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Review Article **Novel drug delivery system**

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PROLIPOSOMES: A BRIEF OVERVIEW OF NOVEL DELIVERY SYSTEM

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ABSTRACT

Liposomes are the most promising and broadly applicable of all the novel delivery systems. The poor stability associated with this system limits its long term storage. To overcome this issue Proliposomes were discovered by Payne et al. in 1986. Proliposomes are dry, free-flowing granular products composed of drug and phospholipid which, upon addition of water, disperse to form a multi-lamellar liposomal suspension. This paper reviews the method of preparation and evaluation of Proliposomes and highlights its potential to be exploited for different routes of administration.

KEYWORDS: **Proliposomes, dry, free flowing, phospholipid, multi-lamellar.**

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INTRODUCTION

Since the discovery of liposomes in 1965 by Bangham et.al, they continue to be the most promising, broadly applicable, and highly researched of all the novel delivery systems[1]. Structurally they are composed of phospholipids which are biodegradable, non toxic and devoid of any antigenic, pyrogenic or allergic reactions, and with careful selection, allows encapsulation of matter that is as small as the lithium ion up to macromolecules as large as genetic material of several hundred thousand Daltons[2,3]. These properties of liposomes have been extensively investigated for drug delivery, drugs targeting, controlled release and increased solubility[4]. However, liposomes are relatively unstable colloidal system manifested by physical and chemical instability[4]. Physical instability is evidenced by vesicle aggregation and fusion, which is associated with changes in vesicle size and loss of entrapped material[5]. Chemical stability is of more importance as it is associated with phospholipids which form the backbone of the bilayer. It is of two types namely- hydrolysis of the ester bonds linking the fatty acids to the glycerol backbone and peroxidation of unsaturated acyl chains (if present) which accelerates liposome breakdown and alters drug-release characteristics[4,5]. These factors influence the in vivo performance and storage behaviour of liposomes[6].

For liposomes to enter the market, they must be stable during the storage period, and remain intact before reaching their targeted tissues to produce action. Various approaches have been used to overcome these problems, some of which include, control of particle size and lamellarity, altering the lipid composition, lyophilisation, electrosteric stabilization etc[4]. One such approach which helped overcome the stability issue associated with liposome and led to the development of a new drug delivery system is the Proliposome (PL). Discovered by Payne[7] et.al in 1986, Proliposomes (PLs) are dry, free-flowing granular products composed of drug(s) and phospholipid(s) which, upon addition of water, disperse to form a multi-lamellar liposomal suspension. It is one of the most cost-effective and widely used methods for producing commercial liposome products. It is based upon the intrinsic property of hydrated membrane lipids to form vesicles on contact with water. Being available in dry powder form, they are easy to distribute, transfer, measure and store making it a versatile system.[8] Liposomes can either be formed in vivo under the influence of physiological fluids or can be formed in vitro prior to administration using a suitable hydrating fluid. The liposomes formed on reconstitution are similar to conventional liposomes and more uniform in size[8]. The present review gives a brief overview of preparation, evaluation and application of PL as novel carrier system.

METHOD OF PREPARATION

Some of the commonly used methods employed in the preparation of PLs are discussed below. They include-

i. Film deposition on carrier method

This is the original method used by Payne et.al in the preparation of PLs. It involves deposition of film of drugs and phospholipids onto a porous, water soluble carrier material. As seen in Fig1, solution of drug and phospholipid/s in a volatile organic solvent is introduced dropwise via feed tube onto a bed of carrier material held in a flask of a rotary flash evaporator under vacuum. At any given time, over-wetting of the matrix is avoided and subsequent aliquot of organic solution is introduced only when a free flowing powder matrix is obtained[9]. The carriers chosen should have high surface area and porosity so that the amount of carrier required can be easily adjusted to support the lipids. It also enables high surfactant to carrier mass ratio in the preparation of PLs. Further, being water soluble they allow rapid formation of liposomal dispersion on hydration and by controlling the size of porous powder, relatively narrow range of reconstituted

liposomes can be obtained. Some of the carriers utilised include- maltodextrin, sorbitol, microcrystalline cellulose, magnesium aluminium silicates, Mannitol, etc[8].

