



Proliposomes: A novel approach to carrier drug delivery system

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ABSTRACT

Liposomes are employed broadly on all the novel drug delivery in current years. Liposomal suspensions were developed and they resulted in limited shelf life and poor stability problems on long term storage, these problems are overcome by Proliposomes. Proliposomes were discovered by Payne et al in 1986. Proliposomes are free flowing granular product composed of drug and phospholipid precursors which on hydration lead to liposomes. This paper reviews different aspects related to liposomes, proliposomes their method of preparation, comparison between liposomes and proliposomes, characterization of proliposomes, applications and major focus is made on Proliposomes employed for different routes of administration.

Keywords: Proliposomes, Carriers, Phospholipids, Unilamellar

INTRODUCTION

The ideal drug delivery system delivers drug at a required by the body during the treatment and the system delivers active entity to the site of action. Currently no available drug delivery systems can achieve all these goals. The targeted drug delivery system leads to the site specific delivery but it fails to control the release kinetics of drug in predictable manner. Many experiments have been carried out to design clinically effective drug delivery system. The carriers used to carry the drug to the site of action include immunoglobulins, serum proteins, synthetic polymers, lipid vesicles (liposomes), microspheres, erythrocytes, reverse micelles, niosomes, pharmacosomes etc [1]. Liposome is a greek word "Lipos" means fat & "soma" means body. British haematologist Dr. Alec D Bangham described them at Babraham institute, in Cambridge [2]. Liposome is a micro vesicle in which an aqueous volume is entrapped within the lipoidal membrane. Drug molecules can be entrapped within the lipid bilayer or in the aqueous space. In current years liposomes are vastly used as the drug carrier to improve stability, therapeutic activity, and reduce side effects [3].

Since the shelf life of liposomal suspensions can be limited, it would be useful to have a method of producing liposomes quickly, at the point of use and without excessive manipulation. These needs are met by the "proliposome" method [4]. Proliposomes (PLs) are covered with liposomal membrane when they are hydrated they produce liposomes. The concept of proliposomes was first introduced by Payne et al., in 1986; they described proliposomes as dry free flowing granular product that forms liposomal dispersion on hydration or on contact with biological fluids in the body the stability problems of liposomes can be overcome by PLs without affecting their intrinsic characteristics. PLs are composed of phospholipid and water soluble porous powder [5].

Proliposomes were developed in large scale for production of liposomal dispersions. The procedure involves intrinsic property of hydrated membrane lipids to form vesicles when they are in contact with water. PLs have been vastly used as the carriers for the site specific drug delivery approaches [6]. Proliposomal formulations also overcome the solubility problems of many drugs and improve the bioavailability of drugs [7].

Advantages of Proliposomes

- Proliposomes include enhanced bioavailability. Protection of drugs from degradation in the GIT.
- Targeting of anti-cancer drugs to tumor sites.
- Proliposomes can be used for controlling release within the vasculature by altering the phospholipid composition of bi-layers.
- Reduced toxicity and for taste masking.
- The proliposomes used for targeted drug delivery and controlled drug release.[8]

2. Comparison between Liposomes and Proliposomes:

Liposomes are Unilamellar or multilamellar spheroid structures composed of lipid molecules, often phospholipids. On oxidation solubility of liposomes is increased and have tendency to aggregate or fuse to hydrolysis. PLs are used as alternatives for liposomes which are composed of water soluble porous powder as carrier phospholipids and drugs dissolved in organic solvent. Drug and phospholipid material is coated on carrier material to form free flowing granular material which shows better stability, greater solubility and shows controlled release [9].

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3. Components Used For the Preparation of Proliposomes

Water soluble carriers: The carrier chosen should be highly porous and of greater surface area. As they being water soluble they on hydration forms liposomal dispersion and on controlling the size of porous powder narrow range of reconstituted liposomes can be obtained. Some of the carriers utilized include- Maltodextrin, Sorbitol, Microcrystalline Cellulose, Magnesium Aluminium Silicates, Mannitol [10].

