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FORMULATION AND ASSESSMENT OF LIPOSOMAL DELIVERY SYSTEMS FOR TARGETED HEPATOPROTECTIVE DRUG DELIVERY TO THE LIVER

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ABSTRACT
This study investigates the development of silymarin-loaded lipid microspheres aimed at enhancing the hepatic delivery and bioavailability of silymarin, a known hepatoprotective agent. Liposomes, niosomes, and lipid emulsions were considered as potential carriers, with lipid emulsions selected due to their favorable properties. Soybean lecithin and Tween 80 were used as surfactants, with soybean oil as the lipid phase. The formulations were characterized in terms of drug release, size distribution, stability, and drug-holding capacity. The silymarin lipid emulsion (Formulation A) exhibited significantly higher drug release compared to a silymarin solution (Formulation B), demonstrating superior stability and drug retention. Transmission electron microscopy (TEM) confirmed the near-spherical shape of the microspheres, with a size range of 0.31–1.24 µm. The drug release from Formulation A was enhanced, indicating its potential for targeted liver delivery with minimized drug loss during transit.

KEY WORDS : Lipid Microspheres, Silymarin, Soya Lecithin.

INTRODUCTION
It is widely acknowledged that the primary challenge with current therapeutic agents lies not in their chemical properties but in the inefficiencies associated with their delivery and distribution within biological systems. Efforts to address this issue have been ongoing for years, resulting in the development of diverse delivery systems and advancements in transportation methods. Among these, lipid-based supramolecular carriers have recently gained significant attention. Liposomes, niosomes, solid lipid particles, and lipid microspheres are notable examples of this class, offering broad therapeutic applications. Notably, lipid emulsions, traditionally used for total parenteral nutrition (TPN), have emerged as promising drug carriers. These phospholipid-based structures, also known as lipid microspheres, are seen as viable alternatives to conventional vesicular systems such as liposomes, niosomes, and ufasomes due to their similar structural and biological behavior. Lipid emulsions carrying corticosteroids, nonsteroidal anti-inflammatory drugs, and prostaglandins have demonstrated superior efficacy compared to their free forms. Their colloidal nature and biological distinctiveness lead to rapid liver uptake upon entering systemic circulation. Additionally, phospholipid-based emulsions offer hepatoprotective properties, with essential phospholipids being marketed for liver protection (e.g., Essentiale™ by Rhone Poulenc). Leveraging their targeted hepatic delivery capabilities, these emulsions can enhance the hepatoprotective effects of silymarin.

Silymarin, a flavonoid complex extracted from Silybum marianum, consists of three isomers—silybin (the major component), silychristin, and silydianin. It is recognized for its hepatoprotective properties, supporting liver cell metabolism and regeneration through antioxidant and protein-restorative mechanisms. Additionally, silymarin fortifies liver cell membranes, preventing the penetration of harmful toxins (Wellington and Jarvis, 2001; Miguez et al., 1994). This study focuses on incorporating silymarin into lipid microspheres designed with optimal characteristics to facilitate efficient hepatic delivery. Furthermore, the research encompasses in vitro characterization and stability assessments to evaluate the efficacy of the lipid-based carrier system.

MATERIALS AND METHODS

The study conducted at Aarupadai Veedu Medical College and Hospital, Puducherry. Received Silymarin as a generous contribution from Ranbaxy Laboratories Ltd. (Gurgaon, India). Soybean lecithin was kindly supplied by Natterman Phospholipids (Germany). The surfactants Span 20, Tween 20, Tween 80, and propylene glycol were procured from S.D. Fine Chemicals (Mumbai, India). Additionally, soybean oil, castor oil, and olive oil were obtained from Protina (India). All other reagents and chemicals used in this research were of analytical grade, ensuring the accuracy and reliability of the experimental outcomes.

General Technique for Preparation

Soybean lecithin was thoroughly dispersed in soybean oil and allowed to dissolve completely by maintaining it overnight at 50°C. The co-surfactants (either hydrophilic or lipophilic) were incorporated into the oily or aqueous phase based on their solubility. The aqueous phase was then gradually introduced into the oily phase, drop by drop, with continuous agitation to form a lipid emulsion. A silymarin solution (10% w/v in 1 M sodium hydroxide) was added to the emulsion to achieve a final concentration of 10 mg/g of lipid emulsion, while stirring at 50°C using a magnetic stirrer. After 10–15 minutes of stirring, the pH was adjusted to 7.4 by adding 1 N orthophosphoric acid (Figure 1) (Moreno, 2001).

