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Research

TPGS Stabilized Silymarin Proliposome: Improve Physico Chemical Properties

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Abstract

Silymarin, a mixture of flavonolignans, exhibits hepatoprotective properties but suffers from poor aqueous solubility (0.04 mg/ml) and low bioavailability (20–50%) due to extensive metabolism, rapid excretion, and low intestinal permeability. To overcome these limitations, a nanoparticulate drug delivery system was developed using proliposomes prepared via the film deposition method. The proliposomes were lyophilized to enhance stability, and characterization studies confirmed homogenous particle size distribution and a zeta potential of approximately ±20 mV, indicating good stability. FT-IR analysis confirmed no chemical interaction between silymarin and polymers. *In-vitro* dissolution studies demonstrated significantly enhanced drug release from proliposomes compared to pure silymarin, likely due to improved solubility and dissolution rate. Furthermore, *in-vitro* permeability studies showed greater drug diffusion across a nitrocellulose membrane for proliposomes than for plain drug. These findings suggest that proliposomes represent a promising drug delivery approach for improving the bioavailability and therapeutic efficacy of silymarin.

Keywords: Silymarin, Proliposome, Poor Bioavailability, Lyophilization, Dissolution Enhancement.

INTRODUCTION

Hepatotoxicity

Chronic liver disease is a global health concern, affecting individuals regardless of age, sex, or region. Cirrhosis, characterized by fibrosis and distortion of hepatic architecture, is a common outcome of prolonged liver injury with severe complications. According to the World Health Organization (WHO), 46% of global diseases and 59% of mortality are due to chronic diseases, leading to approximately 35 million deaths annually.[1] Liver diseases are steadily increasing, ranking as the fifth leading cause of death in the UK[2] and the second among digestive system–related mortalities in the US.[3]

Silymarin

Silybum marianum (milk thistle, family Asteraceae) has long been used for treating liver and gallbladder disorders, hepatitis, cirrhosis, and toxic injuries.[4] Its active extract, silymarin, contains 70–80% flavonolignans (silybin A & B, isosilybin A & B, silydianin, silychristin) and flavonoids (taxifolin, quercetin).[5] Silymarin exhibits antioxidant activity,[6] stimulates protein synthesis and liver regeneration,[7,8] and demonstrates anticancer,[9,10] anti-diabetic,[11] cardioprotective,[12] anti-inflammatory, anti-fibrotic, hypolipidemic, neurotrophic, and immunomodulatory effects.[13] However, its poor solubility (0.04 mg/mL) and low oral bioavailability (20–50%) due to limited intestinal absorption and rapid metabolism restrict its therapeutic potential.[14,15]

Nanotechnology Approach

Improving the solubility and bioavailability of poorly water-soluble drugs is a key focus in pharmaceutical research.[5,6] Nanotechnology enables drug size reduction to the nanoscale (1–1000 nm), preventing agglomeration and enhancing dissolution rate and oral absorption.[7,8]

Rationale of Drug Targeting

Targeted delivery systems aim to achieve higher drug concentrations at the site of action while minimizing systemic exposure and toxicity.[16] Carriers such as liposomes, nanoparticles, and polymeric micelles serve as vectors to encapsulate, protect, and transport drugs to specific sites.

Liposomes and Proliposomes

Liposomes, first described by Bangham in 1961, are bilayered vesicles capable of entrapping hydrophilic and lipophilic drugs.[17] They offer controlled drug release, biocompatibility, and reduced toxicity. However, issues such as instability, aggregation, and leakage limit their clinical application. Proliposomes, developed as a dry, free-flowing alternative, overcome these limitations. Upon hydration, they form uniform liposomes with improved stability, scalability, and ease of storage.[18]

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 most routes 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.

Need for Proliposomal Systems

The versatility of proliposomes makes them suitable for multiple routes of administration, including parenteral, oral, pulmonary, transdermal, and mucosal delivery.[19-29] Their ability to improve solubility, stability, and bioavailability establishes them as promising carriers for poorly water-soluble drugs such as silymarin.

