ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Formulation and Evaluation of Polymeric Micelles for a Poorly Absorbed Drug.

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ABSTRACT

Many of drugs have poor water solubility and so the oral delivery of such drugs is usually associated with limitation of low bioavailability and lack of dose proportionality. Micellar systems are having excellent potential formulation for increasing solubilization and bioavailability of drugs. The aim of this study was to formulate polymeric micellar containing a lipophilic drug, silibinin, using pluronic F-68 (PF-68) by sonication method. Silibinin has demonstrated safety and efficacy as a drug, but its pharmaceutical role is restricted as a result of extremely low aqueous solubility, rapid systemic elimination, inadequate tissue absorption and degradation at alkaline pH; properties that severely curtail its bioavailability. They were characterized for particle size, morphology, zeta potential, and evaluated for its encapsulation efficiency, in vitro dissolution profile, in vitro anti-oxidant, and in vitro cytotoxic activity. The mean particle size was 285.5 nm with PDI of 0.243 and zeta potential of +34mV. FTIR confirms that these polymeric micelles did not show any interaction between drug and PF-68. The XRD and DSC results showed that drug was well incorporated in to the micelles in amorphous form. *In vitro* dissolution profile of NPs shows six times more release than pure silibinin. It was concluded that the prepared polymeric micellar system can be used as a prospective delivery carrier for silibinin with increased solubility.

Keywords: DSC, FTIR, XRD, Micelles

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ISSN: 0975-8585

INTRODUCTION

Silibinin is a mixture of 2 diasteromers (Silybin A and Silybin B) and is used for Hepatoprotective, free radical scavenging, Antioxidant effect, Cytoprotective avtivity, Anticancer effect, hypocholesterolemic activity, Angiogenesis effect, Neuroprotective effect, Chemopreventive agent under prostate, breast and liver cancer. Silibinin is a bioflavonoid comes under the category of flavones and it is a main active constituent isolated from the *Silybum mariana* (Milk thistle) i.e., Silymarin**.** It has been demonstrated to exert in vitro protective effects on endothelial cells and to treat liver related disorders. Moreover, Silibinin has better antioxidant activity than Silymarin. It has poor water solubility (Log P value = 2.63), oral bioavailability is 20-50% and half-life is 3±1 h. These factors are responsible for its reduced biological activity [1].

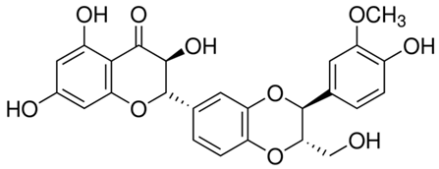

Figure 1: Structure of Silibinin

Conjugation of hydrophilic polymers with small molecule drugs to produce polymer-drug conjugate systems has been demonstrated to be a viable formulation strategy for utilizing hydrophobic drugs in a water-soluble manner, which may offer advantages over the corresponding parent drugs, including fewer side effects, improved solubility, passive tumor targeting, an improved pharmacokinetic profile, and lower plasma concentrations. The quest for building a novel platform has resulted in a number of nanoparticles based formulations which includes inorganic nanoparticles, liposomes, micelles, emulsions, dendrimers etc [2-7]. Polymeric micelles provide an attractive platform in which the formulation can be designed with multiple imaging modalities along with a drug payload. Phospholipid based polymeric micelles is one such system that have shown a great potential in delivering hydrophobic drugs and have been studied extensively because of their attractive features to fulfill the requirements for selective drug delivery [8]. Polymeric micelles are self-assembled amphiphilic copolymers in the form of core/shell nanostructures. The hydrophilic part of the copolymer usually forms the shell and provides stability in the aqueous medium. The hydrophobic core of the micelles has been used extensively for encapsulation of various non-polar drugs like paclitaxel, doxorubicin, amphotericin-B [9-11]. The hydrophobic core of the micelles regulates the release of the encapsulated hydrophobic drug in a sustained manner whereas the hydrophilic polyethylene glycol (PEG) chains on the surface of the micelles impart excellent colloidal stability in aqueous as well as in vivo environment. In most formulations, the hydrophilic chain consists of PEG units. In systemic circulation the hydrophilic PEG chains prevents the binding of the serum proteins (opsonization) on the micellar surface and thus avoids rapid clearance from the blood stream by macrophages. The presence of PEG coatings and small size of the micelles results in a very efficient passive accumulation of these micelles in the tumor by the well-known Enhanced Permeability and Retention (EPR) effect [12, 13].

