

FORMULATION AND EVALUATION OF SILIBININ LOADED SOLID LIPID NANOPARTICLES FOR PERORAL USE TARGETING LOWER PART OF GASTROINTESTINAL TRACT

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ABSTRACT

Objective: The aim of this study was to develop and evaluate silibinin (SIL) loaded solid lipid nanoparticles (SLN) in an attempt to increase its oral bioavailability and targeting the lower part of GI tract.

Methods: Solvent emulsification-evaporation method with slight modification was used to prepare the SLNs, glyceryl monostearate (GMS), trimyristin (TM), tripalmitin (TP) and tristearin (TS) were investigated as solid lipid matrix. Tween 20 (T20), tween 80 (T80), polyvinyl alcohol (PVA), poloxamer 188 (P188), sodium cholate (SC) and sodium deoxycholate (SDC) were investigated as emulsifiers. The formulations were evaluated for entrapment efficiency (EE), particle size distribution and *in-vitro* release profile. Furthermore, the optimized formula (F2) was further investigated by TEM, FTIR and DSC studies.

Results: All the prepared SLNs are within submicronal range and acceptable polydispersity index (PI). The EE of the prepared SLNs were from (64.67±4.51%) to (87.00±2.00%), GMS generally had lower EE than TGs, using P188 or PVA as coemulsifier resulted in SLNs with higher EE%. *In-vitro* release profile show retardation follow the trend TS>TP>TM, and steric stabilizers retard the drug release more than ionic emulsifiers. FTIR and DSC studies were done for the final formula (F2) which contains TS as solid lipid matrix and T80 and P188 as emulsifier combination and it showed no drug – excipient incompatibility and suggests formation of an amorphous solid solution.

Conclusion: It can be concluded that SIL could easily incorporated into SLN containing TS and P188 for oral use.

Keywords: Silibinin; Solid lipid nanoparticles; Tristearin; poloxamer 188; *In-vitro* lipolysis model.

INTRODUCTION

The oral route is considered to be the most convenient route of drug administration involving higher patient compliance, lesser complications and lower cost as compared to parenteral drug delivery systems. Poor solubility and/or poor permeability of drugs are the main causes for their poor oral bioavailability. Physicochemical and metabolic instability in both stomach and liver negatively influence the drug concentration in blood. Hepatic first-pass metabolism is another major cause of poor bioavailability upon perioral administration [1]. Poor solubility of the drugs not only affects oral bioavailability but also encumbers the development of suitable delivery system. Nevertheless, oral formulations are being developed keeping in consideration the basic biological and pharmaceutical approaches of drug delivery via the oral route.

Silybin or silibinin (SIL) (Formal Name: 2-(2R,3R-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-1,4-benzodioxin-6-yl)-2R,3R-dihydro-3,5,7-trihydroxy-4H-1-benzopyran-4-one) is a mixture of two diastereomers (A) and (B) in approximately 1:1 proportion (Figure 1) [2]. The bioavailability and therapeutic efficacy of

SIL is rather limited by its very low solubility in water (430 mg/L). A number of SIL water-soluble semisynthetic derivatives were designed to overcome this problem, e.g., silibinin bis-hemisuccinate (Legalon), silibinin-23-O-phosphate, silibinin-23-O-b-glycosides, and silybinic acid [3]. However, modifications of SIL leading to an increase in its water-solubility usually led to an impairment of its antioxidant (antiradical) activity in the lipophilic milieu [4]. Zhao and Agarwal demonstrated that both free and conjugated SIL shows a rapid tissue distribution [5]. It undergoes phase I and phase II metabolism, especially phase II conjugation reactions and it undergoes multiple conjugation reactions in humans [6], and after oral administration of 560mg of silymarin (equivalent to 240mg SIL), the mean elimination half-life was 6.32 h [7]. Both silymarin and SIL have been used as traditional drugs for more than 2000 years to treat a range of liver disorders, including hepatitis and cirrhosis, and to protect the liver against poisoning from exposure to chemical and environmental toxins, including insect stings, mushroom poisoning, and alcohol [8]. Many *in-vitro* and *in-vivo* studies have reported that SIL possesses antioxidant [9], anti-inflammatory [10], and antiarthritic activities [11], and it has chemopreventive efficacy on prostate cancer [12], hepatic disorder [13], colon carcinoma [14].

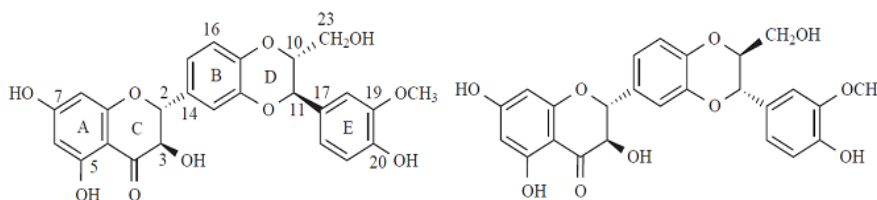


Fig. 1: Silibinin Diastereomers

Solid lipid nanoparticles (SLNs) have been extensively studied with a view to improve bioavailability of poorly soluble drugs and proteins [15]. More recently SLNs loaded with cyclosporine A (lipophilic peptide) have achieved therapeutically effective and reproducible bioavailability in human patients volunteers [16]. Bioavailability of highly lipophilic molecules can be improved. Basically, the body is taking up

the lipid and the solubilized drug at the same time. It can be considered as a kind of "Trojan horse" effect [17]. Oral administration of SLN is possible as aqueous dispersion or alternatively transformed into a traditional dosage forms such as tablets, pellets, capsules, or powders in sachets. Since the stomach acidic environment and high ionic strength favors the particle aggregation, aqueous dispersions of

lipid nanoparticles might not be suitable to be administered as dosage form. In addition, the presence of food will also have a high impact on their performance [18]. The type of the lipid component of the delivery system has a great influence on its capability to enhance absorption. Non-digestible lipids, including mineral oil, sucrose polyesters and others, are not absorbed from the gut lumen. They remain in the gastrointestinal lumen, tend to retain the lipophilic drug within the oil, and thus, may limit the absorption of the drug. Digestible lipids, including triglycerides (TGs), diglycerides, phospholipids, fatty acids, cholesterol and other synthetic derivatives, are suitable oils for drug delivery systems of lipophilic compounds [19]. Various formulation techniques exist for the production of SLNs like: high shear homogenization and ultrasound [20], high-pressure homogenization [21], microemulsion technique [22], solvent emulsification-evaporation [23], solvent diffusion [24], solvent injection [25], double emulsion (w/o/w) [26], supercritical fluid [27], and membrane contactor [28]. Among them, high-pressure homogenization and microemulsion techniques have demonstrated strong potential for scaling up to industrial production scale [29].

Recently, much emphasis is being laid on the development of multiparticulate dosage forms in comparison to single unit systems because of their potential benefits like increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying [30]. Solid dosage forms, especially drug carrier systems with a size larger than 200 µm are strongly subjected to the diarrhea symptoms. This results in a decreased gastrointestinal transit time and leading therefore to a distinct risk of inefficiency. Thus, the efficiency of drug delivery systems can be decreased due to both accelerated carrier elimination and reduced drug release time and drug availability from the delivery system [31].