Formulation Component Selection

Initially, the selection of oil was carried out by using different oils (soybean oil, castor oil, and olive oil) combined with a surfactant (soybean lecithin) and a co-surfactant (Tween 80) to prepare a plain lipid emulsion (Table 1). Next, the surfactant level (soybean lecithin) was optimized by formulating various preparations (Figure 2), keeping the amounts of drug, oil, and co-surfactant constant. Subsequently, different co-surfactants at varying concentrations were evaluated to identify the most suitable co-surfactant and its optimal concentration, maintaining constant levels of drug, oil, and lecithin (Figures 3 and 4). Lastly, the drug concentration within the formulation was optimized (Figure 5).The lipid emulsion components, including the internal oily core, lecithin as a surfactant, and co-surfactants, were tested in various concentrations to refine the formulation's key characteristics, such as globule size distribution, structural stability, longevity, and drug-loading capacity. However, special focus was placed on emulsion stability and drug retention capacity. The formulation demonstrating the highest stability and maximum drug-holding capacity (Formulation A) was selected for further investigation.

Structure and Morphology

The morphology and structural characteristics of the lipid microspheres were examined using transmission electron microscopy (TEM), and photomicrographs were captured at appropriate magnifications (Figures 6a and 6b).

Size and Size Distribution Measurements

The globule size of Formulation A was measured using a Mastersizer (Malvern Instruments Ltd., UK) through the laser light-scattering technique (Figure 7).

Stability Studies

The stability of the prepared lipid emulsion was assessed under various storage conditions, including room temperature (20±5°C), refrigeration (4°C), and elevated temperature (40±5°C) for 45 days (Table 2). Additionally, the emulsions were subjected to repeated centrifugation at 4000 rpm for varying durations (15, 30, 45, 60, 120, 180, and 240 minutes) to evaluate their physical stability (Table 3). The sustainability of Formulation A was further tested across a pH range of 2 to 12 (Table 4). The drug leakage behavior of Formulation A was examined by measuring the percentage of drug retained within the oily phase at different time intervals, extending up to 35 days (Table 5). To determine the amount of drug entrapped within the microdroplets, the lipid emulsion was appropriately diluted with methanol, and the initial drug content was measured using a spectrophotometer. The emulsion was then filtered through a 0.45 µm membrane to separate the unentrapped drug. The filtrate was again analyzed spectrophotometrically after suitable dilution. The difference between the initial and final drug content represented the amount of unentrapped drug, indicating drug leakage.

In Vitro Release Rate Studies

The release studies were conducted using a dialysis bag method with a USP Type 1 dissolution apparatus. The prepared dialysis membrane was soaked in the diffusion medium (phosphate buffer, pH 7.4) for up to 48 hours and stored under refrigeration until use. Before starting the experiment, the membrane was rinsed with distilled water. Formulations A and B (Table 6), each containing the equivalent of 20 mg of silymarin, were placed into separate dialysis bags and positioned in the basket of the dissolution apparatus (USP Type 1). The dissolution process was performed at a rotation speed of 50 rpm, with the temperature of the dissolution vessel maintained at 37±0.5°C. At appropriate time intervals, 5 ml aliquots of the sample were collected up to 36 hours, and an equal volume of fresh diffusion medium was added to maintain a constant volume of 900 ml. The collected samples were analyzed using a UV spectrophotometer at a wavelength of 287 nm (Figure 8).

Table 1: Effect of various oils on the plain lipid emulsion formation and stability characteristics at room temperature (20 ± 5◦C)

Plain lipid emulsion composition				
Lipid (%)	Lecithin (%)	Tween 80 (%)	Degree of cracking	Sustainability
5 (Soyabean) 1.2 4 — >45 daysoil)	1.2	4	-	>45 daysoil
5 (Castor oil)	1.2	4	+	7 days
5 (Olive oil)	1.2	4	+	7 days
—, nil; +, 10–20%				

Table 2: Oil phase separation studies of formulation A at room temperature (20 ± 5◦C), at higher temperature (40 ± 5◦C), and at lower temperature (4◦C).

