CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.7626073

Available online at: http://www.iajps.com *Review Article*

HEXOSOMES: A NEW DRUG DELIVERY SYSTEM AND ITS APPLICATIONS

[1]Vinayak Katekar, [2]Shruti Adhau, [3]Vaishnavi Akotkar, [4]Pooja Sakharkar

[1]Assistant professor Shri R.D. Bhakt College of Pharmacy, Jalna., [2]Assistant professor Vidyabharti College of Pharmacy, Amravati., [3]Assistant professor G.H. Raisoni University school of Pharmacy, Amravati., [4]Assistant professor Ishwar Deshmukh Institute of Pharmacy, Digras.

Article Received: January 2023 **Accepted:** January 2023 **Published:** February 2023

Abstract:

Hexosomes are the opposite hexagonalphases comprised of hexagonally close-packed unbounded water layers covered by surfactants monolayer. Hexosomes (dispersed H_{II} phases) due to their special structural properties have potential to be used as deferent delivery vehicle for pharmaceuticals. Biologically active molecules can either be accommodated within the aqueous domains or can be directly join to the lipid hydrophobic moieties oriented radially outwards from the center of the water rods.Hexosomes are receiving increasing awareness for preparation of pharmaceutical formulations compared with the corresponding inverse nonlamellar phases due to their low viscosity that ensures ease of preparation and handling, particularly in engineering of parenteral dosage forms and the capability of delivering a wide range of diagnostic probesandtherapeutic agents.Hexosomes formulation exhibited high entrapment efficiency, high permeability and better stability on storage, thus proposing itself a novel carrier for various bioactive agents.

Keywords: *Hexosomes, Reverse hexagonal phases,Drug delivery, Transdermal and Parenteral route.*

Corresponding author:

Vinayak Katekar,

Assistant professor,

Shri R.D. Bhakt College of Pharmacy, Jalna.

QR code

Please cite this article in press Vinayak Katekar et al, ***Hexosomes: A New Drug Delivery System And Its Applications.,*** *Indo Am. J. P. Sci, 2023; 10 (02).*

INTRODUCTION:

Explosive growth of research on the use of lipid nanoparticles in the development of Nano carriers for biomedical imaging and drug delivery purposes has been witnessed over the last 28-30 years [1-3]. For diagnostic andtherapeuticapplications, the attractiveness of lipid nanoparticles relies among others on the capability of loading diagnosticand therapeutic agents, protecting them from degradation, enhancing absorption and improving intracellular penetration, modifying pharmacokinetics, minimizing systemic toxicity, and overcoming systemic and tumor barriers [4-7]. They are particularly attractive for loading poorly water-soluble drugs that typically display limited bioavailability, poor pharmacokinetics, and adverse side effects [2, 4]. Surface engineering can also be employed through different surface manipulation strategies on lipid nanoparticles for active drug targeting [5,7].

Beside liposomes, many efforts have been devoted in the utilization of emulsions, micellar solutions including micro emulsions, solid lipid nanoparticles (SLNs), and non-lamellar liquid crystalline nanoparticles (mainly cubosomes and hexosomes) in the development of nanocarriers for drug delivery applications [1, 2,7-12]. Among these nanoparticles, we exclusively focus in the present contribution on major challenges and recent advances in the use of non-lamellar liquid nanoparticles (mainly hexosomes) and SLNs in the development of nanomedicines. We discuss also future opportunities and potential fate of these nano-self-assemblies in vivo [13,14].

Polar amphiphilic lipids that possess a very low aqueous solubility often self-assemble into lipotropic liquid crystalline phases in the presence of excess water [15]. Depending upon the nature of the lipid, the presence of additives, and solution conditions the structures formed often include the reversed hexagonal (H_{II}lamellar (Lα),) and reverse bicontinuous cubic phase (Q_{II}). The bulk liquid crystalline structure may be dispersed to form sub-micrometer particles which retain the internal structure of the monodispersed liquid crystal bulk phase. In the case of hexagonal, lamellar, and cubic phases, these particles have been termed liposomes, hexosomes. The two or three-dimensional liquid crystalline structure consists of discrete lipidic hydrophobic and aqueous hydrophilic domains and imparts a high viscosity to these materials [15]. Hexosomes are submicron sized particles containing internally inverted type hexagonal liquid crystalline phases (H_{II}) which are dispersed in continuous aqueous medium. Hexosomes (dispersed H_{II} phases) due to their special structural properties have potential to be used as alternative delivery vehicle for pharmaceuticals. Biologically active molecules can either be accommodated within the aqueous domains or can be directly coupled to the lipid hydrophobic moieties oriented radially outwards from the center of the water rods. Due to these special properties of hexosomes, they are used to improve solubility of poorly water soluble drugs and to transport therapeutic peptides and proteins by oral, transdermal, and parenteral routes [16, 17]. This review mainly focuses on various methods of preparation of hexosomes, factors affecting hexosomes formation, characterization and their application in drug delivery through various routes.