The major objective of the present work is the development of solid lipid nanoparticles incorporated with silibinin targeting the lower parts of the gastro intestinal tract in an attempt to make use of the fact that the delivery of low water soluble drugs in a presolubilized form will increase its bioavailability, as well as site-specific delivery of drugs to lower parts of the gastro intestinal tract is advantageous for localized treatment of several colonic diseases.

MATERIALS AND METHODS

Chemicals

Dimethyl formamide (DMF), glyceryl monostearate (GMS), sodium deoxycholate (SDC), and potassium dihydrogen phosphate (BDH Chemicals Ltd. Poole England). Dialysis membrane-50nm, HPLC grade methanol, poloxamer 188 (P188), trimyristin extrapure 99% (TM), tripalmitin extrapure 99% (TP), and tristearin extrapure 99% (TS) were purchased from (HiMedia Lab Pvt. Ltd, Mumbai, India). Sodium taurocholate hydrate (M.Wt. 537.68) was from (Hopkin & Williams LTD.-Chandwell Heath. Essex. England). Tween 80 (T80) from (J.T. BAKER). Calcium chloride (M. Wt. 110.99) from (Merck). Tween 20 (T20) and polyvinyl alcohol (PVA) from (Pancreac Quimi-

ca SA. (Barcelona) Spain). Disodium hydrogen phosphate (Riedel De Haen AG Seelze, Honnover, Germany). Lipase from porcine pancreas and sodium cholate (SC) (from Sheep bile) were purchased from (Sigma-Aldrich Chemical Co.-USA).

Method

Preparation of Silibinin Solid Lipid Nanoparticles

Silibinin loaded SLNs (SIL-SLNs) were prepared by solvent emulsification-evaporation method with slight modification [23]. Fifty milligrams of SIL, solid lipid with or without T80 were accurately measured and dissolved in (5 mL) of DMF, forming an organic phase at 40°C ± 2°C. The aqueous phase consisted of coemulsifier in water (25 mL) at 60°C ± 2°C. The organic phase was slowly extruded using a 21G syringe needle into the aqueous phase under mechanical stirring (1000 rpm) at 60°C ± 2°C. After 2 hours, the volume was condensed to approximately (20 ml) to obtain a semitransparent microemulsion. Subsequently, the hot microemulsion was quickly dispersed in the water (100 mL, temperatures ranging from 0 to 2°C) by stirring for 15 minutes, where the microemulsion breaks into ultrafine nanoemulsion droplets which immediately crystallize to form SLNs. The compositions of different batches of the SIL-SLN are given in table (1). Eleven formulas were prepared to study the effect of different factors on the SLNs properties. A fixed amount (50 mg) of SIL as well as a fixed ratio of drug:fat (1:5) was used in all formulas.

Characterization of Solid Lipid Nanoparticles

Determination of Drug Loading and Entrapment Efficiency

The drug loading (DL) and entrapment efficiency (EE) of the prepared SLNs for SIL was evaluated by taking 10 ml of the resultant dispersion, then centrifuged at 12000 rpm for 120 min at 4 °C by using cooling centrifuge (Vision scientific Co. LTD-Korea). The clear part (supernatant layer) was decanted and the remaining part dissolved in 10 ml methanol, sonicated for 30 min then sample was taken, filtered through miliopore filter (0.2 µm) and analyzed for SIL by HPLC method (Mobile phase: HPLC grade methanol and HPLC grade distilled water in a ratio of 90:10 percent (v/v), phenomenox C18 (250 × 4.6mm; 5µm) column,UV- detector; wave length was at 288 nm the running time was 10 min). The percentage EE was calculated as the ratio between actual and theoretical drug content, using equation (1) [32].

$$EE\% = \frac{\text{Amount of drug in precipitate}}{\text{Amount of drug added}} \times 100 \quad \text{Equation 01}$$

Drug loading capacity was calculated as drug analyzed in the nanoparticles versus the total amount of the drug and the excipients added during preparation, according to the following equation:

$$DL\% = \frac{\text{Actual drug content}}{\text{Amount of the drug added} + \text{Amount of excipients added}} \times 100 \quad \text{Equation 0}$$

Table 1: Preparation of Different Formulas of Silibinin Loaded Solid Lipid Nanoparticles

Formula code	Silibinin (mg)	Glyceride Type	Amount (mg)	Surfactant(s)	Amount
F1	50	GMS	250	T80: P188	0.2ml:100mg
F2	50	TS	250	T80: P188	0.2ml:100mg
F3	50	TP	250	T80: P188	0.2ml:100mg
F4	50	TM	250	T80: P188	0.2ml:100mg
F5	50	TS	250	T80	0.2ml
F6	50	TS	250	T80: P188	0.2ml:200mg
F7	50	TS	250	T80:P188	0.2ml:300mg
F8	50	TS	250	T80:SC	0.2ml:100mg
F9	50	TS	250	T80:SDC	0.2ml:100mg
F10	50	TS	250	T80:T20	0.2ml:0.1ml
F11	50	TS	250	T80:PVA	0.2ml:100mg

Microscopic Evaluation

Visualization by light microscope

One drop of each prepared SLN dispersions were examined under the optical microscope using (1000x) magnifying power. Particle

behavior and the external morphology was investigated, as well as sizes of 100-200 particles were determined using a calibrated ocular and stage micrometer fitted in the microscope, the average diameter of the particles and standard deviation was determined. Furthermore, polydispersity index (PI) of the prepared SLN of different

formulas was determined, which indicates the diversity of the particle size. Considering that the PI is calculated from the square of the standard deviation divided by mean diameter as shown in the following equation [33]:

$$(PDI) = \frac{\sigma^2}{m^2} \quad \text{Equation 2}$$

In which, σ : standard deviation and m : mean particle size in the dispersion. Less value of PI indicates enhanced homogeneity of the nanodispersion.

Visualization by transmission electron microscope (TEM)

The size and morphology of the selected formula was examined by TEM (PHILIPS CM 10) with an accelerating voltage of 100 KV. A drop of sample was placed on a carbon coated copper grid and allowed to stand at room temperature for 90 sec to form a thin film. Excess of the solution was drained off with a filter paper. The grid was allowed to thoroughly dry in air, samples were viewed and photomicrographs were taken at suitable magnification.

Fourier-Transform Infrared Spectroscopy (FTIR)

Drug-excipients interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug, pure lipids, physical mixtures of drug and lipids and SLNs formulas using FTIR Shimadzu (Model No. 8400S). Samples were prepared in KBr disk (2 mg sample in 200 mg KBr) with a hydrostatic press at a force of 40 psi for 4 min. The scanning range was 400-4400 cm^{-1} and the resolution was 4 cm^{-1} .