MATERIALS AND METHODS

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.

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

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.

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.

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

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.

RESULTS

Pre-formulation Studies

Characterization of Drug

Silymarin, a BCS Class II drug, exhibits low aqueous solubility (0.04 mg/mL) and high permeability, presenting formulation challenges. Its identity was confirmed by physical observation (yellow powder), solubility profile, melting point (234 °C, consistent with literature), UV absorbance (λmax at 287 nm), and FT-IR spectrum (Figure 5). These results matched reported data, confirming sample purity and suitability for formulation development.[14,15]

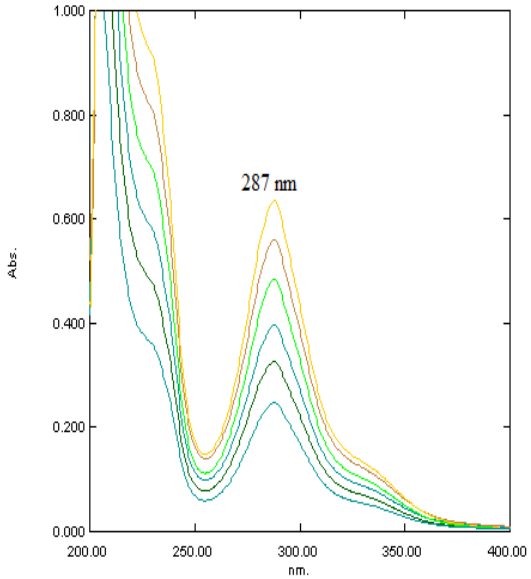


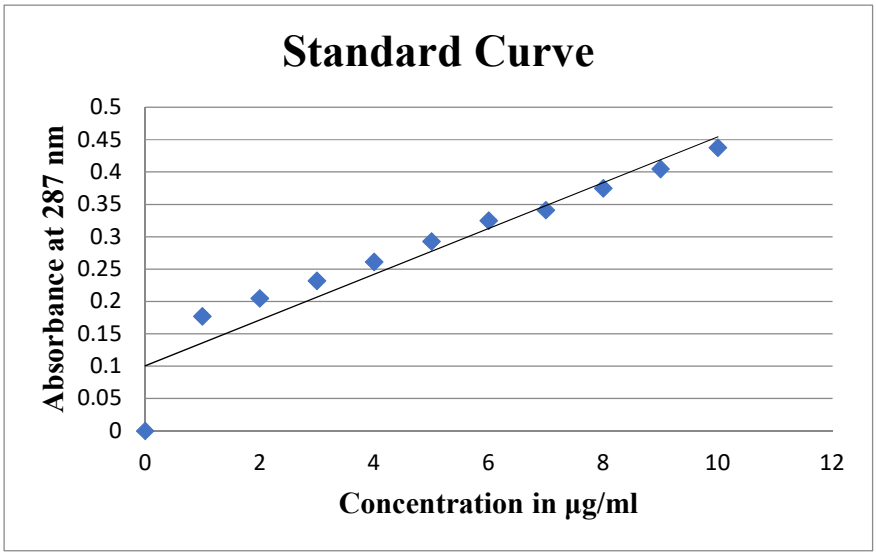
Fig 1: Overlay spectrum of silymarin (UV λmax 287 nm).

Standard Curve of Silymarin

Calibration curves prepared in 0.1 N HCl and phosphate buffer (pH 7.2) showed linearity (10–100 µg/mL), confirming Beer–Lambert's law (Figures 6 & 7). Regression coefficients were $r^2 = 1.009$ in acidic buffer and $r^2 = 1.002$ in phosphate buffer, suitable for drug quantification.

