# TPGS STABILIZED SILYMARIN PROLIPOSOME: IMPROVE PHISICO CHEMICAL PROPERTIES AND HEPATOPROTECTIVE ACTIVITY

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**In partial fulfillment of the requirements for the award of the Degree of**

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**IN**

**BRANCH-I PHARMACEUTICS**

**Submitted by**

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**EERODE – 638 052**

**TAMILNADU.**

**October 2018**

# *CERTIFICATES*

## CERTIFICATE

This is to certify that the work entitled "**TPGS STABILIZED SILYMARIN PROLIPOSOME: IMPROVE PHYSICO CHEMICAL PROPERTIES AND HEPATOPROTECTIVE ACTIVITY**" submitted to the **Tamil Nadu Dr. M.G.R Medical University Chennai**, as partial fulfillment for the award of the Degree of **Master of Pharmacy** was carried out by **Mr. SHAMS ELDEIN AHMED ALI DANGOAL (261610451)** under my direct supervision and guidance in our laboratory during the academic year **2017 - 2018**.

Place : Erode

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## EVALUATION CERTIFICATE

This is to certify that the work embodies in this thesis entitled **"TPGS STABILIZED SILYMARIN PROLIPOSOME: IMPROVE PHYSICO CHEMICAL PROPERTIES AND HEPATOPROTECTIVE ACTIVITY"** submitted to The **Tamilnadu Dr.M.G.R. Medical university**, Chennai was carried out by **Mr. SHAMS ELDEIN AHMED ALI DANGOAL (261610451)** in Nandha college of pharmacy, Erode for the fulfillment of the master degree of pharmacy under direct supervision of **Dr.S.Tamizharasi M.Pharm., Ph.D.,** Professor and Head, Department of Pharmaceutics, Nandha college of pharmacy, Erode-52 during the Academic year **2017 – 2018.**

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any university and the dissertation represent entirely and independent work on the part of candidate.

**INTERNAL EXAMINER** **EXTERNAL EXAMINER**

# *DECLARATION*

## DECLARATION

The work presented in this thesis entitled **"TPGS STABILIZED SILYMARIN PROLIPOSOME: IMPROVE PHYSICO CHEMICAL PROPERTIES AND HEPATOPROTECTIVE ACTIVITY"** was carried out by us in the Department of pharmaceutics, Nandha College of Pharmacy, Erode, under the direct supervision and guidance of **Dr.S.Tamizharasi M.Pharm., Ph.D.,** Professor and Head, Department of Pharmaceutics, Nandha College of Pharmacy, Erode -52.

This work is original and has not been submitted in part or full for any other master degree or diploma of any university.

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# *INTRODUCTION*

# INTRODUCTION

## HEPATOTOXICITY:

Chronic liver disease occurs throughout the world irrespective of age, sex, region or race. Cirrhosis is an end result of a variety of liver diseases characterized by fibrosis and architectural distortion of the liver with the formation of regenerative nodules and can have varied clinical manifestation and complications. According to WHO, about 46 % of global diseases and 59 % of the mortality is because of chronic diseases and almost 35 million people in the world die of chronic diseases.[1] Liver diseases rates are steadily increasing over the years. According to national statistic in the UK, liver diseases have been ranked as fifth most common cause of death.[2] Liver diseases are recognized as the second leading cause of mortality amongst all digestive diseases in US.[3]

## SILYMARIN:

*Silybum marianum*, commonly known as milk thistle in the family Asteraceae, is one of the oldest and thoroughly researched plants of ancient times for the treatment of liver and gallbladder disorders, hepatitis, cirrhosis, jaundice and protection against *Amanita phalloides* mushroom and other toxin poisonings[4]. Silymarin, the active component of this plant, is a standardized extract consisting of approximately 70 to 80 percent silymarin flavanoliganans (silybin A & B, isosilybin A & B, silydianin and silychristin) and flavanoids (toxifolin and quercetin), and the remaining 20 to 30 percent consisting of a chemically undefined fraction comprised of polymeric and oxidized polyphenolic compounds (Figure.1).[5]

Silymarin possess wide range of biological and pharmacological effects, including antioxidant activity,[6] stimulation of protein synthesis and cell regeneration (making it useful in the treatment of toxic liver damage, chronic inflammatory liver diseases and liver cirrhosis),[7,8] and impressive anticancer activity against several human carcinoma cell lines.[9,10] In addition, anti-diabetic activity,[11] cardioprotection,[12] anti-inflammatory, anti-fibrotic, hypolipidemic, neurotrophic and immune modulation effects.[13]

The water solubility of the drug is very poor[14] (0.04 mg/ml). Oral administration of silymarin (silybin) is rapidly absorbed with a $t_{max}$ 2 to 4 hours and its $t1_{/2}$ is 6 hours. Only 20 – 50 percent of oral silymarin is absorbed from the gastrointestinal tract. Therefore, absorption of silymarin from the gastrointestinal tract is low that leads poor bioavailability.[15]

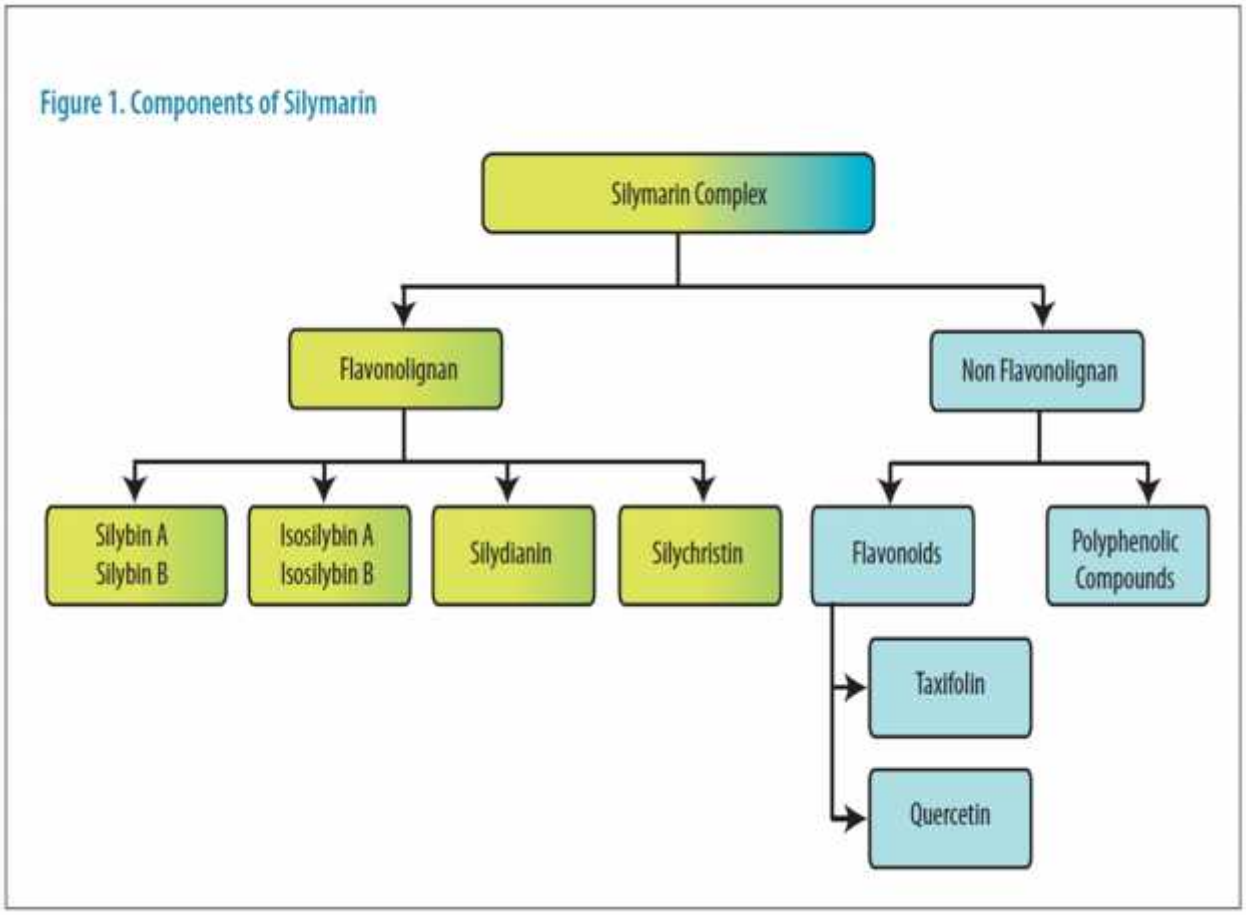



**Figure.1: Components of silymarin.**

Developing novel strategies to enhance the solubility of poor water soluble drugs is one of the mean focuses of pharmaceutical technology[5, 6]. The bioavailability of poorly soluble drugs can be improved by conversion of micro particles to drug nanoparticles. Nanotechnology mainly refers to the study of materials and structures at the nanosized level. In micronization process of drugs the chance for agglomeration is high, so in order to avoid the agglomeration, nano- scale systems are used. A nano- crystal is a material with dimensions measured in nanometers, and particle size ranging from 1- 1000 nanometers. Nanotechnology is a promising strategy for improving the dissolution rate and oral bioavailability of poorly water soluble drugs by reducing the particle size and/or transforming drug from a crystalline to an amorphous state[7, 8].

## RATIONALE OF DRUG TARGETING [16]

The site-specific targeted drug delivery negotiates an exclusive delivery to specific pre-identified compartments with maximum activity of drugs and concomitantly reduced access of drug to irrelevant non-target cells. The controlled rate & mode of drug delivery to pharmacological receptor and specific binding with target cells as well as bioenvironmental protection of the drug in route to the site of action are specific features of targeting.

Invariably, every event stated contributes to higher drug concentration at the site of action and resultant lowers concentration at non-target tissue where toxicity might crop up. The high drug concentration at the target site is relative cellular result of the uptake of the drug vehicle, liberation and efflux of free drug from the target site.

Targeting is signified if the target compartment is distinguished from the other compartments, where toxicity may occur and also if the active drug could be placed predominantly in the proximity of target site. The restricted distribution of the parent drug to the non-target site(s) with effective accessibility to the target site(s) could maximize the benefits of targeted drug delivery.

## CARRIERS USED IN TARGETING DRUG DELIVERY SYSTEMS

Carrier is one of the most important entities essentially required for successful transportation of the loaded drug(s). They are drug vectors, which sequester, transport and retain drug en route. While eluting or delivering it within or in vicinity of target.

**Colloidal carriers:**

- Vesicular systems: liposomes; pharmacosomes; virosomes; immunoliposomes.
- Micro particulate systems: nanoparticles; microparticles; magnetic microspheres; nanocapsules.
- Cellular carriers: resealed erythrocytes; serum albumin; antibodies; platelets; leukocytes.

**Supramolecular delivery system:**

- Micelles; reverse micelles; mixed micelles; polymeric micelles; liquid crystal: lipoproteins.

**Polymer based systems:**

- Signal sensitive; muco-adhesive; biodegradable; bioerodible; solute synthetic polymeric carriers.

**Macromolecular carriers:**

- Proteins, glycoproteins, neo glycoproteins and artificial viral envelops (AVE); Glycosylated water-soluble polymers (poly-L-lysine).
- Mabs; Immunological Fab fragments; antibody enzyme complex & bispesific Abs; Toxins, immunotoxin & rCD4 toxin conjugates, Lecithins (Con A) & polysaccharides.

## INTRODUCTION TO LIPOSOMES [17]

Liposomes have reached the clinical only recently, but they are not a new invention Alec D. Bangham of the Agricultural Research Council's institute of Animal physiology in Cambridge, England, inadvertently produced the first liposome in 1961, while evaluating the effect of phospholipids on blood clotting. When bangham put water in a flask containing a phospholipid film, the water molecules to arrange themselves in to what he discovered. He found vesicles composed of a bilayered phospholipids membrane surrounding water entrapped from the environment.

Phospholipids form closed, fluid-filled spheres when they are mixed with water in part because the molecules are amphipathic; they have a hydrophobic "tail" and a hydrophilic or polar "head". Two fatty acid chains, each composed of 10 to 24 carbon atoms, make up the hydrophobic tail of most naturally occurring phospholipids molecules. Phosphoric acid bound to any of several water soluble molecules composes the hydrophilic head. When a high enough concentration of phospholipids is mixed with water, the hydrophobic tails spontaneously herd together to exclude water, whereas the hydrophilic heads bind to water.

The result is a bilayer in which the fatty acid tails in to the membrane's interior and the polar head groups point outward the polar groups at one surface of the membrane point toward the liposome's interior and those at the other surface point toward the external environment. It is this remarkable reactivity of phospholipids to water that enables workers to load medications in to liposomes. In a liposome form, any water soluble molecules that have been added to the water are incorporated in to the aqueous spaces in the interior of the spheres, whereas any lipid soluble molecules added to the solvent during vesicle formation are incorporated in to the lipid layer.

Liposomes employed for drug delivery typically range in diameter from 250 angstrom units to several micrometers and are usually suspended in a solution. They have two standard forms: "onion-skinned" multilamellar vesicles (MLVs) made up of several lipid bilayers separated by fluid, and unilamellar vesicles, containing of a single bilayer surrounding an entirely fluid core. The unilamellar vesicles are typically characterized as being small (SUVs) or large (LUVs).

**ADVANTAGES OF LIPOSOMES**

The pharmaceutical and pharmacological justification of the use of liposomes as drug carriers is as follows:

- Liposomal supply both a lipophilic environment and aqueous “milleu interne” in one system and are therefore suitable for the delivery of hydrophobic, amphipatic and hydrophilic drugs and agents.
- Liposomes are chemically and physically well characterized entities.
- The biological fate of liposomes after their administration is related to their composition and physical properties.
- Liposomes are biocompatible due to their biodegradability, low toxicity and lack of immunogenicity.
- Liposomes can serve as device for controlled release of drugs in body fluids (micro reservoir concept) and inside cells (after endocytic uptake).
- Liposomes help to reduce exposure of sensitive tissues to toxic drugs.
- Liposomes can be administered through mostnroutes of administration including ocular, pulmonary, nasal, oral, intramuscular, subcutaneous, topical and intravenous.
- Pharmacokinetics and *in-vivo* distribution of liposomes can be controlled by their port of entry combined with their lipid composition and size.

**DISADVANTAGES OF LIPOSOMES**

- Aggregation, fusion and drug leakage during storage.
- Chemically instable i.e., degradable by oxidation and hydrolysis.
- In physiological environment they are destabilized by high density lipoproteins(HDL)
- Purity of natural phospholipids and cost of production.
- They undergo complete mediated phagocytosis and lipid exchange reactions.

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..

## PROLIPOSOMES [18]

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 proliposomes. Proliposomes 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. 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. The present review gives a brief overview of preparation, evaluation and application of proliposomes as novel carrier system.

## INGREDIENTS USED IN THE PREPARATION OF VESICULAR SYSTEMS [19]

### Carrier material

The carrier when used in the proliposomes preparation permits the flexibility in the ratio of components that incorporated. In addition to this, it increases the surface area and hence efficient loading. The carriers should be safe and non-toxic, free flowing, poor solubility in the loaded mixture solution and good water solubility for ease of hydration. Commonly used carriers are listed; they are sorbitol, mannitol, glucose, lactose, sucrose stearate. Another polysaccharide that can be used as carrier is maltodextrin .It has minimal solubility in organic solvent. Thus it is possible to coat maltodextrin. It forms particles by simply adding surfactant in organic solvent. The use of maltodextrin as carrier in proliposomes preparation permitted flexibility in the ratio of surfactant and other components which can be incorporated.

### Membrane stabilizers

Cholesterol and lecithin are mainly used as membrane stabilizer. Steroids are important components of cell membrane and their presence in membrane and their presence in membrane brings about significance changes with regard to bilayer stability, fluidity and permeability. Cholesterol is a naturally occurring steroid used as membrane additive. It prevents aggregation by the inclusion of molecules that stabilize the system against the formation of aggregate by repulsive steric or electrostatic effects. It leads transition from the

gel state to liquid phase in liposomes system. Phosphatidylcholine is a major component of lecithin. It has low solubility in water and can form liposomes, bilayer sheets, micelles or lamellar structures depending on hydration and temperature. Depending upon the source from which they are obtained they are as named as egg lecithin and soya lecithin. It acts as stabilizing as well as penetration enhancer. It is found those vesicles composed of soya lecithin are of larger size than vesicle composed of egg lecithin probably due to the difference in the intrinsic composition. Cholesterol increases or decreases the percentage encapsulation efficiency depending on either the carrier materials or its concentration within the formula. Cholesterol along with the addition of carrier forms homogenous liposomes dispersion rather than only a carrier which forms a gel. Cholesterol is thus usually included in a 1:1 molar ratio in most formulations as it is known to abolish the gel to liquid phase transition of liposomes systems resulting in liposomes that are less leaky. The amount of cholesterol to be added depends on the carriers. It was found that above certain of cholesterol, entrapment efficiency decreased possibly due to a decrease in volume diameter.

**Organic solvents**

Chloroform is an organic solvent which evaporates readily, is convenient to handle and is a popular solvent used in the preparation of vesicle systems. Methanol is also an organic solvent which is mostly used as a mixture with chloroform for compounds that are sparingly soluble in chloroform or methanol alone. The volume ratio of chloroform: methanol used as an organic solvent is usually 8:2. This mixture was used as the solvent in this study as it was predicted that most compounds dissolved readily in the combination.

**Drug**

The drug selection criteria could be based on the following assumptions.

- Low aqueous solubility of drugs.
- High dosage frequency of drugs.
- Short half-life.
- Controlled drug delivery suitable drugs.
- Higher adverse drug reaction drugs.

**Nature of encapsulated drug**

The main factor in the consideration is the influence of an amphiphilic drug on vesicle formation. When drug was encapsulated in liposomes, aggregation occurred and was

overcome by the addition of a steric stabilizer. When more drugs is added the increase in its encapsulation could be the result of saturation of the medium. This suggests that the solubility of certain poorly soluble drugs can be increased by formulation in liposomes but only up to a certain limit above which drug precipitation will occur. Increase in drug concentration showed an increase in both percentage encapsulation efficiency and the amount of drug encapsulated per mole total lipids upon hydration and formation of liposomes.

## METHOD OF PREPARATION [19-29]

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

- Film deposition on carrier method.
- Spray drying method.
- Fluidized bed method.
- Super critical anti-solvent method.

### Film deposition on carrier method

This is the original method used by the preparation of proliposomes. It involves deposition of film of drugs and phospholipids onto a porous, water soluble carrier material. As seen in figure.2, solution of drug and phospholipid's in a volatile organic solvent is introduced drop wise 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 [19]. 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 proliposomes. 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 utilized include- maltodextrin, sorbitol and microcrystalline cellulose. 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 [20]. In order to solve this problem, 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 [21].