Differential Scanning Colorimetry (DSC) Analysis

The DSC analysis of pure drug and drug-loaded SLN was carried out using (DSC 60- Shimadzu-Japan) to evaluate any possible physical interaction. Therefore, changing in the structure of SIL and lipid used in the study during the methods of preparation of SLNs can be predicted by measuring the glass transition temperature before and after loading using DSC. The scanning rate throughout the investigation was (10°C/min) over the range of (40-300°C) under a nitrogen atmosphere.

In-Vitro Drug Release

The in-vitro release of SIL from different SLN formulations was determined using a modified dialysis membrane diffusion technique [34]. An accurately weighed amount of SIL-SLN dispersions equivalent to 50 mg SIL was transferred to a plastic cylinder having the length of 6 cm and diameter of 2.5 cm fitted at its lower end with dialysis membrane (Himedia dialysis membrane pore size limit 50 nm) presoaked with distilled water for 24 hours. The cylinder was then suspended in the dissolution flask of a USP dissolution appara-

tus I using 900 mL of phosphate buffer solution (pH 6.8) containing 0.5% of tween 80 [35], porcine pancreatic lipase 20 USP units/ml, sodium taurocholate 15 mM and calcium chloride 5 mM as dissolution medium [36]. The cylinder was allowed to rotate at a constant speed (25 rpm) at 37°C±0.5 [37]. Aliquots were withdrawn and replaced with dissolution media and then the drug content was determined by HPLC method at predetermined time intervals for a total period of 8 h. All the operations were carried out in triplicate.

Stability on Short-Term Storage

SIL-SLNs dispersion of selected formula was stored in the refrigerator for 90 days under sealed condition. The mean particle size, polydispersity index and drug entrapment efficiency were determined at the end of the period.

RESULTS AND DISCUSSION

Evaluation of Silibinin Loaded Solid Lipid Nanoparticles

Silibinin loaded solid lipid nanoparticles (SIL-SLNs) formulations have been proposed in the present work as a new controlled delivery systems. SIL-SLNs were prepared by solvent emulsification evaporation method. Various process parameters had been optimized; for instance, the rate of addition of organic phase to the aqueous phase was about 1ml/min, it was added using G21 syringe drop wise and the next drop is added only when the previous one is homogeneously mixed.

During the preparation of SIL-SLNs it was found that all the lipids investigated could result in translucent microemulsion at 60°C. However, all batches showed poor physical stability of microemulsion at lower temperatures apparent as turbidity and then solidification at much lower temperature and then translucent microemulsion was reformed upon warming. Many formulas were prepared for SIL-SLNs as illustrated in table (1), and their properties were explained in table (2) as the entrapment efficiency, drug loading, mean particle size distribution, and polydispersity index of these formulas.

Entrapment Efficiency and Drug Loading

In order to attain optimal SIL entrapment efficiency (EE) "which is the ratio of the entrapped drug inside the SLN to the total drug used", several factors were varied including the type of lipid as well as type and amount of surfactant materials used. Relatively high EE of SIL was achieved for most of particles studied, this can be attributed to the fact that SIL is a highly lipophilic drug ($\log P$ 2.99) [38], and thus has a high affinity toward lipid matrix [39].

There is no significant difference ($P < 0.05$) in the EE% among TM, TP and TS as shown in figure (2), however the entrapment efficiency was followed the trend TM>TP>TS. It was previously stated that medium chain triglyceride generally has higher solvent capacity than long chain triglyceride [40, 41].

Table 2: The Entrapment Efficiency Percent, Drug Loading, Mean Particle Size and Polydispersity Index of Different Silibinin Loaded Solid Lipid Nanoparticle Prepared by Emulsification Solvent-Evaporation Method

Formula	Entrapment efficiency %	STD	Drug loading	STD	Average particle size (nm)	STD	Polydispersity index
F1	64.67%	4.51	5.39%	0.38	207.63	91.59	0.195
F2	85.33%	1.53	7.11%	0.13	303.24	137.44	0.205
F3	86.33%	1.15	7.19%	0.10	286.92	118.67	0.171
F4	87.00%	2.00	7.25%	0.17	280.45	150.24	0.287
F5	81.33%	6.51	8.13%	0.25	410.64	189.94	0.214
F6	83.67%	2.31	5.98%	0.16	326.99	135.18	0.171
F7	87.00%	2.00	5.44%	0.13	354.62	139.20	0.154
F8	84.33%	4.51	7.03%	0.38	199.58	60.19	0.091
F9	86.00%	3.00	7.17%	0.25	211.76	86.60	0.167
F10	82.00%	3.61	6.83%	0.30	204.89	81.82	0.159
F11	84.33%	2.52	7.03%	0.21	275.21	73.00	0.070

SIL-SLN prepared from monoglyceride (F1), shows lower EE than those prepared from TGs. Vivek *et al.* showed the effect of lipid matrix on entrapment of olanzapine. According to their report, entrapment efficiency followed this order: tristearin- SLNs > Precirol-

SLNs > Witepsol E85-SLNs > Glyceryl monostearate-SLNs. This order of entrapment efficiency was correlated with partitioning of olanzapine in a system and was comparable to the solubility of the drug in different lipid melts [42].

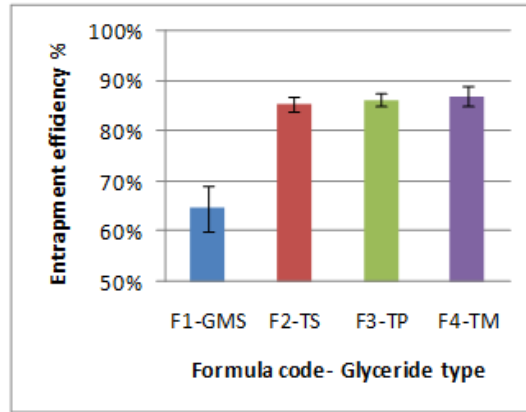


Fig. 2: Entrapment efficiency percent of silibinin loaded solid lipid nanoparticles utilizing different type of glyceride.

Particle Size Distribution

According to table (2), most of the SIL-SLN prepared can be labeled as submicronal size and the particle size showed a wide range of variability ranging from 150nm to 2450 nm depending on the lipid composition, and type and amount of the emulsifier(s). Because most of the researchers accept PI value of less than 0.3 as optimum value [43], we can consider all the prepared SLNs are within the acceptable limit of PI.

Smaller mean particle size was observed for SLNs of GMS (207.63± 91.59) compared with those formulated from TGs, this is may be due to that GMS is not only acted as a lipid matrix but also act as nonio-

nic emulsifier and stabilizer resulted in facilitates emulsification and formation of SLNs of smaller particle sizes[44]. There is no significant effect of TG type on particle size of SLN (P <0.05). TS, TP and TM produce SLNs of mean particle size of (303.24± 137.44), (286.92± 118.67) and (280.45± 150.24) respectively. The mean particle size was found to be approximately 290.20 nm for (F2, F3 and F4), and higher PI had been recorded for TM. This was shown in figure (3).

Using ionic emulsifiers like SDC and SC as in formulas (F8 and F9) mostly resulted in small particle size and lower polydispersity index (figure 4). These results are in agreement with recent one obtained by R. Lopes *et al* during characterization of oryzalin SLNs [45].

