Pharmacosomes are suitable for incorporating both hydrophilic and lipophilic drugs.

The prodrug conjoins hydrophilic and lipophilic properties, and therefore acquires amphiphilic characters, and similar to other vesicle forming components, was found to reduce interfacial tension, and at higher concentrations exhibits mesomorphic behavior [10] Many constraints of various classical vesicular drug delivery systems, such as problems of drug incorporation, leakage from the carrier, or insufficient shelf life, can be avoided by the pharmacosome approach. The idea for the development of the vesicular pharmacosome is based on surface and bulk interactions of lipids with drug. Any drug possessing an active hydrogen atom (-COOH, -OH, -NH2, etc.) can be esterified to the lipid, with or without spacer chain. Synthesis of such a compound may be guided in such a way that strongly result in an amphiphilic compound, which will facilitate membrane, tissue, or cell wall transfer, in the organism [11].

At low concentration the amphiphiles exists in the Monomer State. Further increment in monomers may lead to variety of structures i.e. micelles of spherical or rod like or disc shaped type or cubic or hexagonal shape. Mantelli *et al*., compared the effect of diglyceride prodrug on interfacial tension, with the effect produced by a standard detergent dodecylamine hydrochloride, and observed similar effect on lowering of surface tension. Above the critical micelle concentration (CMC), the prodrug exhibits mesomorphic lyotropic behavior, and assembles in supramolecular structures [12]. The prepared prodrugs are generally characterized for their structural conformation (by IR, NMR spectrophotometry, thin layer chromatography (TLC), melting point determination), partition coefficient, surface tension, and prodrug hydrolysis.

8. Salient Features of Pharmacosomes

- Entrapment efficiency is not only high but predetermined. Because drug itself in conjugation with lipids forms vesicles.
- Unlike liposomes, there is no need of following the tedious, time-consuming step for removing the free, unentrapped drug from the formulation.
- Since the drug is covalently linked, loss due to leakage of drug, does not take place. However, loss may occur by

hydrolysis.
- There is no problem of drug incorporation in the body of the patient.
- Encaptured volume and drug-bilayer interactions do not influence entrapment efficiency, in case of pharmacosome. These factors on the other hand have great influence on entrapment efficiency in case of liposomes.
- The lipid composition in liposomes decides its membrane fluidity, which in turn influences the rate of drug release, and physical stability of the system. However, in pharmacosomes, membrane fluidity depends upon the phase transition temperature of the drug lipid complex, but it does not affect release rate since the drug is covalently bound.
- The drug is released from pharmacosome by hydrolysis (including enzymatic).
- Phospholipid transfer/exchange is reduced, and solubilization by HDL is low. The physicochemical stability of the pharmacosome depends upon the physicochemical properties of the drug-lipid complex.
- Due to their amphiphilic behavior, such systems allow, after medication, a multiple transfer through the lipophilic membrane system or tissue, through cellular walls piggyback endocytosis and exocytosis.
- Following absorption, their degradation velocity into active drug molecule depends to a great extent on the size and functional groups of drug molecule, the chain length of the lipids, and the spacer. These can be varied relatively precisely for optimized *in vivo* pharmacokinetics.
- They can be given orally, topically, extra-or intravascularly.
- Mantelli *et al* [13] compared the effect of diglyceride prodrug on interfacial tension, with the effect produced by a standard detergent dodecylamine hydrochloride, and observed similar effect on lowering of surface tension above the critical micelle concentration (CMC), the prodrug exhibits mesomorphic lyotropic behaviour, and assembles in supramolecular structures. The prepared prodrugs are generally characterized for their structural conformation (by IR, NMR spectrophotometry, thin layer chromatography (TLC), melting point determination), partition coefficient, surface tension and prodrug hydrolysis.

Like other vesicular systems, pharmacosomes are characterized for different attributes such as size and size distribution, nuclear magnetic resonance (NMR) spectroscopy, entrapment efficiency, *in vitro* release rate, stability studies, etc. The approach has successfully improved the therapeutic performance of various drugs i.e. pindolol maleate, bupranolol hydrochloride, taxol, acyclovir, etc.