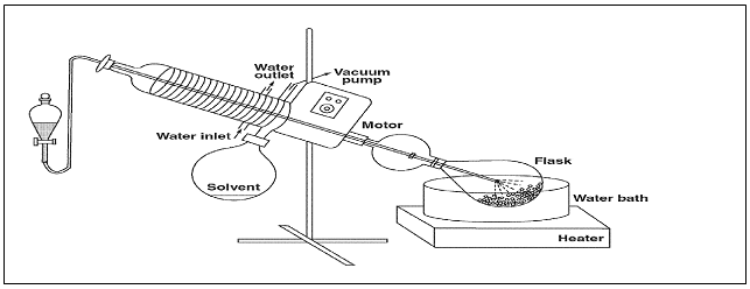

Figure.1
Apparatus for preparing PLs by Film Deposition on carrier method [9]

The manufacturing procedure however appears to be tedious and difficult to control, since the operation requires a discontinuous step of solvent addition and evaporation which is time consuming[11]. In order to solve this problem, Xu[12] et.al modified the method wherein the carrier material was dispersed in organic solution of drug and phospholipid/s in flask of rotary evaporator, and subjected to vacuum evaporation. The suspension made the lipid distribution more uniform and efficient and the process is continuous and time saving compared to the original method[12].

ii. Spray drying method

The unique feature of spray drying process lies in its ability to involve both particle formation and drying in a continuous single step, allowing better control of particle formation. Spray drying is not only limited to aqueous solutions, but can also be used for non-aqueous systems to prepare particles. This method is mainly used when particles of uniform size and shape are required and can be easily scaled up it is cost effective and suitable for large scale production of PLs[13,14].

As seen in Fig.2, the spray drying process involves four stages: atomization of the product into a spray nozzle, spray-air contact, drying of the spray droplets and collection of the solid product[15]. Initially liquid dispersions containing pure lipid or lipids and carrier in organic solvent are prepared and pumped into the drying chamber. The dispersions are atomized into the drying chamber using a spray nozzle and are dried in a concurrent air flow which is then collected in a reservoir[15].

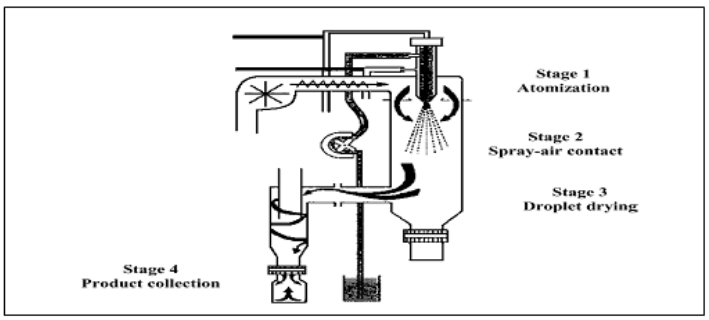

Figure.2
Apparatus for preparing PLs by Spray drying method [15]

Major concerns to spray drying are high working temperatures, shearing stresses and absorption phenomenon that may lead to thermal and mechanical degradation of the

active molecules. This can be improved by optimising the operating parameters such as drying air temperature and liquid spraying rate. Stabilising adjuvants such as disaccharides, cyclic oligosaccharides and polyols can also be used to protect the integrity of the active molecules and enhance the efficiency of hydration by increasing the surface area of lipids[13,14].

iii. Fluidised bed method

This method can be employed for the large scale production of PLs and works on the principle of particle coating technology. The carrier material used here can vary from crystalline powder to non pareil beads. When using beads as carrier material, initial seal coating is applied to the beads to provide a smooth surface for further coating of phospholipids. This ensures formation of thin uniform coating of phospholipid around the core and formation of smaller sized liposomes upon hydration. Solution of drug and phospholipid in organic solvent is sprayed onto the carrier material through a nozzle. At the same time, the organic solvent is removed by applying vacuum to the fluid bed. To remove the trace amount of residual solvent the finished lipid-coated powder/beads can be dried under vacuum overnight. The method offers following advantages: a] It utilizes Film coating technology which is well established and processable. b] Various cores and coating materials are available or easy to prepare. c] It is a cost-effective method to prepare liposomes for drug delivery[16,17].

iv. Super critical anti-solvent method:

Supercritical anti solvent method utilises Supercritical Carbon dioxide ($SCCO_2$) in the preparation of PLs.$SCCO_2$ is a fluid state of carbon dioxide where it is held at or above its critical temperature and pressure. Anti-solvent technology is widely used in food industry and was developed to prepare PLs because of its lower residual solvents, simpler steps and mild operation temperatures. As shown in Fig.3, the apparatus used in the preparation of PLs include three parts: a sample delivery unit, a precipitation unit and a separation unit. The sample delivery unit consists of two pumps: one for CO_2 and the other for solution. CO_2 is supplied from the CO_2 cylinder (1) which is cooled down by a refrigerator (2) and introduced via a high-pressure pump (3) to the buffer tank (4), in which it is preheated. The drug solution is introduced via HPLC pump (11).The solvent used for dissolving the drug should be completely miscible with CO_2. Opening the valves A and B allows the entry of solution and CO_2 into the vessel through the nozzle (B). As seen in Fig 3B, solution is sprayed through the inner tubule whereas CO_2 is sprayed through the outer tubule of the nozzle. The precipitation unit consists of a vessel (9) heated by an air bath. The separation unit consists of a separator (13) and a wet gas meter (14). The organic solvent is separated from $SCCO_2$ in the separator because of lower pressure whereas volumetric flow rate of CO_2 is measured by the wet gas meter.

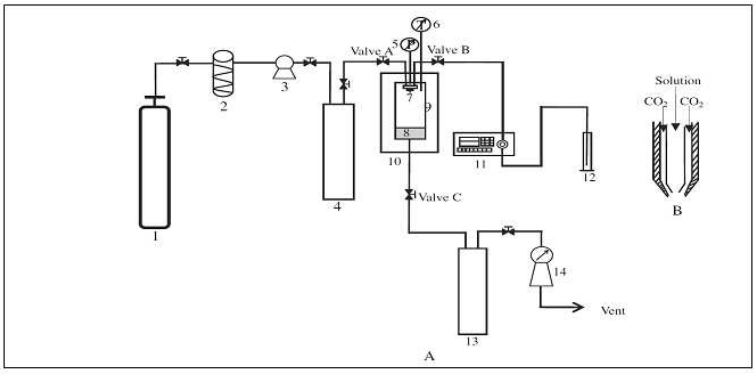

Figure.3
Apparatus for preparing PLs by Supercritical Anti Solvent Method[18]

After the temperature and pressure of the separating vessel reaches the preset value, valve A is opened to allow entry of CO_2 followed by opening of valve B allowing the entry of drug solution. $SCCO_2$ and solution are mixed and diffused into one another rapidly as they are sprayed through the coaxial nozzle. This causes the solutes dissolved in organic solvent to reach supersaturation in a very short period of time because the solubility of solutes in the organic solvent decreases greatly. As a result, the PLs are precipitated in the vessel. Once the solution is completely utilised, valves A and B are closed while valve C is opened in order to depressurize the vessel at the operating temperature. The samples are collected on the filter (8) at the bottom of the vessel. The pressure, temperature and flow rate of the drug solution need to be optimized to obtain high drug loading in PL[18,19].

EVALUATION

Following are the parameters used in the evaluation of PL –

i. Scanning Electron Microscopy (SEM)

SEM is mainly used to view the surface morphology of the PL powder. This involves comparing the image of the pure carrier material with that of the PL. The illegibility of the image of the carrier material in the formulation confirms the deposition of phospholipid on the carrier and thus confirming the formation of PL[20].

ii. Transmission Electron Microscopy (TEM)

TEM is mainly used to study the morphology of the liposomes formed after hydration of the PL powder. The process involves hydrating PLs with purified water and observing the shape and lamellarity of the liposome vesicles formed under the microscope[8,9,20].

iii. Hydration Study

Hydration study is done to evaluate the ability of PLs to form liposomes on hydration. It is done by placing a small amount of PL powder on glass slide or on cavity slide and slowly adding water drop wise while observing it under the microscope to view the formation of vesicles. It should rapidly (less than 30 seconds) form liposomes on hydration, ensuring rapid conversion into the same when it comes in contact with hydration fluids[8,9,20].

During the hydration process dissolution/disintegration occurs. It involves hydration of lipid surface to form liposomes which tend to bud off from the central core of PL. The process continues till hydration of lipid and dissolution of carrier is complete[21].

iv. Flow Property

Flow property of a powder formulation dictates its content uniformity and is vital in handling and processing operations. Further, dose handling and ease of filling into container depends on the same. Being a solid powder based formulation it is important to evaluate the flow property of PL. It also ensures that despite the deposition of phospholipids on carriers, the flowability of particles is not affected.