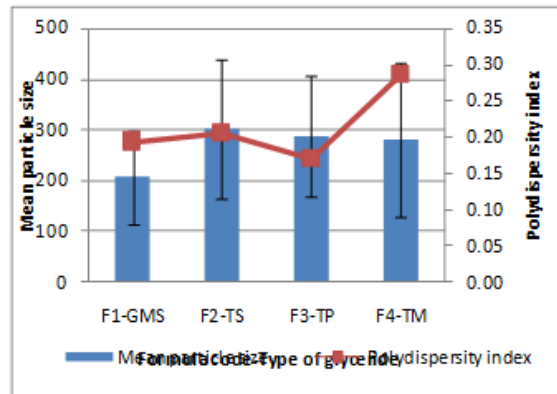


Fig. 3: Mean particle size and polydispersity index of silibinin loaded solid lipid nanoparticles utilizing different type of glyceride.

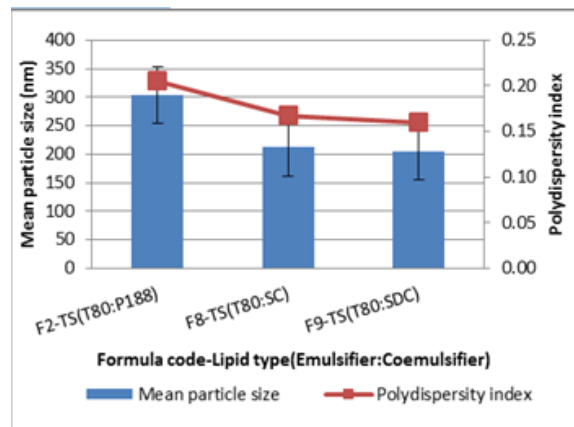


Fig. 4: Mean particle size and polydispersity index of silibinin loaded solid lipid nanoparticles formulated from tristearin with different emulsifiers' combination.

Higher mean particle size was found with increasing P188, this might be attributable to the effect of overly large quantity of the polymer in the formulation [46]. Similar observations were described by Alaa Eldeen B. Yassin *et al* [47], and by Sanjula B. *et al* [48].

Mean particle size of SIL-SLNs formulated with TS as solid matrix without P188 (F5) was (410.64 ± 189.94) while those prepared with 100 mg P188 as coemulsifier as in formula (F2) resulted in decrease of mean particle size of SLNs to about (303.24 ± 137.44) , increasing the amount of P188 to 200mg and 300mg leads to increase in mean particle size to (F6= 326.99 ± 135.18) and (F7= 354.62 ± 139.20) respectively. This might indicates that presence of P188 in relatively small amount is sufficient to produce stable SLNs.

Microscopic Evaluation

Visualization by light microscope

The investigation of the formulated SIL-SLN dispersions in a light microscope revealed that the particles show a round or nearly round shape with a certain variety in particle size is given. Figure (5) shows images of SIL-SNPs of some selected formulations. Imaging analysis of formula (F23) as shown in figure (5-A) showed that these

particles exhibit a spherical shape, and a dense lipid matrix without aggregation.

When only T80 was used (e.g. F5), irregular aggregates were obtained and resulted in non-homogeneous formulation (figure 5-B). The instability of SLN suspensions to aggregation and gelation could be attributed to α -to- β polymorphic transitions of emulsified TGs. These polymorphic transitions lead to a change in particle shape (from roughly spherical to disc-like), which causes a large increase in the oil-water surface area. Consequently, particle aggregation can occur between exposed hydrophobic patches on different particles. For this reason, it is important to include sufficient levels of an emulsifier that rapidly forms a protective coating around the lipid droplets after they crystallize [49]. In particular, it has been suggested that forming a thick steric layer around the lipid particles may be an effective means of avoiding particle aggregation and gelation [50].

Visualization by transmission electron microscopy

Transmission electron microscopy (TEM) is a microscopic technique commonly used for the analysis of materials on the nanoscale. Because it uses electrons, which have a shorter wavelength than light, it is capable of achieving resolution a thousand times better than can be achieved with a light microscope [51].

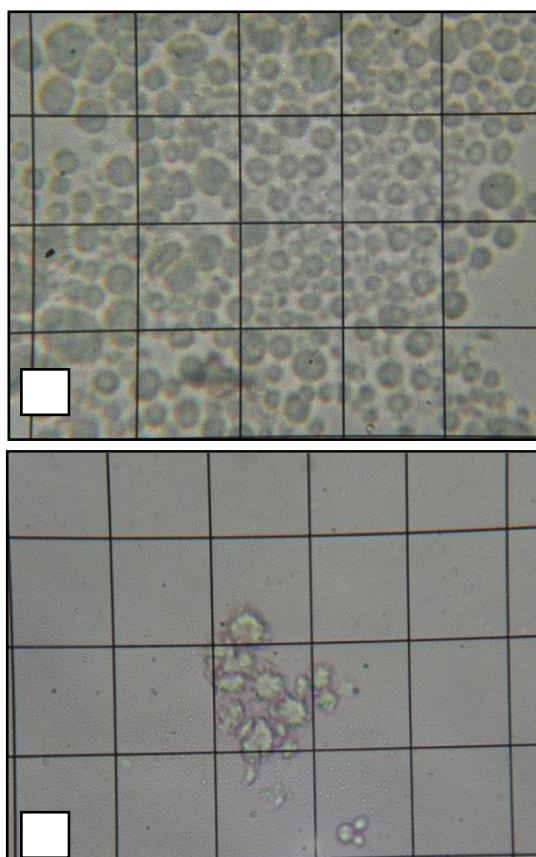


Fig. 5: Light microscope images of A: Formula (F23) and B: Formula (F26). Magnification (1000x)

It was observed in the TEM photographs of formula (F2) in figure (6-A) that particles were almost spherical. Furthermore, the imaging analysis showed that the particle was surrounded by homogeneous shading figure (6-B arrow), this suggest that formulated SLNs in formula (F2) resembled the drug-enriched core model (the drug occupies the core of the particles), SIL was dispersed well due to their miscibility in the lipid matrix, In such a model the core is surrounded by a practically drug-free lipid shell and homogenous hydrophilic polymer [52]. By contrast, the drug-enriched core is formed in case cooling of the hot oil/water emulsion leads to precipitation of the drug first. This takes place preferentially in lipid solutions with drug dissolved at its saturation solubility in the lipid at production temperature. During cooling, a super saturation and

subsequent drug precipitation prior to lipid recrystallization are achieved. Due to the increased diffusional distance and hindering effects by the surrounding solid lipid shell, the drug has a sustained release profile [53, 54].

FTIR Spectroscopy of the Optimized Formulation

FTIR spectroscopy was used to investigate the interactions between lipid, drug and other excipients. From the FTIR graphs of pure drug and optimized formulation (F2), it is confirmed that there are no particular interactions between the lipids and drug .FTIR Spectra of pure drug and drug loaded SLN are shown in the figures (7) and (8) respectively.