Based on this background the objectives of present investigation were set in an attempt to develop a novel, safe, effective and stable micellar system comprised of Pluronic F68 (PF-68) incorporating silibinin. The physicochemical properties of the formulations were evaluated, and its in vitro drug release was also investigated.

EXPERIMENTAL

Materials

Pluronic F-68 (PF-68) and silibinin were purchased from Sigma Aldrich, USA. All other chemicals and reagents were of analytical grade. Milli Q water (Millipore) was used throughout the studies.

Methods

Preparation of polymeric micelles

The formulation of micelles was executed by sonication method. The sonication method consisted of the following steps; weighing of the PF-68 (200.0 mg) and silibinin (5.0 mg) into a screw-top glass vial, addition of 3.0 mL distilled water and subsequent sonication using a sonicator (Vibracell, USA) at for 10 minutes. Micelles formed were centrifuged at 13000 rpm for 3 min and filtered through 0.45 µm filter. Empty micelles were prepared according to the same method in the absence of drug [14, 15].

Solubilization efficiency of polymeric micelles

The solubilization efficiency of polymeric micelles was investigated for their solubilization enhancement capacity as compared to pure drug. The weighed amount of drug and drug loaded polymeric micelles were suspended in distilled water. The vials were placed on a mechanical shaker at ambient temperature. After equilibrium (24 h), the obtained suspension was centrifuged at 10,000 rpm for 10 min (Remi PR-24 Centrifuge, India) and filtrate analyzed for silibinin concentration at 287 nm using UV-visible spectrophotometer (1600, Shimadzu, Japan) [16-19].

Particle size, polydispersity and zeta potential measurement by dynamic light scattering (DLS)

The average particle size distribution and charge of the resulting polymeric micelles was determined by dynamic light scattering (Zetasizer ZEN 3600, Malvern, UK). The experiment was performed using clear disposable zeta cell, water as a dispersant which has refractive index (RI) - 1.330 and viscosity (cP) - 0.88 and the temperature was kept constant at 25 °C. The sample was analyzed for three times to minimize the error.

Encapsulation capacity of polymeric micelles

Weighed amount of drug loaded polymeric micelles were dissolved in methanol, sonicated for 5 min to break the micelles, diluted suitably and then analysed by UV spectrophotometer at 287 nm [20]. The encapsulation capacity was determined by the following equation:

$$\text{Encapsulation capacity} = \frac{\text{Actual weight of the drug}}{\text{Theoritical weight of the drug}} \text{ X } 100$$

Fourier transform infrared spectroscopy (FT-IR)

The samples were subjected to FT-IR analysis by KBr pellet method using Fourier-Transform Infrared (FT-IR) spectrophotometer, (Shimadzu, FT-IR 8400S, Japan) in the region of 4000 cm^{-1} - 400 cm^{-1}.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed on pure sample of drug and its formulation using DSC-60 apparatus (Shimadzu, Japan). Calorimetric measurements were made with empty cell (high purity alpha alumina discs) as the reference. The instrument was calibrated using high purity indium metal as standard. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10 °C min^{-1}.

X-ray powder diffraction (XRD)

Samples were subjected to XRD studies using an X-ray diffraction meter (Rigaku miniflex, Japan) with Cu-NF filtered Cu Kα radiation. Quartz was used as an internal standard for calibration. Samples were scanned in the 2θ range of 5-100°. The scanning speed used for the recording was 3°/min with step size of 0.02°. The percentage crystallinity was calculated.

***In vitro* release studies**

Release of silibinin from the micelle formulation in vitro was monitored by a dialysis method. Dialysis was carried out at 37°C using Spectra/Pordialysis membranes (Spectrum Laboratories, Inc, Rancho Dominguez, CA, USA) with a molecular weight cutoff of 1 kDa and phosphate-buffered saline (pH 7.4) as the sink solution. The molecular weight cutoff of the dialysis membrane only allows for diffusion of the free drug. Briefly, 1 mL of the drug loaded micellar dispersion was placed in a dialysis bag. The end sealed dialysis bag was immersed into 100 mL of PBS (pH 7.4) at 37 °C which was stirred at 100 rpm speed. At scheduled intervals, 3 mL of the dialysis medium was collected and the same volume of fresh medium was added immediately. The samples from each time interval were analyzed spectrophotometrically at 287 nm for silibinin content [15-17].