9. Advantages of Pharmacosomes
- Entrapment efficiency is not only high but predetermined, because drug itself in conjugation with lipids forms vesicles.
- Unlike liposomes, there is no need of following the tedious, time-consuming step for removing the free, unentrapped drug from the formulation.
- Since the drug is covalently linked, loss due to leakage of drug, does not take place. However, loss may occur by hydrolysis.
- No problem of drug incorporation.
- Encaptured volume and drug-bilayer interactions do not influence entrapment efficiency, in case of Pharmacosomes. These factors on the other hand have great influence on entrapment efficiency in case of liposomes.
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- Due to their amphiphilic behavior, such systems allow, after medication, a multiple transfer through the lipophilic membrane system or tissue, through cellular walls piggyback endocytosis and exocytosis.
- Following absorption, their degradation velocity into active drug molecule depends to a great extent on the size and functional groups of drug molecule, the chain length of the lipids, and the spacer. These can be varied relatively precisely for optimized in vivo pharmacokinetics.
- They can be given orally, topically, extra-or intravascularly Preparation and characterization: The aqueous solution of these amphiphiles typically exhibits concentration dependent aggregation.

10. Disadvantages of Pharmacosomes

The disadvantages are [14]
- Pharmacosomes can only encapsulate the water insoluble drugs in relatively small hydrobic regions within membrane bilayer rather than relatively large surface.
- Pharmacosomes on storage undergo fusion and aggregation as well as chemical hydrolysis.

11. Method of Preparation of Vesicle

Pharmacosomes are usually self vesiculating. The two well established procedures for preparation of pharmacosomes are:

1. Hand-shaking method

In hand-shaking method, the dried film of drug lipid complex deposited in a round bottom flask upon hydration with aqueous medium readily gives vesicular suspension.

In the drug lipid complex usually, lecithin is added many a times to reduce surface tension of the complex, so when reconstituted in an aqueous medium gives good surface wetting properties.

Water is usually used as an aqueous phase.

2. Ether injection method

In ether injection method, organic solution of drug lipid complex was injected slowly into the aqueous medium, wherein the vesicles were readily formed.

Here the drug lipid complex is mixed with ether which acts as a solvent and then it is slowly injected in the aqueous medium and spontaneous formation of vesicles takes place.

12. Evaluation of Pharmacosomes

i) Size

The size of the vesicles is in the nanorange. The size of the vesicles is usually measured using an instrument known as Zetasizer XS [15]. The principle of this instrument is based on scattering of light. The light ray is passed through the solution containing the vesicles. The size of the vesicles is then measured on the basis of scattered of light.

ii) *In vitro* release rate

In the bulk equilibrium reverse dialysis bag technique described here, emulsion is introduced inside the dialysis bag and the continuous (receiver) phase is placed outside. Dialysis bags containing the continuous phase (receiver phase) alone are suspended in a vessel containing the donor phase (diluted emulsion) and the system is stirred. At predetermined time intervals, each dialysis bag is removed and the contents are analyzed for released drug. An advantage of this technique is the increase in the membrane surface area available for transport from the donor to the receiver phases. Another advantage of this method is the increased efficiency in terms of staffing as a consequence of the reduction in the number of steps.

iii) Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) is the name given to a physical resonance phenomenon involving the observation of specific quantum mechanical magnetic properties of an atomic nucleus in the presence of an applied, external magnetic field.

The principle of NMR usually involves two sequential steps:

- The alignment (polarization) of the magnetic nuclear spins in an applied, constant magnetic field H_0.
- The perturbation of this alignment of the nuclear spins by employing an electro-magnetic, usually radio frequency (RF) pulse. The required perturbing frequency is dependent upon the static magnetic field (H_0) and the nuclei of observation.

NMR spectroscopy is one of the principal techniques used to obtain physical, chemical, electronic and structural information about molecules due to either the chemical shift Zeeman Effect, or the Knight Shift effect, or a combination of both, on the resonant frequencies of the nuclei present in the sample. It is a powerful technique that can provide detailed information on the topology, dynamics and three-dimensional structure of molecules in solution and the solid state.

iv) Surface morphology

The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity.

The types of signals produced by an SEM include secondary electrons; back scattered electrons (BSE), characteristic x-rays, light (cathodoluminescence), specimen current and transmitted electrons. The signals result from interactions of the electron beam with atoms at or near the surface of the sample. A wide range of magnifications is possible, from about x 25 (about equivalent to that of a powerful hand-lens) to about x 250,000, about 250 times the magnification limit of the best light microscopes.