The FTIR chart obtained for pure SIL powder is illustrated in figure (7). Bands at the region 2900-3600 cm^{-1} the broad intense band for H-bonded phenolic OH stretching was seen at the expe- nce of the free hydroxyl band , other principle peaks are at 1718.63 cm^{-1} for keton C=O stretching , 1641.48 cm^{-1} , 1595.18 cm^{-1} and 1467.88 cm^{-1} for C=C ring stretching and 1363.72 cm^{-1}

for symmetric bending vibration of CH₃ group also 1274.99 cm^{-1} represents the stretching vibration of C-O-C while 1161.19 cm^{-1} ,1085.96 cm^{-1} and 1030.2 cm^{-1} for in plane aromatic C-H and C-C bending and 825.56 cm^{-1} for out of plane =C-H bending , 644.25 cm^{-1} and 624.96 cm^{-1} are the absorption bands of out of plane for C=C of benzene ring.

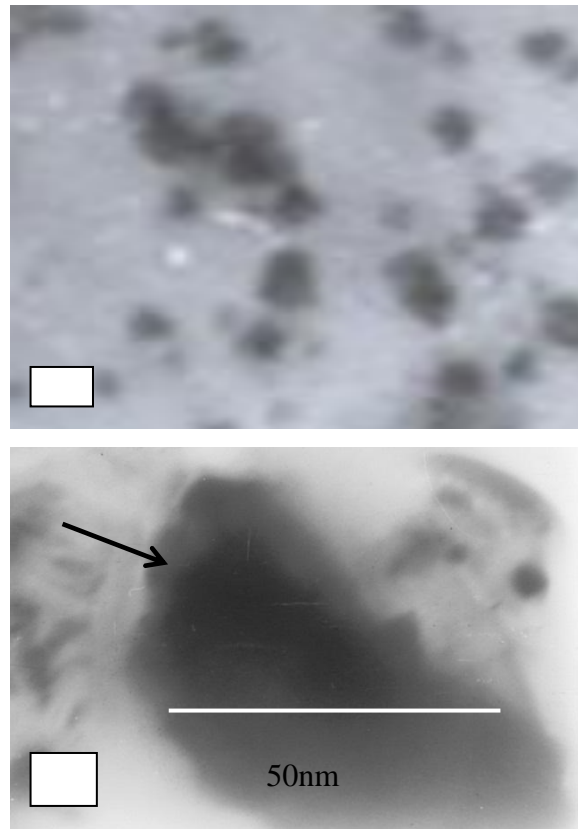


Fig. 6: Transmission electron microscopy micrographs of formula (F23). A: 13000x and B: 48000x.

Differential Scanning Calorimetry

As shown in figure (9), the sharp melting endothermic peak of SIL was observed at 94.72°C, and the endothermic peak of tristearin used as solid lipid was observed at 66.05 °C (figure 10) is indicative to the highly ordered arrangement of TS molecules. Melting endothermic peaks of a physical mixture of drug and excipients, with the same composition as in formula (F2) was shown in figure (11). The

melting peak of 103.45 °C for SIL was observed in the physical mixture, although the peak was markedly shifted, it indicate that the drug was still in crystalline form [55].

While the thermograms of the SIL-SLN (F2) in figure (12) did not show the endothermic peak for SIL. This suggests that SIL was not in crystalline state but in amorphous state or molecularly dispersed structure of the drug in lipid matrix [56].

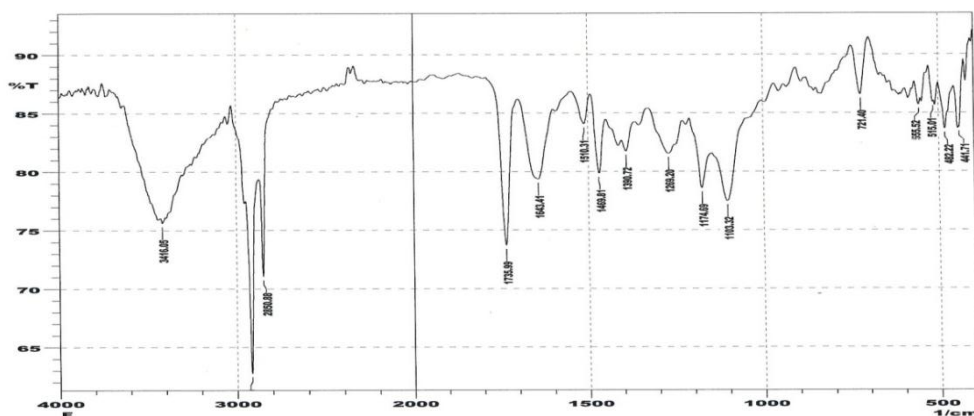


Fig. 7: FT-IR spectrum of silibinin

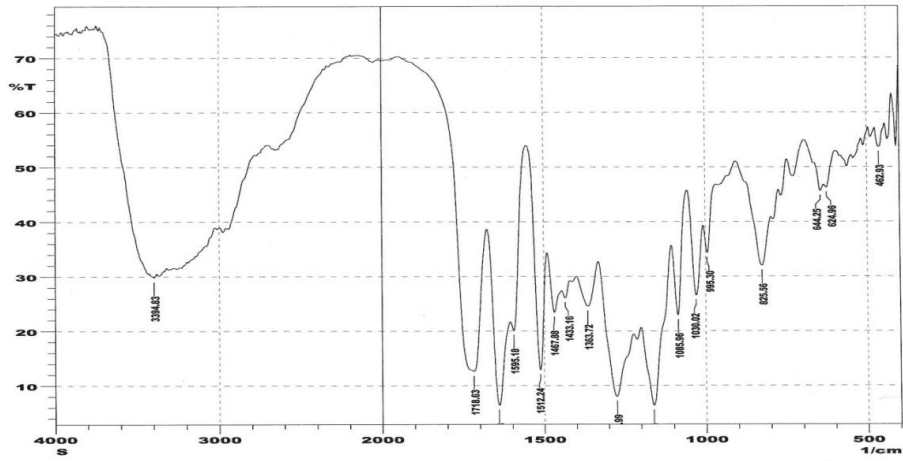


Fig. 8: FT-IR spectrum of formula (F2)