Kinetic analysis of *in vitro* release data

In order to determine the release mechanism that provides the best description to the pattern of drug release, the *in vitro* release data were fitted to zero-order, first-order, Higuchi matrix model and Korsmeyer-Peppas model using the software, PCP Disso v2.08. The model with the highest correlation coefficient values or determination coefficient (R^2) was considered as the best fit model. The release data were also kinetically analyzed using the Korsmeyer–Peppas model and the release exponent (n) describing the mechanism of drug release from the matrices was calculated by regression analysis using the following equation:

$$M_t/M_\infty = kt^n$$

Where M_t/M_∞ is the fraction of drug released at time t and k is a constant incorporating the structural and geometric characteristics of the release device. When n = 0. 5, Case I or Fickian diffusion is indicated, 0.5<n<1 for anomalous (non-Fickian) diffusion, n = 1 for Case II transport (Zero order release) and n>1 indicates Super case II transport [21].

Stability studies

Formulations were packed in a screw capped bottle and studies were carried out for 12 months by keeping at

- 25° ± 2°C and 60 ± 5% RH
- 30° ± 2°C and 65 ± 5% RH

And for 6 months for accelerated storage condition at

- 40° ± 2°C and 75 ± 5% RH

Samples were withdrawn on 0, 3, 6 and 12 months for long term storage condition and 0, 3 and 6 months for accelerated storage condition and checked for changes in physical appearance and drug content as per ICH Q1A(R_2) guidelines [22].

RESULTS AND DISCUSSION

Solubilization efficiency of polymeric micelles

It was carried out with a rationale of comparing the solubilization efficiency of pure drug and drug in polymeric micelles. Solubility of silibinin increased 20-fold when formulated as polymeric micelles (Figure 2). The total solubility effect exerted by micelles may be because of entrapment of drug in the hydrophobic core with exterior hydrophilic surrounding. This investigation showed that the primary purpose of synthesizing polymeric micelles i.e. enhancement in solubilisation efficiency of silibinin is served.

ISSN: 0975-8585

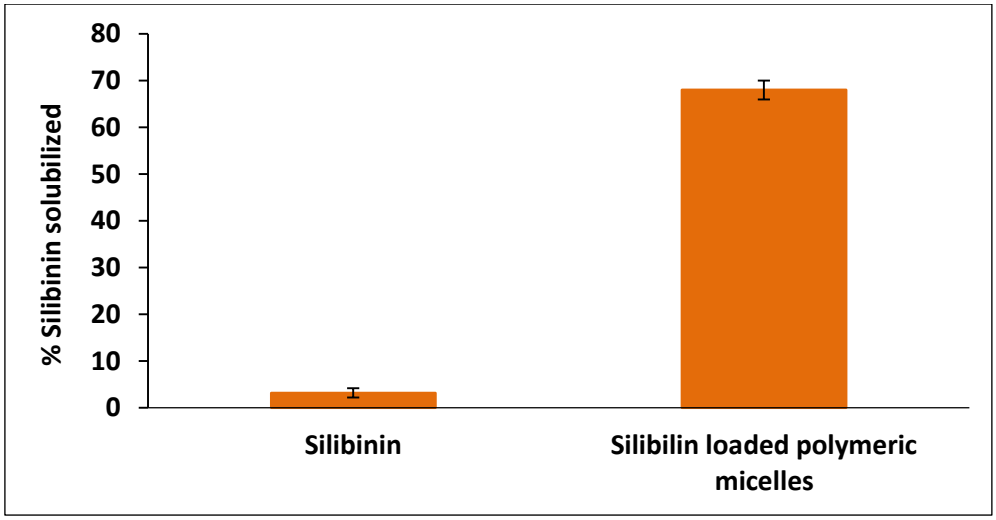

Figure 2: Solubilization efficiency of silibinin and drug loaded polymeric micelles in distilled water

Particle size, polydispersity and zeta potential measurement by dynamic light scattering (DLS)

The average particle size, polydispersity index and zeta potential for polymeric micelles were determined by dynamic light scattering.

The particle size analysis revealed that the average particle size is 285.8 nm with low polydispersity index value of 0.243. As known, the polydispersity index is a parameter used to define the particle size distribution of nanoparticles. It is a dimensionless number and it values ranges from 0.5 – 0.7 for monodispersed particles, Values greater than 0.7 are characteristic of samples with a broad size distribution. Therefore, it can be stated that the particle size distribution is unimodel, having a narrow range and a homogeneous size distribution.

The zeta potentials of silibinin polymeric micelles was sufficiently high (34.4 mV) to prevent agglomeration of micelles.