Advantages of Hexosome as a drug delivery system:[18-20]

1. Used for the delivery of large molecules like peptide and proteins.
2. Stability enhancement (improved shelf-life and/or in vivo stability).
3. Means of solubilizing, encapsulating and transporting active pharmaceutical ingredients.
4. High drug loading.
5. Reduction in overall dose but maintaining the effectiveness of the drug thus decreases toxicity.
6. Enhances permeation of drug through skin and mucosal membrane for transdermal, dermal and transmucosal delivery.

STRUCTURE OF HEXOSOME:

The hexagonal unit cell (the smallest periodic group from which the liquid crystal can be built) is of a primitive (P) type, which represents one infinite rod per unit cell corner. The hexagonal unit cell is described in terms of three sides of the cell: a, b, and c, where a = b represents the space between each corner of the cylinder micelles (termed the lattice parameter, α), and c is the infinite length of each rod. As a result, the angles between a and b are 60° and 120° and between a (or b) and c are 90°. Reverse hexagonal (type II, termed H_{II}) consists of water-filled cylinders arrangedin a continuous lipid matrix [21] (Fig. 1 and fig. 2).

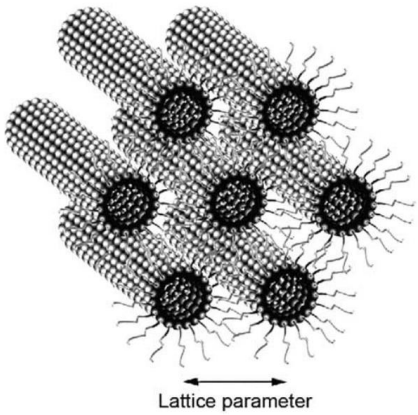

Fig no.1: Schematic presentation of H_{II} mesophase.

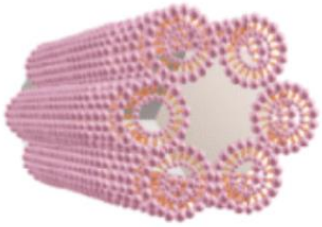

Fig no.2: Structure of Hexosome.

FACTORS AFFECTING HEXOSOMES FORMATION:

Critical Packing Parameter:

A local constraint upon the curvatures of the interface can be attributed to the geometric features of the lipid. These can be described by the effective critical packing parameter (CPP).

$CPP = Vs/ a_0 l$

Where Vs is the hydrophobic chain volume, a_0 is the cross polar head group area and l is the hydrophobic chain length of the molecule in its molten state. The CPP is affected by the lipid polarity, temperature, water content and addition of hydrophilic or hydrophobic additive. The packing parameter is useful in predicting which phases can be preferentially formed by a given lipid, since it connects the molecular shape and properties to the favored curvature of the polar-a polar interface, and therefore the topology and shape of the aggregate.

It is known that reversed-type mesophases are formed from amphiphiles with CPP>1 as illustrated [22,23].

Additives:

Hydrophobic additives like oil when added to the formulation increases the critical packing parameter by increasing hydrocarbon chain space (Vs) [20-22]. The solubilization of hydrophobic additives such as triglyceridesand tetradecane also induces a significant reduction in the amount of solubilized water in the internal structure of these dispersions [13]. Oleic acid when added to GMO formulation, oleic acid gets solubilized in nonpolar tail of GMO and thus increases the hydrocarbon chain space (Vs) which increases the CPP>1 which in turn favors the formation of H_{II} phases which are stable at room temperature [24].

METHODS OF PREPARATION:

Materials:

Oleyl glycerate and phytanyl glycerate were manufactured at CSIRO-Molecular Science, Australia as described previously [25] and were 99% and 96% pure by reverse phase HPLC, respectively. Myverol 18-99 (GMO) was obtained from Quest International. Myverol 18-99 is known to have very similar phase behaviour to pure glyceryl monooleate [26], most importantly that it forms cubic phase in excess water at physiological temperatures, and therefore is considered to be a good model for GMO. Irinotecan base was obtained from Dabur, India and was used as received. Oleic acid (99%) and sorbitol were sourced from Sigma (St. Louis, MO). Pluronic F127 (Poloxamer 407) was injectable grade material sourced from BASF (Ludwigshafen, Germany) [27].

Solubility:

Due to the limited quantities of the phytanyl glycerate and oleyl glycerate and the cytotoxicity of irinotecan, solubility studies of irinotecan were conducted in small 250 µL amber vials. Samples were prepared by weight. Using a micro spatula, excess irinotecan was added to approximately 200 mg of lipid in triplicate. The mixtures were manually mixed and stored at 37 ◦C before centrifugation and sampling. An accurately weighed mass of the solution (~20 mg) was removed and dissolved in 1 mL of an acetonitrile: chloroform: methanol mixture (45:45:10%, v/v/v). Irinotecan content was determined by HPLC as described previously [25].