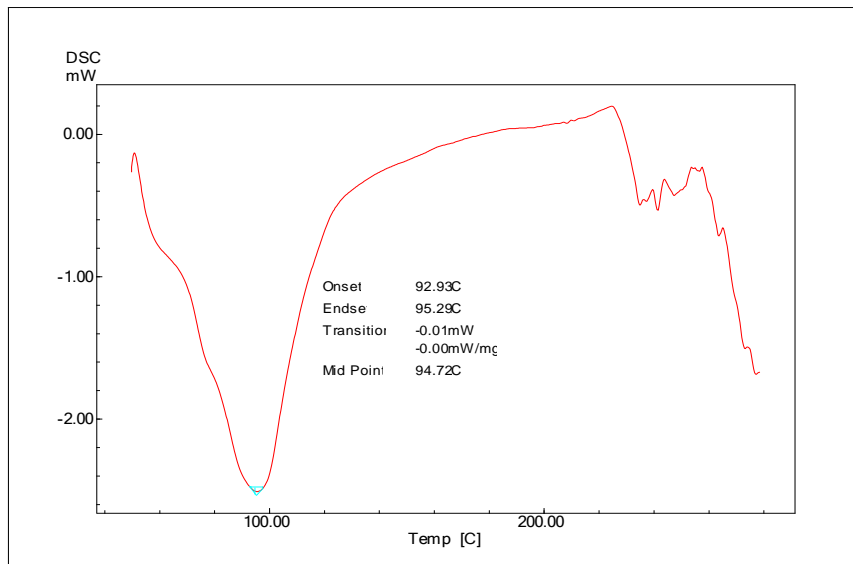


Fig. 9: DSC thermogram of silibinin

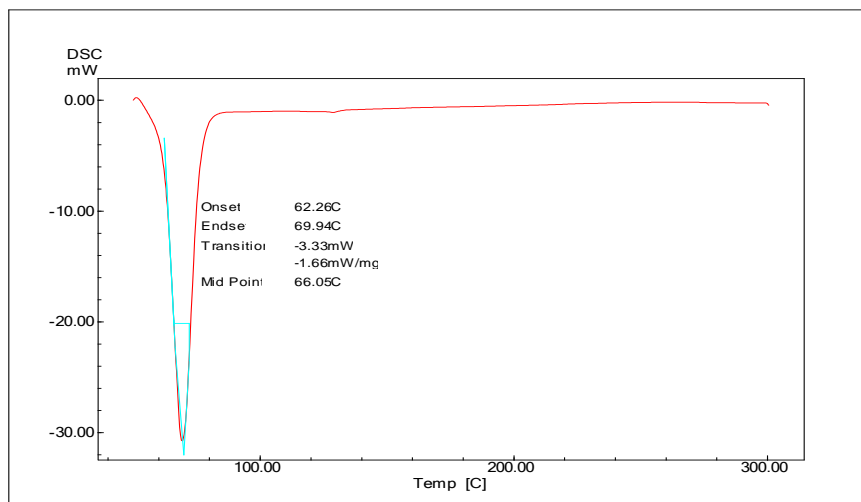


Fig. 10: DSC thermogram of tristearin

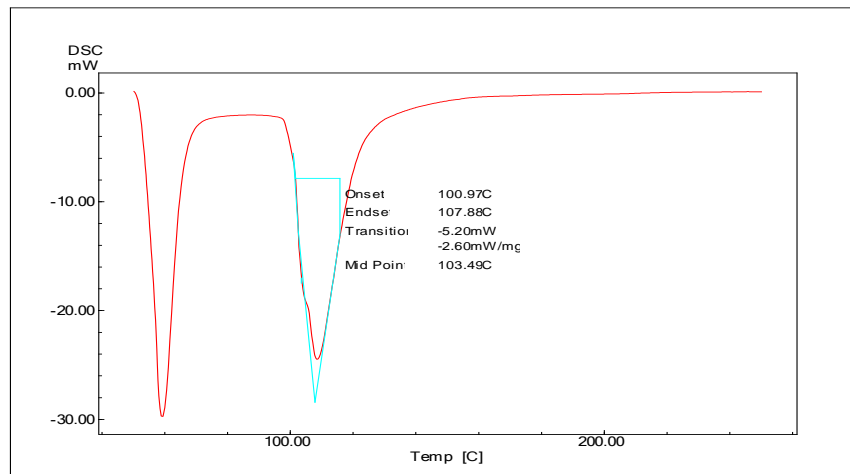


Fig. 11: DSC thermogram of physical mixture as in formula (F2)

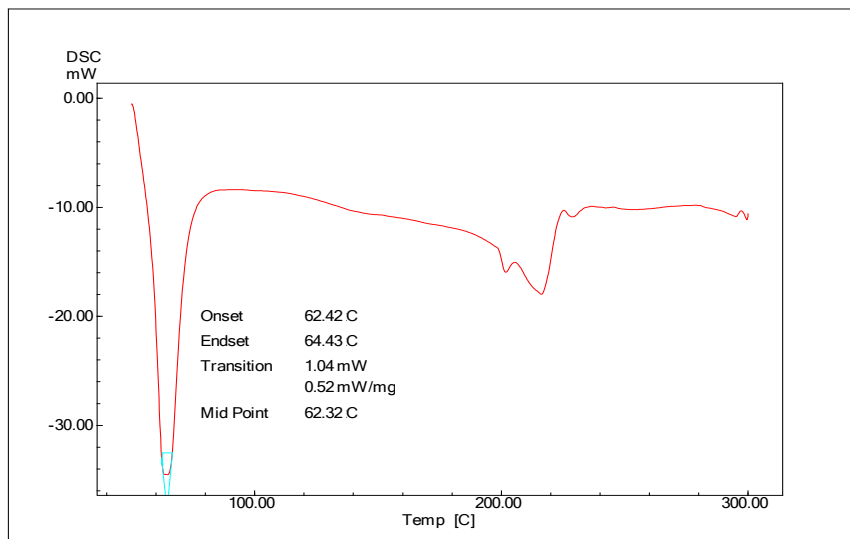


Fig. 12: DSC thermogram of formula (F2)

Reduction in the melting point and enthalpy of the melting endotherm was also observed for tristearin when formulated as SLNs. Freitas and Muller also observed that the crystallization behavior of Compritol SLNs differed distinctly from that of the bulk lipid [57]. Incorporation of the drug inside the lipid matrix results in an increase in the number of defects in the lipid crystal lattice, and hence causes a decrease in the melting point of the lipid in the final SLN formulations [58].

***In-vitro* Release from SLNs**

The most important factors influencing the drug release *in-vitro* from lipid-based drug delivery systems in biorelevant media are the concentration of bile salts, pancreatic lipase and calcium in the medium. Bile salts solubilize drug and lipolytic products; the pancreatic lipase directly digests the lipid carrier and calcium removes free fatty acids from the surface of digesting oil droplets as calcium soap [59]. Lipase from porcine pancreas is very similar to that of human pancreas lipase. Both display the same molecular weight, the same composition of amino acids and a similar reactivity in the sulphide groups [60], so that it was used throughout this study.

In-vitro lipolysis model mimics *in-vivo* physiology by adding digestive enzyme (pancreatic lipase) to a simulated digestion buffer. The model is widely applied to evaluate lipid-based drug delivery system

performance *in-vitro*. Despite the wide acceptance of the lipolysis model, a standard experimental protocol is not yet established and to accommodate the study purposes, different specifications are defined by different research groups [61].

A key issue investigated in this study was the feasibility of using SLN to deliver SIL to the lower part of GIT and this was examined by determining the drug release. The *in-vitro* release results of certain SLN formulations prepared by solvent emulsification-evaporation method are shown in ongoing figures. The amount of SIL released from the SLNs was plotted as a function of time. It could be noted that certain formulations were chosen in order to evaluate their *in-vitro* drug release; this choice was based upon the entrapment efficiencies of the formulations, where a %EE of (80%) or higher was an inclusion criterion for the release study.