S. No.	Time (days)	% phase separated (oil) at room temperature (20 ± 5˚C)	% phase separated (oil) at higher temperature (40 ± 5˚C)	% phase separated (oil) at lower temperature (4˚C)
1	1	—	—	—
2	7	—	—	—
3	14	—	—	—
4	21	—	—	—
5	28	—	—	—
6	30	—	—	—

7	35	—	—	—
8	40	—	0.8	—
9	45	—	1.3	—

Table 3: Effect of centrifugation (4000 rpm) on oil phase separation

S. no.	Centrifugation time (min)	% oil phase remaining after centrifugation treatment (%)
1	0	100
2	15	100
3	30	100
4	45	100
5	60	98
6	120	96
7	180	92
8	240	90

Table 4: Effect of different pH on formulation A (at 20 ± 5◦C)

S. no.	pH of the silymarin lipid emulsion	Sustainability (days) mean
1	2.0	3
2	4.0	7
3	6.0	45
4	6.4	>45
5	7.4	>45
6	8.0	>45
7	10.0	8
8	12.0	5

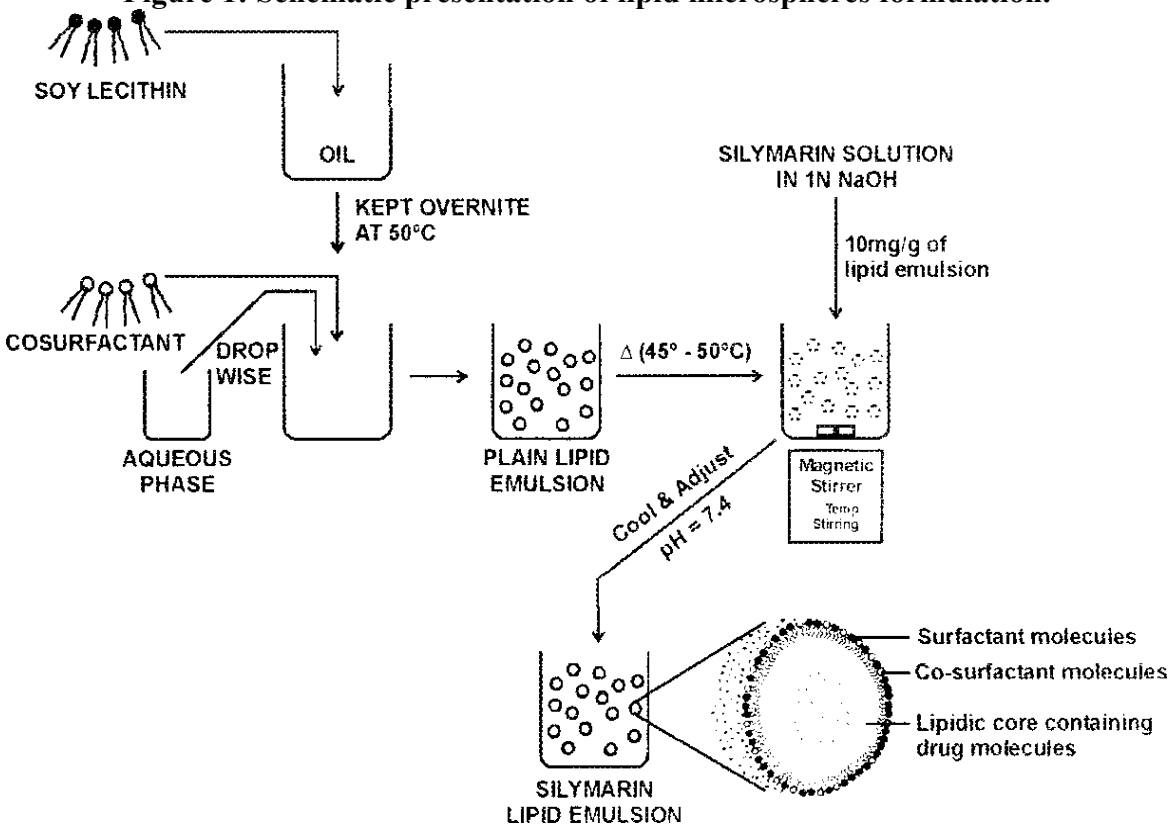
Table 5: Drug leakage studies of formulation A at room temperature (20 ± 5◦C) during storage period.