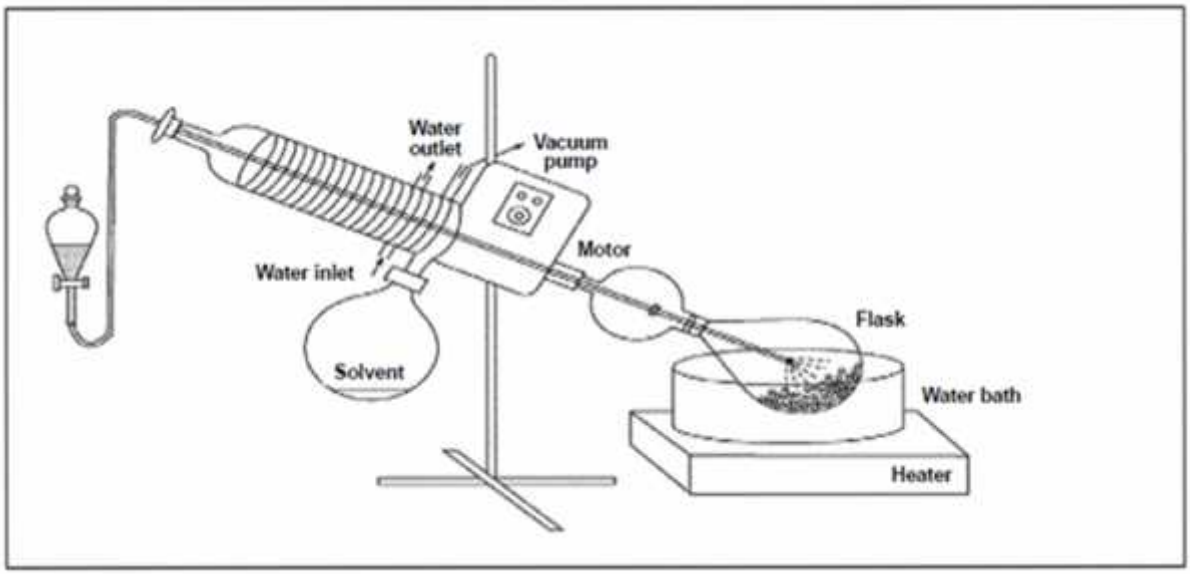



**Figure.2 Apparatus for the preparing PLs by flim deposition on carrier method**

**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 proliposomes [22-23]. As seen in Figure.3, 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 [24]. 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 [25]. 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 optimizing the operating parameters such as drying air temperature and liquid spraying rate. Stabilizing adjuvant 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 [26-27].

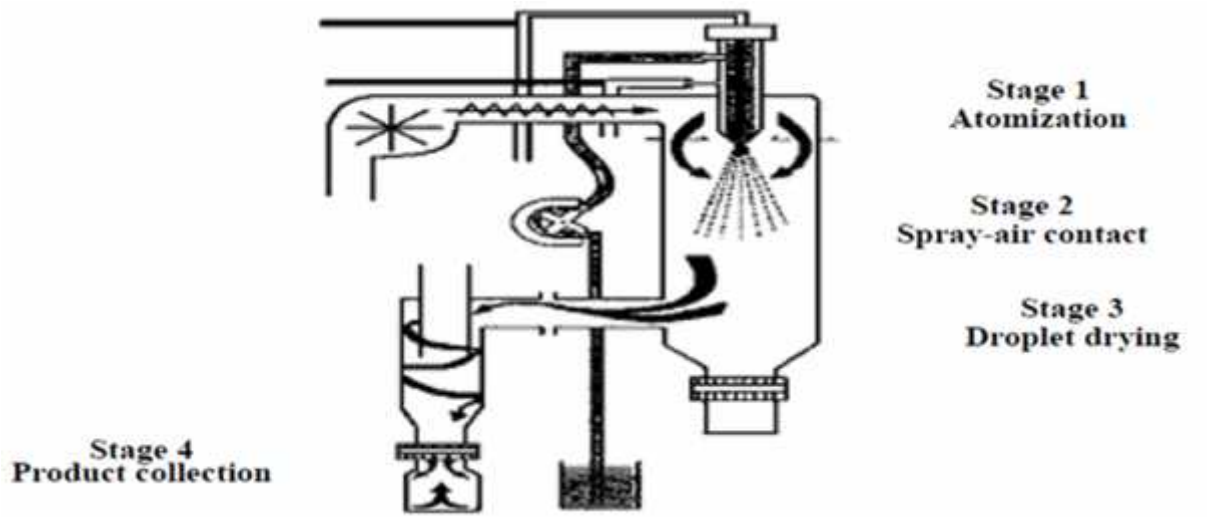



**Figure.3 Apparatus for the preparing PLs by spray drying method**

**Fluidised bed method**

This method can be employed for the large scale production of proliposomes 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:

- It utilizes film coating technology which is well established and processable.
- Various cores and coating materials are available or easy to prepare.
- It is a cost-effective method to prepare liposomes for drug delivery [28-29].

**Super critical anti-solvent method** [28-29]

Supercritical anti solvent method utilizes Supercritical Carbon dioxide ($SCCO_2$) in the preparation of proliposomes. $SCCO_2$ is a fluid state of carbon dioxide where it is held at or above its critical temperature and pressure. Antisolvent technology is widely used in food industry and was developed to prepare proliposomes because of its lower residual solvents, simpler steps and mild operation temperatures. As shown in figure.4, the apparatus used in the preparation of proliposomes 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. After the temperature and pressure of the separating vessel reaches the pre-set 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 super saturation in a very short period of time because the solubility of solutes in the organic solvent decreases greatly. As a result, the proliposomes are precipitated in the vessel. Once the solution is completely utilized, 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 proliposomes.

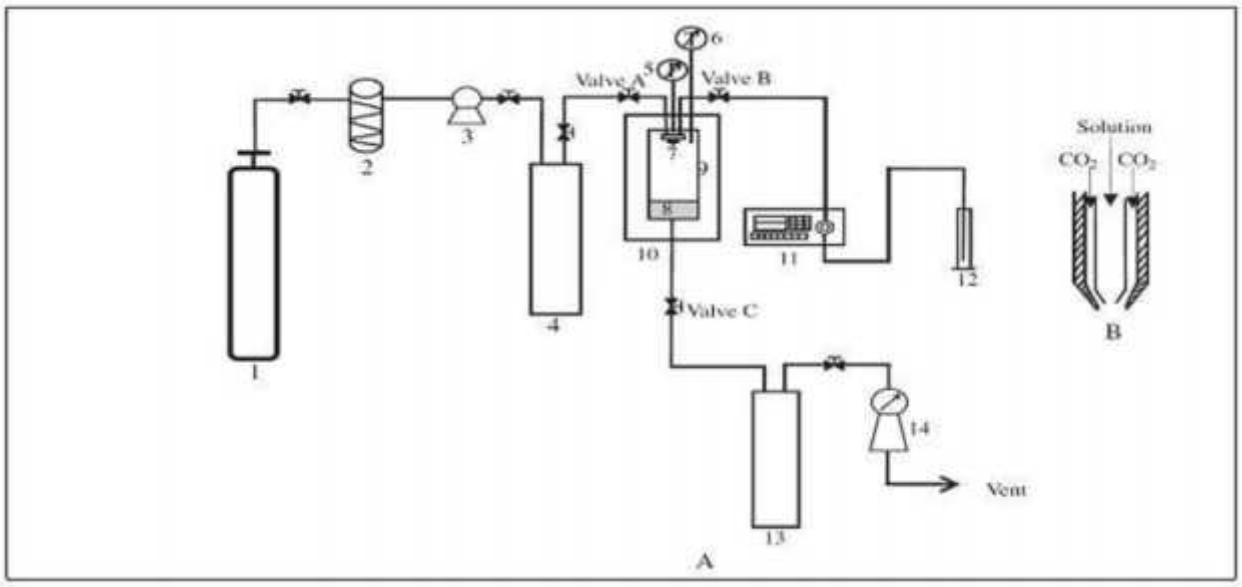



**Figure.4 Apparatus for preparing proliposomes by Supercritical Anti-solvent method [29]**

## CHARACTERIZATION OF PROLIPOSOMES [30 - 34]

**Mean particle size and particle size distribution:**

Particle size distribution analysis of proliposome determines the physicochemical behavior of the formulation, such as saturation solubility, dissolution velocity, physical stability etc. The particle size distribution can be determined by using different particle size analyzers such as photon correlation spectroscopy (PCS), laser diffraction (LD) and coutler counter multisizer.

**Zeta potential:**

Zeta potential is an important parameter in characterization study which indicates the stability of formulated proliposomes. The suspensions stabilized only by electrostatic repulsion, a minimum zeta potential of ± 30 mV is required, and if the suspension is stabilized by combined electrostatic and steric stabilizer a zeta potential of ± 20 mV is sufficient.

**Saturation solubility and dissolution velocity:**

Proliposomes have an important advantage over other techniques used for solubilization that it can increase the dissolution velocity and also the saturation solubility. The assessment of saturation solubility and dissolution velocity of proliposomes helps in determining the *in-vitro* behavior of the formulation.

***In-vitro* drug release studies-USP paddle method:**

An *in-vitro* drug release study is an integral part of characterization for any drug delivery system. It is considered an indicator of batch to batch variability associated with quality control. It can discriminate between various batches for the same formulation consisting of the same ingredients at various levels. Most interestingly, it can be used as an indicator for the *in-vivo* performance of the formulation. There are several factors which can govern drug release from a polymeric nanoparticulate carrier such as: solubility; diffusion; desorption; matrix erosion; degradation; and particle size. No standardized methods are available to study the drug release from nanosized carries.

***In -vitro* release and permeation studies**

*In-vitro* release and skin permeation studies for proliposomes were determined by different techniques like Franz diffusion cell, dialysis tubing and reverse dialysis. In case of dialysis, the prewashed dialysis tubing is used which can be hermatically sealed, the proliposomes are placed in it and then dialysed against a suitable dissolution medium at a

room temperature. The samples are withdrawn from the medium at suitable interval, centrifuged and analysed spectrophotometrically (UV, HPLC).

## APPLICATIONS OF PROLIPOSOMES [35-55]

Proliposomes can be exploited for the following routes of administration

- **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 121 ºC 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 [35-36].

Proliposomes are well suited for parenteral application of liposomes. The main advantage associated with proliposomes is that it allows sterilization without affecting the intrinsic characteristics [36]. Besides, they can be stored as sterilized in dry state and can be hydrated prior to administration to form multilamellar liposomal suspension [37]. 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 proliposomes [38].

- **Oral Delivery**

Oral drug delivery continues to be the preferred route of administration, but liposomes have limited success in delivering drugs through oral route [39-40]. 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, proliposomes represents the first example of delivering liposomes into solid dosage form such as tablets or capsules [40]. Further, liposomes are formed on contact with biological fluids at the site of absorption ensuring the retention of liposome integrity. Proliposomes 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 [41]. 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 [42]. Further, the phospholipid molecules which form the backbone of the bilayer structure help to enhance the solubility of drug molecule.

- **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 [43-44]. Drug delivery to the pulmonary route is achieved by three types of devices namely

- **Pressurised Metered Dose Inhalers (pMDI)**

As the name suggests it consists of solution or suspension of drugs in liquefied propellants. Use of Hydro fluroalkanes as non-ozone depleting propellants over CFCs has the limitation for liposome delivery as they are poor solvents for phospholipids. Proliposomes help overcome this limitation as they can be suspended in these propellants and serve as carrier for pulmonary delivery of liposomes through pMDI [45].

- **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 and reduced toxicity, uniform deposition of drugs locally, patient compliance, stability and high dose carrying capacity. Being available as dry powder form, Proliposomes are the best alternative for delivering liposomes through DPIs [43-45]. It was developed by spray dried liposome encapsulated dapsone DPI for prolonged drug retention in lungs to prevent pneumocystis carinii pneumonia. Prolonged drug release of up to 16 hours was observed *in-vitro*.

- **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, proliposomes serve as a stable

alternative for delivering liposomes through nebulization. Besides, the ready formation of an isotonic liposome formulation in situ from proliposomes seems to offer advantages over other formulation approaches [43-44].

- **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 proliposomes 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 [37-46]. Exemestane, a novel steroidal aromatase inactivator has limited bioavailability of 42% due to poor solubility and extensive first-pass metabolism. The utilised proliposomes system for transdermal delivery of Exemestane and found a 2.4 fold increase in bioavailability from proliposomes gel compared to oral suspension [46]. Proliposomes have also been developed for sustained delivery of nicotine [37] and aceclofenac [48] transdermally.

- **Mucosal delivery**

Proliposomes 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 [48]. 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 proliposomes 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 proliposomes for vaginal and nasal drug delivery [49].

- 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 proliposomes makes them excellent carriers to molecularly disperse the drug [49]. 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. It was developed by a proliposoms formulation of Clotrimazole and compared the fungicidal efficacy with the standard ointment in rats. The results indicated that Clotrimazole containing vaginal proliposomes 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 [55].

- Nasal mucoadhesive delivery has been used to improve local and systemic delivery of therapeutic compounds [51]. It is a promising alternative for systemic administration of drugs that are poorly absorbed via the oral route [52]. 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 [52-53]. 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 proliposomes plays a role in sustaining the plasma concentration of drugs with short half-life in systemic circulation [54-55].
- 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. The utilised proliposomes for nasal delivery of propranolol. Sustained plasma concentration of propranolol was obtained due to the slow hydration process of proliposomes in nasal cavity. It was given by the Mean hydration time (MHT) of proliposomes which was defined as the difference of Mean Residence time between liposomes and proliposomes. It was found to be 80.4 minutes which confirmed longer residence time of proliposomes in nasal cavity [55].

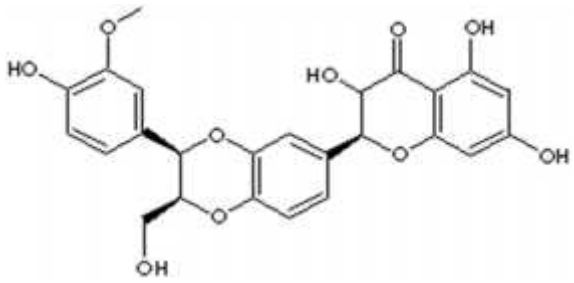
# *DRUG PROFILE*

## DRUG PROFILE

**SILYMARIN:** [56-58]

**PROPERTIES:**

- **Structural Formula:**




- **Chemical name:**

  (2*R*,3*R*)-3,5,7-Trihydroxy-2-[(2*S*,3*S*)-3-(4-hydroxy-3-methoxyphenyl)-2-hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]-2,3-dihydrochromen-4-one.

- **Molecular Formula** : $C_{25}H_{22}O_{10}$
- **Molecular Weight** : 482.44
- **Appearance** : Yellow powder
- **Density** : 1.527 gm/$cm^3$
- **pH** : 5.5 to 7.0
- **Melting point** : 230-233 $^0$C
- **Solubility** : Soluble in DMSO and in ethanol
- **Functional Categories** : Anti-oxidant, estrogenic and apoptotic agent.
- **Stability and storage conditions** : Keep container tightly closed and dry in a well-ventilated place.
- **Incompatibilities** : Incompatible with strong oxidizing agent.
- **Handling precautions** : Provide appropriate exhaust ventilation at places where dust is formed.

**PHARMACOKINETICS:**

Silymarin is not soluble in water and is usually administered in an encapsulated form. Silymarin is absorbed when given orally. Peak plasma concentration is achieved in 6-8 pH. The oral absorption of silymarin is only about 23-47 %, leading to low bioavailability of the compound; it is administered as a standard extract (70-80 % silymarin). After oral administration the recovery in bile ranges from 2-3 %. Silybin and the other components of silymarin are rapidly conjugated with sulfate and glucuronic acid in the liver and excreted through the bile. The poor water solubility and bioavailability of silymarin led to the development of enhanced formulations.

**2.1.3 DOSE:**

Now food silymarin 300mg. Adult: Recommended dose is 140mg 2 – 3 times/day.

**2.1.3 MECHANISM OF ACTION:**

Silymarin hepatoprotective effects are purportedly accomplished via several mechanisms; these include:

- Antioxidationt.
- Inhibition of lipid peroxidation.
- Stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration.
- Enhanced liver detoxification via inhibition of phase I detoxification.
- Enhanced glucuronidation and protection from glutathione depletion.
- Anti-inflammatory effects, including inhibition of leukotriene and prostaglandin synthesis, Kupffer cell inhibition, mast cell stabilization, and inhibition of neutrophil migration.
- Slowing or even reversing of fibrosis by reduction of the conversion of hepatic stellate cells into myofibroblasts.
- Anticarcinogenesis by inhibition of cycline-dependent kinases and arrest of cancer cell growth.
- Silymarin is also found to have immunomodulatory effects on the diseased liver.

**2.1.4 ADVERSE EFFECTS:**

Abdominal bloating, diarrhea, flatulence, loss of appetite, nausea and stomach upset.

**2.1.5 DRUG – FOOD INTERACTION:**

Food drug interaction mainly involves the interaction of drug with food. Herbs, dairy products, alcohols and caffeine.

**2.1.5 CONTRAINDICATIONS:** Hypersensitivity.

**2.1.6 PHARMACOLOGICAL ACTION:**

Hepatoprotective, immunomodulatory, antioxidant, anti-inflammatory activities neuroprotective, and antiproliferative effects. It is also used for hypercholesterolemia, amanita mushroom poisoning and psoriasis.

# *STABILIZER PROFILE*

**STABILIZER PROFILE**

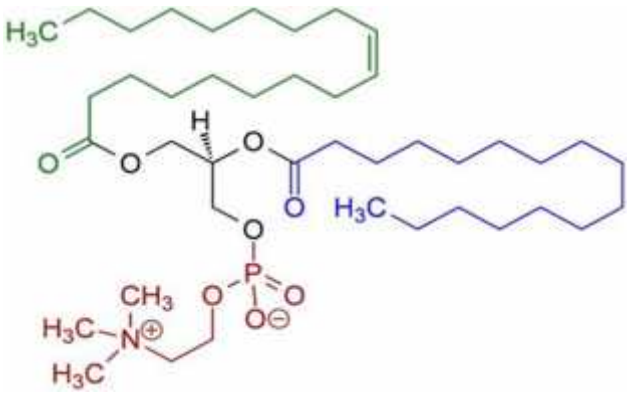
**SOYA LECITHIN [59]**

**PROPERTIES:**

**Chemical Name:**

(2R)-2, 3-di (tetradecanoyloxy)propyl) 2-(trimethylazaniumyl) ethyl phosphate.

**Chemical Structure:**



| | |
|---|---|
| **Molecular Formula** | **:** $C_{44}H_{86}NO_8P$ |
| **Molecular Weight** | **:** 789 |
| **Appearance** | **:** Cream to yellowish light brown powder |
| **Density** | **:** 1.03 to 1.08 gm/cm$^3$ |
| **pH** | **: 7** |
| **Melting point** | **:** 226-230 $^0$C |
| **Solubility** | **:** It has low solubility in water. |
| **Functional categories** | **:** Natural emulsifier or stabilizer, anti-oxidant, wetting agent. |

Soya lecithin has emulsification and lubricant properties, and is a surfactant. It can be totally metabolized by humans, so is well tolerated by humans and non-toxic when ingested; some emulsifiers can only be excreted via the kidneys.

**STABILITY AND STORAGE CONDITIONS:**

Stable, but light, heat, moisture and air sensitive.

Keep container tightly closed. Store in a cool, well-ventilated area.

**INCOMPATIBILITIES:**

Incompatible with strong oxidizing agent.

**HANDLING PRECAUTIONS:**

If overheated, remove source of heat.

**APPLICATION IN PHARMACEUTICAL TECHNOLOGY:**

It acts as a wetting, stabilizing agents and choline enrichment carrier, helps in emulsifications and encapsulation, and is good dispersing agent. It can be used in manufacture of intravenous fat infusions and for therapeutic use.

## TPGS [(60)]

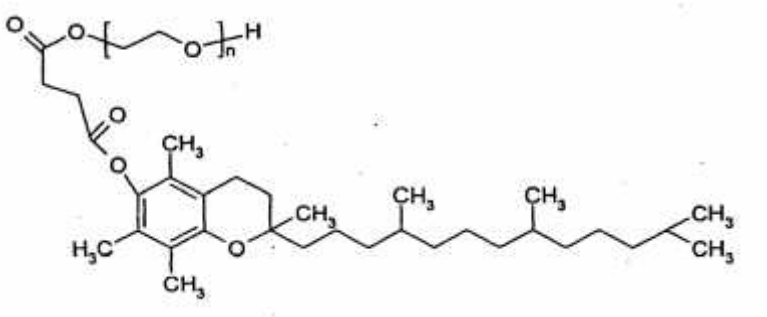
## (D-α-tocopheryl polyethylene glycol 1000 succinate)

**PROPERTIES:**

**Chemical name:**

α-[4-[[(2*R*)-3,4-dihydro-2,5,7,8-tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-2*H*-1-benzopyran-6-yl]oxy]-1,4-dioxobutyl]-ω-hydroxy-poly(oxy-1,2-ethanediyl)

**Structural formula:**



| | |
|---|---|
| **Molecular formula** | **:** $C_{35}H_{58}O_6$ |
| **Molecular weight** | **:** 574.83 g/mol |
| **Appearance** | **:** White to light tan waxy solid |
| **pH** | **:** 4-10 |
| **Melting point** | **:** 37 – 41 $^0$C |
| **Solubility** | **:** Water soluble (1 g/10 ml) |
| **Functional categories** | **:** Tocopherol polyethylene glycol 1000 succinate may be used to create biodegradable polymers and anti-oxidant, surfactants. |

**STABILITY AND STORAGE CONDITION:**

Keep container closed. Protect from contamination.