Encapsulation capacity of polymeric micelles

The percentage entrapment efficiency of Silibinin loaded polymeric micelles was found to be 78.2±2.1% suggesting that Silibinin can be effectively loaded into polymeric micelles.

Fourier transform infrared spectroscopy (FT-IR)

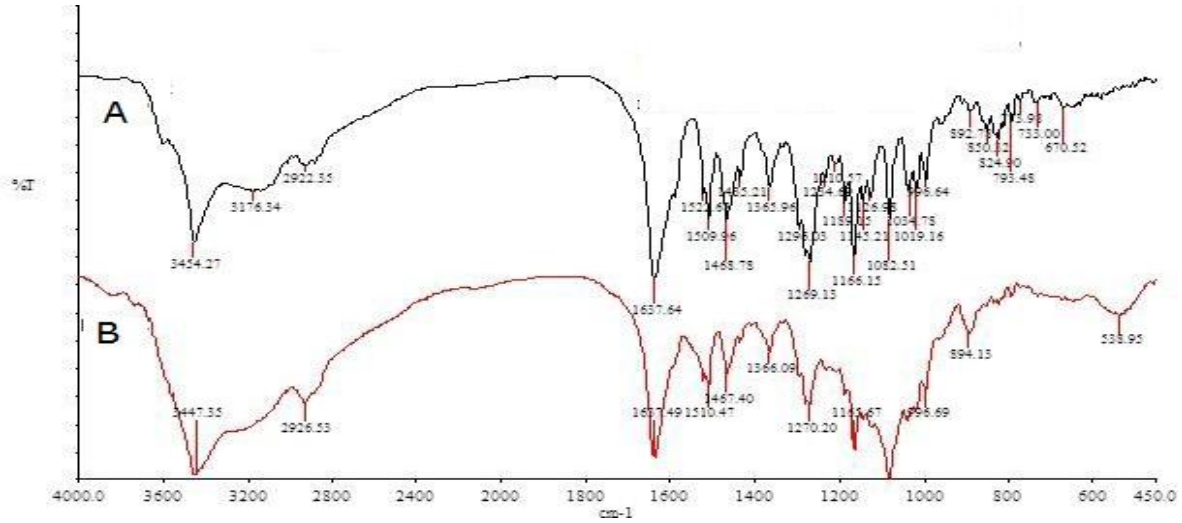

Figure 3: FT-IR spectra of (A) pure silibinin and (B) silibinin loaded polymeric micelles

ISSN: 0975-8585

This study is done to find the information regarding chemical bonding and molecular structure of a material and intermolecular interaction in solid material. FT-IR spectrum of Pure Silibinin and Silibinin loaded polymeric micelles were compared (Figure 3). Pure Silibinin showed the characteristic peaks at 3435.35 cm^{-1} due to -OH stretching, 2927.62 cm^{-1} due to -CH stretching, 1641.42 cm^{-1} due to -C=O stretching, 1512.16 and 1472.62 cm^{-1} due to -C=C skeleton vibration of aromatic ring stretching, 1268.18 cm^{-1} due to -C-O-C stretching. The spectra of drug loaded polymeric micelles did not show any changes in peak position from pure silibinin spectra. These result revealed that there is an absence of chemical interaction between silibinin and PF-68.

Differential scanning calorimetry (DSC)

As evident from figure 4, Silibinin showed a broad peak at 165-178 ^{o}C. The physical state of drug in the polymeric matrix influences drug release. The thermogram of silibinin loaded polymeric micelles did not show any peaks of silibinin. This shows that silibinin is completely encapsulated without any traces of drug on the surface of the polymeric micelles. From the figure, it is seen that the area and the enthalpies of the drug decreased in polymeric micelle formulation. This could be due to change in the state of the drug form crystalline to amorphous.