Table 1: Standard curve of silymarin in 0.1 N 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

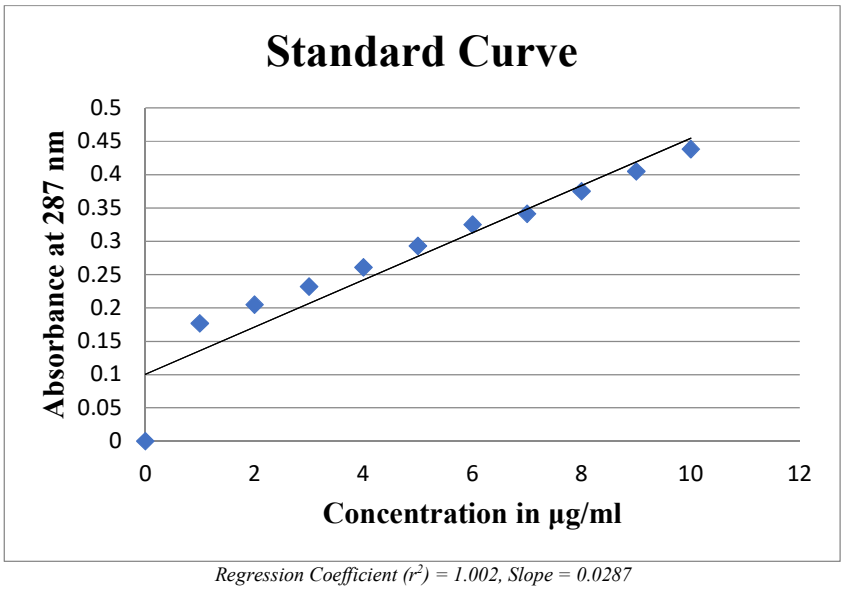


Regression Coefficient (r^2) = 1.009, Slope = 0.0149

Fig 2: Standard curve of silymarin in 0.1N HCl.

Table 2: 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



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

Fig 3: Standard curve of silymarin in phosphate buffer.

Drug–Polymer Compatibility

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. (Figures 8–12, 15–16).

Table 3: Interpretation of IR spectra of drug and excipients.

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

Table 4: IR spectra of physical mixtures (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

Proliposomes were prepared using the film deposition method. Two formulations were designed:

- **SF1:** Without TPGS
- **SF2:** With TPGS (0.5 g) as stabilizer

Table 5: Composition of silymarin proliposome formulations

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

LYOPHILIZATION

Lyophilization was performed using 1% mannitol as cryoprotectant, enhancing stability.

Evaluation of Proliposomes

Particle Size and PDI

The mean particle size was 100.6 nm (SF1) and 80.52 nm (SF2), with PDI values 0.265 and 0.284, respectively. Smaller particle size in SF2 indicates improved homogeneity.

Table 6: Particle size and PDI 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.