3.1 Phospholipids

They are the major structural component of biological membranes, where two type of phospholipids exist- Phosphodiglycerides and Sphingolipids. The most common phospholipid is phosphatidylcholine (PC) molecule. Molecule of phosphatidylcholine are not soluble in water and in aqueous media they align themselves closely in planar bilayer sheets in order to minimize the unfavourable action between the bulk aqueous phase and long hydrocarbon fatty chain. The Glycerol containing phospholipids are most common used component of liposome formulation and represent greater than 50% of weight of lipid in biological membranes. These are derived from Phosphatidic acid. Examples of phospholipids are:

- Phosphatidyl choline (Lecithin) – PC
- Phosphatidyl ethanolamine (cephalin) – PE
- Phosphatidyl serine (PS)
- Phosphatidyl inositol (PI)
- Phosphatidyl Glycerol (PG).

3.2 Steroids

Cholesterol and its derivatives are quite often included as components of liposomal membrane. Its inclusion in liposomal membranes has three recognized effects. Increasing the fluidity or micro viscosity, reducing the permeability of the membrane to water soluble molecules and stabilizing the membrane in the presence of biological fluids such as plasma.

3.3 Solvents

They are used for providing the softness for vesicle membrane. The volatile organic solvents or solvent mixtures such as ether, chloroform, methanol, ethanol [11].

4. Method of Preparation

4.1. Film deposition

This is the original method used by Payne et.al in the preparation of PLs. The method includes settling of film of drug and phospholipid on to a carrier material which is porous and water soluble. Organic solvent which includes drug and phospholipid combination which is introduced drop wise via feed tube onto a bed of carrier material held in a flask of a rotary flash evaporator under vacuum. Over wetting of a matrix is avoided. It also includes high surfactant to carrier mass ratio in the preparation of PLs which on hydration lead to liposomal dispersion. Some of the carriers utilized include- maltodextrin, sorbitol, microcrystalline cellulose, magnesium aluminium silicates, Mannitol etc [12]. The manufacturing procedure is found to be tedious as the operation requires discontinuous step

of solvent addition and evaporation which is time consuming. Hence the method was modified where the carrier material was dispersed in organic solution of drug and phospholipids 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 [8].

4.2. Spray drying

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. Spray drying process involves particle formation and drying in a continuous step. Working of a spray dryer involves following stages: Atomization of the liquid, drying of the liquid particles, recovery of dried product [13]. Spray drying method is carried out for aqueous and non aqueous systems. It leads to particles of uniform size and shape and it is suitable for large scale production of PLs [14, 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 [13]. Major areas of focus in 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 [14, 15].