**INCOMPATIBILITY:**

Reacts with strong oxidizing agents and alkali.

**HANDLING PRECAUTIONS:**

Avoid keeping TPGS with oxidizing materials.

**APPLICATION IN PHARMACEUTICAL TECHNOLOGY:**

TPGS has been widely used for its emulsifying, dispersing, gelling, and solubilizing effects on poorly water-soluble drugs. It can also act as a P-glycoprotein (P-gp) inhibitor and has been served as an excipient for overcoming multidrug resistance (MDR) and for increasing the oral bioavailability of many anticancer drugs.

# CHOLESTEROL [60]

**PROPERTIES:**

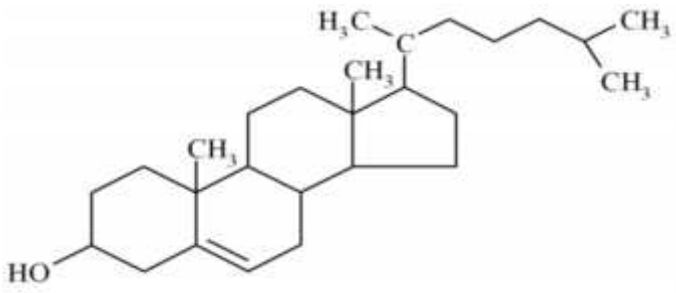
**Synonym**

Cholesterolum; Cholesterin

**Chemical name**

Cholest- 5-en-3β- ol

**Structural formula**




| | |
|---|---|
| **Molecular formula** | **:** $C_{27}H_{46}O$ |
| **Molecular weight** | **:** 386.67 g/mol |
| **Appearance** | **:** White or faintly yellow, almost odourless, pearly leaflets, needles, powder or granules. |
| **pH** | **:** 7.35 -7.45 |
| **Melting point** | **:** 147-150 $^0$C |
| **Solubility** | **:** Soluble in acetone and vegetable oils |
| **Functional categories** | **:** Cholesterol may be used to emulsifying agent, Emollient. |

**STABILITY AND STORAGE CONDITION:**

It is stable, and should be stored in a well- closed container and protected from light.

**SAFETY:**

It is generally regarded as an essentially non- toxic and non- irritant material at the levels employed as an excipients

**HANDLING PRECAUTIONS:**

Rubber or plastic gloves, eye protection and a respirator are recommended.

**APLICATION IN PHARMACEUTICAL TECHNOLOGY:**

It is used in cosmetics & topical pharmaceutical formulations as an emulsifying agent. It imparts water absorbing power to ointments. It is the major sterol of higher animals, & is found in all body tissues, especially in brain & spinal cord.

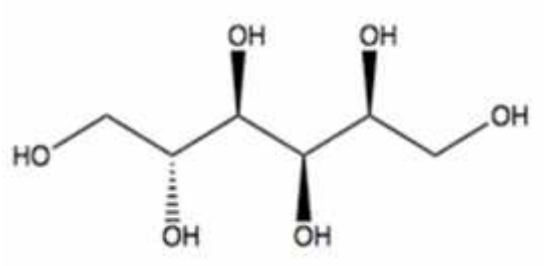
# SORBITOL [62]

**PROPERTIES:**

**Chemical name:**

(2S,2R,4R,5R,)-Hexane-1,2,3,4,5,6-hexol

**Structural formula:**




**Molecular formula** : $C_6H_{14}O_6$

**Molecular weight** : 182.17 g/mol

**Appearance** : White crystalline powder

**pH** : 5.0 – 7.5

**Melting point** : 94-96 $^0$C

**Solubility** : Soluble in water and slightly soluble in methanol

**Functional categories** : Sorbitol is used as substitute for sugar, laxative.

**STABILITY AND STORAGE CONDITION:**

It is stable stored in a well closed container at room temperature

**SAFETY:**

It is generally regarded as an essentially non- toxic and non- irritant material at the levels employed as an excipients.

**HANDLING PRECAUTIONS:**

If overheated it melts avoid. Respirator recommended

**APPLICATION IN PHARMACEUTICAL TECHNOLOGY:**

Sorbitol often is used in modern cosmetics as a humectants and thickener. It is also used in mouthwash and toothpaste. Some transparent gels can be made only with sorbitol, because of its high refractive index.

# *LITERATURE REVIEW*

## LITERATURE REVIEW

**Theresa Kott, *et al* (1994)** [63] investigated the hepatoprotective activity by using animal (rat) with acetylsalicylic acid (150 mg/kg b.w.per os daily) for six weeks. Half of the rats received silymarin (17.5 mg/kg b.w. per os daily) in the last three weeks of the experiment. It was found that administration of acetylsalicylic acid led to the signs of hepatic damage. Administration of silymarin diminished the extent of the hepatic damage. The obtained results suggest that silymarin be administered to the patients undergoing long-term treatment with non-steroidal anti-inflammatory drugs in order to prevent hepatic damage, but further studies are needed to elaborate on the clinical aspects of silymarin treatment in those patients.

**Om Parkash Katare, *et al* (2005)** [64] studied synergistic hepatoprotective effect of silymarin with phospholipids when it is engaged in microspheres so as to passively target it to liver and to compare these silymarin formulations with silymarin solution. Various silymarin loaded lipid emulsions were formulated which includes: formulation A soyabean oil as an internal oily phase, soya lecithin as surfactant and tween 80 as co-surfactant; formulation B which was same as formulation A but was filtered through 0.45 micro membrane filter and finally steam sterilized for intravenous administration; formulation C containing soyabean oil as an internal oily phase, soya lecithin as surfactant, tween 80 and propylene glycol as cosurfactant/ cosolvent.while comparing to three formulations , the best formulation was found formulation B , it may confirmed by practically.

**Rajesh Agarwal, *et al* (2006)** [65] studied silymarin consists of a family of flavonoids (silybin, isosilybin, silychristin, silydianin and taxifoline) commonly found in the dried fruit of the milk thistle plant Silybum marianum. Although silymarin's role as an antioxidant and hepatoprotective agent is well known, its role as an anticancer agent has begun to emerge. Extensive research within the last decade has shown that silymarin can suppress the proliferation of a variety of tumor cells (e.g., prostate, breast, ovary, colon, lung, bladder); this is accomplished through cell cycle arrest at the G1/S-phase, induction of cyclin-dependent kinase inhibitors (such as p15, p21 and p27), down-regulation of anti-apoptotic gene products (e.g., Bcl-2 and Bcl-xL), inhibition of cell-survival kinases (AKT, PKC and MAPK) and inhibition of inflammatory transcription factors (e.g., NF-κB). Silymarin can also down-regulate gene products involved in the proliferation of tumor cells (cyclin D1, EGFR, COX-2, TGF-,, IGF-IR), invasion (MMP-9), angiogenesis (VEGF) and metastasis (adhesion molecules). The anti- inflammatory effects of silymarin are mediated through

suppression of NF-κB-regulated gene products, including COX-2, LOX, inducible iNOS, TNF and IL-1.

**Sarala yanamandra, *et al* (2014)** [66] Objective of the present study was to develop a proliposomal formulation to decrease the hepatic first-pass metabolism of a highly metabolized drug. Lovastatin was chosen as the model drug. Proliposomes were prepared by mixing different ratios of phospholipids such as soy phosphatidylcholine (SPC), hydrogenated egg phosphatidylcholine (HEPC) and dimyristoyl phosphatidylglycerol (DMPG) individually with drug and cholesterol in an organic solvent.In- vivo studies were carried out in male Sprague–Dawley rats. Following single oral administration of the selected formulation (F9), a relative bioavailability of 162% was achieved compared to pure lovastatin. The study demonstrated that proliposomes can be used as a drug delivery system to decrease the hepatic first-pass metabolism.

**Grishma M Patel, *et al* (2017)** [67] present work was to apply quality by design (QbD) principles for the development of proliposome of poorly soluble lopinavir (LPV). The patient-centric quality target product profile (QTPP) was defined and critical quality attributes (CQAs) earmarked. Risk assessment studies were carried out to identify the probable risks affecting the CQAs of the product. On the basis of preliminary study, lipid:drug ratio and amount of carrier were selected as critical material attributes (CMAs) and were optimized by face centered central composite design. Liposome vesicle size, drug entrapment efficiency and % drug release after 60 min were selected as CQAs and mathematical relationship between CQAs and CMAs was derived using multiple linear regression analysis. Solid state characterization studies (DSC, SEM and X-RD) were performed for optimized proliposome, suggested transformation of crystalline to amorphous form. Oral bioavailability study in wistar rats revealed that LPV proliposome exhibited 2.24 and 1.16 fold higher bioavailability than pure LPV and available commercial formulation of LPV/RTV (lopinavir + ritonavir), respectively. Stability study of the optimized LPV loaded proliposome was performed as per ICH guideline and was found to be stable for period of 6 months. Overall results of the study indicate that the proliposome offers advantages of enhanced oral bioavailability for poorly soluble.

**D. V. Gowda, *et al* (2016)** [68] investicate 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.

**Osama A Badary, *et al* (2005)** [69] evidenced cisplatin-induced nephrotoxicity and confirm the antioxidant potential of silymarin. The effect of silymarin (NAR), a naturally occurring citrus flavanone, on the acute nephrotoxicity produced by cisplatin (7 mg/kg, i.v.) was investigated in the rat. Oral administration of NAR (20 mg/kg/day) for 10 days, starting 5 days before cisplatin single i.v. injection, produced significant protection of renal function. NAR reduced the extent of cisplatin-induced nephrotoxicity, as evidenced by significant reduction in serum urea and creatinine concentrations, decreased polyuria, reduction in body weight loss, marked reduction in urinary fractional sodium excretion and glutathione S-transferase (GST) activity, and increased creatinine clearance. Cisplatin-induced alterations in renal cortex lipid peroxides and GST activity were markedly improved by NAR. Cisplatin-induced alterations in renal cortex antioxidant defense system were greatly prevented by NAR. In cisplatin-NAR combined treatment group, antioxidant enzymes namely superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were significantly increased to 54.5, 30.3 and 35.6%, respectively compared to cisplatin treated group. Platinum renal content was not affected by NAR treatment. The results provide further insight into the mechanisms of cisplatin-induced nephrotoxicity and confirm the antioxidant potential of NAR.

**Yingjun Liao, *et al* (2008)** [70] studied to explore the optimal combination of agents used along with cisplatin for protection of hepatotoxicity. The objective of this study was to explore the optimal combination of agents used along with cisplatin for protection of hepatotoxicity. Animal experiment was carried out based on the orthogonal design L8 (27) setting seven factors with two different levels of each and eight groups of mice were needed. The agents tested in this study were zinc, selenium, fosfomycin, sodium thiosulfate (STS), N-acetyl-cysteine (NAC), methionine and taurine. Mice were supplemented by gavage with various combinations of agents as designed in the orthogonal table once a day for nine days beginning two days before cisplatin administration. 3.5 mg/kg body weight of cisplatin was given intraperitoneally once a day for five days simultaneously. After cessation of cisplatin administration, the agents were supplemented continuously for two days. Activities of alanine aminotransferase (ALT) in serum, levels of glutathione (GSH) and malondialdehyde (MDA) in liver were analyzed after cessation of supplementation. Results showed zinc, fosfomycin

and methionine were the effective factors for protection of weight loss; fosfomycin and methionine were the effective factors for prevention of decreased liver ratio; selenium, fosfomycin and STS were the effective factors for prevention of increased ALT activities in serum. On the other hand, methionine was the only effective factor for prevention of decreased GSH levels in liver; zinc, selenium and fosfomycin were the effective factors for prevention of increased MDA levels in liver. Based on the data observed in this study, the optimum combinations of agents were selenium, fosfomycin, methionine and taurine, and zinc, selenium, STS and methionine. In conclusion, each agent used in this study could play a beneficial role for prevention of cisplatin hepatotoxicity; however, none could play the crucial role. The potentiated actions for prevention of cisplatin hepatotoxicity could be achieved via combined use of these agents.

**Abdelmeguid, *et al* (2010)** [71] suggested that silymarin possess protective effects against cisplatin hepatotoxic action in animal models. The benefit of silymarin, a plant extract with strong antioxidant activity against hepatotoxicity induced by cisplatin in rats was investigated in this study. Cisplatin is one of the most effective chemotherapeutic drugs, yet it alone does not achieve a satisfactory therapeutic outcome and at high doses it can produce undesirable side effects. Five equal-sized groups (18 rats each) of male Sprague dawley rats [control, vehicle; cisplatin; silymarin 2 hrs after cisplatin injection; and silymarin 2 hrs before cisplatin injection] were used. Histopathological and ultra-structural observations of livers were carried out using light and electron microscopy. Results documented that cisplatin produced behavioral, external features animal changes, as well as hazard pathological picture changes in liver where most hepatocytes appeared diminutive with vacuolated cytoplasm, sinusoids dilated and organelle disorganized. These results revealed that cisplatin may be toxic and terminates in complex liver damage. Administrations of silymarin 2 hrs after cisplatin significantly increase the body weight returning it to normal, yet it failed in complete protection against the pathological alteration caused by cisplatin. Pretreatment with silymarin 2 hrs before cisplatin significantly decreased the pathological changes induced by cisplatin and appeared highly protective. These results suggested that silymarin possess protective effects against cisplatin hepatotoxic action in animal models. Since, no significant toxicity of silymarin is reported in human studies, this plant extract can be used as a dietary supplement by patients taking anti-cancerous medications and might serve as a novel combination agent with cisplatin since it plays a significant role in reducing its toxicity.

**Marija Petrovic, *et al* (2016)** [72] evidenced that cisplatin can activate in cancer cell, the mechanism of resistance and clinical toxicities. Cisplatin (cis-Diamminedichloroplatinum II) is one of the most important chemotherapeutic agents widely used in treatment of many types of solid cancer. Accumulating evidence suggests that the cytotoxic activity of cisplatin involves both nuclear and cytoplasm component, but its biochemical and molecular mechanisms of action are still unclear. Its mode of action is linked to the ability of cisplatin to interact with purine bases on the DNA, causing DNA damage, interfering with DNA repair mechanisms and inducing apoptotic cell death in cancer cells. The major limitations in the clinical application of cisplatin are the numerous side effects and the development of cisplatin resistance by tumors. Mechanisms that can explain cisplatin resistance include the reduction in drug accumulation inside the cell, higher concentration of glutathione and metallothioneins, faster repair of cisplatin adducts and modulation of apoptotic cell death in various cells. In this article we review the pathways that cisplatin can activate in cancer cell, the mechanisms of resistance and clinical toxicities. A deep knowledge of mechanisms of action may lead to design of more efficient platinum-based antitumor drugs and provide new therapeutic strategies in cancer treatment.

# *AIM AND OBJECTIVE*

## AIM AND OBJECTIVE

Silymarin is one of the best known hepatoprotective drugs obtained from the seeds of Silybum marianum L., Family: Asteraceae or Compositae. Since ages, this plant, also known as milk thistle, is being used as a herbal cure for liver and biliary tract diseases. This plant has been known to safeguard and also regenerate the liver cells in various diseases affecting liver such as cirrhosis, jaundice and hepatitis. It exhibits strong anti-oxidant action via free radical scavenging activity and inhibits lipid peroxidation. It also prevents the entry of harmful toxicants such as heavy metals, pesticides, alcohols, medicines, $CCL_4$ etc. in liver, thereby protecting the liver cells from further damage.

Silymarin is a mixture of flavonolignans, which comprises of silybin, isosilybin, silydianin and silychristine. It exerts hepatoprotective action at an oral dose of 240-800 mg/day in two or three divided dose. The water solubility of the drug is very poor (0.04 mg/ml). Oral administration of silymarin is rapidly absorbed with a $t_{max}$ 2 to 4 hours and its $t_{1/2}$ is 6 hours. Only 20 – 50 percent of silymarin is absorbed from the gastrointestinal tract. Therefore, absorption of silymarin from the gastrointestinal tract is low that leads poor bioavailability.

The Silymarin poor bioavailability is mainly due to extensive metabolism, poor aqueous solubility and rapid excretion through urine and bile as well as low permeability across intestinal epithelial cells. The short half-life, poor bioavailability and high frequency of administration of silymarin, thus nesessery for the development of nanoparticulate drug delivery systems.

Nanotechnology based drug delivery systems improve the aqueous solubility; enhance the dissolution, permeation and bioavailability of poorly soluble drugs. Many hepato protective drugs offer challenging problems in drug formulation and it is generally associated to poor solubility and dissolution characteristics and thus leads to low bioavailability. In order to enhance these characteristics, preparation of proliposomes has been achieved using film deposition method.

The aim of the present investigation is to formulate and optimize TPGS modified silymarin proliposomes by film deposition method in order to overcome its poor dissolution behavior, minimize physical instability problems, prolonging the circulation time of the entrapped drug and to improve oral absorption, altering its organ distribution as well as metabolic instability followed by improve its therapeutic potential in terms of hepatoprotective activity.

So the objective of the present study is to carry out formulation and evaluation of TPGS modified proliposomes of silymarin by using suitable polymer to improve its solubility, dissolution, permeation bioavailability and to increase it biological half-life followed by evaluation of its therapeutic potential in terms of hepatoprotective activity using suitable animal model.

The following objectives are outlined to achieve the aim and need of the study:

- To select appropriate drug and polymers
- To perform Pre-formulation studies
- To develop the preparation of proliposomes.
- To perform *in-vitro* release studies and other evaluation parameters for formulations.
- Pharmacological activity.

# *PLAN OF WORK*

## PLAN OF WORK

The aim of our work is to formulate and evaluate the TPGS stabilized proliposome of the drug silymarin by using different polymers and surfactants. The following experimental protocol is therefore designed to allow a systemic approach to the study.

- Literature survey.
  - Procurement of drug and raw materials.
  - Preformulation studies.
  - Physical evaluation.
  - Solubility studies.
  - Melting point determination.
- Drug and polymer interaction study.
  - FT-IR
- Formulation of proliposome by film deposition method and lyophilisation of the prepared proliposomes.
- Evaluation of prepared proliposomes.
  - Drug content.
  - Particle size analysis.
  - Poly dispersity index.
  - Zeta potential analysis.
  - Solubility studies
  - *In-vitro* drug release.
  - *In-vitro* permeation studies.
- Pharmacological activity
  - Hepatoprotective activity.