Bulk phase behaviour and water content:
Crossed polarised optical microscopy was utilised to determine phase identity by observation of birefringence when in contact with excess aqueous solution [28,29]. An Axiovert inverted microscope (Melbourne, Zeiss, Australia) and heating stage (Melbourne, Mettler Toledo, Australia) were used for this purpose, and conducted as described previously [30]. Water content in equilibrated liquid crystal samples was determined by Karl Fisher coulometry, also described previously [30].

Production and characterization of hexosomes:
Briefly, the lipid components and Poloxamer 407, and drug where present, were heated to 60 ◦C to provide a clear solution. The aqueous medium (25 mL) was also heated to 60 ◦C. A coarse dispersion was produced by injecting the lipidic solution into the aqueous medium whilst homogenizing with an UltraTurrax T25 tissue homogenizer (IKA, Staufen, Germany) at 10,000 to 11,000 rpm using an S25N18G dispersing element for 90 s [31]. The dispersion was immediately transferred to a thermostatted Avestin C5 homogenizer (Avestin, Ottawa, Canada) at 60 ◦C, and the coarse dispersion refined through 10 passes at 10,000 psi. The final dispersion was collected into a glass vial containing a magnetic stirrer bar and cooled to room temperature under stirring for 30 min before particle size analysis [31].

Zeta potential andparticle size were measured using a Malvern,Malvern Instruments, UK and dispersions were diluted 1 in 100 with water prior to measurement for optimal measurement sensitivity. Analysis used the Contin algorithm and particle size values reported are Z average values for particle diameter. SAXS and cryo-TEM were described in the previous publication on dispersed glycerate surfactant systems [30,31].

Drug release:
Drug release from the bulk liquid crystalline phase was as described previously [30]. Briefly, the lipid(s) and irinotecan were combined and equilibrated with a slight excess of Milli-Q water (40%, w/w) at 37 ◦C for 100 h on a roller mixer. The bulk phase was loaded into a micro beaker (approximately 500 mg) and immersed in a thermostatted beaker at 37^0Ccontaining the release medium (500 mL of Milli-Q water), and stirred by means of a digital overhead propeller stirrer (Eurostar) at 100 rpm (30 mm tri-prop stirrer shaft). The release medium was sampled periodically (100 µL) and replaced with fresh release medium. The samples were analyzed for drug content by HPLC. Irinotecan was analyzed by HPLC as previously described [30].

Drug release from Hexosomes was conducted by pressure ultrafiltration using a Millipore YM10 membrane in an Amicon 8050 magnetically stirred pressure ultrafiltration cell (Millipore, Australia). This method has been previously described in detail for drug release from Cubosomes [32]. Briefly, the Hexosome dispersion was diluted 1 in 100 in water to stimulate drug release from the Hexosomes, and at required time points the cell was pressurized to force free drug solution through the membrane for collection and analysis. In each case 900 µL was discarded prior to collection of the 100 µL sample as this amount of irinotecan far exceeded that required to saturate the membrane in previous experiments [32].

Analytical methods:
Irinotecan was quantified by HPLC based on the method using an Altima C8 5µm, 250 mm × 4.6 mm column (Alltech, Melbourne, Australia). Irinotecan was eluted with mobile phase comprising 38% ACN/62% (50 mM Na2HPO4, 5 mM heptane sulfonic acid, adjusted to pH 6.4 with 85% orthophosphoric acid) in isocratic mode on a Waters Alliance HPLC system with flow rate of 1 mL/min and UV detection at 370 nm. The method was validated by standard methods for precision and accuracy[33]. In order to trap the lactone and carboxylate forms of irinotecan, the samples were immediately diluted in HPLC mobile phase to prevent conversion between the two forms as described previously by Rivory. Oleyl alcohol was quantified using a Zorbax Extend C18 column, 150 mm × 4.6 mm, 5µm (Alltech Associates, Melbourne, Australia) and eluted with a mobile phase comprising 90% acetonitrile, 10% Milli-Q water with UV detection at 200 nm [33].

VARIOUS METHODS OF CHARACTERIZATION OFHEXOSOMES:

Visualization:

Visualization of hexosomes can be done using Transmission Electron Microscopy (TEM). Small-angle X-ray scattering (SAXS), cryo-transmission electron microscopy (cryo-TEM) and cryo-field emission scanning electron microscopy (cryo-FESEM) are use to probe internal structure of hexosomes [34].

Small-Angle X-Ray Scattering (SAXS):

Small angle X-ray scattering (SAXS) is the most recognized method to study the structural features of reversed mesophases, both in their bulk or dispersed form. In general the position and intensity of the diffraction peaks can be used to identify a certain type of self-assembled structure and even to identify the corresponding space group. [35,36]

Cryo-TEM:

Cryo-TEM is an ideal tool to study routinely the morphology and structure of mesophase particles with a resolution down to about 2–3 nm. In particular, it allows one to obtain more detailed information on the crystallographic structure of particles. [35] Cryo-FESEM Cryo-FESEM technique is applied to get 3-D images of hexosomes [35]. 2. Vesicle Size and Zeta Potential Particle size and zeta potential can be determined by Dynamic light scattering (DLS) using a computerized inspection system [2] and by Photon Correlation Spectroscopy (PCS) [36, 37].