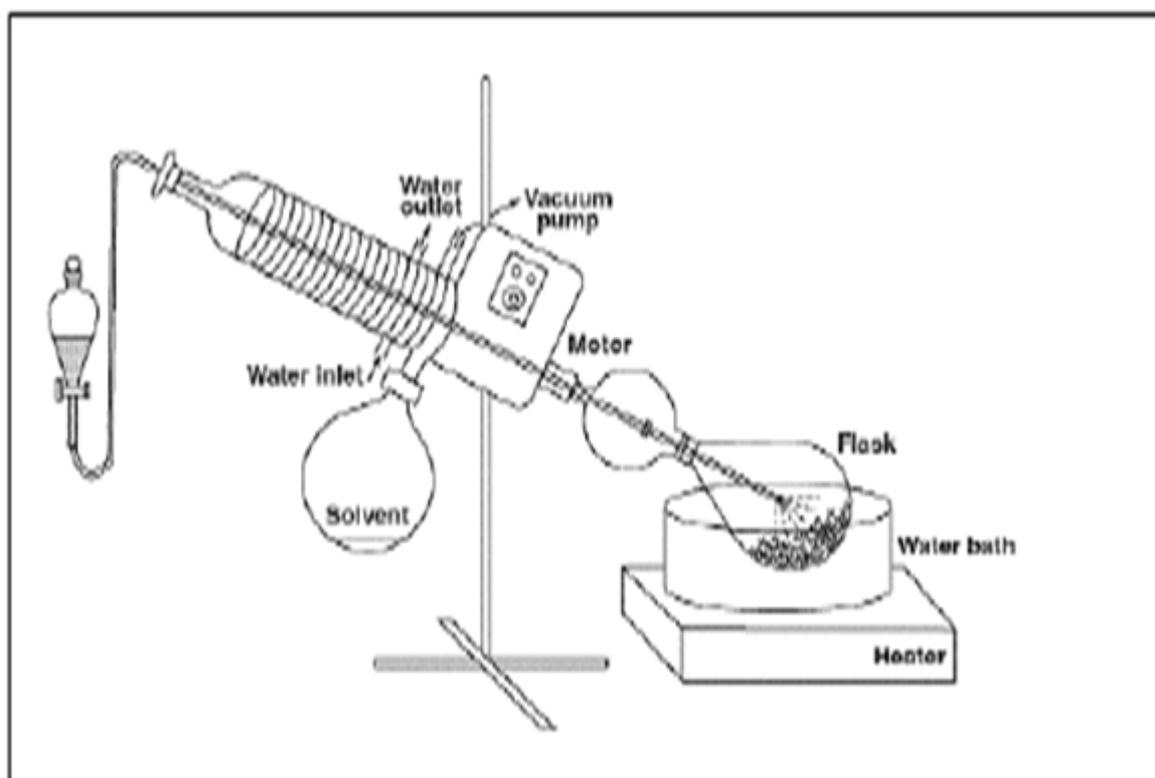


Figure 1: Film deposition apparatus

4.3. 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, simple 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). 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 SCCO₂ in the separator because of lower pressure whereas volumetric flow rate of CO₂ is measured by the wet gas meter.