# *MATERIALS AND INSTRUMENTS*

# MATERIALS AND INSTRUMENTS

## MATERIALS

**Table: 1. List of materials used.**

| S.NO | NAME | COMPANY |
|---|---|---|
| 1. | Silymarin | Himedia, Mumbai. |
| 2. | Soya lecithin | Glen mark Generic Limited, Mumbai. |
| 3. | TPGS | Ludwigshafen, Germany. |
| 4. | Hydrochloric Acid | Himedia, Mumbai. |
| 5. | Potassium Dihydrogen Phosphate | Himedia, Mumbai. |
| 6. | Disodium Hydrogen Phosphate | Himedia, Mumbai. |
| 7. | Distilled water | Leo scientific, Erode. |
| 8. | α-Tocopherol | Loba Pvt., Mumbai. |
| 9. | Sodium chloride | Nice Chemicals, Coimbatore. |
| 10. | Nitro cellulose membrane | Loba Pvt., Mumbai. |
| 11. | Cisplatin | Alkem, Mumbai. |
| 12. | Cystone | Himalayas, India. |

**INSTRUMENTS**

**Table: 2. List of instruments used.**

| S.NO | NAME | COMPANY |
|---|---|---|
| 1. | Melvern Zetasizer | Malvern Nano ZS-90, UK. |
| 2. | Freeze Dryer | Lyodel (Delvac), India |
| 3. | Cold centrifuge | Remi, Mumbai. |
| 4. | Deep freezer | Blue Star |
| 5. | Research centrifuge | Remi |
| 6. | Rotary shaker | Genuine |
| 7. | Hot air oven | Genuine |
| 8. | Refrigerator | Godrej |
| 9. | Analytical balance | Shimadzu, Japan |
| 10. | IR- Spectrometer | Ftir-8400 S Shimadzu, Japan |
| 11. | PH-meter (Digital) | Li 613,Elico |
| 12. | UV spectrophotometer | UV 1800 Shimadzu, Japan |
| 13. | BTS 350 Semiautomatic analyser | Bio system, Spain |
| 14. | Magnetic Stirrer | Remi, Vasai(India) |

# *METHODOLOGY*

# METHODOLOGY

## METHODS:

1. Procurement of drug, polymers and excipients for formulation development.
2. To carry out preformulation study.

   Preformulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to developing the stable and bio-available dosage form obviously, the type of information needed will be depends on the dosage form to be developed. The use of preformulation parameter minimizes the chances in formulation and acceptable, safe, efficacious and stable product and at same time provides the basis for optimization of the drug product quality.
3. Drug and polymer interaction studies: Infrared spectroscopy.
4. To formulation of proliposomes:

   Film deposition method. Lyophilization of the prepared proliposomes.

- Pre-formulation study
  - Solubility studies
  - Characterization of the drug, excipients and its mixture using melting point determination, UV spectroscopy and Infrared spectroscopy.
  - Preparation of calibration curve of drug in 0.1 N Hcl (pH 1.2) and phosphate buffer (pH 7.4)
  - Compatibility study of drug, polymer and its mixture
  - Preliminary development of trial batches to establish the required profiles.
- Evaluation of proliposome formulations.
  - Physical evaluation: Morphology and surface topography of the formulation using Particle size analysis, Poly dispersity index, Zeta potential and IR study.
  - *In-vitro* dissolution study and other evaluation parameters to study of best formulation.
- Pharmacological activity
  - Hepatoprotective activity.

**FORMULATION OF PROLIPOSOMES:**

Based on the composition given in table. 7, using film deposition method, by using different stabilizers like soya lecithin, cholesterol, TPGS and carrier like sorbitol, in entire formulation drug, stabilizers concentration are constant. Only TPGS differ in formulations. Carriers sorbitol taken in a round bottom flask. Then silymarin powder (1 gm), soya lecithin (2 gm) and cholesterol (2 gm) added according to the formula. It was dissolved by addition of chloroform. Further to make slurry, chloroform added. The round bottom flask was fitted and the solvent was evaporated at 60 rpm under reduced pressure at a temperature of 45±2 °C, until the product become free flowing, dry condition. After that they obtained product were dried overnight at room temperature in a desiccators under vacuum. The obtained final preparation of proliposomes was stored in a sealed container at 5 °C and kept it for evaluation process.

**LYOPHILIZATION OF FORMULATIONS:**

The proliposome formulations were freeze dried to increase the shelf life and to study the dissolution behavior. 1 % mannitol was added to each formulation as a cryoprotectant at the time of lyophilization. Virtis freeze drier is used for lyophilization of proliposomes. At first the sample was kept overnight in deep freezer at -70 °C and then sample was kept in Virtis freeze drier for two days at -50 °C at 2 millitorr.

**EVALUATION OF PROLIPOSOME:**

**PARTICLE SIZE DISTRIBUTION:**

The particle size analysis of different formulations of proliposome was carried out using Microtac Blue wave particle size analyzer. Before measurement of the samples, they have to be diluted with de-ionized water to obtain a suitable concentration for measurement. The results obtained for particle size distributions were used to confirm the formation of nano - sized particles.

**ZETA POTENTIAL ANALYSIS:**

The particle charge was one of the most important parameter in assessing the physical stability of proliposome. The large numbers of particles were equally charged, then electrostatic repulsion between the particles was increased and thereby physical stability of the formulation was also increased. Typically, the particle charge of colloidal system was measured as zeta potential measured via the electrophoretic mobility of the particles in an

electrical field. Zeta potential analysis of prepared proliposome formulation was carried out using Malvern Zetasizer (Malvern instruments). Before measurement the samples were diluted with de-ionized water and conductivity was adjusted by addition of sodium chloride.

**FOURIER TRANSFORM INFRA- RED SPECTROSCOPY:**

FT- IR spectra were recorded on the sample prepared in KBr disks (2 mg sample in 200 mg KBr disks) using Shimadzu Fourier Transform Infra-Red spectrometer. The samples were scanned over a frequency range 4000-400 $cm^{-1}$.

**RE-DISPERSIBILITY & PERCENTAGE DRUG CONTENT DETERMINATION:**

The prepared proliposomes were analyzed for drug content by UV spectroscopic method. Different batches of proliposome equivalent to 10 mg of silymarin weighed accurately and dissolved in 10 ml ethanol. The stock solutions were diluted with distilled water and analyzed by UV spectroscopy at 287 nm.

**SATURATION SOLUBILITY STUDIES:**

The saturation solubility studies were carried out for both the unprocessed pure drug and different batches of lyophilized proliposomes. 10 mg of unprocessed pure drug and proliposome equivalent to 10 mg of silymarin was weighed and separately introduced into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks were sealed and placed in rotary shaker for 24 hours at 37 °C and equivalent for 2 days. The samples were collected after the specified time interval and it is filtered and analyzed. The samples were analyzed using UV spectrophotometer at 287 nm.

***IN-VITRO* DRUG RELEASE STUDIES:**

The *in- vitro* release of silymarin drug and its proliposome formulation was carried out in USP dissolution test apparatus using paddle method at a rotation speed of 50 rpm. The dissolution profile was carried out in freshly prepared acidic buffer (pH 1.2) and also in phosphate buffer (pH 7.2) 10 mg of pure drug and proliposome containing 10 mg of silymarin equivalent was taken and placed in dissolution medium. The volume and temperature of dissolution medium were 900 ml and 37.0 ± 0.2 °C, respectively. Samples were withdrawn at fixed time intervals and were filtered. The filtered samples were analyzed at 287 nm using Shimadzu UV-Visible spectrophotometer. The results obtained for different proliposome formulations were compared with the dissolution profile of unprocessed drug.

**PERMEATION STUDIES:**

Permeation study was carried out for both unprocessed drug and different proliposome formulations using cellulose nitrate membrane. The membrane was attached to the franz diffusion cell and then it was dipped in a beaker containing phosphate buffer pH 7.2. The pure drug sample and equivalent quantity of lyophilized proliposome were weighed and placed in the different diffusion cell containing the specific quantity of buffer. The samples were withdrawn at specific time intervals in 10 minutes and replaced with fresh buffer solution. Finally the samples were analyzed using UV spectrophotometer at 287 nm.

**PHARMACOLOGICAL ACTIVITY OF PROLIPOSOME FORMULATIONS**

**HEPATOPROTECTIVE ACTIVITY**

This study was approved by animal ethical committee from Nandha College of pharmacy. The animal ethical committee approval number is **NCP/IPEC/2015-16-04**.

Albino Wister strain rats were divided into five groups of six each. CIS-toxicity was induced by a single dose intraperitoneal injection (i.p.) of Cis (i.p., 7 mg/kg b.w.).

**Group I:** (Control group) animal served as normal control.

**Group II:** (CIS treated group) rats received a single dose of cisplatin i.p on fifth day of the experiment at a dose of 7 mg/kg [73].

**Group III:** (CIS-NAR) rats of this group were administrated with naringenin (10 mg/kg i.p; once daily) for 9 days and on 5th day CIS (7 mg/kg b.w) was injected by i.p route. [74]

**Group IV:** (CIS-SILY) rats of this group were administered with silymarin (10 mg/kg) for 9 days and on 5th day CIS was injected by i.p at a dose of 7 mg/kg.

**Group V: (**CIS-SF2) rats of this group were administered with SF2 (10 mg/kg i.p; once daily) for 9 days and CIS at a dose of 7 mg/kg was injected by i.p. on the fifth day of the treatment. [75]

At the end of the experiment, rats were euthanized under light anesthesia with diethyl ether; rats were sacrificed by cervical decapitation. For biochemical assays, blood samples were collected and serum was separated by centrifugation at 2500 rpm for 15 mins and analyzed for various biochemical parameters. Total bilirubin and direct bilirubin was evaluated by Jendrassik & Grof's Method [76]. Triglycerides activity of serum was tested by the method defined by Glycrokinase peroxidase. Total cholesterol activity was evaluated in accordance with the assay of CHOD-PAP. Albumin and total protein was evaluated by biuret

method [77]. AST, ALT and ALP activities were assessed in serum with commercially available kits (IFCC) by using an auto analyze [78]; livers were excised, rinsed clean in saline and preserved in 10% formalin for histopathological study [79].

# *RESULTS AND DISCUSSION*

## RESULT AND DISCUSSION

Silymarin is BCS Class – II drug with low solubility and high permeability. Thus, it was challenging to enhance the solubility and dissolution rate of silymarin particles in an aqueous solution. Film deposition method was employed to produce proliposome of silymarin.

# PREFORMULATION STUDIES:

### CHARACTERIZATION OF DRUG:

The sample of silymarin was evaluated by physical character and by determining the melting point. The Ultra-violet (UV) and FT-IR absorption spectrum of pure silymarin was recorded, which was compare and matched with IP reference spectra.

### PHYSICAL OBSERVATION:

Physical observation of the drug revealed that silymarin is yellow powder.

### SOLUBILITY STUDIES:

Silymarin is insoluble in water (0.04 mg/ml) and slightly soluble in dimethyl sulfoxide, and dimethyl formamide.

### MELTING POINT DETERMINATION:

The melting point of Silymarin was found to be 234 °C and it was matched with literature, which assured the identity of the received sample.

### ULTRA-VIOLET (UV) ABSORPTION SPECTRA:

The silymarin was analyzed by spectrophotometrically in between 200 nm – 400 nm. The maximum absorbance ($\lambda_{max}$) was found at 287 nm which was used for quantitative analysis. The overlay spectrum of silymain was mentioned in figure.5.

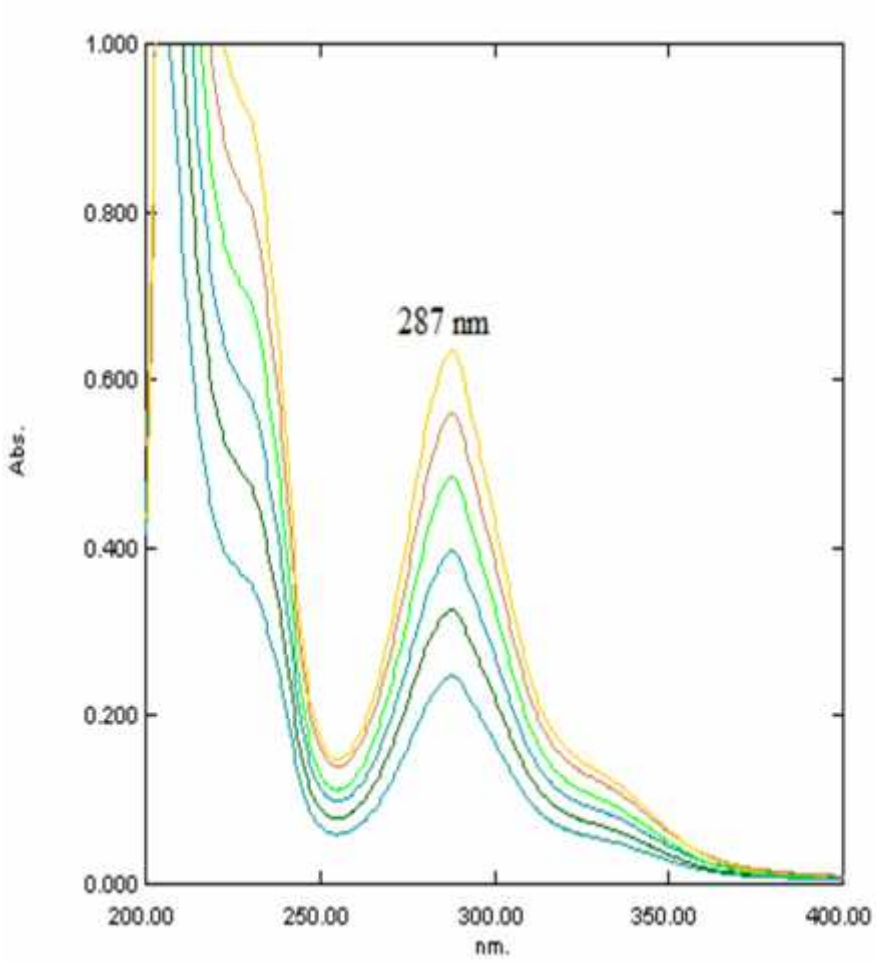



**Figure.5: Overlay Spectrum of Silymarin.**

**PREPARATION OF STANDARD CURVE OF SILYMARIN:**

**Standard curve in 0.1 N HCl & phosphate buffer (pH 7.2):**

The standard curve was prepared by using various concentrations versus absorbance at 287 nm. The calibration curve was found linear at different concentration at range in 10 – 100 µg/ml.

From the below data (Table. 3) it was observed that the drug obeys beer's law in concentration range of 10 -100 µg/ml in 0.1 N HCl. The slope is 0.0149, the correlation coefficient is 1.009 it follows fit curve since $r^2$ is around 1.

**Table. 3: Standard curve of silymarin in 0.1N HCl**

| S.No | Concentration in µg/ml | Absorbance at 287 nm |
|---|---|---|
| 1. | 10 | 0.157 |
| 2. | 20 | 0.171 |
| 3. | 30 | 0.185 |
| 4. | 40 | 0.198 |
| 5. | 50 | 0.212 |
| 6. | 60 | 0.227 |
| 7. | 70 | 0.241 |
| 8. | 80 | 0.258 |
| 9. | 90 | 0.273 |
| 10. | 100 | 0.295 |

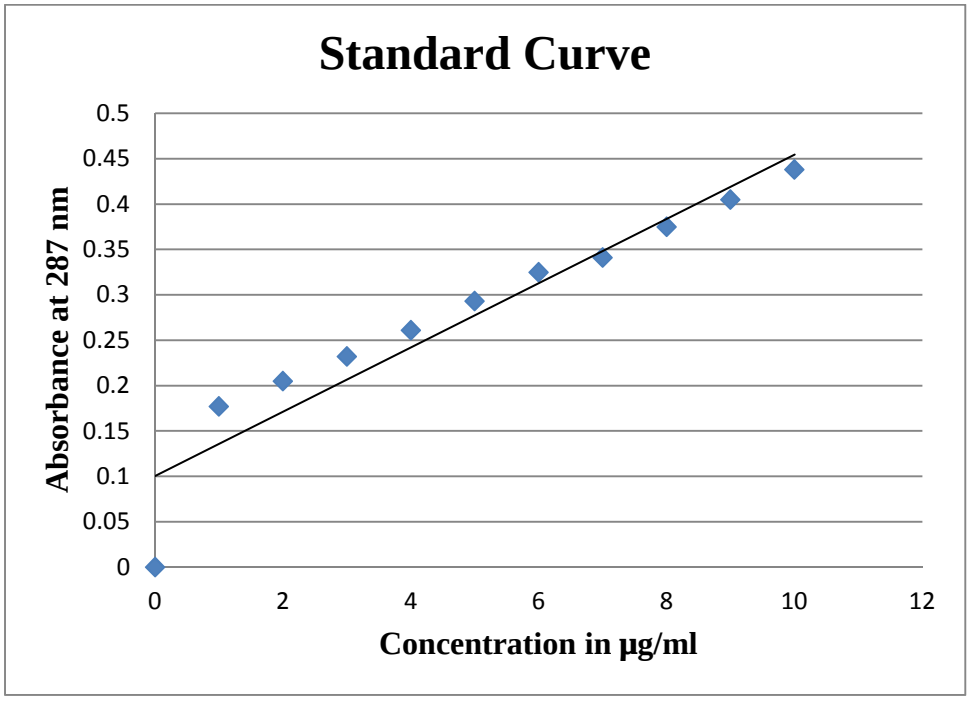



**Figure.6: Standard curve of silymarin in 0.1N HCl.**

Regression Coefficient ($r^2$) = 1.009, Slope = 0.0149

From the below data (Table. 4) it was observed that the drug obeys beer's law in concentration range of 10 -100 µg/ml in pH 7.2 phosphate buffer. The slope is 0.0287, the correlation coefficient is 1.002 it follows fit curve since $r^2$ is around 1.

**Table. 4: Standard curve of silymarin in phosphate buffer**

| S.No | Concentration in µg/ml | Absorbance at 287 nm |
|---|---|---|
| 1. | 10 | 0.177 |
| 2. | 20 | 0.205 |
| 3. | 30 | 0.232 |
| 4. | 40 | 0.261 |
| 5. | 50 | 0.293 |
| 6. | 60 | 0.325 |
| 7. | 70 | 0.341 |
| 8. | 80 | 0.375 |
| 9. | 90 | 0.405 |
| 10. | 100 | 0.438 |

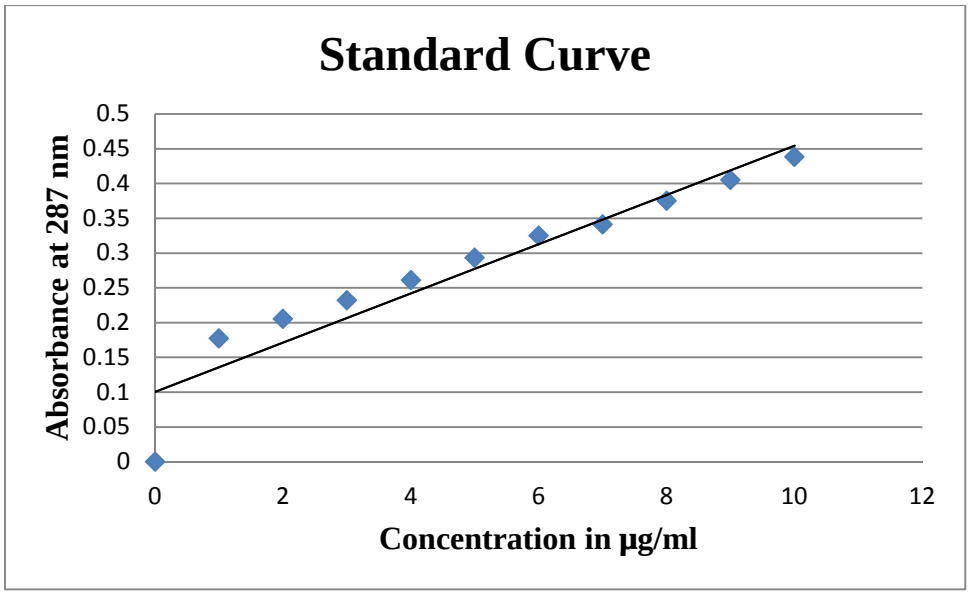



**Figure 7: Standard curve of silymarin in phosphate buffer.**

Regression Coefficient ($r^2$) = 1.002, Slope = 0.0287

**DRUG AND POLYMER COMPATIBILITY STUDY:**

**FOURIER TRANSFORM INFRA RED SPECTROSCOPY:**

FTIR Spectroscopy was used to study the possible interaction between pure drug and polymers. The FT-IR spectra of pure silymarin, soya lecithin, cholesterol, sorbitol, TPGS and physical mixture of the drug were recorded. The characteristic peaks for silymarin can be observed. Similar peak were seen in physical mixture of silymarin and polymers. There was no discrimibile shift/disappearance/appearance of peaks in combined spectra that indicated good drug – polymer compatibility and no chemical interaction between silymarin and polymers. Hence, all the polymers were found suitable for development of the proliposome. The values are representated in the Table. 5 and Table. 6.