Vesicle Size and Zeta Potential

Particle size and zeta potential can be determined by Dynamic light scattering (DLS) using a computerized inspection system [38] and by Photon Correlation Spectroscopy (PCS) [36, 37].

Drug Content

Amount of drug can be quantified by a modified high performance liquid chromatographic method [36, 37].

Penetration and Permeation

Infrared spectroscopy (FT-IR) can be used to study the permeation of formulation. Depth of penetration and pathway of penetration can be assessed using confocal laser scanning microscopy (CLS) [38].

Ex-Vivo Studies

Drug release from the hexosomal formulation can be studied by using Franz diffusion cells [39].

Drug Release

Drug release pattern from hexosomes is obtained by pressure ultrafiltration technique using a Millipore YM10 membrane in an Amicon 8050 magnetically stirred pressure ultrafiltration cell. [40,41]

Percent Drug Loading

The amount of drug loaded in to the dispersion can be determined with Centricon® and the amount of drug present in the filtrate is assayed through HPLC [42].

$$\text{Percent drug loading} = \frac{\text{Entrapped drug(mg)}}{\text{Total drug added(mg)}} \times 100$$

Physical Stability of Dispersion:

The ability of hexosomal dispersion to retain the drug (i.e., drug-retentive behaviour) can be assessed by keeping the dispersion at 25ºC (room temperature; RT) for a period of 16 weeks. Samples are withdrawn periodically and analyzed for the drug content by HPLC. Stability of hexosomes can also be assessed by the measurement of size and structure over time using photon correlation spectroscopy along with TEM visualization [28]. The stability of the hexosomes can also be examined with a LUMiFuge 114 a microprocessor-controlled analytical centrifuge that detects a large variety of demixing phenomena (floating and clarification) of the dispersed systems during centrifugation over the whole sample length (25 mm). Centrifugation at 300–3000 rpm, which corresponds to 12–1200g (gravimetric factor), results in an accelerated migration of the dispersed particles. Local variations in particle concentration are detected due to changes in light transmission. [43].

Rheological Measurements:

Rheological measurements can be performed using the Rheoscope 1 rheometer. A cone-plate sensor was used with a diameter of 35 mm, cone angle of 1°, and a gap of 0.024 mm. The linear viscoelastic range (LVR) of a material was determined before carrying out the oscillatory measurements. The storage and loss moduli are plotted as a function of stress at frequency = 1 Hz, at temperatures of 25 °C. The shear moduli is independent of stress up to a critical applied stress and generally were observed to fall off sharply beyond the values of 100–120 Pa. These results indicate that the samples possess linear viscoelastic properties up to about 100–120 Pa. Above these typical values the microstructure of the HII phases breaks down, which was reflected by the

rapid decrease of the moduli. According to the determined LVR, the viscoelasticity measurements are generally performed at 75 Pa. Frequency-dependent rheological measurements are conducted in the range of 0.01–100 rad/s. The viscoelasticity of the HII phases can be characterized in terms of the elastic modulus G, the loss modulus G, the complex viscosity η*, and the time max, according to the Maxwell model. The impact of drug and other additives on the viscoelasticity properties of H_{II} phases can be studied by this method [43].

Molecular Interaction of Drug with HII Phases:
Attenuated total reflectance Fourier transform infrared (ATR-FTIR) measurements can be used for the detailed analysis of molecular interactions occurring in the reverse hexagonal mesophases as well as to clarify the structure and interactions of the peptide drugs with the mesophase components [44].

Diffusion NMR:
Diffusion NMR can be used to study the diffusion of single molecules within a specific liquid crystalline phase. It is powerful method since the diffusion coefficient of water, emulsifier (such as monoglycerides) and guest molecules can be directly extracted [30].

APPLICATIONS OF HEXOSOME NANOCARRIERS:
Hexosomes are receiving increasing attention for preparation of pharmaceutical formulations compared with the corresponding inverse nonlamellar phases due to their low viscosity that ensures ease of preparation and handling, particularly in engineering of parenteral dosage forms and the capability of delivering a wide range of therapeutic agents and diagnostic probes. In addition to this, due to their versatility, these carriers can be made multifunctional through an array of surface engineering strategies [45-49]. However, pharmaceutical applications of hexosomes are still in infancy and sporadic. (Table 1) provides representative examples of the potential pharmaceutical uses of these soft nonlamellar liquid crystalline nanocarriers.

Table no.1: Representative examples of the applications of Hexosome

Applications	Drug or Imaging probe	Advantages
Intravenous (theranostic)	Nitroxide	Effective for MRI contrast in vivo
Theranostic	Technetium-99m (^{99m}Tc)	Noninvasive visualization tool applicable for SPECT/CT
Topical	siRNA	Optimization of skin penetration without skin irritation
Mucosal	^{3}H-oleic acid (radiolabeling agent)	Successful in situ transformation from Lα to mucoadhesive H2 with enhanced adsorption onto the mucosal surface

Hexosomes have largely been investigated as effective carriers for anticancer drugs through different routes of administration [50,51,52] They facilitate the accumulation of anticancer drugs in tumor cells through the enhanced permeability and retention (EPR) effect [53].