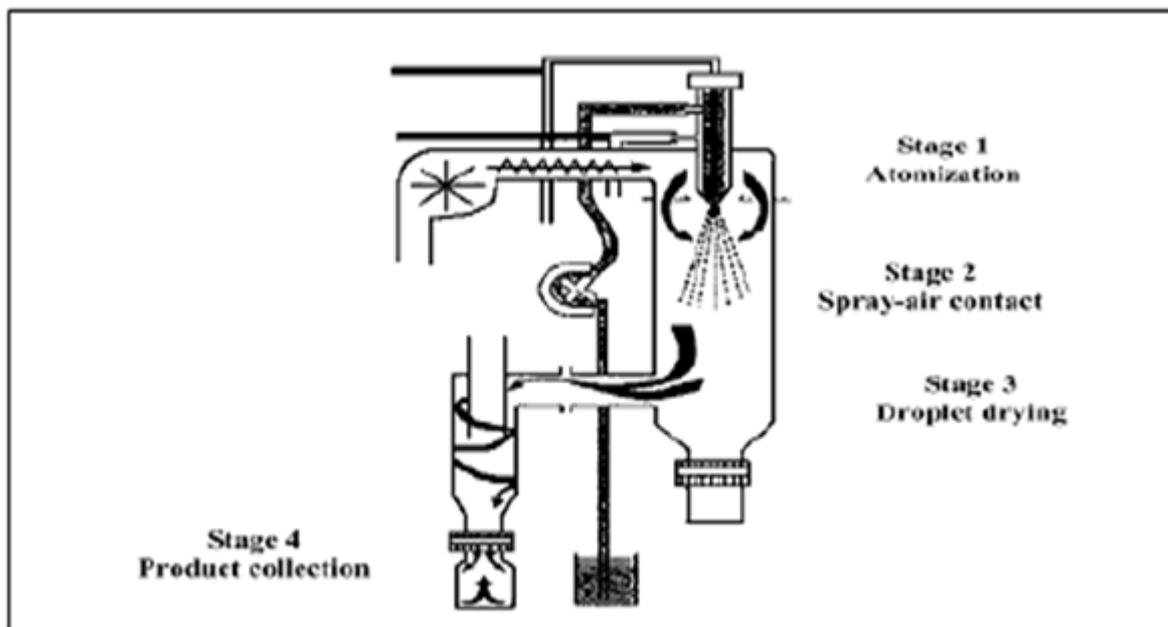


Figure 2: Spray drying apparatus

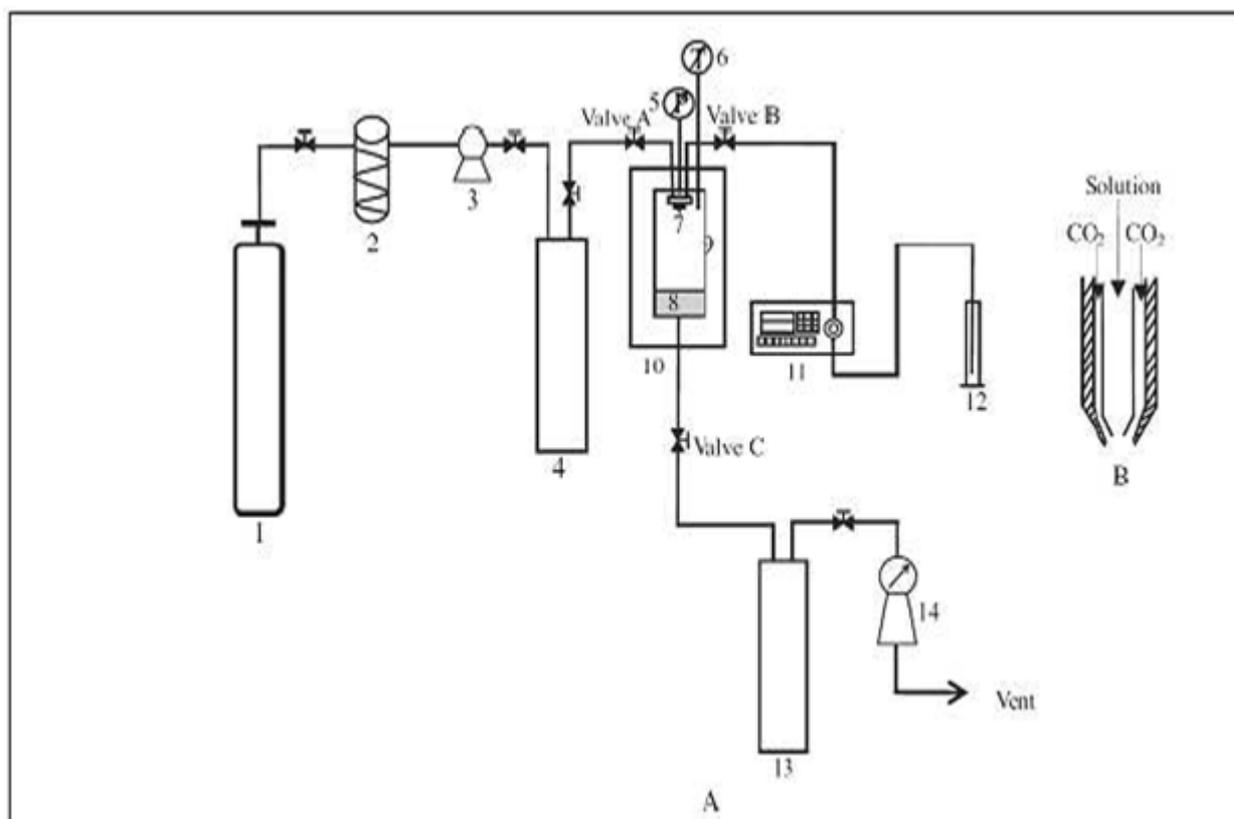


Figure 3: Super critical anti-solvent apparatus

After the temperature and pressure of the separating vessel reaches the preset value, valve A is opened to allow entry of CO₂ followed by opening of valve B allowing the entry of drug solution. SCCO₂ 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 PLs [16, 17].