S. No.	Time (days)	Total Drug initially (mg)%	% remaining	% drug lost
1	1	49.0	100	-
2	2	48.0	100	-
3	3	48.5	100	-
4	7	48.0	100	-
5	14	47.8	100	-
6	21	47.6	98.6	1.4
7	28	47.0	97.8	2.2
8	35	47.0	97.0	3.0

Table 6: Composition of silymarin formulations

Formulations / Components	A	B
Silymarin (10% solution in 1 M NaOH)	1%	1%
Soyabean oil	5%	×
Soya lecithin	1.2%	×
Tween 80	4%	×
Propylene glycol	×	q.s
Water	q.s	×

Figure 1: Schematic presentation of lipid microspheres formulation.



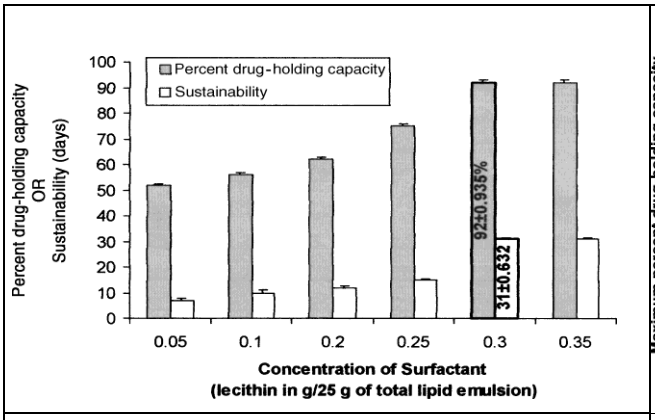


Figure 4: Effect of varying concentrations of cosurfactant (Tween 80) on per- cent drug-holding capacity and sustainability of silymarin lipid emulsion.

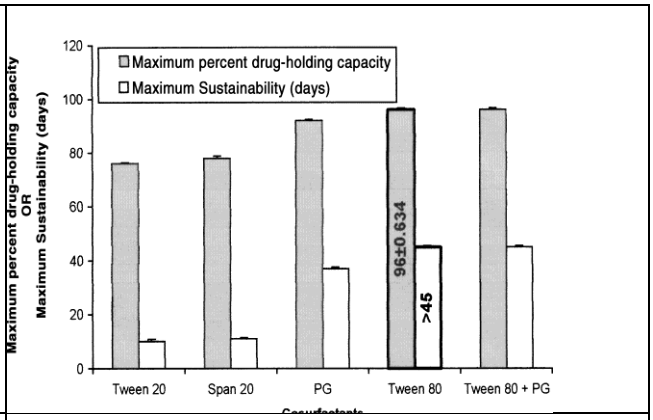
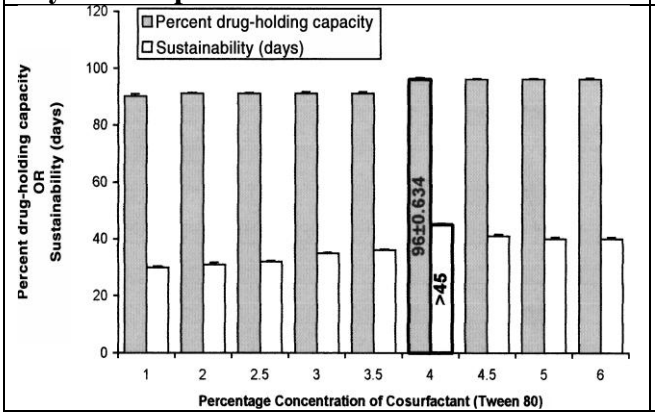


Figure 5: Effect of different levels of drug on the percent drug-holding capacity and sustainability in final composition of the lipid emulsion