13. Physicochemical Stability of Pharmacosomes

Like other vesicular systems, Pharmacosomes are characterized for different attributes such as size and size distribution, nuclear magnetic resonance (NMR) spectroscopy, entrapment efficiency, in vitro release rate, stability studies, etc. The approach has successfully improved the therapeutic performance of various drugs i.e. Pindolol maleate, Bupranolol hydrochloride, Taxol, acyclovir, etc [16]. Kaiser studied the effect of different electrolyte media on physicochemical stability of Bupranolol hydrochloride Pharmacosomes. Since polar hydrophilic head group is very sensitive towards different electrolytes, spontaneous aggregation was observed at different concentration depending upon the valency of the electrolyte. However aggregation in the presence of non-electrolyte moderate to indifferent. Best candidate for isotonization was found to be 5% glucose. The approach has successfully improved the therapeutic performance of various drugs i.e. Pindolol maleate, Bupranolol hydrochloride, Taxol, acyclovir, etc. yang *et al.* Found that CDP – diacyl prodrug initially forms large vesicles, which diminish in size and finally form micelles. They shows that slow kinetics are essential requirement for Phospholipid on bimembrane in order to confer stability to the lipid bilayer and prevent the rapid exchange of lipids between membranes of living cells. The phase transition temperature of pharmacosomes in the vesicular and Micellar state could have significant influence on their interaction with membranes. Pharmacosomes can interact with bimembranes enabling a better transfer of active ingredient this interaction leads to change in phase transition temperature of bimembranes thereby improving the membrane fluidity leading to enhance permiations.

Table 1: Comparison between Liposomes and Pharmacosomes [17]

	Liposomes	Pharmacosomes
Principle	Incorporation of drug in the aqueous or lipid phase of a mixture of lipid where the physicochemical properties of the carrier and release of drug will be functions of different lipids used.	Covalent binding of a drug to a lipid where the resulting compound is the carrier and the active compound at the same time. The physicochemical properties depend on drug as well as the lipid.
Loss of drug	Through leakage	No leakage, since drug is covalently bound but loss of drug by hydrolysis is possible.
Manufacturing	Cast fill method Extrusion/sonication Injectable method Reverse phase evaporation etc.	Self dispersion through moderate mixing and sonication.

Separation of free drug	By gel filtration, dialysis, ultrafilteration, ultracentrifugation.	Not necessary since the drug covalently linked.
Volume of inclusion	Decisive in incorporation of drug molecules.	Irrelevant, since the drug is covalently bound.
Surface charge	Achieved through lipid combination.	Depends on the physicochemical structure of the drug lipid complex.
Membrane fluidity	Depends on lipid combination and presence of cholesterol fluidity influences the rate of drug release and physical stability of system.	Depends on phase transition temperature of drug lipid complex. No effect on release rate since the drug is covalently bound.
Release of drug	Diffusion through the bilayer, desorption from the surface or release through degradation of liposomes.	Hydrolysis (including enzymatic).
Physical stability	Relatively good Aggregation through double valenced cation.	Depends on physicochemical properties of the drug-lipid complex.

Pharmacosomes bearing unique advantages over liposome vesicles have come up as potential alternative to conventional vesicles. The system yet requires greater efforts towards investigating the non-bilayer phases, and exploring the mechanism of action. Furthermore, the effect of covalent linkages and addition of spacer group on rate of *in vivo* Hydrolysis and subsequent pharmacokinetics is to be exhaustively studied, in order to exploit more advantages of this system. Like other vesicular drug delivery systems, Pharmacosomes, on storage, undergo fusion and aggregation, as well chemical hydrolysis [18].

14. Marketed Preparations

Human Iron Dextran is manufactured as low molecular weight Iron Dextran by Pharmacosmos. As the only injectable iron product, CosmoFer® offers the flexibility of iron repletion by total dose iron infusion, intravenous and intramuscular iron injection. [19]

Veterinary Iron Dextran is used as iron supplement for prevention of iron deficiency anemia in piglets. Uniferon® Iron Dextran drug products are marketed in several countries.

Dextran polymers from Pharmacosmos are manufactured according to Good Manufacturing Practice (GMP). Our Dextran products include clinical and reagent grade Dextran and GPC Standards for GPC chromatography.

15. Conclusion

Pharmacosomes bearing a unique advantages over liposomes and niosomes vesicles, have come up as potential alternative to conventional vesicles. The drug shows excellent entrapment efficiency and there is minimal loss of drug due to leakage. Like other vesicular drug delivery systems, Pharmacosomes, on storage, undergo fusion and aggregation, as well chemical hydrolysis. Similar to other vesicular system Pharmacosomes still play an important role in the selective targeting, and the controlled delivery of the controlled delivery of various drugs. Current research trends are generally based on using different approaches like pegylation, biotinyzation etc. for cellular targeting.

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