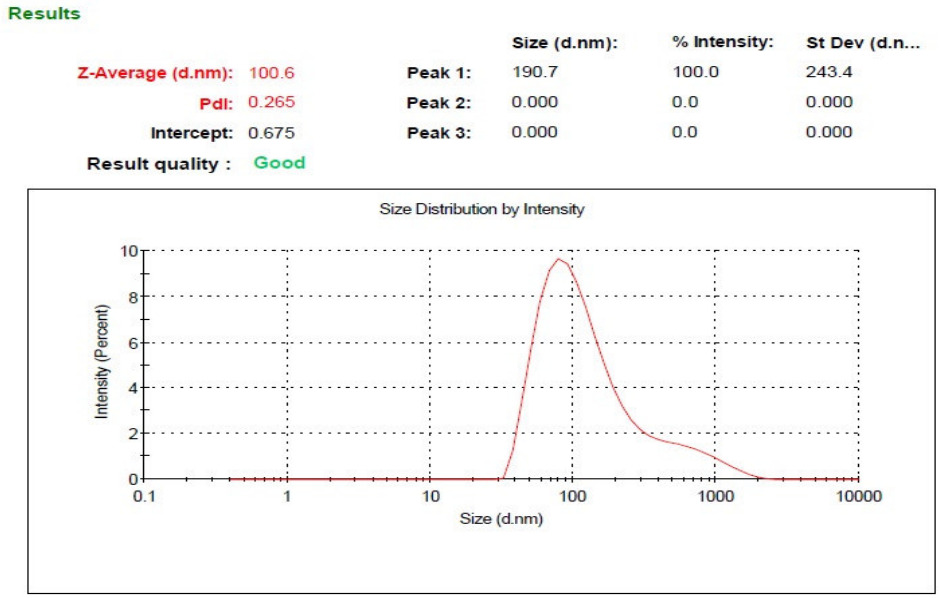


Fig 4: Particle size distribution and poly dispersity index of proliposomes SF1.

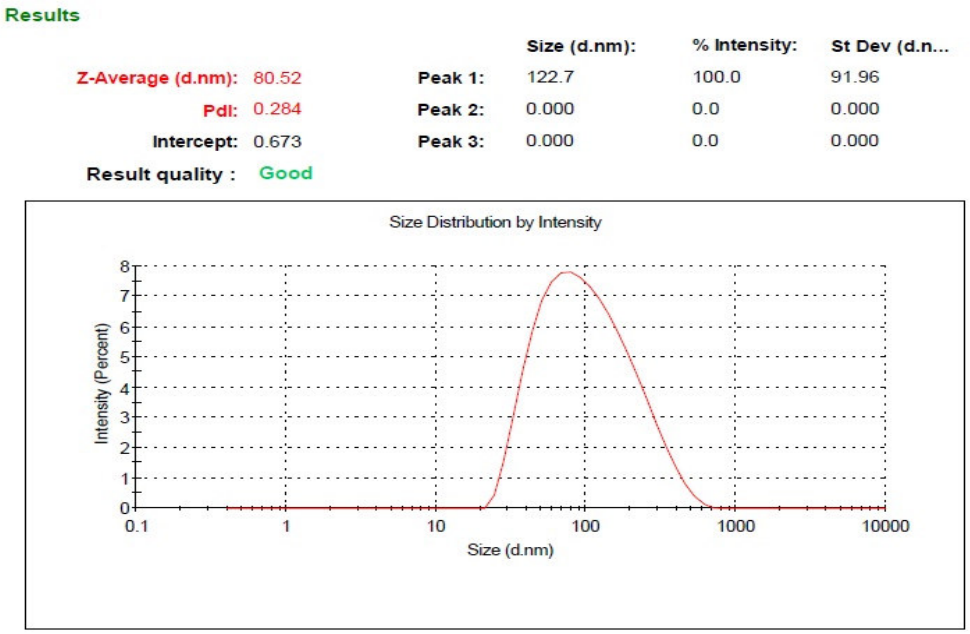


Fig 5: Particle size distribution and poly dispersity index of proliposomes SF2.

Zeta Potential

The zeta potential values were −18.6 mV (SF1) and −4.75 mV (SF2), indicating good stability due to electrostatic repulsion and steric hindrance.

Table 7: Zeta potential of proliposomes.