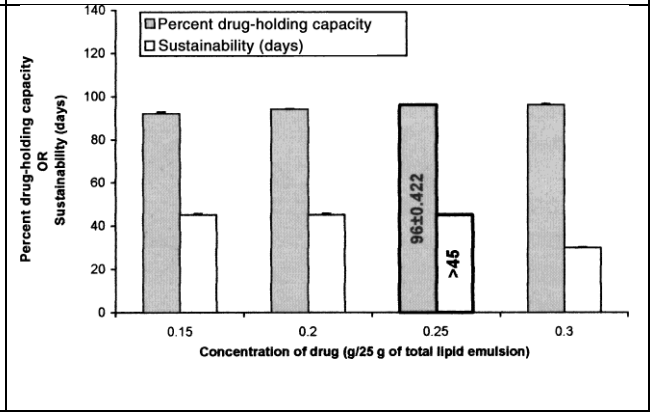


Figure 6: Transmission electron photomicrographs of silymarin-loaded lipid microspheres.

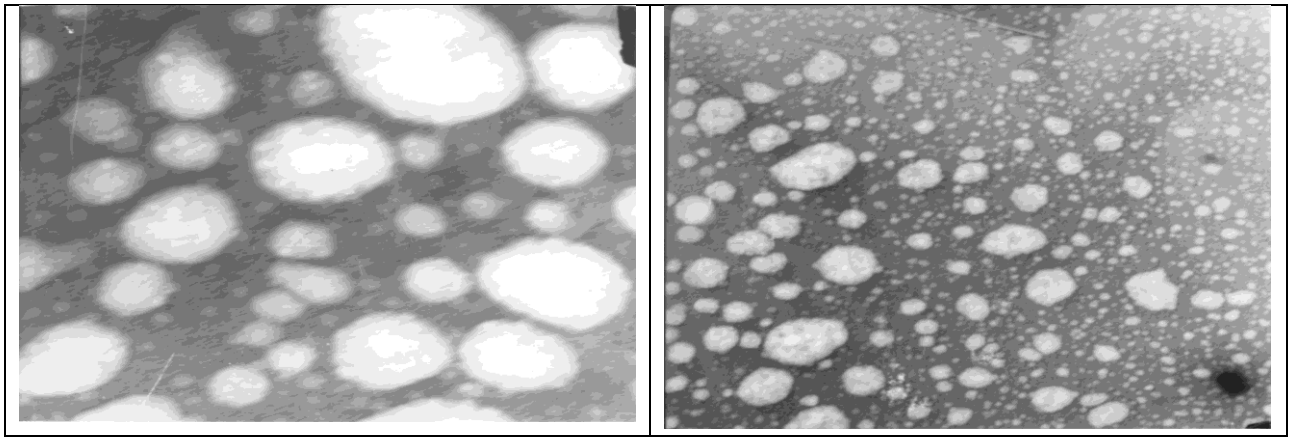


Figure 7: Graph depicting particle size distribution.