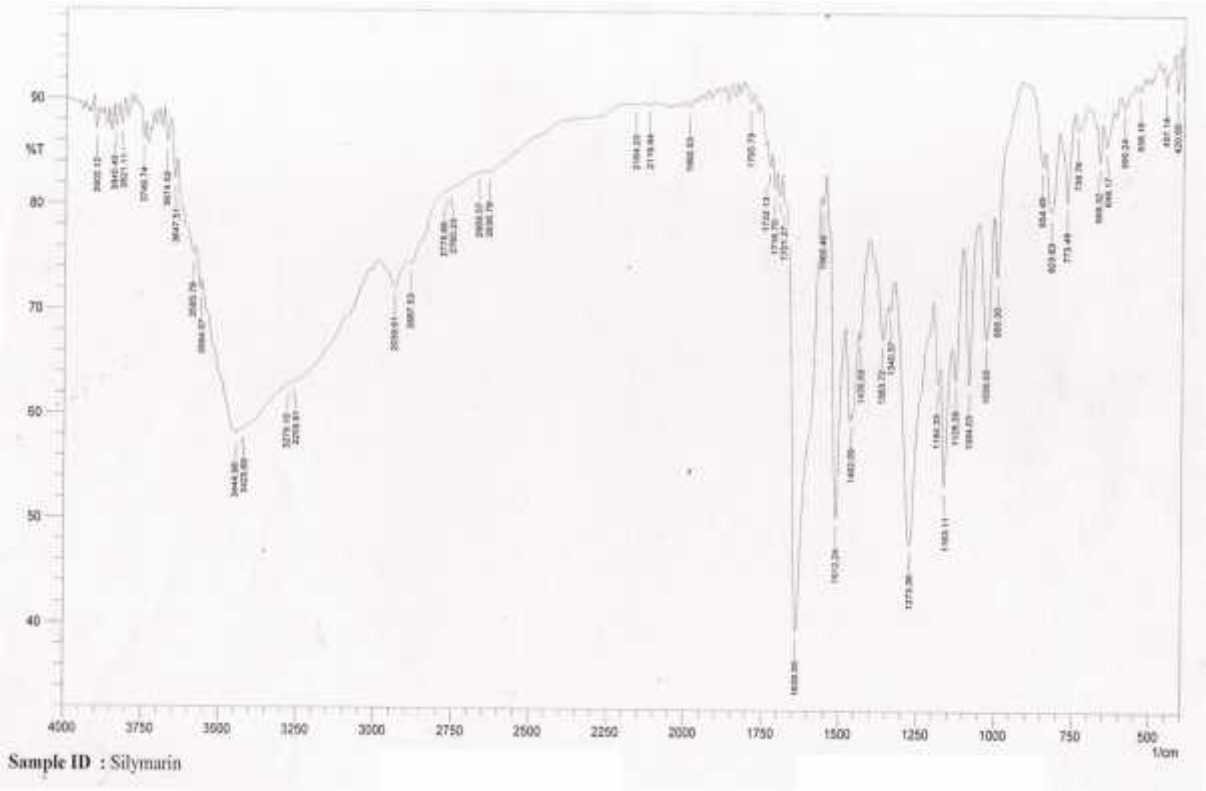



**Figure.8: FTIR spectrum of silymarin.**

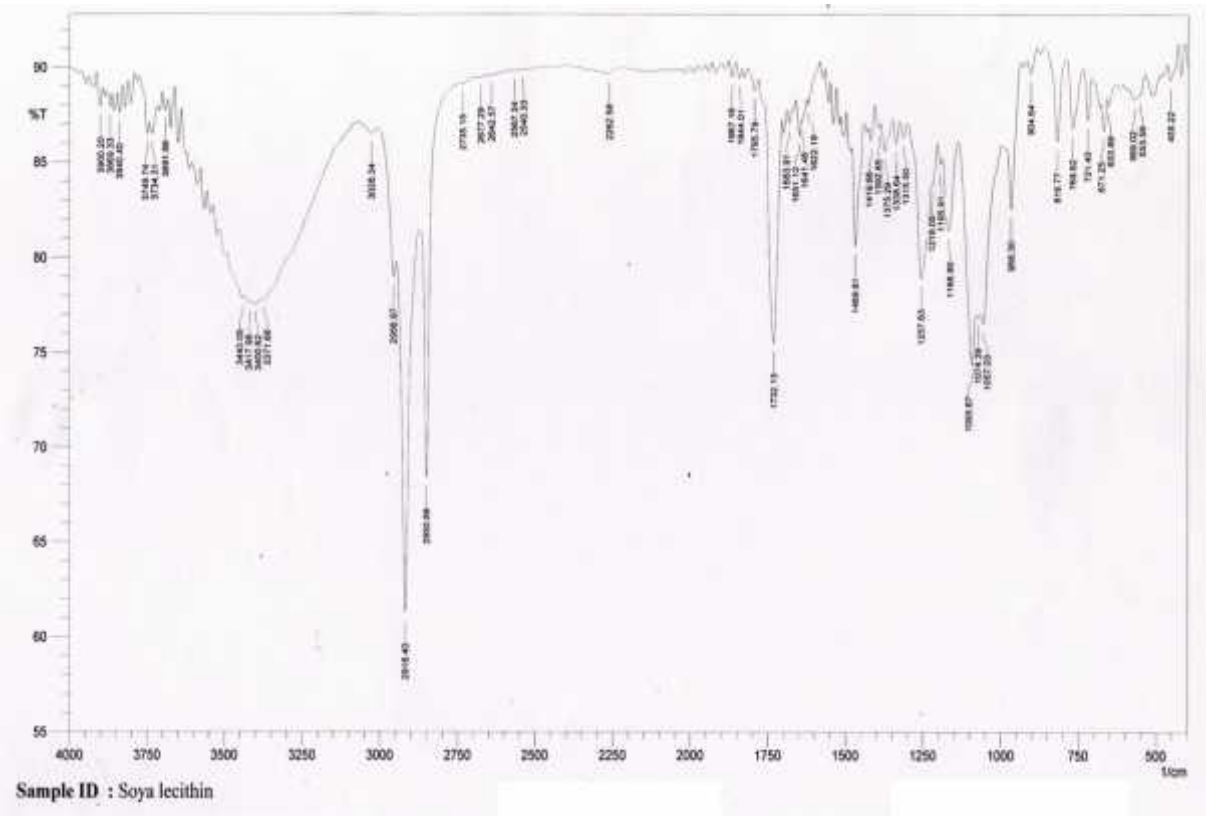



**Figure.9: FTIR spectrum of soya lecithin.**

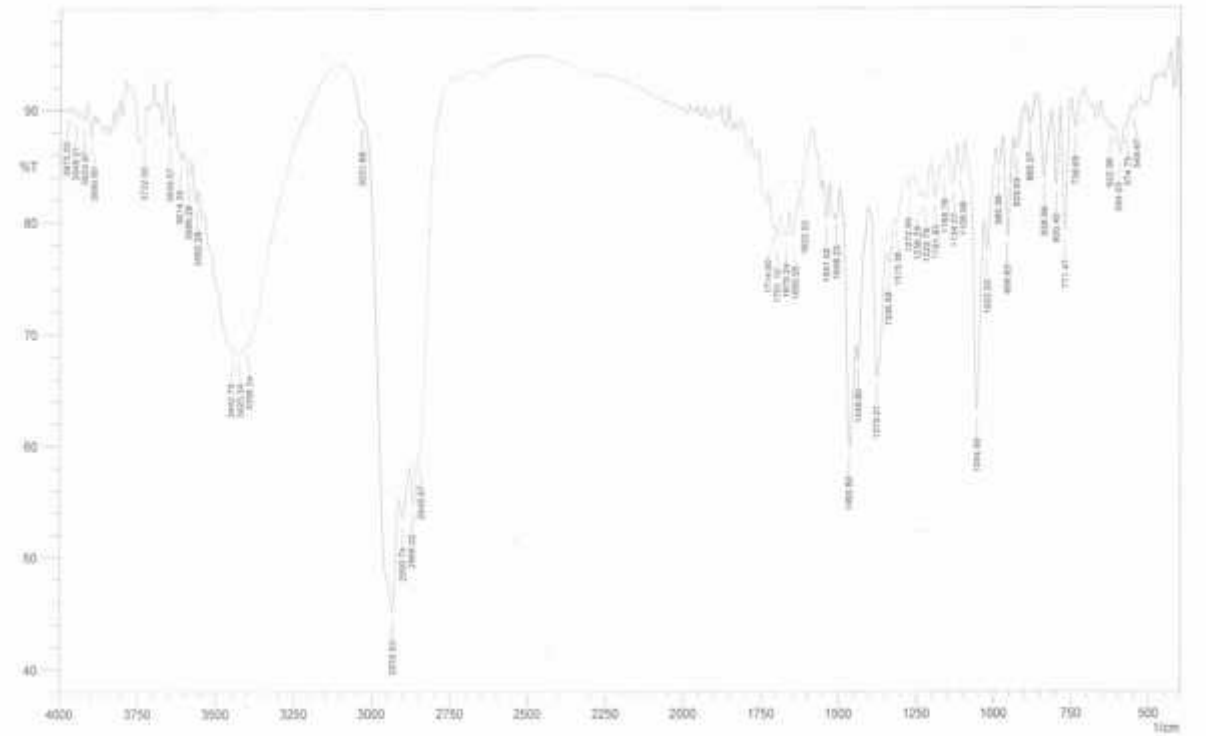



**Figure.10: FT-IR spectrum of cholesterol.**

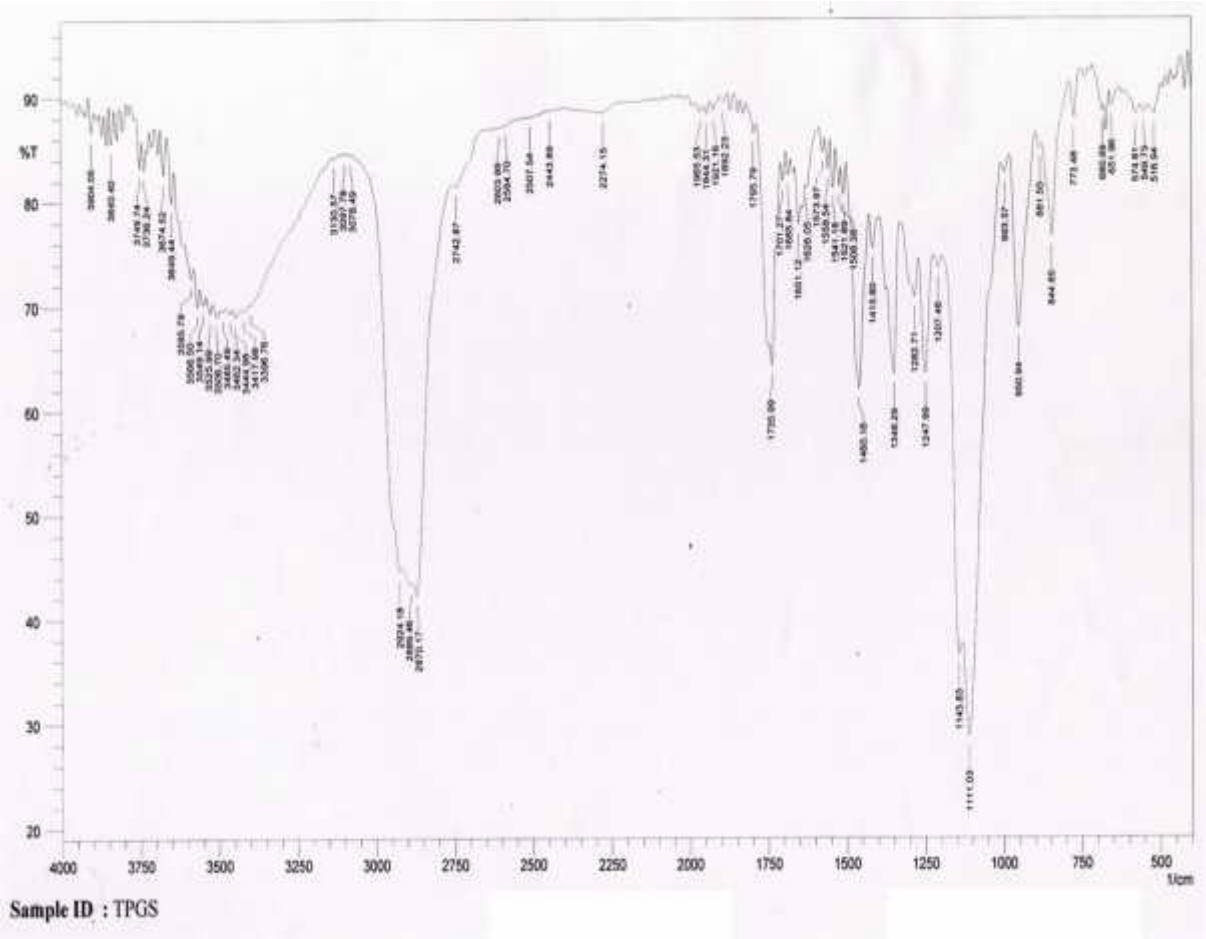



**Figure.11: FTIR spectrum of TPGS.**

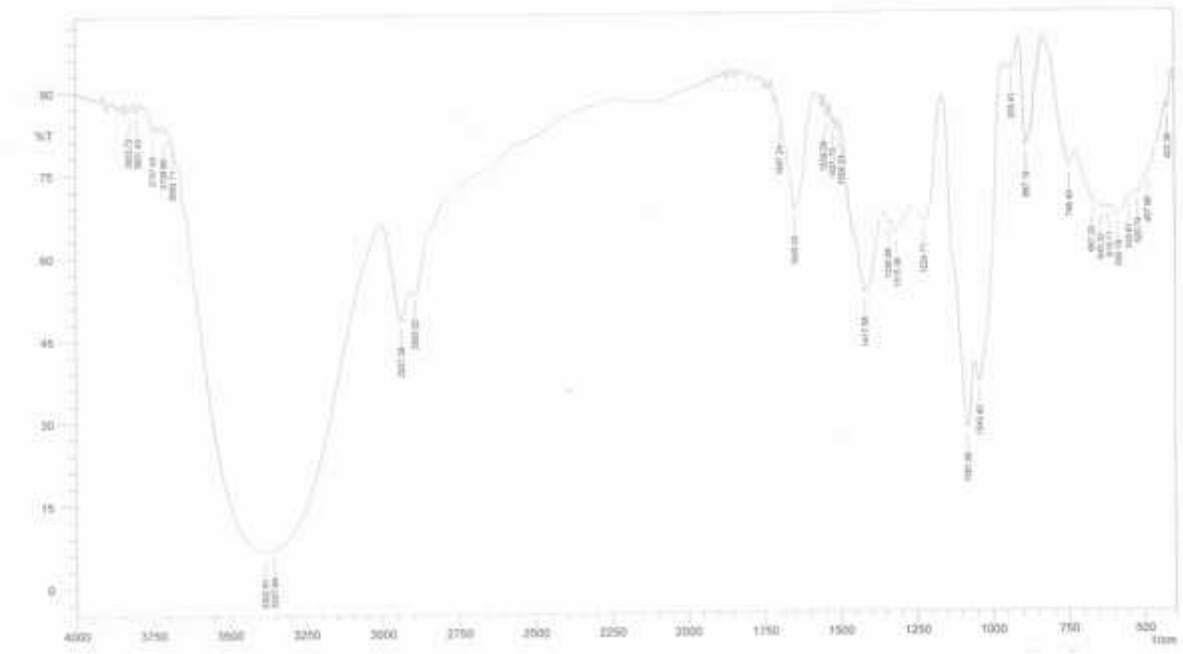


**Figure.12: FTIR spectrum of sorbitol.**

**Table. 5: Interpretation of IR spectrum of silymarin, soya lecithin, cholesterol, sorbitol and TPGS.**

| Transition | IR Range ($cm^{-1}$) | Absorption wave number ($cm^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | Silymarin | Soya Lecithin | Cholesterol | Sorbitol | TPGS |
| **O-H Stretching Alcohols, phenols** | **3500 - 3200** | 3444.98 – 3259.81 | 3443.05 – 3371.68 | 3442.70 , 3425.34, 3398.34 | 3382.91 ,3357.84 | 3485.49 – 3396.76 |
| **O-H Stretching Carboxylic acid** | **3300 - 2500** | 3279.10 – 2636.78 | 2956.97- 2850.88 | 3031.89 – 2848.67 | 2937.37 ,2893.02 | 2924.18 – 2870.17 |
| **C-H Stretching Alkane** | **3000 - 2850** | - | - | 2933.53 , 2900.74 , 2866.02 | 2937.37, 2893.02 | 2742.87 |
| **HC≡CH Stretching Alkynes** | **2260 - 2100** | 2164.20, 2119.84 | 1732.13 | - | - | 1735.99 – 1685.84 |
| **C=O Stretching Carbonyl** | **1760 - 1665** | - | 1469.81 | 1714.60 – 1670.24 | 1697.24 | 1460.16 |
| **-C=C- Stretching Alkenes** | **1680 - 1640** | 1639.55 | 1375.29 – 1338.64 | 1670.24 , 1650.95 | 1649.02 | 1348.29 |
| **C=C Stretching Heterocyclic aromatic** | **1550 - 1475** | 1512.24 | 1257.63 | 1541.02 , 1508.23 | 1539.09 , 1521.73, 1508.23 | 1282.71, 1247.99 |
| **C-H Bending Alkanes** | **1470 - 1450** | 1462.09 | - | 1465.80 | - | 1247.99 – 1111.03 |
| **C-O Stretching Alcohol, Carboxylic acid** | **1320 - 1000** | 1184.33 – 1128.39 | 1093.67 – 1057.03 | 1315.36 – 1022.20 | 1315.36- 1043.42 | - |
| **=C-H Bending Alkenes** | **1000 - 650** | 823.63 | 968.30 - 653.89 | 985.56 – 738.69 | 935.41- 667.32 | 993.37 – 651.96 |