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

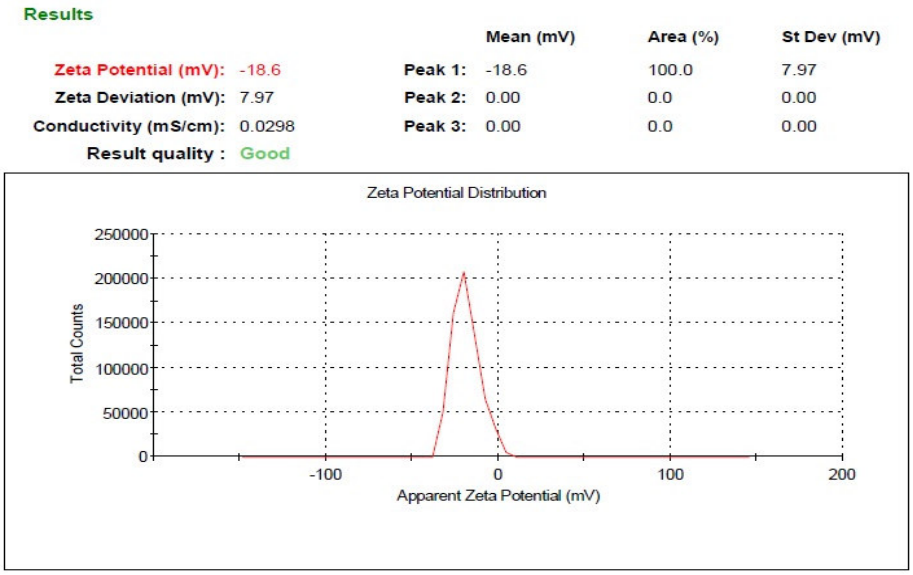


Fig 6: Zeta potential of proliposome SF1.

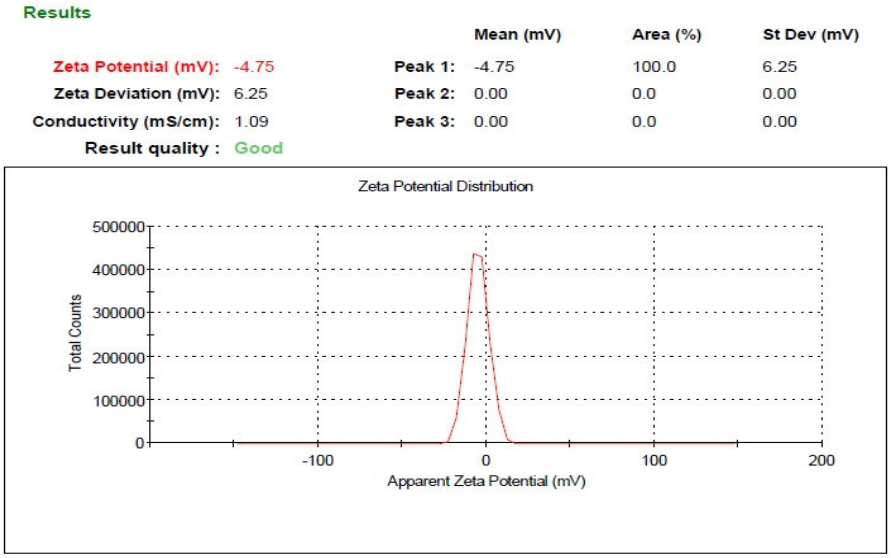


Fig 7: Zeta potential of proliposome SF2.

Drug Content

Drug content was within acceptable range: 95.49% (SF1) and 99.61% (SF2), confirming uniform drug dispersion.

Table 8: Percentage drug content of proliposomes

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

Mean of three observations ± SD.

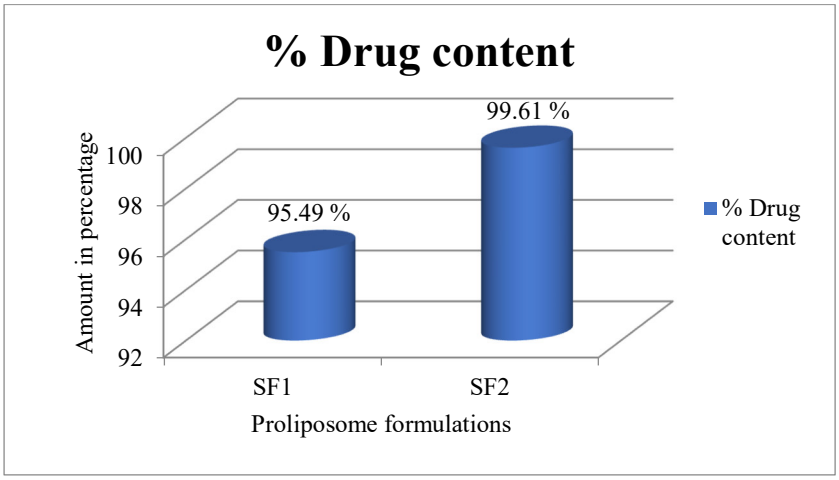


Fig 8: Drug content analysis of SF1 and SF2.