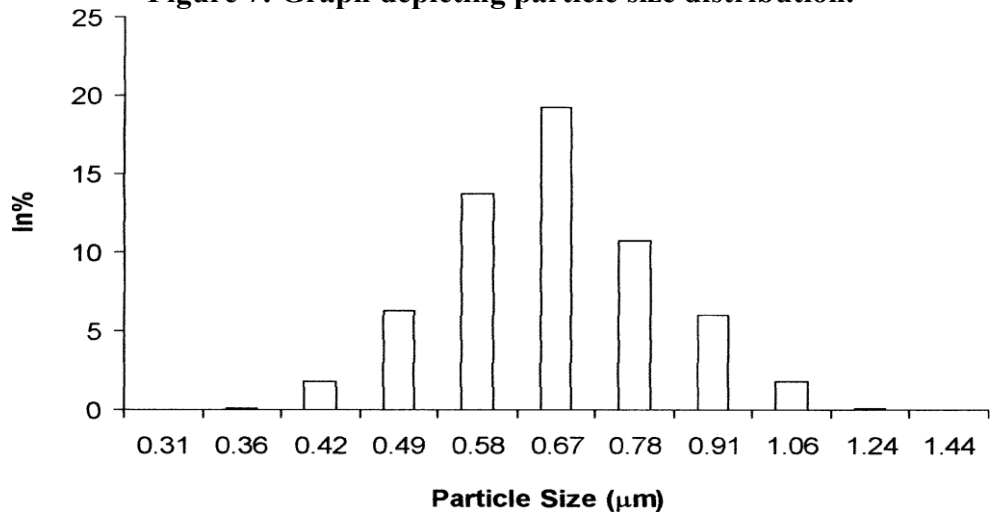
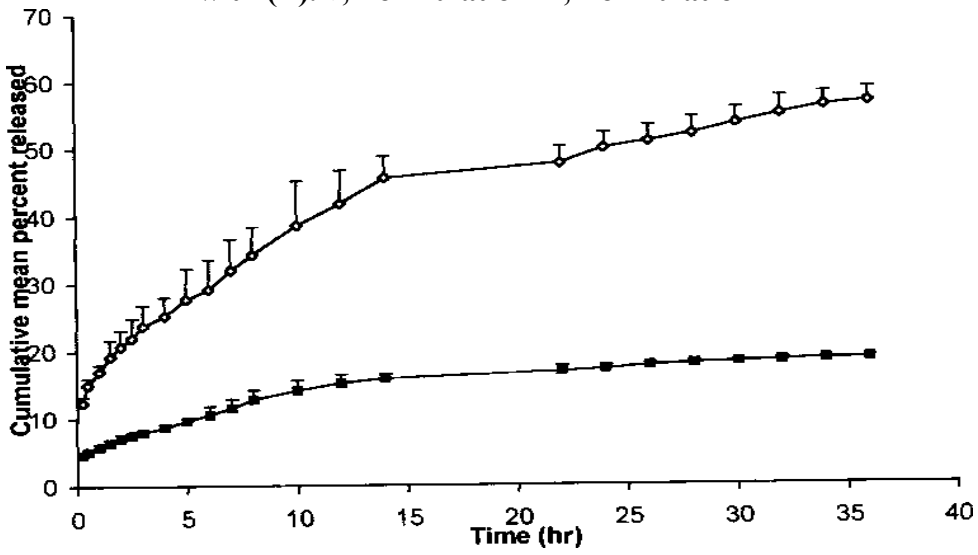


Figure 8: Comparison of cumulative mean percent released vs time (h) for formulation (A) with (B). ♦, Formulation A; Formulation B



RESULTS AND DISCUSSION

Formulation Considerations and Process Conditions

The process parameters, including stirring duration, stirring speed, and the method of combining the aqueous and oily phases, were optimised for the preparation of silymarin lipid emulsion. The optimal stirring time at 3000 rpm was determined to be 2 hours. It was observed that the rate of aqueous phase addition into the oily phase played a pivotal role in achieving a stable emulsion. Specifically, the first

25% of the total aqueous phase should be added drop by drop, ensuring that each drop is thoroughly and uniformly mixed before adding the next drop. This gradual addition method was found to be critical for obtaining a homogeneous lipid emulsion.