Flow property is assessed by measuring the parameters such as Angle of Repose, Carr's Compressibility Index and Hausner's Ratio[8,20].

v. Number of vesicles per cubic mm

Distinctive advantage of PL formulation is speculated only when abundant number of vesicles are derived from hydration of PLs which form an important prerequisite for optimizing the composition of the same. This is done by counting the liposomes formed after hydration under optical microscope using haemocytometer[8].

Total no. of liposomes per mm^3 is given by-

$$\frac{\text{Total number of liposomes counted X Dilution factor X 4000}}{\text{Total number of Squares counted}}$$

vi. Measurement of Vesicle Size

One of the important parameter for vesicular system is vesicle size and size distribution. This can be done by hydrating the PL powder followed by manual agitation and determining particle size using suitable Particle size analyser[1,8,20].

vii. Entrapment Efficiency

Entrapment efficiency is determined by hydrating the PLs to form liposome dispersion

followed by separation of unentrapped drug and determining the amount of drug entrapped. Unentrapped drug can be separated by ultracentrifugation[10, 18, 19], and ultrafiltration [8,12] . Percent drug entrapment is given by-

$$\frac{\text{Entrapped Drug X 100}}{\text{Total Amount of Drug added}}$$

APPLICATIONS

PLs can be exploited for the following routes of administration-

i. Parenteral Delivery

For liposomes to be developed for parenteral application, their sterilization is mandatory. Routinely employed sterilization techniques in Pharmaceutical industry include- Steam sterilization, γ-irradiation, Aseptic manufacturing and filtration sterilization. Terminal sterilization using steam at 121ºC may not be suitable for liposomal formulations, since high temperature may disrupt the liposome architecture due to hydrolysis of lipids, leading to physical destabilization. γ-irradiation is also unsuitable for liposomal dispersions, since radiation causes hydrolysis and accelerates the peroxidation of unsaturated lipids. Aseptic manufacturing is not commonly used due to the expense and difficulty in validation. Filtration sterilization of the final product can be challenging due to the structural complexity of these vesicles and loss of lipids by their non-specific adsorption to filters[22, 23].

PLs are well suited for parenteral application of liposomes. The main advantage associated with PLs is that it allows sterilization without affecting the intrinsic characteristics[9]. Besides, they can be stored as sterilized in dry state and can be hydrated prior to administration to form multilamellar liposomal suspension[10]. In addition, several recent studies have reported that γ- irradiation sterilisation is not as detrimental to liposomes as previously assumed, particularly when irradiated in the dry state. Since hydroxyl radicals (resulting from exposure of water to radiation), are a major source of the free radicals which cause the damage. Thus water content plays a key role in the stability of liposomes during this process. Being available in dry form, γ- irradiation may be used as a sterilization technique for PLs[24].

ii. Oral Delivery

Oral drug delivery continues to be the preferred route of administration, but liposomes have limited success in deliver ing drugs through oral route[8,25]. This is due to the absence of a stable dosage form for oral delivery and erratic and unpredictable absorption profiles shown by liposomes. This is due to their inability to retain their integrity at the site of absorption. Being available as free flowing powder, PL represents the first example of delivering liposomes into solid dosage form such as tablets or capsules[9]. Further, liposomes are formed on contact with biological fluids at the site of absorption ensuring the retention of liposome integrity. PLs act as one of the most promising vehicles for enhancing the dissolution efficiency of poorly soluble drugs. It forms multi-lamellar vesicles on hydration which ensures higher incorporation of insoluble drugs due to increased hydrophobic volume within the liposomal lamellae. It also allows conversion of drug from crystalline to amorphous form[1.] The larger particle size of multi lamellar liposomes formed on hydration ensures lymphatic uptake and improves the bioavailability of drugs undergoing high first pass metabolism[26]. Further, the phospholipid molecules which form the backbone of the bilayer structure help to enhance the solubility of drug molecule.