Delivery of Anti-Cancer Agent:
Irinotecan is an anticancer drug, which is highly effectivein the treatment of metastatic colorectal cancer. Irinotecandisplays a pH dependent equilibrium between its activelactone and inactive carboxylate forms, with rapidconversion to the carboxylate form occurring at neutral pHand concurrent cleavage of the bipiperidino-side chain to thehighly cyctotoxic SN-38 (Fig. 3). As a consequence, thecurrently marketed product Camptosar®, containingirinotecan for infusion, is formulated at a low pH of 3.5 inorder to maximize the lactone content at the time ofintravenous infusion. However it is anticipated that dilutionwith infusion liquids and the time required for slowintravenous infusion, often 1.5 h in the clinical setting, will result in conversion of significant proportions of theirinotecan to its inactive form. Minimizing this conversionmay be beneficial in facilitating lower effective dose regimeswith shorter infusion times [54]. The formulation of irinotecan inglycerate-based hexosomes can improve the retention ofirinotecan in the lactone form at near neutral pH. The particlesize of the hexosomes is suitable for intravenousadministration [55].

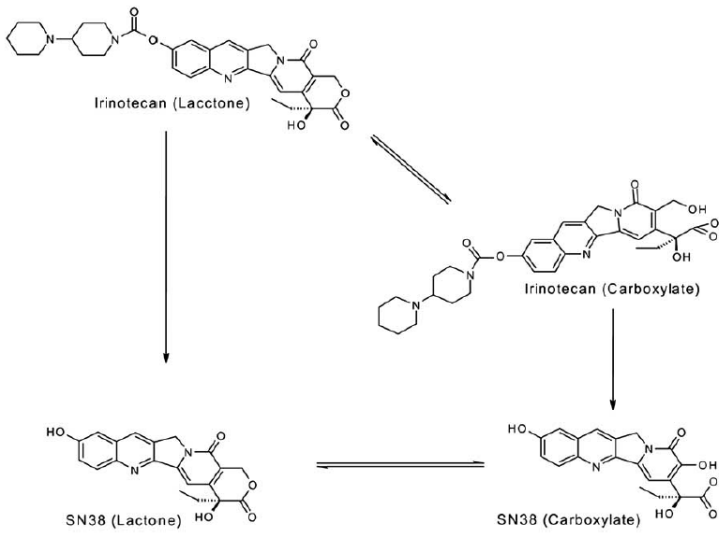

Fig no.3: Structures of irinotecan and SN-38 in the lactone and carboxylate forms.

Trans mucosal Delivery of Hormones:
Oral administration of hormones is associated with problemslike high first pass metabolism, low oral bioavailabilityand several dose dependent side effects. These hormones canbe given by other routes. E.g. Oromucosal delivery of progesteronevia hexosomes. Preparedhexosomal dispersion loaded with progesterone and dispersedit in Carbopol 934P gel for oromucosal delivery system [19].As hexosomes composition contains oleic acid whichhelps in hexosomes formation as well as act as penetrationenhancer, bioavailability is increased [56,57].

Parenteral Sustained Drug Delivery System:
Parenteral dosage forms of hormone regulating peptides,proteins and antibodies are almost always required as thesetherapeutic agents are often unstable in and/or poorly absorbedfrom the gastrointestinal tract. Sustained release platformsare particularly attractive for such agents due principallyto their often short biological half-lives. Reversehexagonal phases can provide sustained release matrices fordepot drug delivery. Boyd *et al.* [30] used oleyl glycerateand phytanyl glycerate as the surfactants separately for thepreparation of H_{II} phases. Of the two surfactants, matrixformed of phytanyl glycerate produced more sustainedrelease of drug as compared to oleyl glycerate.

Transdermal Delivery of Peptide:
Peptides incorporated into an HII phases are protectedfrom enzymatic degradation. HII phases can also includepenetration enhancer so that it increases flux across thestratum corneum to increase the concentration of drug at thesite of action. Cyclosporin A and three dermal penetrationenhancers (phosphatidylcholine, ethanol, or Labrasol) can besolubilized into reverse hexagonal (H_{II}) liquid crystallinestructures composed of monoolein, tricaprylin, and water.Cyclosporin a (CSA) is a cyclic undecapeptide which isused clinically as an efficient immunosuppressive agent. It ishence widely used in transplantation medicine anddermatology. However, a major drawback of CSA is its toxicity, mainly a reversible nephrotoxicity, limiting itsclinical use. Another problem is the low oral bioavailabilityof CSA which is attributed to poor drug absorption andintestinal metabolism. Additionally, topical and transdermaladministration of CSA is limited by its profoundhydrophobicity and the barrier property of the stratumcorneum (SC). In principle, such limitations can beaddressed and modified via the application of a penetrationenhancer that may increase the diffusion coefficient of thedrug into the SC. Dispersed H_{II} phases have large surfacearea to interact with the skin and high fluidity and so can beused as transdermal drug delivery system without causingskin irritation [18, 40].