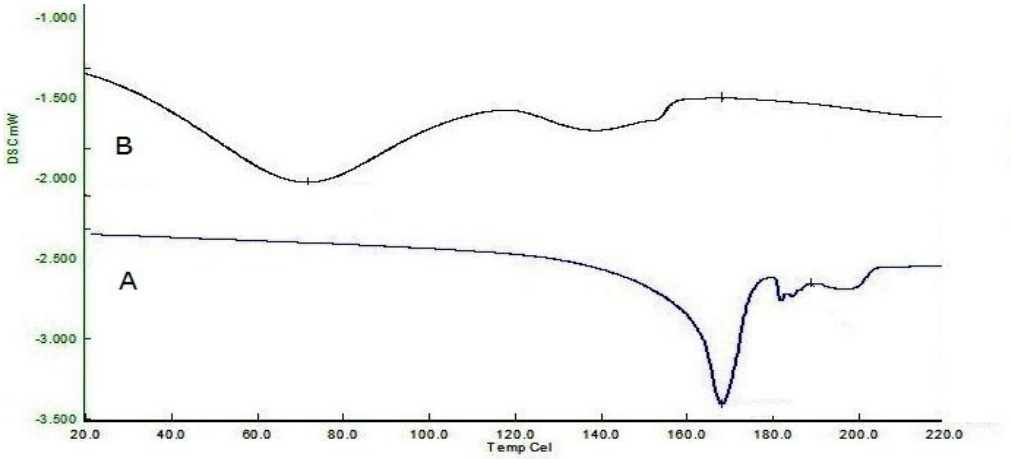

Figure 4: DSC spectra of (A) pure silibinin and (B) silibinin loaded polymeric micelles

X-ray powder diffraction (XRD)

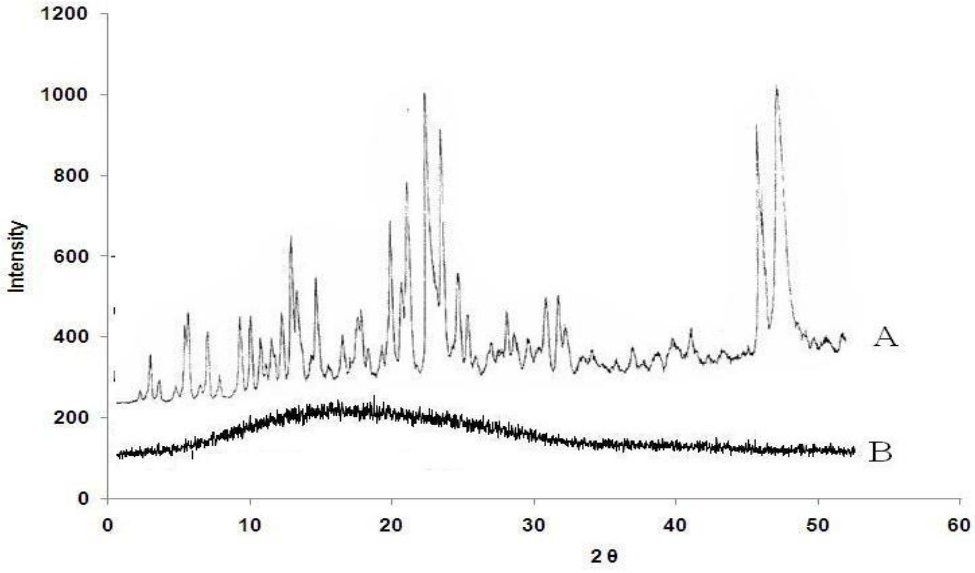

Figure 5: XRD diffractograms of (A) pure silibinin and (B) silibinin loaded polymeric micelles

The XRD difractograms of silibinin and silibinin loaded polymeric micelles are reported in figure5. The diffractogram of pure silibinin shows some intense peaks, which are indicative of crystallinity. When they

are prepared to nanoparticles there is an occurrence of phase transition, i.e., changes to amorphous state. This indicates that drug is completely entrapped in polymeric micelles. The PXRD pattern of polymeric micelles showed complete disappearance of the diffraction peak at the same 2θ value, confirming the existence of the amorphous form of silibinin.

***In vitro* release studies**

The release pattern of silibinin was studied in PBS (pH 7.4) at 37±1 °C. Figure 6 shows the drug release behavior of the PF-68 micelles and drug solution. Cellophane membrane with molecular weight cut off around 1 kDa was used for the experiments that retains polymeric micelles and only permits transfer of drug in solution form. The release of incorporated drug molecules form the micellar system is governed by transfer of drug from micelles to the surrounding aqueous medium followed by diffusion through the cellophane membrane into the receptor medium. In case of silibinin solution drug was released at a slow rate due to its limited solubility whereas significantly more ($p<0.05$) amount of drug was released from micellar formulations. Silibinin incorporated in the hydrophobic core is retained firmly by the micelles as observed by the controlled release with *in vitro* sink condition [15].