Zaleplon is a hypnotic drug indicated in insomnia and is a potential anticonvulsant. Due to its limited aqueous solubility and extensive first pass metabolism it shows poor bioavailability of 30%. Janga[8] et al. utilised PLs for oral delivery of Zaleplon and found 2-5 fold improvement in oral bioavailability in rats compared to pure drug. Vinpocetine is used in the prevention and treatment of ischemic stroke and other cerebrovascular diseases. Due to its poor aqueous solubility and high first pass metabolism, it has low oral bioavailability of 7% in humans. PLs utilised by Xu[12] et.al improved the oral bioavailability of vinpocetine by 3.5 fold in rabbits compared to the pure drug.

Silymarin is widely used to maintain liver health and treat hepatic disorders but is slightly soluble in water and in oil and shows poor permeation across the intestinal epithelial cells. PL utilised by Xiao[20] et.al for Silymarin showed 3.4 fold increase in oral bioavailability in beagle dogs compared to the pure drug.

PLs have also been successfully used for other poorly soluble drugs such as, Exemestane[1], Salamon Calcitonin [9],Glyburide [26], Halofantrine[27] and Progesterone [29]. PLs also have the ability to enhance the lipophilicity of highly hydrophilic drugs. This is due to the similarity between the liposomal bilayers and biomembranes. In addition, their relatively small size and bioadhesive nature help facilitate the absorption of poorly absorbed and poorly permeable drugs through endocytosis[8,29]. Cromolyn sodium is an anti-inflammatory drug used in prophylactic treatment of bronchial asthma and allergic rhinitis. It is poorly absorbed from the gastrointestinal tract (bioavailability < 1%). Increasing the lipophilic character of Cromolyn could facilitate passive transport and, thereby, improves its absorption across the barrier membranes. PL utilised by Deshmukh et.al showed 4-7 fold increase in transepithelial flux compared to pure drug indicating enhanced lipophilicity of Cromolyn by liposome encapsulation[25]. A patent titled "Enteric-coated proliposomal formulations for poorly water soluble drugs" is present relating to the oral delivery of PL. The advantage of this invention is that it provides a simple and inexpensive system to facilitate the oral administration of poorly water soluble drugs and enhancing their stability and bioavailability. Examples of drugs utilizing the invention are Halofantrine, Testosterone and Famotidine[30].

iii. Pulmonary Delivery

Major advantage of liposomes as pulmonary drug delivery system is that they are prepared from phospholipids which are endogenous to lungs as component of lung surfactant. Drug encapsulation in liposomes provides modulated absorption, resulting in localized drug action in the respiratory tract and prolonged drug presence in circulation and reduced systemic adverse effects[5,31]. Drug delivery to the pulmonary route is achieved by three types of devices namely-

a. Pressurised metered dose Inhalers (pMDI)

As the name suggests it consists of solution or suspension of drugs in liquefied propellants. Use of Hydrofluroalkanes as non-ozone depleting propellants over CFCs has the limitation for liposome delivery as they are poor solvents for phospholipids. PLs help overcome this limitation as they can be suspended in these propellants and serve as carrier for pulmonary delivery of liposomes through pMDI[5].

b. Dry Powder Inhalers (DPIs)

These disperse the drug into the patient's airstream as a fine powder during inhalation. Delivering liposomes through DPI have many advantages such as controlled delivery, increased potency, reduced toxicity, uniform deposition of drugs locally, patient compliance, stability and high dose carrying capacity. Being available as dry powder form, PLs are the best alternative for delivering liposomes through DPIs[5, 32]. Chougule[32] et.al developed spray dried liposome encapsulated Dapsone DPI for prolonged drug retention in lungs to prevent Pneumocystis carinii pneumonia. Prolonged drug release of up to 16 h was observed in vitro.

c. Nebulizers

Nebulization offers the simplest means, for delivering liposomes to the human respiratory tract but it is concerned with liposome leakage and drug stability. Use of dry powder formulations has been suggested to overcome these issues. Lyophilisation and jet milling may be used to obtain dry powder but tend to have deleterious effect on liposomes due to the stresses involved in these processes. Thus, PLs serve as a stable alternative for delivering liposomes through nebulization. Besides, the ready formation of an isotonic liposome formulation in situ from PLs seems to offer advantages over other formulation approaches[5,31].

iv. Transdermal delivery

Phospholipids, being the major component of liposomal system, can easily get integrated with the skin lipids and maintain the desired hydration conditions to improve drug permeation. When PLs are applied to mucosal membrane, they are expected to form liposomes on contact with mucosal fluids whereby the resulting liposomes act as sustained release dosage form for loaded drugs. Liposomes formed on hydration have the ability to modulate diffusion across the skin. They do so by fusing with the skin surface and establishing concentration gradient of the intercalated drug across the skin. Thus they enhance skin permeation. Also, the vesicle intercalation into the intracellular lipid layers of the skin results in fluidization and disorganization of the regular skin structure, obviating the barrier function of the stratum corneum [10,33].