Desmopressin, a hydrophilic peptide loaded hexosomaldispersion can be used as sustained and controlled releasetransdermal drug delivery system [17].

Oral Sustained Release Drug Delivery System:
Lipid based mesophases enhances the bioavailability ofpoorly water soluble drugs after oral administration. Somelipids like oleyl glycerate and phytantriol are not digested bygastrointestinal (GI) enzymes thus HII phases formed ofthese lipids are stable in GI fluids and sustain the release ofdrug.

Oleyl glycerate is an amphiphile which is poorly digestedin stomach. Phytantriol is a lipid which does not possessester bond thus it is not digested. The phases formed byoleyl glycerate and phytantriol are stable and can be used inoral sustained release formulation [58].

CONCLUSION:

Hexosomes are promising alternative lipid-based nanocarriers offering solubilization of a plethora of hydrophilic,hydrophobic and zwitterionic drugs and biopharmaceuticals. They are amenable to surface functionalization with polymers and targeting ligands and may be tuned for development of theranostic entities. They are also increasing interest for solubilization and delivery of antigens as an alternative vaccination strategy.Hexosomes has attracted great interest for new potential applications in the area of pharmaceutical technology.Hexosomes formulation exhibited high entrapment efficiency, high permeability and better stability on storage, thus proposing itself a novel carrier for various bioactive agents.

REFERENCES:

1. Couvreur P, Vauthier C. Nanotechnology: intelligent design to treat complex disease. Pharm Res (N Y) 2006;23:1417e50.
2. Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. Faseb J 2005;19:311e30.
3. Wibroe PP, Ahmadvand D, Oghabian MA, Yaghmur A, Moghimi SM. An integrated assessment of morphology, size, and complement activation of the PEGylated liposomal doxorubicin products Doxil, Caelyx, DOXOrubicin, and SinaDoxosome. J Control Release 2016;221:1e8.
4. Garcia-Pinel B, Porras-Alcala C, Ortega-Rodriguez A, Sarabia F, Prados J, Melguizo C, et al. Lipid-based nanoparticles: application and recent advances in cancer treatment. Nanomaterials 2019;9:638.
5. Bor G, Mat Azmi ID, Yaghmur A. Nanomedicines for cancer therapy: current status, challenges and future prospects. TherDeliv 2019; 10:113e32.
6. Azmi ID, Moghimi SM, Yaghmur A. Cubosomes and hexosomes as versatile platforms for drug delivery. TherDeliv 2015;6:1347e64.
7. Steichen SD, Caldorera-Moore M, Peppas NA. A review of current nanoparticle and targeting moieties for the delivery of cancer therapeutics. Eur J PharmaceutSci 2013;48:416e27.
8. Murgia S, Biffi S, Mezzenga R. Recent advances of non-lamellar lyotropic liquid crystalline nanoparticles in nanomedicine. CurrOpin Colloid Interface Sci 2020;48:28e39.
9. Souto EB, Baldim I, Oliveira WP, Rao R, Yadav N, Gama FM, et al. SLN and NLC for topical, dermal, and transdermal drug delivery. ExpetOpin Drug Deliv 2020;17:357e77.
10. Yingchoncharoen P, Kalinowski DS, Richardson DR. Lipid-based drug delivery systems in cancer therapy: what is available and what is yet to come. Pharmacol Rev 2016;68:701e87.
11. Sastri KT, Radha GV, Pidikiti S, Vajjhala P. Solid lipid nanoparticles: preparation techniques, their characterization, and an update on recent studies. J ApplPharmaceutSci 2020;10:126e41.
12. Pucek A, Tokarek B, Waglewska E, Bazylinska U. Recent advances in the structural design of photosensitive agent formulations using “soft” colloidal nanocarriers. Pharmaceutics 2020;12:587.
13. Mahant S, Rao R, Souto EB, Nanda S. Analytical tools and evaluation strategies for nanostructured lipid carrier based topical delivery systems. ExpetOpin Drug Deliv 2020;17:963e92.
14. Tapeinos C, Battaglini M, Ciofani G. Advances in the design of solid lipid nanoparticles and nanostructured lipid carriers for targeting brain diseases. J Control Release 2017;264:306e32.
15. Kaasgaard, T.; Drummond, C.J. Ordered 2-D and 3-D nanostructured amphiphile self-assembly materials stable in excess solvent. Phys. Chem. Chem. Phys., 2006, 8, 4957-4975.
16. Amar-Yuli, I.; Wachtel, E.; Ben Shoshan, E.; Danino, D.; Aserin,A.; Garti, N. Hexosome and hexagonal phases mediated by hydration and polymeric stabilizer. Langmuir, 2007, 23, 3637-3645.
17. Libster, D.; Ben Ishai, P.; Aserin, A.; Shoham, G.; Garti, N. From the microscopic to the mesoscopic properties of lyotropic reverse hexagonal liquid crystals. Langmuir, 2008, 24, 2118-2127.
18. Lopes, L. B.; Ferreira D. A.; Paula D.; Garcia J.; Thomazini A.; Fantini C. A.; Bentley B. Reverse hexagonal phase Nano dispersion of monoolein and oleic acid for topical delivery of peptides: in Vitro and in Vivo skin penetration of cyclosporin A. Pharm. Res., 2006, 23(6), 1332-1342.
19. Swarnakar, N. K.; Jain V.; Dubey V.; Mishra D.; Jain N.K. Enhanced oromucosal delivery of progesterone via hexosomes. Pharm. Res., 2007, 24(12), 2223-2230.
20. Boyd, B. J.; Whittaker D. V.; Khoo S. M.; Davey G. Hexosomes formed from glycerate