Formulation Component Variables

Among the various oils (castor oil, olive oil, and soyabean oil), the soyabean oil in the phase volume ratio of 8.8:1 with respect to aqueous and nonaqueous phase, has shown maximum stability of the formed emulsion (Table 1). The suitability of soybean oil for emulsion formation can be attributed to its long-chain fatty acid components, such as arachidonic acid and linoleic acid, which, when combined with phospholipids, contribute to the development of an effective interfacial barrier within the emulsion. In formulations prepared using varying amounts of lecithin as a surfactant, while maintaining constant levels of drug (1%) and soybean oil (5%), the most stable emulsion (sustainability of 31 days with a value of 0.632) and the highest drug-holding capacity (92% with a value of 0.935) was achieved when the lecithin concentration was set at 0.3 g per 25 g of total lipid emulsion.The improved stability observed at this concentration is likely due to the complete encapsulation of the oily core by phospholipid layers, which effectively act as a barrier against droplet coalescence. However, increasing the lecithin concentration beyond this point did not further enhance stability (Figure 2). Formulations prepared with different co-surfactants at varying concentrations (Figures 3 and 4), while keeping other components constant, displayed variations in stability and drug-holding capacity. The highest stability of 15 ± 0.684 days and 15 ± 0.329 days, along with drug-holding capacities of 76 ± 0.424% and 78 ± 0.954%, were achieved with 4% concentrations of Tween 20 and Span 20, respectively. Both propylene glycol and Tween 80 were found to enhance the emulsification efficiency of lecithin. Among them, propylene glycol demonstrated the highest stability of 37 ± 0.452 days and a drug-holding capacity of 92 ± 0.835% at a 10% concentration. This improvement is attributed to propylene glycol's short molecular chain, which allows rapid diffusion between phases, providing flexibility to the interfacial film, enabling it to deform easily around the oily droplets (Miyata et al., 1996). The optimal concentration of Tween 80 was determined to be 4% of the total lipid emulsion, resulting in a stability of over 45 days and a drug-holding capacity of 96 ± 0.634%. The effectiveness of Tween 80 is due to its role as an auxiliary surfactant, forming a mixed interfacial film with lecithin around the oil core, making the surface hydrophilic due to its long polyoxyethylene glycol chains, thereby preventing coalescence (Karison, 1984; Heller and Pugh, 1960). The enhanced drug-holding capacity (96 ± 0.628%) observed with Tween 80 may be due to its ability to create drug-accommodating domains. However, increasing the Tween 80 concentration beyond this level slightly reduced stability to 40 ± 0.628 days, which was likely due to phase separation caused by leaching of excess Tween 80 (Figures 3 and 4). The combination of Tween 80 (4%) and propylene glycol (10%) did not further improve emulsification efficiency beyond that achieved with Tween 80 (4%) alone, which was therefore selected as the best co-surfactant. Based on these findings, the levels of lipid, surfactant, and co-surfactant for the formulation were fixed, and the impact of varying drug concentrations on stability and drug-holding capacity was evaluated. Formulations were prepared with different drug amounts, specifically 0.15/25 g (0.6%), 0.20/25 g (0.8%), 0.25/25 g (1%), and 0.3/25 g (1.2%) (Figure 5).The results indicated that increasing the drug concentration up to 1% enhanced the drug-holding capacity of the lipid emulsion. This improvement is likely due to the solubilizing capacity of the lipid core and the drug-accommodating properties of the mixed surfactant and co-surfactant domains. However, when the drug level exceeded 1%, the stability of the emulsion decreased to 30 ± 2.84 days, which was likely due to the saturation of both oily and aqueous domains, preventing any additional drug accommodation. Based on stability and drug-holding capacity outcomes, Formulation A was identified as the optimum formulation and selected for further characterization studies.

Shape, Size, and Size Distribution Measurements

The results of transmission electron microscopy (TEM), as observed in the photomicrographs (Figures 6a and 6b), showed that the lipid emulsion microdroplets were nearly spherical in shape, with a coated surface appearance. This coated surface indicates the formation of a flexible interfacial film, created through the interaction of the emulsifier (lecithin) and co-emulsifier (Tween 80). The size distribution of the microdroplets ranged from 0.31 to 1.24 μm, with the most frequently occurring size between 0.632 and 0.732 μm. The median diameter (the size at which 50% of particles are smaller) was recorded as 0.46 μm. Additionally, 10% of particles measured were smaller than 0.33 μm, and 90% were smaller than 0.89 μm. The specific surface area of the droplets was calculated to be 10.7592 m^2/g. The submicron particle size range and consistent size distribution can be attributed to the composition of the lipid emulsion, where the optimal concentration of co-surfactant (Tween 80) integrates with the surfactant (lecithin). This combination produces microdroplets with a well-defined oily core, surrounded by an interfacial barrier that provides the necessary strength and stability, ensuring a uniform droplet size distribution.