Exemestane, a novel steroidal aromatase inactivator has limited bioavailability of 42% due to poor solubility and extensive first-pass metabolism. Jukanti[33] et.al utilised PL system for transdermal delivery of Exemestane and found a 2.4 fold increase in bioavailability from PL gel compared to oral suspension. PLs have also been developed for sustained delivery of Nicotine[10] and Aceclofenac[34] transdermally.

v. Mucosal delivery

PLs form vesicular structures (liposomes) in vivo, triggered by the aqueous environment found on the mucosal surfaces. Phospholipids present in them have natural affinity for biological membranes. Besides they are generally nontoxic and non-irritant[2]. The presence of drug as molecular dispersion in the bilayers offers improved drug activity. Further, the difficulties associated with liposomal preparations such as stability and loading are circumvented because the PLs convert to vesicular structures in vivo, i.e., on the mucosa. Liposomes formed on hydration with the mucosal fluid, get deposited on the mucosa as drug reservoirs thereby increasing the drug retention capacity. The significantly higher mucosal retention of the liposomes, results in higher partitioning of the drug into the mucosa. This is responsible for prolonged and enhanced drug activity. This led to the utilization of PLs for vaginal and nasal drug delivery[35].

Vaginal delivery systems are frequently required to treat local fungal infections. The poor aqueous solubility of antifungal and steroid compounds in conventional formulations limits their presence as molecular dispersion and consequently affects the drug concentration at active sites. The association of these lipophilic agents with the phospholipid molecules of PLs make them excellent carriers to molecularly disperse the drug[35]. Clotrimazole is widely and effectively used for the treatment of vulvovaginal candidiasis but has low aqueous solubility. Commercially available conventional Clotrimazole vaginal delivery systems, such as creams, foams, and gels, are considered to reside the drug for a relatively short period of time at the targeted site. Ning[36] et.al developed a PL formulation of Clotrimazole and compared the fungicidal efficacy with the standard ointment in rats. The results indicated that Clotrimazole containing vaginal PL prolonged drug release and increased the drug retention into the mucosa. This resulted in higher antifungal efficacy compared to the standard ointment and in addition it did not affect the morphology of vaginal tissues confirming the non toxic and non irritant nature of the carrier.

Nasal mucoadhesive delivery has been used to improve local and systemic delivery of therapeutic compounds [37]. It is a promising alternative for systemic administration of drugs that are poorly absorbed via the oral route [38]. Limitations associated with this route are mucociliary clearance which limits the residence time of drug in the nasal cavity and lack of sustained release of drugs with short half life[38, 39]. Proliposomal delivery helps to overcome these limitations. Liposomes formed on hydration decrease the mucociliary clearance of drugs due to their surface viscosity and provide intimate and prolonged contact between the drug and mucus membrane. Hydration process of PL plays a role in sustaining the plasma concentration of drugs with short half life in systemic circulation[40,41].

Propranolol is a β-blocker which shows rapid absorption when administered intranasally as an aqueous solution. Due to this, it is eliminated very rapidly from the systemic circulation needing frequent dosing. Ahn[41] et.al utilised PL for nasal delivery of Propranolol. Sustained plasma concentration of Propranolol was obtained due to the slow hydration process of PL in nasal cavity. It was given by the Mean hydration time (MHT) of PLs which was defined as the difference of Mean Residence time between liposomes and PLs. It was found to be 80.4 minutes which confirmed longer residence time of PL in nasal cavity.

CONCLUSION

PLs have provided a major breakthrough in solving the stability issues associated with liposomes. It has also opened newer areas of liposome application especially in the area of oral delivery. Utilising methods such as Spray drying and Fluidised bed drying PLs can be produced on a large scale. They also have the ability to be delivered as conventional formulations. Thus, PLs have a high commercial value to enter the market as drug delivery vehicle.

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