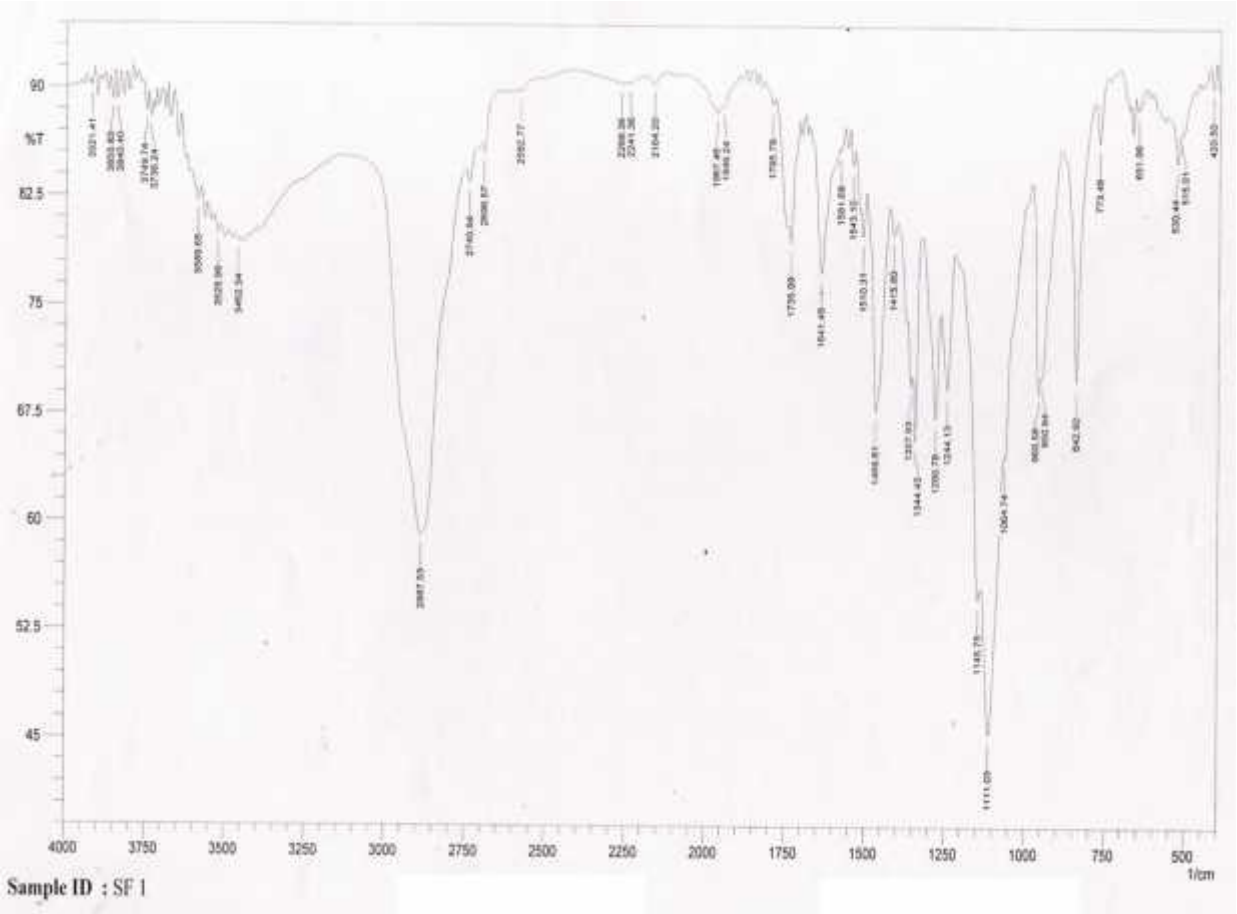



**Figure.15: FTIR spectrum of physical mixture of silymarin formulation 1.**

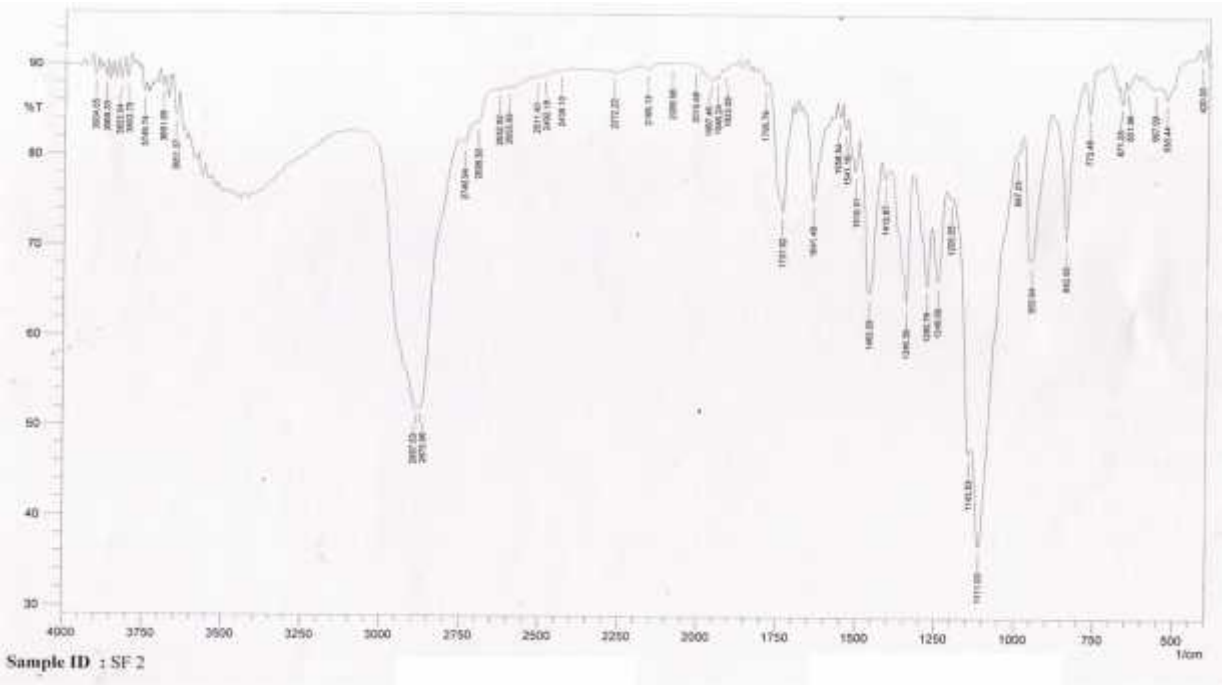



**Figure.16: FTIR spectrum of physical mixture of silymarin formulation 2.**

**Table. 6: Interpretation of IR spectrum of silymarin proliposome formulation physical mixtures of SF1 and SF2.**

| Transition | IR Range ($cm^{-1}$) | Absorption wave number ($cm^{-1}$) | | |
|---|---|---|---|---|
| | | Silymarin | SF1 | SF2 |
| **O-H Stretching Alcohols, phenols** | **3500 - 3200** | 3444.98 – 3259.81 | 3462.34 | 3483.56 – 3240.52 |
| **O-H Stretching Carboxylic acid** | **3300 - 2500** | 3279.10 – 2636.78 | 2740.94, 2696.57 | 3119.00 |
| **C-H Stretching Alkane** | **3000 - 2850** | - | 2887.53 | 2887.53 |
| **HC≡CH Stretching Alkynes** | **2260 - 2100** | 2164.20, 2119.84 | 2268.36 – 2164.20 | 2166.13 |
| **C=O Stretching Carbonyl** | **1760 - 1665** | - | 1735.99 | 1737.92 |
| **-C=C- Stretching Alkenes** | **1680 - 1640** | 1639.55 | 1641.48 | 1641.48 |
| **C=C Stretching Heterocyclic aromatic** | **1550 - 1475** | 1512.24 | 1543.10, 1510.31 | 1541.18 – 1510.31 |
| **C-H Bending Alkanes** | **1470 - 1450** | 1462.09 | 1469.81 | 1462.09 |
| **C-H Rocking Alkanes** | **1370 - 1350** | 1363.72 | 1357.93 | 1346.36 |
| **C-O Stretching Alcohol, Carboxylic acid** | **1320 - 1000** | 1184.33 – 1128.39 | 1111.03 | 1143.83, 1111.03 |
| **=C-H Bending Alkenes** | **1000 - 650** | 823.63 | 960.58 – 842.92 | 950.94, 842.92 |

**FORMULATION OF PROLIPOSOMES:**

Silymarin proliposomes was prepared by film deposition method using different polymers such as soya lecithin, cholesterol, sorbitol and TPGS respectively. The polymers were selected based on literature review and preformulation studies. The ratio of silymarin proliposome formulations are representated in Table.7.

| S.No | Formulation Code | Silymarin (gm) | Soya lecithin (gm) | Cholesterol (gm) | Sorbitol (gm) | TPGS (gm) |
|---|---|---|---|---|---|---|
| 1. | SF1 | 1 | 2 | 2 | 5 | - |
| 2. | SF2 | 1 | 2 | 2 | 5 | 0.5 |

**Table. 7: Composition of drug loaded using different polymers.**

**LYOPHILIZATION:**

The lyophilized proliposomes was performed to increasing the stability. The prepared silymarin proliposomes was lyophilized using freeze dryer at -70 °C for 3 days. Then vacuum was applied. 1 % mannitol solution was added as a cryoprotectant. Degassing was carried out in between to prevent explosion.

**EVALUATION OF PROLIPOSOMES:**

**PARTICLE SIZE AND POLY DISPEERSITY INDEX:**

The particle size distribution has most important characteristics affecting the *in-vivo* fate of proliposomes. The particle size was measured by Malvern particle size analyzer. The proliposomes mean particle size ranges from SF1 (100.6nm) and SF2 (80.52nm) for without stabilizer and with stabilizer respectively as shown in Table. 8.

Polydispersity index gives degree of particle size distribution and promotes the physical stability of proliposomes. The ranges of the formulations SF1 and SF2 from 0.265 and 0.284 respectively. The formulation SF2 showed lowest particle size (80.52nm) that indicates good uniformity in particle size distribution.

**Table. 8: Particle size and poly dispersity index of proliposomes**

| S.No | Formulations | Average Particle size (d.nm) | Poly dispersity index |
|---|---|---|---|
| 1. | SF1 | 100.6 ± 0.19 | 0.265 ± 0.07 |
| 2. | SF2 | 80.52 ± 0.11 | 0.284 ± 0.03 |

**Mean of three observation ± SD.**

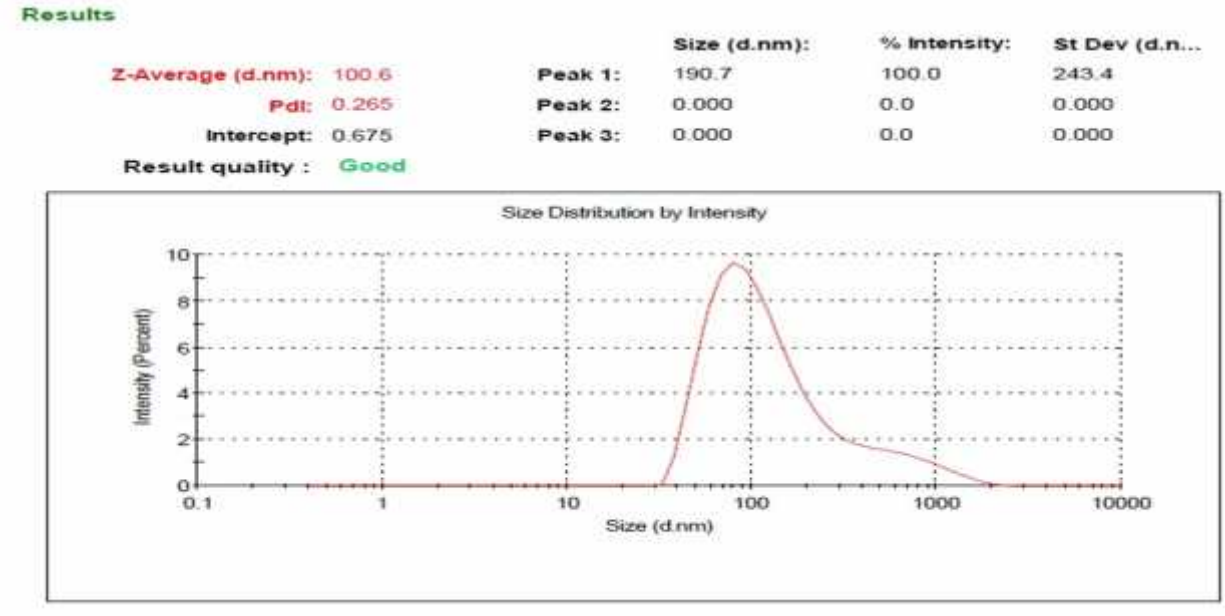



**Figure.17: Particle size distribution and poly dispersity index of proliposomes SF1.**

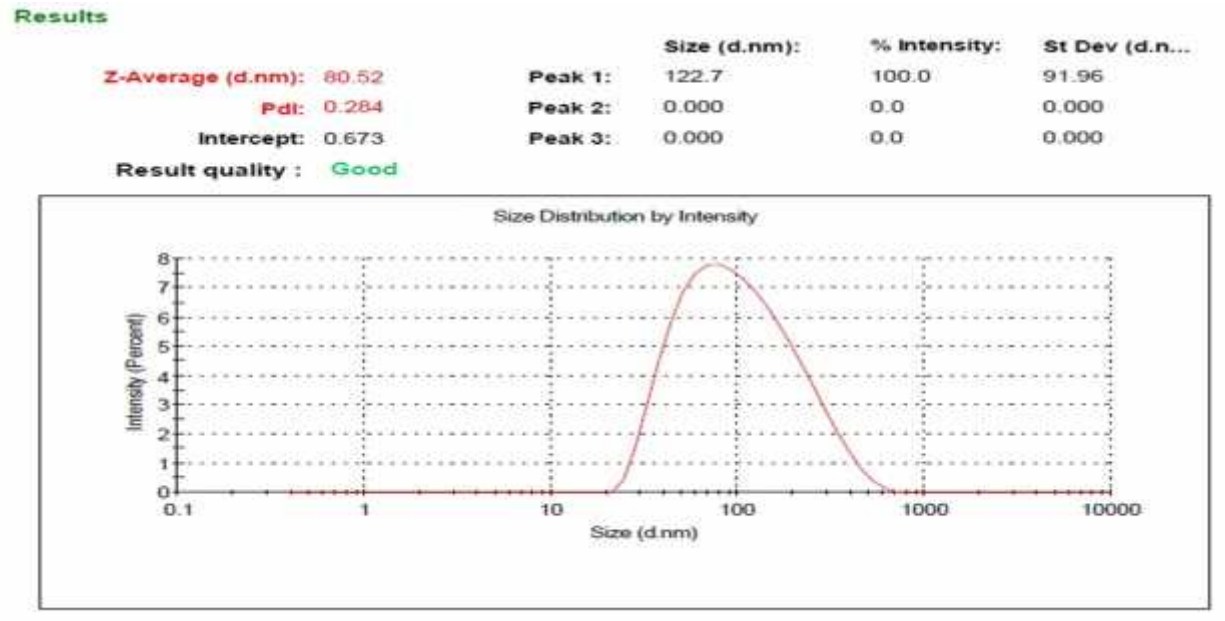



**Figure.18: Particle size distribution and poly dispersity index of proliposomes SF2.**

**ZETA POTENTIAL ANALYSIS:**

The determination of the zeta potential parameter (properly related to the double electric layer on the surface of colloidal particles) of a proliposomes is an essential as it provides an indication about the physical stability of proliposomess. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensures easy redispersion. In the case of combined electrostatic and steric stabilization, a minimum zeta potential of ± 20 mV is desirable. In the study of zeta potential of proliposomes formulations SF1 and SF2 was found to be in the range of SF1 (-18.6 mV) and SF2 (-4.75 mV) respectively, which indicates good physical stability of proliposomes. The zeta potential graphs are presented in Figures 19 and 20.

**Table. 9: Zeta potential of proliposomes.**

| S.No | Formulations | Zeta potential (mV) |
|---|---|---|
| 1. | SF1 | -18.6 |
| 2. | SF2 | -4.75 |

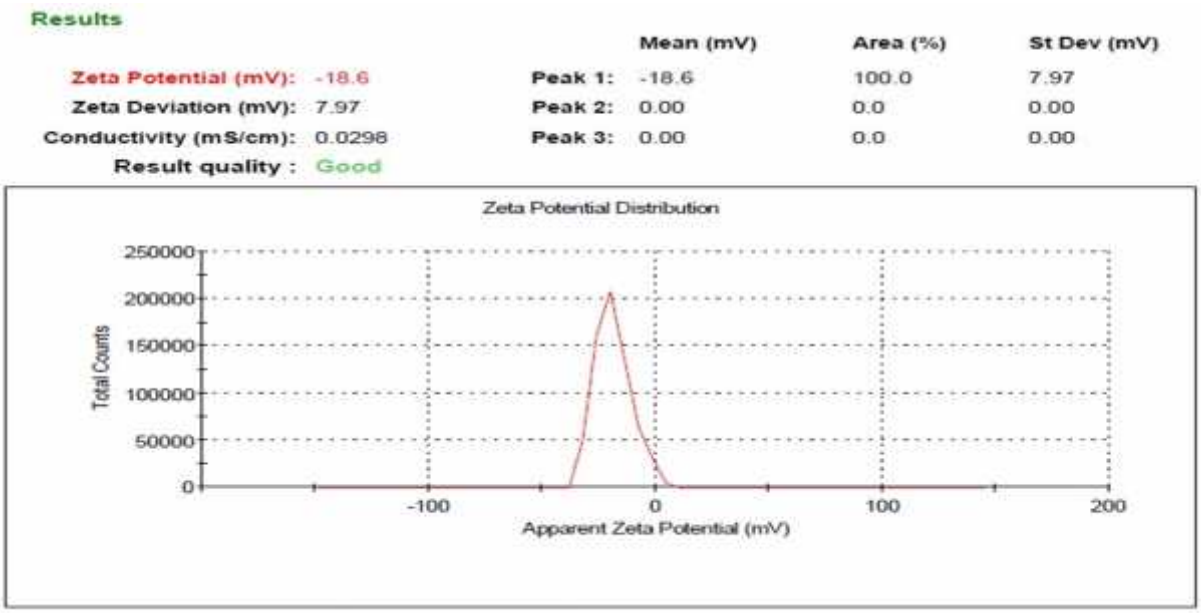



**Figure.19: Zeta potential of proliposome SF1.**

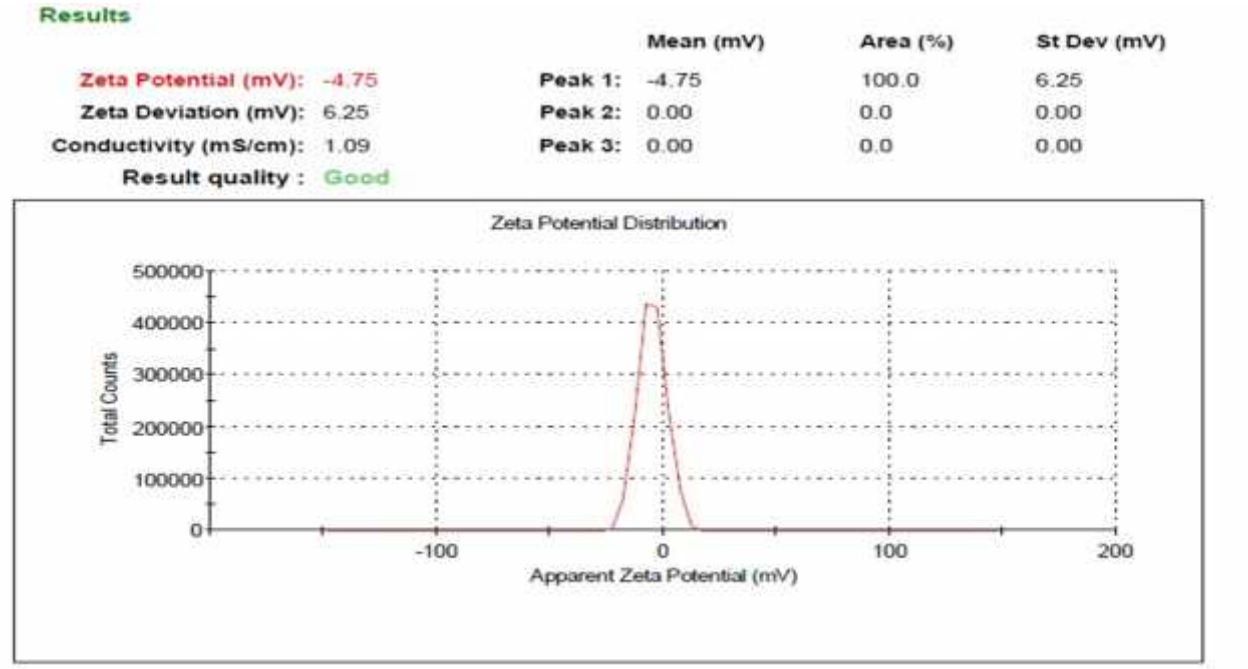



**Figure.20: Zeta potential of proliposome SF2.**

**FOURIER TRANSFORM INFRA RED SPECTROSCOPY:**

The FT-IR analysis was used to evaluate the possible intermolecular interaction between silymarin and the excipients. The FT-IR spectra of formulated lyophilized were recorded. Due to similarities in molecular structure of soya lecithin, cholesterol, sorbitol and TPGS showed similar absorption bands, in which the IR spectra of formulation showed all the characteristics peaks without any markable change in their position after successful lyophilized proliposome, it indicate there is no chemical interaction between silymarin, soya lecithin, cholesterol, sorbitol and TPGS in proliposomes. In proliposome formulation (SF2), a characteristic peak of silymarin was observed. The values are representated in the Table.10.