surfactants-formulation as a colloidal carrier for irinotecan. Int. J. Pharm., 2006, 318,154-162.
21. Amar-Yuli, I.; Wachtel, E.; Shalev, D.E.; Aserin, A.; Garti, N. Solubilization of food bioactives within lyotropic liquid crystalline mesophases. Curr. Opin. Colloid Interface Sci., 2009, 14, 21-32.
22. Israelachvili, J. N.; Mitchell D. J.; Ninhan B. W. Theory of self assembly of hydrocarbon amphiphiles into micelles and bilayers. J. Chem. Soc. Faraday Trans., 2, 1976, 72, 1525-68.
23. Mitchell, D. J.; Ninham, B. W. Micelles, vesicles and microemulsions. J. Chem. Soc. Faraday Trans., 2, 1981, 77, 601- 29.
24. Chang, C. M.; Bodmeier, R. Effect of dissolution media and additives on the drug release from cubic phase delivery systems. J. Control. Release, 1997, 46, 215-222.
25. Boyd, B.J., Davey, G., Drummond, C.J., et al. Surfactants and Lyotropic Phases Formed There from, International Patent WO 2004/022530.
26. Clogston, J., Rathman, J., Tomasko, D., et al. Phase behaviour of a monoacylglycerol (Myverol 18–99 K)/water system. Chem. Phys. Lipids 2000; 107, 191–220.
27. Rosevear, F.B. Liquid crystals: the mesomorphic phases of surfactant compositions. J. Soc. Cosmetic Chem. 1968; 19, 581–594.
28. Rosevear, F.B. The microscopy of the liquid crystalline neat and middle phases of soaps and synthetic detergents. J. Am. Oil Chem. Soc. 1954: 31, 628–639.
29. Rosevear, F.B. Liquid crystals: the mesomorphic phases of surfactant compositions. J. Soc. Cosmetic Chem. 1968; 19, 581–594.
30. Boyd, B.J., Whittaker, D.V., Khoo, S.-M., Davey, G. Lyotropic liquid crystalline phases formed from glycerate surfactants as sustained release drug delivery systems. Int. J. Pharm. 2006; 309, 218–226.
31. Gustafsson, J., Ljusberg-Wahren, H., Almgren, M., Larsson, K. Submicron particles of reversed lipid phases in water stabilized by a nonionic amphiphilic polymer. Langmuir 1997; 13, 6964–6971.
32. Boyd, B.J. Characterization of the drug release from cubosomes using the pressure ultrafiltration method. Int. J. Pharm. 2003; 260, 239–247.
33. Rivory, L.P., Robert, J. Reversed-phase high-performance liquid chromatographic method for the simultaneous quantitation of the carboxylate and lactone forms of the camptothecin derivative irinotecan, CPT-11, and its metabolite SN-38 in plasma. J. Chromatogr. B: Biomed. Appl. 1994; 661, 133–141.
34. Borne, J.; Nylander, T.; Khan, A. Phase behaviour and aggregate formation for the aqueous monoolein system mixed with sodium oleate and oleic acid. Langmuir, 2001, 17, 7742-7751.
35. Chang, C. M.; Bodmeier, R. Effect of dissolution media and additives on the drug release from cubic phase delivery systems. J. Control. Release, 1997, 46, 215-222.
36. Gustafsson, J.; Ljusberg-Wahren, H.; Almgren, M.; Larsson, K. Submicron particles of reversed lipid phases in water stabilized by a Non-ionic amphiphilic polymer. Langmuir, 1997, 13, 6964-6971.
37. Salentinig, S.; Yaghmur, A.; Guillot, S.; Glatter, O. Preparation of highly concentrated nanostructured dispersions of controlled size. J. Colloid Interface Sci., 2008, 326, 211-220
38. Boyd, B.J. Characterisation of the drug release from cubosomes using the pressure ultrafiltration method. Int. J. Pharm., 2010, 260, 239-247.
39. Chung, H.C.; Kim. J.; Um, J.Y.; Kwon, I.C.; Jeong, S.Y. Selfassembled “nanocubicle” as a carrier for peroral insulin delivery. Diabetologia, 2002, 45, 448-451.
40. Libster, D.; Aserin, A.; Wachtel, E.; Shoham, G.; Garti, N. An HII liquid crystal-based delivery system for cyclosporin A: Physical characterization. J. Colloid Interface Sci., 2007, 308, 514-524.
41. Libster, D.; Ishai, P. B.; Aserin, A.; Shoham, G.; and Garti, N. Molecular interaction in reverse hexagonal mesophae in the presence of cyclosporin A. Int. J. Pharm., 2009, 367, 115-126.
42. Sagalowicz, L.; Mezzenga, R.; Leser, M.E. Investigating reversed liquid crystalline mesophases. Curr. Opin. Colloid Interface Sci., 2006, 11, 224-229.
43. Turunen, T. M.; Urtti, A.; Paronen, P.; Audus, K. L.; Rytting, J. H. Effect of some penetration enhancers of epithelial membrane lipid domains: Evidence from fluorescence spectroscopy studies. Pharm. Res., 1994, 11, 288-294.
44. Lee, J.; Kellaway, I. W. Combined effect of oleic acid and polyethylene glycol 200 on buccal permeation of [D-Ala2, D-Leu5] enkephalin from a cubic phase of glyceryl monooleate. Int. J. Pharm., 2000, 204, 137-144.
45. Fong C, Le T, Drummond CJ. Lyotropic liquid crystal engineering-ordered nanostructured small molecule amphiphile self-assembly materials by design. Chem. Soc. Rev. 41(3), 1297–1322 (2012).
46. Yaghmur A, Rappolt M. Chapter five – the micellar cubic Fd3m phase: recent advances in