Saturation Solubility

Proliposomes exhibited higher solubility compared to pure drug:

- **Pure drug:** 19.67 µg/mL (acidic buffer), 25.45 µg/mL (phosphate buffer)
- **SF2:** 91.73 µg/mL (acidic buffer), 98.21 µg/mL (phosphate buffer)

This improvement is attributed to particle size reduction, enhanced wetting, and amorphous conversion.

Table 9: 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.

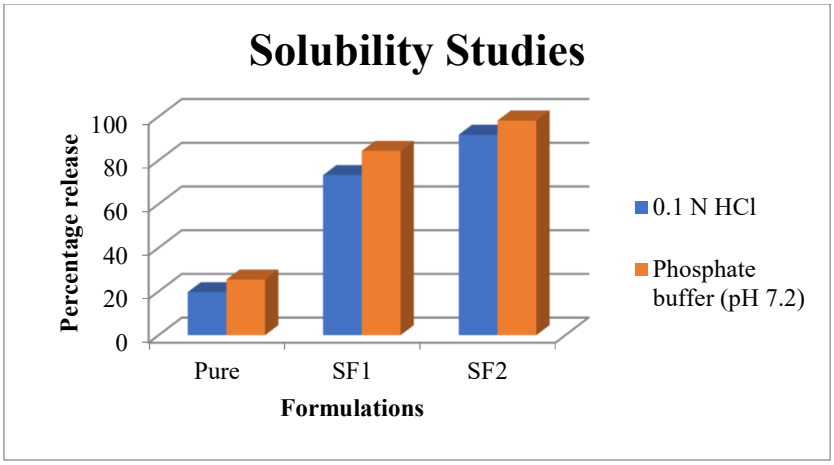


Fig 9: Solubility comparison of pure drug, SF1, and SF2.

In-Vitro Dissolution Studies

Silymarin showed poor dissolution (31% in 0.1N HCl; 37% in phosphate buffer at 12 h). In contrast, proliposomes achieved significantly higher release:

- SF1: 79.71% (acidic buffer), 89.78% (phosphate buffer)
- SF2: 93.73% (acidic buffer), 98.35% (phosphate buffer)

These results confirm enhanced dissolution rate due to nanosizing and improved wettability.

Table. 10: 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. 11: 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.

***In-Vitro* Permeability**

Franz diffusion studies demonstrated higher permeation for proliposomes:

- Pure drug: 26.61%
- SF1: 50.33%
- SF2: 64.83% at 60 min

The enhancement can be attributed to nanosizing, surfactant effect of TPGS, and improved solubility.

Table 12: Permeability studies

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 %

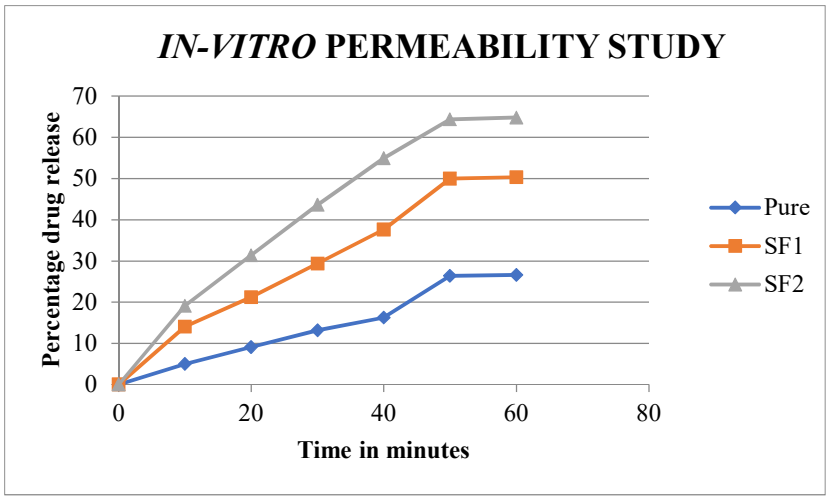


Fig 10. Comparative permeability profiles of proliposomes and pure drug.