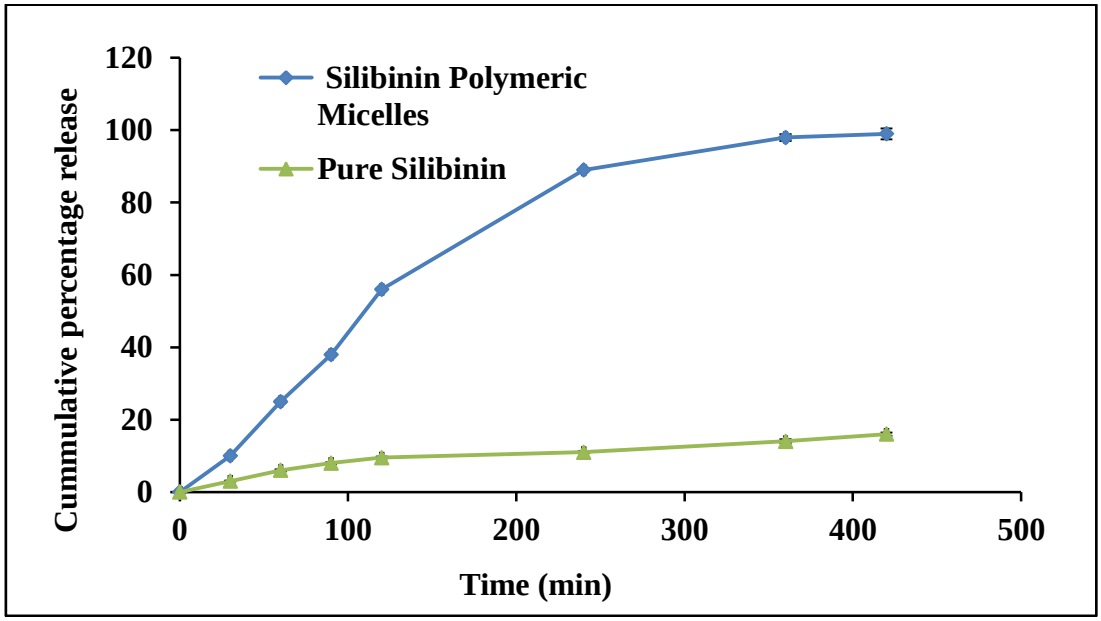

Figure 6: *In vitro* release study of pure silibinin and polymeric micelles

Kinetic analysis of *in vitro* release data

The best fit model with the highest correlation coefficient values or determination coefficients (R^2) for the formulations was the Korsmeyer-Peppas model. When the *in vitro* release data from the formulations were fitted to the Korsmeyer and Peppas equation, the values of n obtained were >1 in all the cases. The n value ranged from 1.5476. Since the n value was >1, this indicated a super case-II transport wherein multiple release mechanisms exists, predominant being swelling and relaxation. This is the ideal method of drug release in order to achieve a pharmacological prolonged, sustained and controlled action.

Stability studies

The observations of long-term storage conditions and accelerated conditions are shown in the table 1. Results indicate no significant changes in the parameter even when it was subjected to stress testing for a period of six months.

When the polymeric micelle formulation was studied for long-term storage conditions and accelerated conditions, the drug content in the formulation within the 95% confidence interval and hence the slight decrease in the drug content was statistically not significant.

Table1: Stability study data of the silibinin loaded polymeric micelles

Stability condition	Sampling interval (months)	Physical appearance	% Drug content*
25°±2°C/60±5% RH	**0**	No change	99.30±0.10
	3	No change	99.12±0.14
	6	No change	98.98±0.08
	12	No change	98.85±0.12
30°±2°C/65±5% RH	**0**	No change	99.30±0.10
	3	No change	99.02±0.11
	6	No change	98.92±0.10
	12	No change	98.78±0.14
40º±2°C/75±5% RH	**0**	No change	99.30±0.10
	3	No change	98.40±0.32
	6	No change	97.80±0.39

*Mean±SD, n=3

CONCLUSION

In the present study, silibinin loaded polymeric micelles were prepared by sonication method and characterized for the delivery of silibinin. Polymeric micelles showed high solubility than pure silibinin. Silibinin was encapsulated in polymeric micelles and characterized by different techniques, which proved its encapsulation within the micelles. The polymeric micelles showed high drug encapsulation efficiency, enhanced dissolution and sustained drug release. Due to more solubilization, the bioavailability of silibinin can be expected to be more as compared to plain drug. The micelles were stable under the conditions of staorage. All of these properties are desirable features in effective therapeutic formulations, and these results strongly suggest that the prepared PF-68 polymeric micelles could be used as a functional drug carrier for silibinin.

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