In order to compare the drug release profile from the prepared SLN formulations, the percentage of drug released after 2 hours and 8 hours were statistically analyzed using one-way ANOVA and shown in table (3). It was clear that the drug release rate is high in the initial stage which is may be due to more drug content located on the SLN surface due to the large specific surface area [62], or it is probably caused by the drug adsorbed on the nanoparticle surface or precipitated from the superficial lipid matrix [63].

Table 3: Comparative Cumulative Release Percent for Time 2 Hour and Time 8 Hour of Selected Silibinin Loaded Solid Lipid Nanoparticles in Phosphate Buffer (pH: 6.8)

Formula code	Entrapment Efficiency (%)	Mean particle size	Cumulative release (%) t= 2 hour	Cumulative release (%) t= 8 hour
F2	85.33%	303.24	30%	55%
F3	86.33%	286.92	33%	67%
F4	87.00%	280.45	35%	75%
F5	81.33%	410.64	32%	61%
F6	83.67%	326.99	28%	50%
F7	87.00%	354.62	27%	49%
F8	84.33%	199.58	38%	80%
F9	86.00%	211.76	35%	77%
F10	82.00%	204.89	33%	83%
F11	84.33%	275.21	31%	60%

After that the drug has a sustained release profile which may be due to the increased diffusional distance and hindering effects by the surrounding solid lipid shell [64].

Different release pattern was observed from different TGs as solid matrix. Slower release of SIL from SLNs prepared from TS as solid matrix compared with those prepared from TP and TM, 55% of total loaded SIL was released at the end of experiment from SLNs containing TS as lipid matrix, SLNs prepared from TP and TM there was 67% and 75% drug release respectively. TGs are esters derived from glycerol and three fatty acids, these FAs are stearic (18C) for TS, palmitic (16C) and myristic (14C) for TP and TM respectively. Pancreatic lipase digests triglycerides to monoglycerides and free fatty acids. It was previously stated that drug release from SLNs can take place either by diffusion or by degradation of the lipid matrix which occurs mainly by the enzyme, lipase [65]. TS, TP and TM- SLN show different degradation rates by the lipolytic enzyme pancreatic lipase as a function of their composition (lipid matrix, stabilizing surfactant) and it has been demonstrated that the longer the fatty acid chains of the TGs are, the slower is their degradation [66]. This clearly confirmed that

higher solubility of drug in a more lipophilic matrix is sufficient to prolong the drug release for longer period of time [67]. Figure (13) shows the release pattern of SIL from SLNs prepared from different TGs as lipid matrix. Figure (14) shows the effect of the amount of P188 on the release pattern of SIL from the SLNs. Although incorporation of P188 resulted in smaller particle size, it results in a more sustained in the release profile of SIL from SLN. 61% of total SIL was released at the end of the experiment from (F5) which contains only T80 as emulsifier, incorporation of P188 in 100mg, 200mg and 300mg result in 55%, 50% and 49% drug release respectively. However, incorporation of P188 in amounts of (200mg) and (300mg) resulted in SLNs with DL% (5.98%) and (5.44%) respectively, which considered to be much less than that of (F2); (7.11%).

As shown in figure (15), slower release profile from formulations contain P188 (F2) compared with those contain T20, SC or SDC (F36, F37 and F38 respectively). This was in consistent with that observed by Olbrich and Muller when they used poloxamer 407 as steric stabilizer in studying the effect of surfactant and surfactant mixtures on the enzymatic degradation of SLN [66].

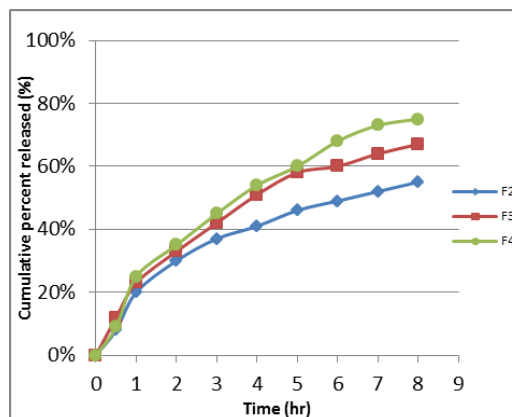


Fig. 13: In-vitro release profile of silibinin from SLNs in pH (6.8) phosphate buffer prepared with (♦) tristearin, (■) tripalmitin and (●) trimyristin.

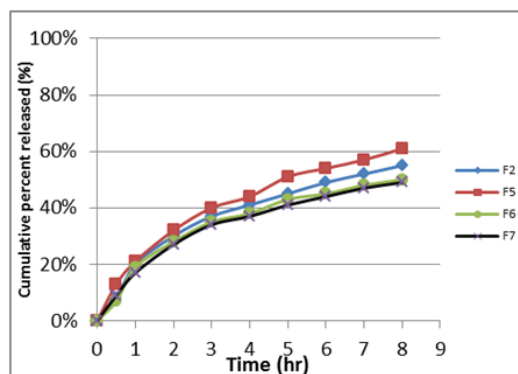


Fig. 14: In-vitro release profile of silibinin from SLNs in pH (6.8) phosphate buffer prepared with (♦) tween80 (0.2ml) +poloxamer 188(100mg), (■) tween80 (0.2ml), (●) tween80 (0.2ml) +poloxamer 188(200mg), and (x) tween80 (0.2ml) +poloxamer 188(300mg).

Muller *et al* have discussed similar concepts in another study using poloxamer 188 [68], and it was explained by that ethylene oxide chains in the poloxamer molecule hindered the anchoring of the lipase (described as the *windscreen wiper effect*) and consequently the degradation and release from the SLN, this phenomenon can be useful for the preparation of slow biodegradable SLNs for oral administration. Although in a later study they found that P188 had no effect on enzymatic degradation of SLN prepared from TM [69], the difference from the current results may be explained by that we used different lipid matrix, different emulsifiers combination, different drug incorporation model which has crucial effect on the drug

release pattern, as well as different method in the preparation of SLN [70]. However, there is no significant effect of incorporation of PVA instead of P188 on the release profile of SIL from SLNs. Both of PVA and P188 are steric stabilizers that can form coat surrounding the particle and hence provide a controlled release profile [71]. Current findings were demonstrated in figure (15). The slow release of the SIL from most of selected formulations suggests homogeneous entrapment of the drug throughout the systems, Paliwal R. *et al* have also discuss similar concept, and the concluded that drug release can be achieved when the drug is homogeneously dispersed in the lipid matrix[72].