DISCUSSIONS

Silymarin is a BCS Class II drug characterized by poor aqueous solubility (0.04 mg/mL) and low oral bioavailability (20–50%) due to extensive metabolism, rapid elimination, and limited intestinal permeability.[21,22] Improving solubility and dissolution is therefore essential for enhancing its therapeutic efficacy. In this study, proliposomes were successfully formulated using the film deposition method with soya lecithin, cholesterol, sorbitol, and TPGS. Lyophilization further improved stability, consistent with earlier reports that proliposomes offer superior stability compared to conventional liposomes.[25,26]

Particle Size and Stability

The average particle size of formulations SF1 and SF2 were 100.6 nm and 80.52 nm, respectively, with narrow PDI values (<0.3), indicating uniform size distribution. Smaller particle size in SF2 suggests that TPGS acted as an effective stabilizer, reducing aggregation and improving homogeneity. Similar findings were reported by Payne et al., where nanosized proliposomes enhanced solubility and stability.[18] Zeta potential values (−18.6 mV for SF1 and −4.75 mV for SF2) indicated moderate stability. The slightly reduced charge for SF2 may be attributed to steric stabilization by TPGS, which compensated for electrostatic repulsion. This observation aligns with previous studies where combined steric–electrostatic stabilization improved nanoparticle stability. [23,24]

Drug Content and Compatibility

Drug content was >95% in all formulations, indicating uniform drug distribution and reproducibility of the preparation method. FT-IR spectra revealed no significant chemical interaction between silymarin and excipients, confirming compatibility, consistent with earlier studies on silymarin–lipid complexes.[5,7]

Saturation Solubility and Dissolution

Proliposome formulations showed markedly higher solubility compared to pure silymarin (19.67 µg/mL in 0.1 N HCl; 25.45 µg/mL in phosphate buffer). SF2 achieved maximum solubility (91.73 µg/mL and 98.21 µg/mL, respectively), likely due to nanosizing, amorphization, and surfactant-mediated wetting. This enhancement is consistent with Woo et al., who demonstrated that lipid-based systems significantly improve silymarin solubility and dissolution.[21]

Dissolution studies confirmed a dramatic increase in drug release from proliposomes. While pure silymarin released only 31–37% over 12 h, SF2 achieved >90% release, highlighting the role of TPGS in improving dissolution. Jain (2001) emphasized that targeted nanocarriers significantly enhance dissolution and bioavailability of poorly soluble drugs, supporting our findings.[23]

Permeability Enhancement

Franz diffusion studies revealed that proliposomes significantly enhanced drug permeation across nitrocellulose membrane. SF2 achieved 64.83% diffusion at 60 min compared to only 26.61% for pure drug. This improvement is attributed to nanosizing, large surface area, and surfactant action of TPGS, which is known to inhibit efflux transporters and enhance intestinal absorption.[27]

Overall Implications

The findings collectively demonstrate that proliposomes particularly TPGS-stabilized systems are highly effective in improving the physicochemical properties, dissolution rate, and permeability of silymarin. These results align with earlier reports that nanocarrier-based proliposomal systems can overcome limitations of poor solubility and bioavailability in phytoconstituents.[4, 5, 18] Thus, proliposomes offer a promising delivery system for silymarin, potentially enhancing its therapeutic efficacy in hepatoprotective applications. Further in-vivo studies are warranted to confirm pharmacokinetic and pharmacodynamic improvements.

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. 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.

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