4.4. Fluidised bed method

The principle here involves particle coating technology. The carrier material used here involves crystalline powder to non pore beads. When beads act as carrier material, initial seal coating is applied to the beads to provide a smooth surface for further coating of phospholipids. This leads to provide a smooth surface for further coating of phospholipids. It leads to thin uniform coating of phospholipid around the core and forms smaller sized liposomes on hydration.

Procedure: Phospholipid in organic solvent and drug solution is sprayed on carrier material via nozzle. Simultaneously the organic solvent is removed by applying vacuum to fluid bed.

The finished lipid coated powder/beads can be dried under vacuum overnight to remove the trace amount of residual solvent [18, 19].

5. Parameters used in the evaluation of PLs

5.1 Hydration Study

The ability of PLs to form liposomes on hydration is carried out by hydration study. The procedure involves placing a small amount of PL powder on glass slide and slowly adding water, drop wise and it is observed under microscope to view formation of vesicles. Liposomes are formed rapidly on hydration during this process dissolution and disintegration occurs. This involves hydration of lipid surface to form liposomes which are formed by the bud off central core of PL. The process is undergone till the hydration of the lipid and dissolution of carrier is complete [20].

5.2 Flow Property

Flow property of a powder formulation. It mainly explains content uniformity and handling processing operations and also ease filling. Since it is a solid powder based formulation it is important to analyse the flow property of PLs. Flow property is assessed by measuring the parameters such as Angle of repose, Carr's compressibility index and Hausner's ratio.[10,21]

5.3 Zeta potential

This determines the surface charge of particles. It is the difference in potential between surface of tightly bound layer (shear plane) and electro neutral region of the solution [22].

5.4 Transmission Electron Microscopy (TEM)

TEM plays role in the morphological study of liposomes. Hydration of proliposomes with purified water and the shape and lamellarity of the liposomes formed is observed under microscope [10, 23, 24].

5.5 Scanning Electron Microscopy (SEM)

SEM is mainly used to view the surface morphology of the PLs powder. Here the image of the pure carrier material is compared with the PLs. The deposition of phospholipid on the carrier material is confirmed by illegibility of the image of the carrier material in the formulation of PLs [21].

6. Applications

6.1 Parenteral delivery

Liposomes for the parenteral administration should be sterilized that is mandatory. Sterilization techniques include steam sterilization, γ -irradiation, Aseptic manufacturing and filtration sterilization. Terminal sterilization using steam at 121°C may not be suitable for liposomal formulations. Since high temperature may lead to destruction of liposome structure due to lipid hydrolysis and increases the per oxidation of unsaturated lipids [25,26].

PLs are most suited for parenteral administration of liposomes. Advantage with the PLs is that it allows sterilization without affecting the intrinsic properties. PLs can be stored as sterilised in dry state and can be hydrated before administration to liposomal dispersions. Recent studies have found that γ - irradiation sterilisation is not as detrimental to liposomes as previously assumed, particularly when irradiated in the dry state. Hydroxyl radicals (resulting from exposure of water to radiation) are major source of free radicals which leads to damage. Thus water content is main factor in the stability of liposomes during this process. PLs are available in dry form γ -irradiation may be used as sterilisation technique for PLs [27]

6.2 Oral delivery

PLs are developed to improve stability of liposomes and increase solubility and bioavailability of poorly soluble drugs for ex: Domperidone is a 5HT₃ receptor antagonist used in the treatment of nausea and vomiting. Domperidone has low aqueous solubility and after oral administration it undergoes excessive hepatic first pass metabolism which leads to low oral bioavailability of domperidone which may not decrease rate of vomiting. Hence PLs of domperidone were developed to improve bioavailability by increasing intestinal permeability which would transport drug through lymphatic transport system bypassing first pass metabolism [28].