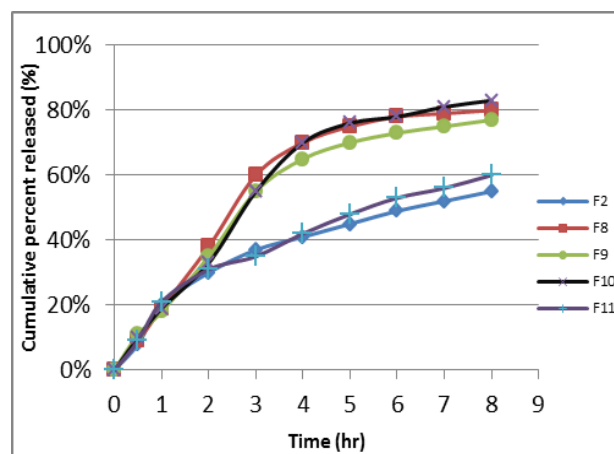


Fig. 15: *In-vitro* release profile of silibinin from SLNs in pH (6.8) phosphate buffer prepared with different emulsifier combination. (♦) tween80+poloxamer188, (■) tweee80+sodiumchololate, (●) tween80+sodium deoxycholate, and (×) tween80+tween20, and (×) tween80+PVA.

Release Kinetics Studies

In-vitro release profiles for the selected SIL-SLN formulas were applied on various kinetic models (zero order, first order, Higuchi and Korsmeyer–Peppas models). The rate constant as well as the highest correlation and the best fitted line was obtained in order to find out the mechanism of drug release, the release kinetics data of the selected SIL-SLNs was given in table (3-3).

The release profiles of the tested SLNs formulations were best fitted into the Higuchi equation. The Higuchi equation describes the diffusion of drug from homogenous and granular matrix systems. The drug release from a matrix system is said to follow Higuchi's release kinetics if the amount of drug released is directly proportional to the square root of time and the resulted slopes are proportional to an apparent diffusion coefficient [42], in which drug release shows first burst effect followed by a slow and continuous release [73]. Furthermore Korsmeyer–Peppas model described the drug release kinetics in a most befitting manner, where the values of the diffusional exponent (n) was more than 0.5 confirming that the formulations followed non-Fickian diffusion kinetics (anomalous transport), i.e. the release is ruled by both diffusion of the drug and dissolution/ erosion of the lipid matrix. This indicates that the release mechanism shifted from diffusion-controlled to an anomalous transport in which both diffusion and erosion are governing the release. From table (3) one can conclude that the best fit for formula (F23) with the highest correlation coefficient (R^2) was resulted with Higuchi and followed by first order and Korsmeyer–Peppas model; which indicates that drug release occurred through diffusion of the drug through the lipid matrix. The Higuchi model has been adjusted to the silibinin release profile from SLN in formula (F2) and is represented in figure (16).

Optimum formulation

The formulation was optimized for the three responses: entrapment efficiency, particle size and release behavior. Formula (F23) was selected for stability test on short term storage.

Table 3: The Release Kinetics Data of the Selected Silibinin Loaded Solid Lipid Nanoparticles

Formula Code	Zero order		First order		Higuchi model		Korsmeyer–Peppas model		
	k_0	R^2	k_1	R^2	k_H	R^2	K	R^2	n
F23	0.1	0.8	0.1	0.9	0.3	0.9	29.7	0.9	0.6
	16	95	30	78	72	85	36	41	36
F24	0.1	0.9	0.1	0.9	0.3	0.9	31.0	0.9	0.6
	18	16	66	76	77	91	19	82	05
F25	0.1	0.9	0.2	0.9	0.3	0.9	25.7	0.9	0.7
	22	36	07	10	84	87	70	53	10
F26	0.1	0.9	0.1	0.9	0.3	0.9	33.6	0.9	0.5
	14	10	27	87	64	95	13	91	49
F27	0.1	0.8	0.1	0.9	0.3	0.9	30.0	0.9	0.6
	17	80	43	73	77	79	10	25	45
F28	0.1	0.8	0.1	0.9	0.3	0.9	32.3	0.9	0.5
	16	94	38	66	73	88	50	71	89
F36	0.1	0.8	0.3	0.9	0.4	0.9	23.9	0.9	0.8
	30	66	14	88	18	47	14	50	06
F37	0.1	0.8	0.2	0.9	0.4	0.9	25.7	0.9	0.7
	28	87	44	92	08	60	08	66	42
F38	0.1	0.9	0.2	0.9	0.4	0.9	23.1	0.9	0.7
	31	01	58	82	13	56	69	71	96
F39	0.1	0.9	0.1	0.9	0.3	0.9	28.8	0.9	0.6
	16	27	29	71	68	91	90	61	31

Stability on Short-Term Storage

SIL-SLNs dispersion of formula (F23) was stored in the refrigerator for 90 days under sealed condition. The mean particle size, polydispersity index and drug entrapment efficiency were determined at the end of the period.

A thin layer of solid was found to sediment at the base of the glass vial containing the dispersion which rapidly redispersed and homogeneous state was reformed upon agitation, so high physical stability of (F23) was suggested upon storage at refrigerator 90 days.

The mean particle size was increased slightly from 303.24±137.44 to 314.34±142.40 nm at the end of the period. Here, the contributing factor for the stability of the system may be due to the protective nature and viscosity imparted by the P188. Being hydrophilic polymer it acts as a coat to shield the particle surface charge responsible to cause their agglomeration and can easily compensate for the missing electrostatic repulsion and stabilize the dispersion for a longer time [74].

The EE of the batch after 90 days (83.28%) indicated that the drug was retained within the nanoparticles throughout the stability period. The lowered entrapment efficiency observed during storage may be due to drug expulsion during lipid modification (ie, transformation of higher-energy α and β' modifications to the lower-energy β modification) of tristearin [75, 76].

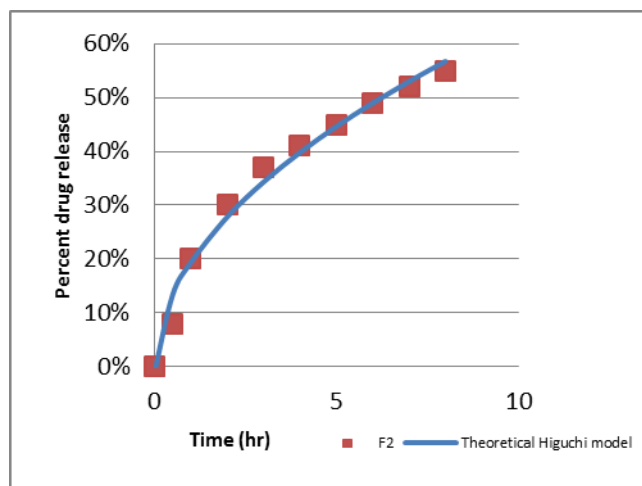


Fig. 16: Comparison between the release profile of silibinin obtained from (F2) (■) and the theoretical Higuchi model (—).

CONCLUSIONS

Silibinin loaded SLN was easily prepared using different types of glycerides and emulsifiers. Entrapment efficiency, particle size and release profile are greatly affected by glyceride type and emulsifiers used. Tween 80 and poloxamer 188 emulsifier combination and tristearin as lipid matrix could produce stable solid lipid nanoparticles of silibinin with no chemical modification. Drug-enriched core model was proposed for the prepared solid lipid nanoparticles. Silibinin loaded solid lipid nanoparticles may imply enhanced oral bioavailability due to the fact that the delivery of low water soluble drugs in a presolubilized form will increase its bioavailability and absorption from the gastro intestinal tract.

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