Stability Studies

Formulation A demonstrated good stability at room temperature (20 ± 5°C) and at lower temperature (4°C). However, at higher temperature (40 ± 5°C), the lipid emulsion remained stable for 35 days. After this period, slight phase separation occurred, with 0.8% separation observed on the 40th day and 1.3% on the 45th day of storage (Table 2). This behavior can be attributed to the interfacial film potentially becoming destabilized due to the thermal effects at elevated temperatures, which may have affected the fluid state of the emulsion. As a result, conformational changes in the lecithin layer at the surface of the oily droplets could have led to the separation of the oil phase from the aqueous phase, resulting in phase separation. The emulsion stability was considered acceptable, as only a small amount of the oil phase was lost after 60 minutes of centrifugation at 4000 rpm. Further centrifugation, up to 240 minutes, caused only minimal additional loss of the oil phase (Table 3). All formulations exhibited stability within a pH range of 6–8 (Table 4). Below pH 6 and above pH 8, the lipids in the emulsion may have been compromised, preventing the formation of an effective interfacial film around the oil droplets. In Formulation A, at room temperature (20 ± 5°C), the drug remained entrapped within the oil phase, with only 3% of the drug leaking out over a 35-day period (Table 5).

In Vitro Release Rate Studies

The prepared silymarin lipid emulsion (Formulation A) was compared with the silymarin solution (Formulation B) in terms of their drug release profile. The average percent release for Formulation A was found to be 56.70 ± 2.039 after 36 hours, while for the silymarin solution, the release was only 18.67 ± 0.192 during the same time period (Figure 8). The enhanced drug release from the silymarin lipid emulsion compared to the silymarin solution can be attributed to the presence of surfactants on the surface of the lipid emulsion. These surfactants help to solubilize the drug and facilitate its movement towards the outer domain of the oily core, improving its transport from the surface of the system. This observation indicates that the silymarin lipid microspheres, within the submicron size range, are capable of carrying the drug in a manner that enhances its bioavailability, enabling it to reach the systemic circulation at the desired concentration.

CONCLUSION

This study demonstrated an improved release of silymarin from the silymarin-loaded microspheres compared to the silymarin solution. It was concluded that silymarin can be effectively incorporated into lipid microspheres with the desired characteristics in terms of size, shape, entrapment efficiency, and reasonable stability. The synergistic effect of phospholipids and silymarin, when arranged and incorporated into an appropriate carrier system, is anticipated. This system could passively target silymarin to the liver, minimizing the drug loss during transit

REFERENCES

1. Heller, W., and Pugh, T. L. Steric stabilization of collidal solutions by adsorption of flexible macromolecules. J. Polym. Sci. 1960; 203–217.
2. Jaeschke, H., Werner, C., and Wendel, A. Disposition and Hepatoprotec- tion by phosphatidyl Choline liposomes in mouse liver. Chem. Biol. Interact. 64: 1987; 127–137.
3. Karison, P. Kurzes Lehrbuch der Biochemic fu¨r Mediziner and Naturwis- senschaftler. Thieme Verlag. 1984; 134–141.
4. Miguez, M. P., Anundi, I., Sainz-Pardo, L. A., and Lindros, K. O. Hepatoprotective mechanism of Silymarin: No evidence for involvement of Cytochrome P450 2E1. Chem. Biol. Interact. 91: 1994; 51–63.
5. Miyata, I., Miyamoto, H., and Yonese, M. Effect of chain lengths of n-Alcohol on the formation of Single-phase microemulsions in n-Heptane/n-Alcohol/sodium Dodecyl Sulfate/water systems. Chem. Pharm. Bull. 1996; 1049–1055.
6. Mizushima, Y., Hamano, T., and Yokoyama, K. Use of lipid emulsions as a novel carrier for consticosteroids. J. Pharm. Pharmacol. 34: 1982; 49–50.
7. Mizushima, Y., Wada, Y., Etoh, Y., and Watanabe, K. Antiinflammatory effect of indomethacin ester incorporated in lipid microsphere. J. Pharm. Pharmacol. 35: 1983; 398–399.
8. Moreno, M. A., Frutos, P., and Ballesteros, M. P. Lyophilized lecithin based oil-in-water microemulsions as new and low toxic delivery system for Amphotericin B. Pharm. Res. 18: 2001; 344–351.
9. Wellington, K., and Jarvis, B. Silymarin: A review of its clinical properties in the management of hepatic disorders. Bio. Drugs. 15: 2001; 465–489.
10. Yamaguchi, T. Lipid microspheres for drug delivery from the pharmaceu- tical viewpoint. Crit. Rev. Ther. Drug Carrier Sys. 11: 1994; 215–229