6.3 Pulmonary Delivery

The Levofloxacin (LEV)-proliposomes in a dry powder aerosol form for pulmonary delivery. Spray drying method was used to prepare LEV-PLs containing LEV, soybean phosphatidylcholine, cholesterol and porous mannitol. LEV-PLs were found less toxic for respiratory cells did not activate Antimetabolites to produce inflammatory mediators. LEV-PLs were more effective against *M bovis* than that of free LEV [29].

6.4 Dry Powder Inhalers (DPIs)

Proliposomes powder including isoniazid (INH) in a dry powder aerosol form were prepared by spray drying method which were not toxic to respiratory cells and did not activate AM to produce inflammatory response including interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and nitric oxide at a toxic level. INH-PLs were to show greater efficacy against AM infected with *M.bovis* which was significantly higher than that of free INH. INH-PLs are main candidates in treatment of tuberculosis [30].

6.5 Ocular drug delivery

It has been difficult due to poor bioavailability of drugs from conventional ocular dosage forms due to precorneal loss effects. Vesicular drug delivery system like liposome is used to improve bioavailability of drugs and also therapeutic efficacy. Active drug ingredients which are encapsulated in lipid vesicles show better solubility and they pass through cornea and liposomes offer convenience of an ophthalmic drop.

Ex: Ciprofloxacin containing liposomal hydrogels are effective in prevention of bacterial adhesion to catheters.

6.6 Mucosal delivery

Proliposomes are converted to liposomes on hydration by the aqueous environment found on the mucosal surfaces. PLs include phospholipids which are non toxic and non irritant and they have natural affinity for biological membranes. The molecular dispersion of drug in bilayer leads to improved therapeutic activity [31]. The Clotrimazole (CT)-containing vaginal proliposomes prolonged drug release and may increase amount of drug retention into the mucosa to result in more antifungal efficacy [32]. CT-proliposomes will not affect the morphology of vaginal tissues. Hence the dosage form is more effective and convenient [32].

6.7. Transdermal delivery

Phospholipids are major component of liposomes which can get in to skin lipids and improve skin permeation by maintaining hydration conditions. PLs form liposomes on contact with mucosal fluids and resulting liposomes act as sustained release dosage forms for loaded drugs. Liposomes formed on hydration can modulate diffusion across the skin. Hence the skin permeation is enhanced and 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 [33,34]. Ex: Proliposomes have been developed for sustained delivery of Aceclofenac [34], Nicotine [24] transdermally.

7. RECENTLY REPORTED PROLIPOSOMES:

- Proliposome tablet containing Nimodipine was prepared and drug release from proliposome was studied [3].
- The sustained transdermal drug delivery of Nicotine-PLs was examined and found to be feasible. They were prepared by standard method using sorbitol and lecithin. The formulations are topically applied under occlusive conditions [24].
- The mesophasic, a proliposome system for Levonorgestrel was developed and evaluated both *in vitro* and *in vivo*. It was found to be more efficient than PEG- based ointment system which was employed as the control formulation [36].
- Vitamin E Proliposomes were prepared to increase the stability of vitamin E, lyophilization and thin-film ultrasonic dispersion method was used to prepare vitamin E proliposomes [37].
- Chloramphenicol was encapsulated within proliposomal gel was prepared for the local treatment of bacterial vaginosis, which delivers the entrapped drug efficiently during an extended period of time [38].
- Proliposome-vitaminD₃ prepared by supercritical antisolvent method. PL-vitamin D plays important role in calcium absorption, prevention of cancer and metabolism for bone health [39].

CONCLUSION

Proliposomes have played a major role in solving the problems related to stability and bioavailability and solubility of poorly soluble drugs. PLs are produced on a large scale by utilising methods of spray drying and fluidised bed drying. PLs are administered orally, parenterally, topically and also they have been in greater demand in cosmetic industry. PLs are used as versatile carriers for targeted and sustained drug delivery. Based on all this criteria PLs have proved their greater position in modern drug delivery system.

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