**Table.10: Interpretation of IR spectrum of proliposome formulations of SF1 and SF2.**

| Transition | IR Range ($cm^{-1}$) | Absorbance wave number ($cm^{-1}$) | |
|---|---|---|---|
| | | SF1 | SF2 |
| **O-H Stretching alcohol, phenol** | **3500 - 3200** | 3504.77 – 3363.97 | 3483.56 – 3331.18 |
| **C-H Stretching alkenes** | **3100 - 3000** | 3101.64 – 3032.20 | 3119.00 |
| **C-H Stretching alkanes** | **3000 – 2850** | 2918.40 – 2856.67 | 2891.39, 2874.03 |
| **HC≡CH Stretching alkynes** | **2260 - 2100** | 2164.20, 2115.98 | 2266.43, 2164.20 |
| **C=O Stretching carboxylic acid** | **1760 - 1690** | 1739.85 | 1741.78 |
| **-C=C- Stretching alkenes** | **1680 - 1640** | 1639.55 | 1643.41 |
| **C-C Stretching aromatic** | **1500 - 1400** | 1458.23 | 1458.23 |
| **C-H Rocking alkanes** | **1370 - 1350** | 1356.00 | 1348.29 |
| **C-O Stretching alcohols ester** | **1320 – 1000** | 1107.18 | 1111.03 |
| **=C-H Bending alkenes** | **1000 - 650** | 995.30 – 740.69 | 950.94 – 773.48 |

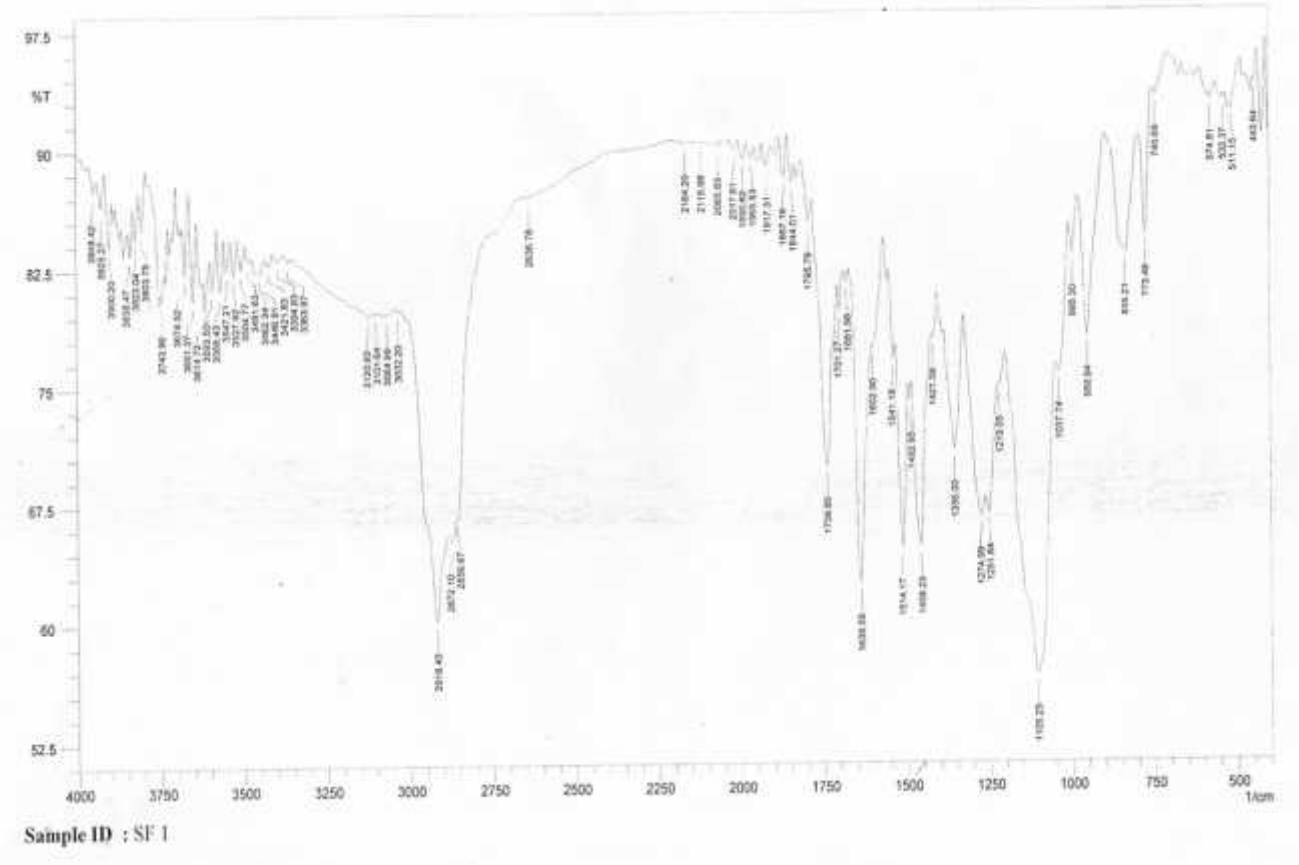



**Figure.21: FT-IR spectrum of proliposome silymarin formulation 1.**

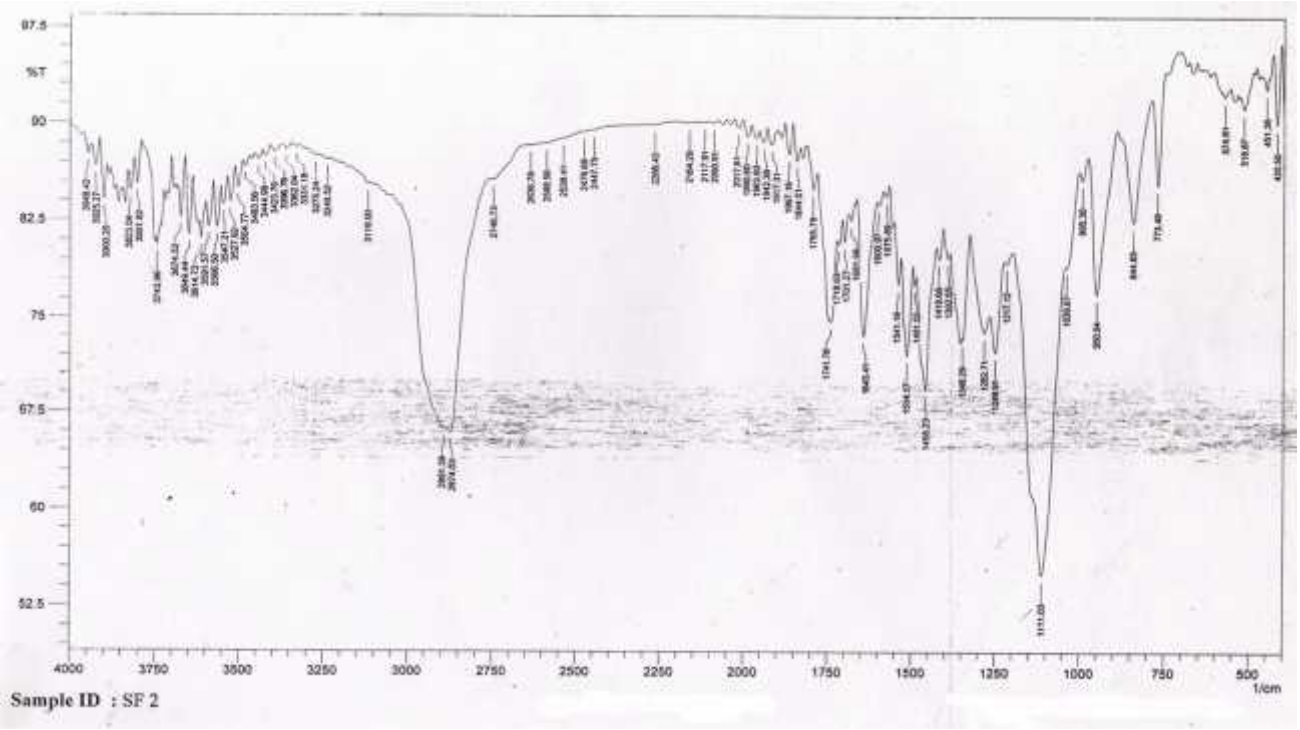



**Figure.22: FTIR spectrum of prolioposome silymarin formulation 2.**

**DRUG CONTENT DETERMINATION:**

In proliposome formulation the drug particles were reduced to nano size. During the formulation, the drug content of silymarin proliposome formulations was found to be in the range of 95.49, 99.61 respectively, which was found to be within the range of ± 5% of the theoretical claim. The results showed the all proliposome formulations have shown the presence of high drug content low standard deviation and loss of drug was lower during preparation process. It indicates that the drug is uniformly dispersed in the powder formulation. Therefore, the method used in the study appears to be reproducible for preparation of proliposomes. The results are as given in Table. 11.

**Table. 11: Percentage drug content of all lyophilized silymarin proliposome formulations.**

| S.No | Formulation code | % drug content |
|---|---|---|
| 1. | SF1 | 95.49 ± 0.63 |
| 2. | SF2 | 99.61 ± 0.32 |

**Mean of three observations ± SD**.

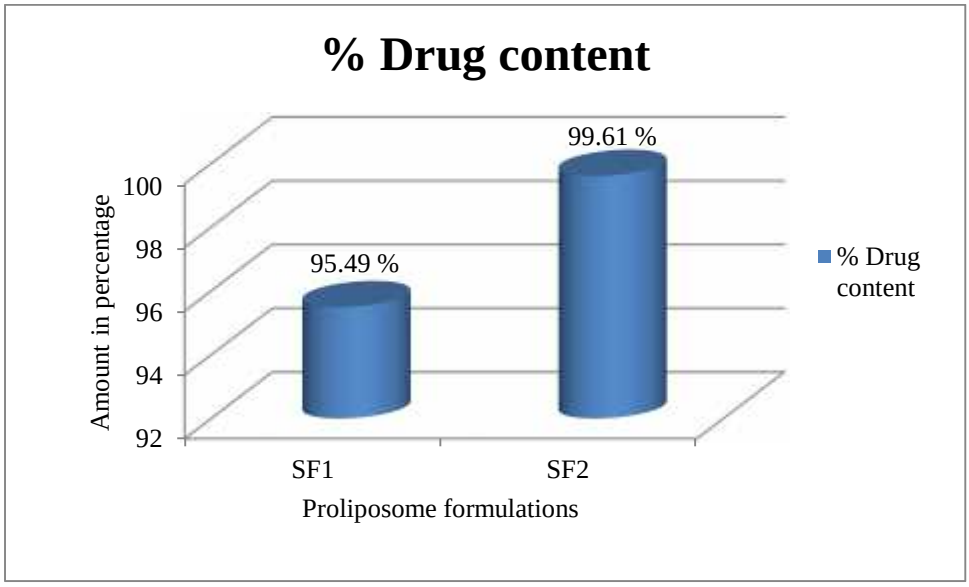



**Figure.23: Percentage drug content of all lyophilized silymarin proliposome formulations.**

**SATURATION SOLUBILITY STUDIES:**

The solubility profile of silymarin proliposome formulations increases dissolution velocity and saturation solubility, size reduction leads to increase in dissolution rate. The solubility of silymarin showed 19.67 µg/ml in 0.1 N HCl and 25.45 µg/ml in phosphate buffer (pH 7.2). The saturation solubility studies of crystallization investigation, indicating that proliposome showing maximum solubility compared to unprocessed drug is due to the amorphous nature of drug after film deposition method.

The saturation solubility increased with an increase in carrier proportion of all carriers. This might be due to better wetting ability associated with soya lecithin (2 gm), cholesterol (2 gm), sorbitol (5 gm) and TPGS (0.5 gm) of proliposomes. With stabilizer proliposome formulation (SF2) have very fine particle size and hence large surface area, so as the proportion of carrier increases; a large surface is presented for adsorption of the drug crystals. Enhancement in saturation solubility was found to in order of SF2 > SF1. The solubility of prepared proliposomes in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.2) are represented in Table. 12 figure. 24.

**Table. 12: Saturation solubility studies of pure drug and proliposomes.**

| S.No | Formulation Code | Absorbance at 287 nm | |
|---|---|---|---|
| | | 0.1 N HCl BUFFER | PHOSPHATE BUFFER |
| 1. | Pure | 19.67 ± 0.04 | 25.45 ± 0.23 |
| 2. | SF1 | 73.24 ± 0.48 | 84.31 ± 0.16 |
| 3. | SF2 | 91.73 ± 0.17 | 98.21 ± 0.53 |

**Mean of three observation ± SD.**

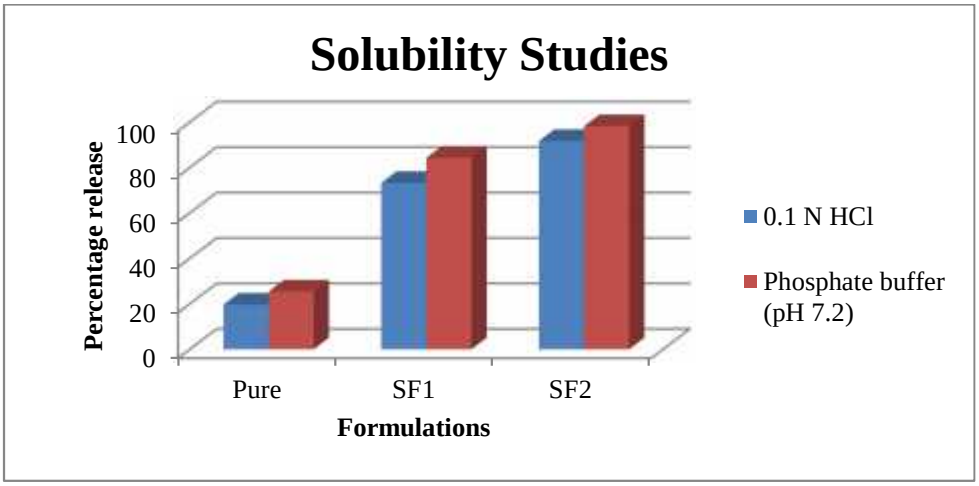



**Figure. 24: Saturation solubility studies of pure drug and proliposomes.**

***IN – VITRO* DISSOLUTION STUDIES:**

The most important feature of proliposome is the increase in the dissolution velocity, not only because of increase in surface area but also because of increasing saturation solubility. Silymarin is a poorly soluble drug. Its solubility is pH dependant increasing with 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.2) was selected for dissolution studies to stimulate gastric condition and allows the greater discrimination of our processing effects. In order to assess the goal of improving the dissolution rate of lyophilized silymarin in proliposome was achieved. *In-vitro* dissolution profile of the percentage release versus time profile of pure silymarin and different silymarin proliposome samples were determined in 0.1 N HCl and phosphate buffer under sink condition.

*In-vitro* drug release data from the proliposome were carried out for 12 hours and graphically represented as percentage drug release versus time profile (Figure 25 and 26).

The dissolution rate of pure drug is very low. Only 31% of the drug was released in 0.1 N HCl and 37 % of the drug were released in phosphate buffer at the end of 12 hours. On the contrary, proliposome dissolution rate is increased more than the pure drug. This could be due to the increased surface area of the drug and possible better contact between the proliposome and dissolution medium. In case of proliposome formulations SF1 and SF2 showed that 79.71% (SF1), 93.7 % (SF2) (in 0.1 N HCl) (table. 13) and 89.78% (SF1), 98.35% (SF2) (in phosphate buffer) (table. 14) drug was released at the end the end of 12 hours with increasing carrier proportion. Based on the above data SF2 shows maximum *in-vitro* release in 0.1 HCl and phosphate buffer.

**Table. 13: Comparative dissolution profile of lyophilized proliposome and pure drug in acid buffer (pH 1.2)**

| S.No | Time in Hours | Percent of (±SD) drug release | | |
|---|---|---|---|---|
| | | PURE | SF1 | SF2 |
| 1. | 0 | 0.00 ± 0.0 | 0.00 ± 0.00 | 0.00 ± 0.0 |
| 2. | 1 | 5.43 ± 0.04 | 12.68 ± 0.31 | 16.30 ± 0.12 |
| 3. | 2 | 13.29 ± 0.46 | 21.75 ± 0.37 | 24.78 ± 0.52 |
| 4. | 3 | 16.93 ± 0.28 | 27.82 ± 0.46 | 32.66 ± 0.27 |
| 5. | 4 | 21.18 ± 0.32 | 32.08 ± 0.32 | 41.50 ± 0.18 |
| 6. | 5 | 23.01 ±0.26 | 40.57 ± 0.07 | 49.65 ± 0.01 |
| 7. | 6 | 23.64 ± 0.17 | 46.05 ± 0.01 | 59.37 ± 0.12 |
| 8. | 7 | 25.48 ± 0.05 | 53.95 ±0.14 | 69.10 ± 0.15 |
| 9. | 8 | 26.72 ± 0.32 | 62.47 ± 0.07 | 80.05 ±0.05 |
| 10. | 9 | 27.35 ± 0.21 | 72.20 ± 0.26 | 88.60 ± 0.32 |
| 11. | 10 | 28.59 ± 0.14 | 77.12 ± 0.18 | 90.51 ± 0.08 |
| 12. | 11 | 29.22 ± 0.42 | 79.62 ± 0.25 | 93.63 ± 0.13 |
| 13. | 12 | 31.07 ± 0.29 | 79.71 ± 0.07 | 93.73 ± 0.03 |

**Mean of three observation ± SD.**

**Table. 14: Comparative dissolution profile of lyophilized proliposome and pure drug in phosphate buffer (pH 7.2)**

| S.No | Time in Hours | Percent of (±SD) drug release | | |
|---|---|---|---|---|
| | | PURE | SF1 | SF2 |
| 1. | 0 | 0.00 ± 0.0 | 0.00 ± 0.0 | 0.00 ± 0.0 |
| 2. | 1 | 1.56 ± 0.03 | 29.47 ± 0.02 | 35.12 ± 0.07 |
| 3. | 2 | 5.33 ± 0.07 | 35.15 ± 0.08 | 40.49 ± 0.14 |
| 4. | 3 | 8.78 ± 0.14 | 39.89 ± 0.16 | 47.43 ± 0.19 |
| 5. | 4 | 12.24 ± 0.15 | 45.27 ± 0.23 | 54.70 ± 0.34 |
| 6. | 5 | 16.02 ± 0.28 | 52.84 ± 0.24 | 62.60 ± 0.38 |
| 7. | 6 | 17.92 ± 0.34 | 62.62 ± 0.33 | 71.45 ± 0.45 |
| 8. | 7 | 22.64 ± 0.39 | 67.40 ± 0.41 | 77.17 ± 0.36 |
| 9. | 8 | 27.37 ± 0.28 | 72.49 ± 0.35 | 84.47 ± 0.32 |
| 10. | 9 | 31.16 ± 0.31 | 79.78 ± 0.37 | 89.58 ± 0.06 |
| 11. | 10 | 34.02 ± 0.25 | 85.51 ± 0.16 | 92.50 ± 0.22 |
| 12. | 11 | 37.51 ± 0.08 | 89.66 ± 0.13 | 98.25 ± 0.18 |
| 13. | 12 | 37.55 ± 0.18 | 89.78 ± 0.07 | 98.35 ± 0.09 |