the structural characterization and potential applications. In: Advances in Planar Lipid Bilayers and Liposomes Aleš I, Chandrashekhar VK (Eds). Academic Press, 111–145 (2013).
47. Muir BW, Acharya DP, Kennedy DF et al. Metal-free and MRI visible theranostic lyotropic liquid crystal nitroxidebased nanoparticles. Biomaterials 33, 2723–2733 (2012).
48. Nilsson C, Barrios-Lopez B, Kallinen A et al. SPECT/ CT imaging of radiolabeled cubosomes and hexosomes for potential theranostic applications. Biomaterials 34, 8491–8503 (2013).
49. Lakshmi NM, Yalavarthi PR, Vadlamudi HC et al. Cubosomes as targeted drug delivery systems – a biopharmaceutical approach. Curr. Drug Discov. Technol. 11, 181–188 (2014).
50. Zhai J.; Fong C.; Tran N.; Drummond C. J. Non-Lamellar Lyotropic Liquid Crystalline Lipid Nanoparticles for the Next Generation of Nanomedicine. ACS Nano 2019, 13, 6178–6206. GuoC.; Wang J.; Cao F.; Lee R. J.; Zhai G. Lyotropic Liquid Crystal Systems in Drug Delivery. Drug Discovery Today 2010, 15, 1032–1040.
51. Madheswaran T.; Kandasamy M.; Bose R. J.; Karuppagounder V. Current Potential and Challenges in the Advances of Liquid Crystalline Nanoparticles as Drug Delivery Systems. Drug Discovery Today 2019, 24, 1405–1412.
52. Godlewska M.; Majkowska-Pilip A.; Stachurska A.; Biernat J. F.; Gaweł D.; Nazaruk E. Voltammetric and Biological Studies of Folate-Targeted Non-Lamellar Lipid Mesophases. Electrochim. Acta 2019, 299, 1–11.
53. Boyd, B. J.; Whittaker D. V.; Khoo S. M.; Davey G. Hexosomesformed from glycerate surfactants-formulation as a colloidal carrierfor irinotecan. Int. J. Pharm., 2006, 318,154-162.
54. Yaghmur A, Sartori B, Rappolt M. The role of calcium in membrane condensation and spontaneous curvature variations in model lipidic systems. Phys. Chem. Chem. Phys. 2011; 13(8), 3115–3125.
55. Neto C, Aloisi G, Baglioni P et al. Imaging soft matter with the atomic force microscope: cubosomes and hexosomes. J. Phys. Chem. B 1999; 103(19), 3896–3899.
56. Turunen, T. M.; Urtti, A.; Paronen, P.; Audus, K. L.; Rytting, J. H.Effect of some penetration enhancers of epithelial membrane lipiddomains: Evidence from fluorescence spectroscopy studies. Pharm.Res., 1994, 11, 288-294.
57. Lee, J.; Kellaway, I. W. Combined effect of oleic acid and polyethyleneglycol 200 on buccal permeation of [D-Ala2, D-Leu5]enkephalin from a cubic phase of glyceryl monooleate. Int. J.Pharm., 2000, 204, 137-144.
58. Boyd, B. J.; Khoo, S.M.; Whittaker, D.V.; Davey, G.; ChristopherJ.H. Porter. A lipid-based liquid crystalline matrix that providessustained release and enhanced oral bioavailability for a modelpoorly water soluble drug in rats. Int. J. Pharm., 2007, 340, 52-60.