**Mean of three observation ± SD.**

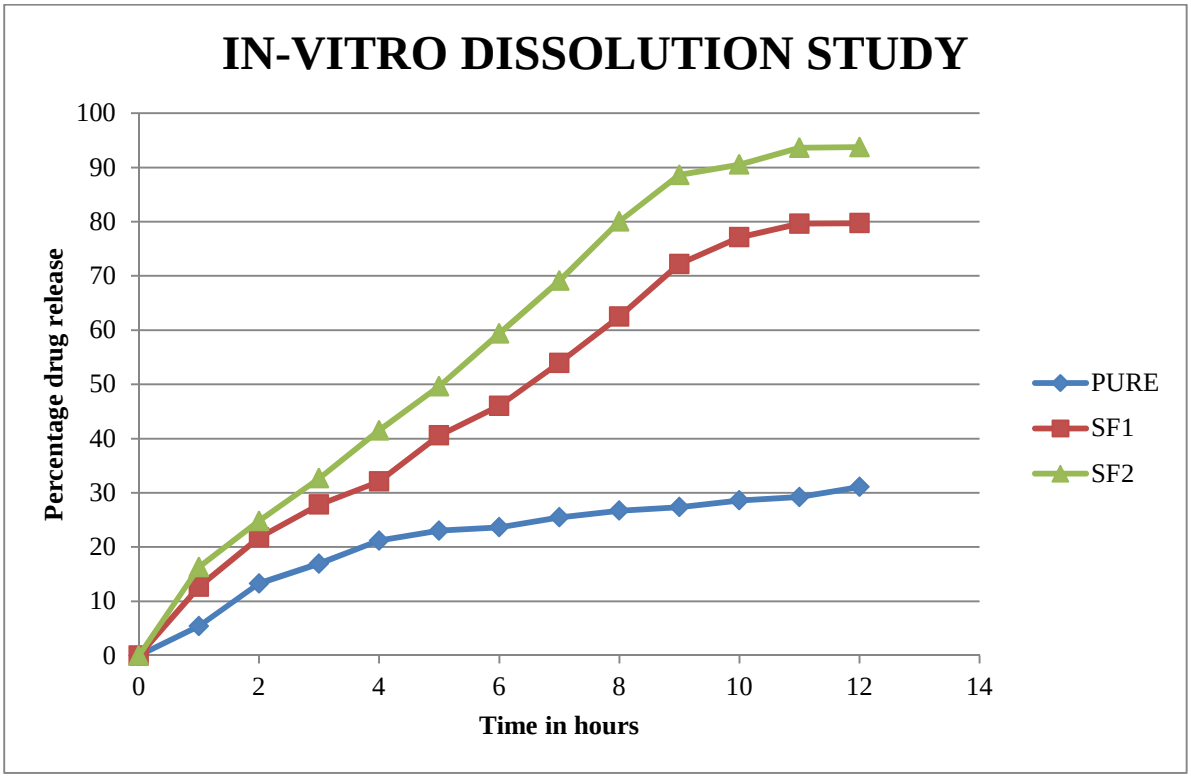



**Figure. 25: Comparative dissolution study of different silymarin formulations in acid buffer (pH 1.2)**

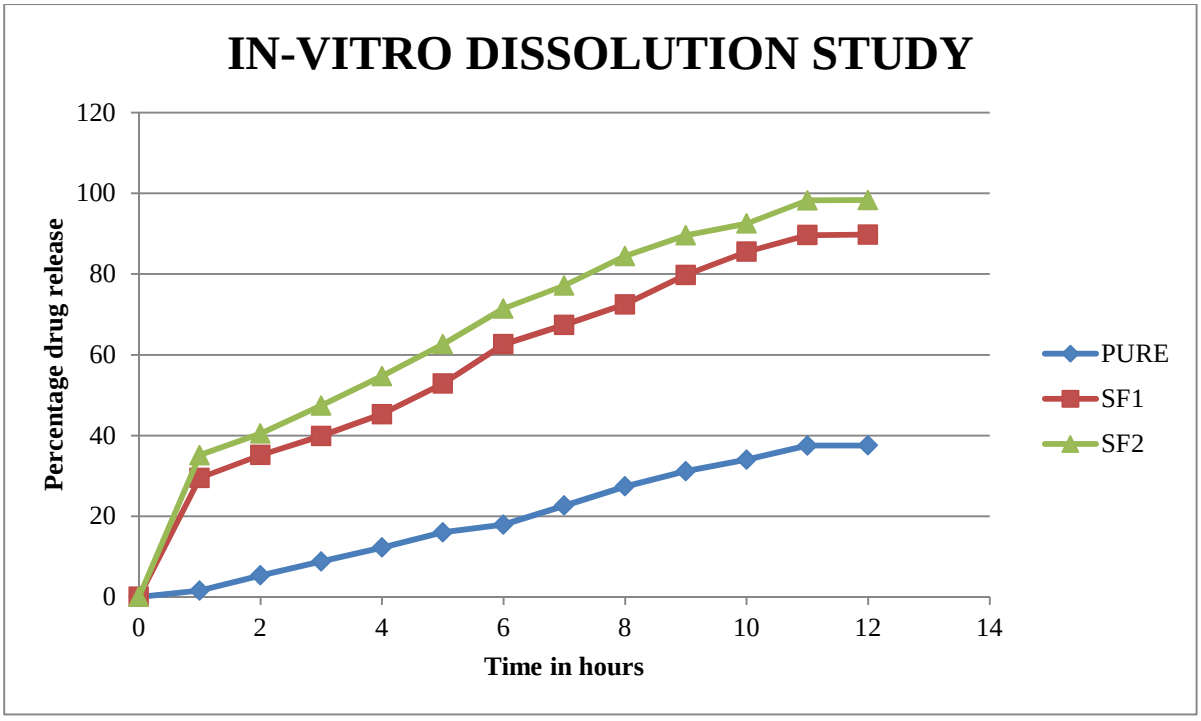



**Figure. 26: Comparative dissolution study of different silymarin formulations in phosphate buffer (pH 7.2)**

## *IN-VITRO* PERMEABILITY STUDIES:

The *in*-vitro permeability study was carried out using Franz Diffusion Cell. After 1 hour of diffusion, 50.33% (SF1) and 64.83% (SF2) of the drug was diffused from the lyophilized proliposome respectively, while from pure drug, the diffusion was found to be 26.61%. Thus, the amount of the drug diffused through the nitro cellulose membrane has doubled when it is given in the form of a proliposome. It can be clearly seen that the permeation of the drug from lyophilized proliposome is much faster than the pure drug. The enhanced diffusion may be explained in terms of the huge specific surface area of the proliposome droplets and improved permeation of the silymarin because of the presence of surfactant, which reduces the interfacial tension of formulation. The results are shown in Table. 15 and Figure. 27.

**Table. 15: Comparative permeability studies of lyophilized proliposome and pure drug in phosphate buffer (pH 7.2).**

| S.No | Time in minutes | Silymarin | SF1 | SF2 |
|---|---|---|---|---|
| 1. | 10 | 05.03 % | 14.09 % | 19.12 % |
| 2. | 20 | 09.06 % | 21.23 % | 31.33 % |
| 3. | 30 | 13.15 % | 29.42 % | 43.62 % |
| 4 | 40 | 16.26 % | 37.67 % | 54.98 % |
| 5. | 50 | 26.43 % | 50.00 % | 64.40 % |
| 6. | 60 | 26.61 % | 50.33 % | 64.83 % |

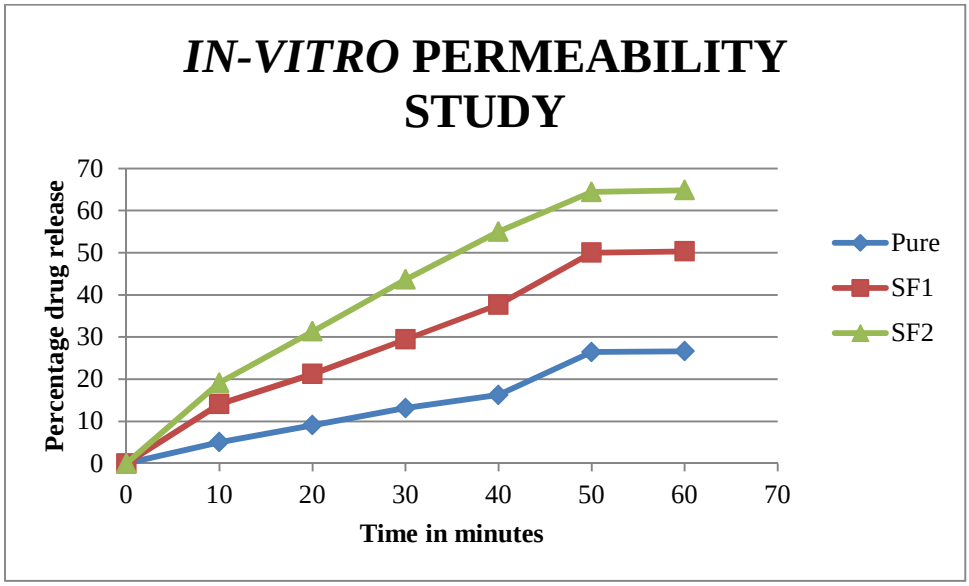



**Figure. 27: Comparative permeability studies of lyophilized proliposomes and pure drug in phosphate buffer (pH 7.2).**

## SELECTION OF BEST FORMULATIONS

From the above data of characterization studies, SF2 shows leading potential formulation compared to SF1. Pharmacological activities were performed by using SF2.

## PARAMETERS ASSESSED FOR LIVER FUNCTIONS

CIS administration resulted in significant elevation of bilirubin, triglycerides, total cholesterol, AST, ALT and ALP and bilirubin levels and that of protein levels were significantly decreased compared to the normal control group indicating liver damage. Pretreatment with naringenin, silymarin and SF2 remarkably prevented the biological changes induced by CIS. The effect of silymarin and SF2 on liver parameters was presented in Table.16 & 17, figure. 28 & 29. Administration of silymarin and SF2 at the doses of 10 mg/kg notably (* $p < 0.05$;**$p < 0.01$) prevented hepatotoxicity induced by CIS.

**Table: 16 & 17. Effect of Silymarin and SF2 on liver function of CIS-induced liver injury in rats.**

| Animal Group | Total bilirubin (mg/dl) | Direct bilirubin (mg/dl) | Total protein (g/dl) | Albumin (g/dl) |
|---|---|---|---|---|
| **I** | 1.42 ± 0.02 | 0.84 ± 0.01 | 8.25 ± 0.20 | 5.26 ± 0.06 |
| **II** | 3.28 ± 0.05 | 1.54 ± 0.03 | 4.75 ± 0.40 | 2.57 ± 0.33 |
| **III** | 2.02 ± 0.01 | 0.74 ± 0.01 | 7.23 ± 0.04 | 4.78 ± 0.02 |
| **IV** | 3.16 ± 0.01** | 1.13 ± 0.02** | 6.33 ± 0.06* | 4.90 ± 0.09** |
| **V** | 3.41 ±0.02** | 0.92 ± 0.04** | 5.90 ± 0.12** | 3.57 ± 0.03** |

| Animal Group | Triglycerides (mg/dl) | Total cholesterol (mg/dl) | AST (U/L) | ALT (U/L) | ALP (U/L) |
|---|---|---|---|---|---|
| **I** | 155±3.24 | 203.5±3.41 | 106.83± 0.98 | 43.35±1.88 | 105.66±0.55 |
| **II** | 232±4.39 | 379.66±5.02 | 168.5±1.2 | 276.15±6.05 | 213.0±1.36 |
| **III** | 167±3.92 | 244.0±5.01 | 115.±1.07 | 104.2±3.71 | 165.0±1.75 |
| **IV** | 205±4.96** | 308.16±3.5** | 137.16±1.07** | 196.1±3.21 | 197.66±1.11** |
| **V** | 180±5.04* | 276±4.42** | 123.5±1.05** | 174.4±6.12 | 174.66±1.54** |

Value represents as mean ± S.E.M. * *p < 0.01, significant difference compared with control group, * p < 0.05, significant difference compared with control group (n = 6).

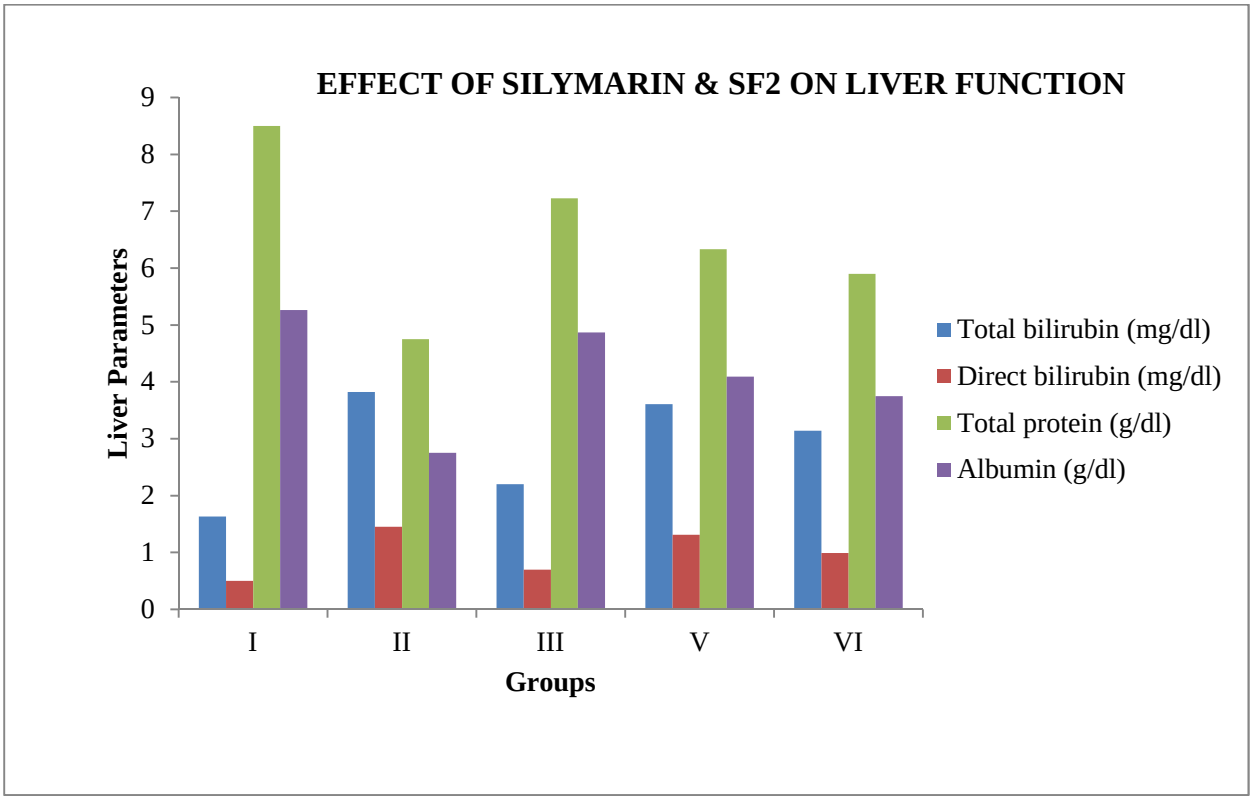



**Figure: 28. Effect of silymarin & SF2 on liver function.**

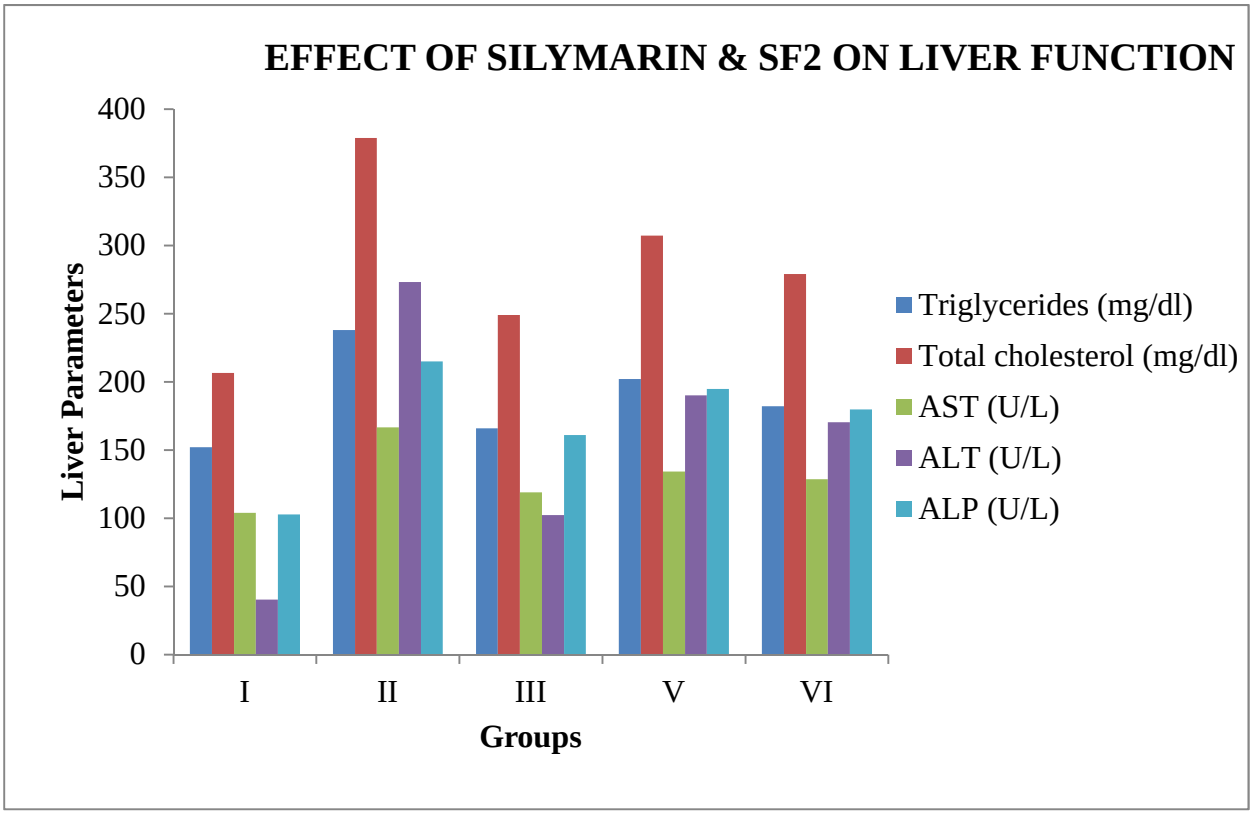



**Figure: 29. Effect of silymarin & SF2 on liver function.**

# *SUMMARY AND CONCLUSION*

## SUMMARY AND CONCLUSION

Silymarin proliposome was successfully prepared by film deposition method. This method of manufacturing was found to be simple, did not require specialized equipments and has scale – up feasibility. The proliposome was converted into dry powder by lyophilization in order to increase its stability. From the reports, the particle size and zeta potential values were measured immediately after preparation of proliposome. The particle size of the lyophilized proliposome is homogenous in size and size distribution. All the formulation showed lower particle sizes. Zeta potential is an indication of the stability of the proliposomes. The Zeta potential of formulation was around ± 20 mV. The zeta potential of best formulation (SF2) indicating good quality.

In FT-IR study proliposome showed the characteristic peeks due to pure silymarin without any markable change in their position, indicating no chemical interaction between silymarin and polymers. *In-vitro* dissolution studies indicated that the dissolution rate of the drug from the lyophilized proliposomes is significantly higher than that of the pure drug. This study indicated higher drug diffusion from proliposome, possibly due to higher increases in saturation solubility and dissolution rate than plain drug. The *in-vitro* permeability results show that the drug diffusion across the nitrocellulose membrane from proliposome is significantly higher than the plain drug.

From the previous reports the hepatotoxicity play a critical role in pathogenesis of CIS induced hepato toxicity. Pretreatment with SF2 significantly attenuated CIS-induced functional liver compared to silymarin. One possible reason of SF2-mediated preservation is that, before CIS administration, pretreatment with SF2 could permit inception of free radicals produced by CIS prior to reaching DNA and causing damage. The study provides strong evidence for the use of the SF2 has hepato protective activity against CIS induced damage in liver. Therefore, SF2 could be an encouraging chemoprotective agent to approach CIS-mediated toxicity.

These observations lead us to the conclusion that proliposome seems to be a promising drug delivery system, which can provide an effective and practical solution to the problem of formulating drugs with low aqueous solubility, poor systemic bioavailability and its hepatoprotective activity.

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