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Research Article

### FORMULATION, CHARACTERIZATION AND *IN-VITRO* RELEASE STUDY OF SILYMARIN NANOSUSPENSION

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**Abstract:**

**Objective:** The main objective of this research was to carry out formulation and evaluation of polymeric nanosuspension for silymarin drug by using suitable surfactants to improve its bioavailability.

**Methods:** The silymarin nanosuspension was prepared and lyophilized for enhancing the dissolution of poorly soluble drug. The high pressure homogenization technique was adapted to produce the silymarin nanosuspension respectively using polymers such as Poloxamer188, Poloxamer 407 and Soya lecithin with TPGS as stabilizers in various proportions in combinations. Further, the prepared silymarin nanosuspension were characterized for particle size, poly dispersity index (PDI), zeta potential, drug content, solubility study, in vitro release study, compatibility studies (FT-IR, DSC), in vitro permeability studies and stability studies.

**Results:** The silymarin nanosuspension were prepared and lyophilized. The mean particle size ranged from  $100.6 \pm 0.16$  d.nm to  $275.0 \pm 0.21$  d.nm and the drug content ranged from 94.73 % to 99.61 %. Drug content of polymeric nanosuspension was increased by increasing drug to polymer ratio. The FTIR study confirmed the stable nature of silymarin in the drug-loaded polymeric nanosuspension. In DSC thermogram of pure silymarin a short melting endothermic peak was observed at  $148.3^{\circ}\text{C}$  due to melting point of the pure silymarin. At  $263^{\circ}\text{C}$  owing the presence of amorphous form of the drug in nanosuspension or the dissolution of crystalline drug into the molten carriers. From thermogram it was concluded that the drug and the surfactant do not interact with each other. All the silymarin nanosuspension showed good dissolution property ranging from 90.49 % to 97.96 % in the in-vitro release study after 12 hours. The in-vitro permeability study were shows enhanced diffusion due to huge specific surface area of the nanosuspension droplets and improved permeation of the drug because of the presence of surfactant, which reduces the interfacial tension of formulation. Stability studies were carried out for the best formulation, SF2 indicates that there is no change in drug content and dissolution profile of the formulation.

**Conclusion:** The results obtained in this research work clearly indicated that nanosuspension 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 and poor systemic bioavailability.

**Key words:** Nanosuspension, high pressure homogenization, Poloxamer 188, poloxamer 407, soya lecithin, TPGS.

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## INTRODUCTION:

One of the major problems associated with poorly soluble drugs is very low bioavailability. The problem is even more complex for drugs which are poorly soluble in both aqueous and nonaqueous media, belonging to BCS class II as classified by biopharmaceutical classification system. Formulation as nanosuspension is an attractive and promising alternative to solve these problems. *Silybum marianum*, commonly known as milk thistle in the family Asteraceae, is one of the oldest and thoroughly researched plants of ancient times for the treatment of liver and gallbladder disorders, hepatitis, cirrhosis, jaundice and protection against *Amanita phalloides* mushroom and other toxin poisonings. Silymarin, the active component of this plant, is a standardized extract consisting of approximately 70 to 80 percent silymarin flavonolignans (silybin A & B, isosilybin A & B, silydianin and silychristin) and flavonoids (toxifolin and quercetin), and the remaining 20 to 30 percent consisting of a chemically undefined fraction comprised of polymeric and oxidized polyphenolic compounds [1].

Silymarin possesses wide range of biological and pharmacological effects, including antioxidant activity [2], stimulation of protein synthesis and cell regeneration (making it useful in the treatment of toxic liver damage, chronic inflammatory liver diseases and liver cirrhosis) [3,4], and impressive anticancer activity against several human carcinoma cell lines [5,6]. In addition, antidiabetic activity [7], cardioprotection [8], anti-inflammatory, antifibrotic, hypolipidemic, neurotrophic and immune modulation effects [9]. Developing novel strategies to enhance the solubility of poorly water soluble drugs is one of the main focuses of pharmaceutical technology [1, 2]. The bioavailability of poorly soluble drugs can be improved by conversion of micro particles to drug nanoparticles. Nanotechnology mainly refers to the study of materials and structures at the nanosized level. In micronization process of drugs the chance for agglomeration is high, so in order to avoid the agglomeration, nano-scale systems are used. A nanocrystal is a material with dimensions measured in nanometers, and particle size ranging from 1- 1000 nanometers. Nanotechnology is a promising strategy for improving the dissolution rate and oral bioavailability of poorly water soluble drugs by reducing the particle size and/or transforming drug from a crystalline to an amorphous state [3, 4]. In the present study, silymarin nanosuspension were developed using a hydrophilic polymer, Polaxamer 188, Polaxamer 407 and Soya lecithin with TPGS in combination.

## MATERIALS AND METHODS:

### Materials

Silymarin was a gift sample from HIMEDIA, Mumbai. TPGS, Soya lecithin, Poloxamer 188, Poloxamer 407 polymers were received as the gift sample from Ludwigshafen Germany, Glenmark Generic Limited, Dr.Reddy's Generics, India. All other ingredients used were of analytical grade.

### Formulation of Silymarin Nanosuspension

Nanosuspension of silymarin was prepared by high pressure homogenization technique by using the different concentration of the stabilizers like poloxamer 188 (SF1), poloxamer 407 (SF2) and soya lecithin (SF3) with 0.5 % and TPGS (1%) respectively, but in all the formulation drug concentration remain constant. Silymarin powder (1 % w/v) was dispersed in aqueous surfactant solution using magnetic stirrer. After drug dispersion in the surfactant solution first size reduction step was carried out using an Ultra- Turax T25 basic homogenizer at 9500 rpm for 10 minutes. The obtained mixtures were homogenized using Micron-LAB 40 high pressure homogenizer, the homogenization step includes first two cycles at 100 bar and next two cycles at 500 bars pressure as initial step. Finally the suspension was homogenized for 15 cycles at 1500 bar pressure [10].

**Table 1: Composition of silymarin nanosuspension formulation**

Code	Drug: Polymer: TPGS ratio
SF1	1:0.5:1 (Silymarin: Poloxamer188: TPGS)
SF2	1:0.5:1 (Silymarin: Polaxamer407: TPGS)
SF3	1:0.5:1 (Silymarin: Soya lecithin: TPGS)

### Formulation of Dry Nanoparticles

The homogenized nanosuspensions were freeze dried to increase the shelf life of suspension 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 nanosuspension. 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[11].

### Particle Size Distribution and Polydispersity Index

The particle size analysis of different batches of nanosuspension 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 [12].

#### **Zeta potential analysis**

The particle charge was one of the most important parameter in assessing the physical stability of emulsion and suspensions. 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 nanosuspension 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 [12].

#### **Fourier Transform Infra- Red Spectroscopy**

FT- IR spectra were recorded on the sample prepared in KBr disks (2mg sample in 200mg KBr disks) using Shimadzu Fourier Transform Infra-Red spectrometer. The samples were scanned over a frequency range 4000-400  $\text{cm}^{-1}$ [13].

#### **Re-Dispersibility and Percentage Drug Content Determination**

The prepared nanosuspension was analyzed for drug content by UV spectroscopic method. Different batches of nanosuspension 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 [14].

#### **Saturation Solubility Studies**

The saturation solubility studies were carried out for both the unprocessed pure drug and different batches of lyophilized nanosuspension. 10mg of unprocessed pure drug and nanosuspension equivalent to 10 mg of silymarin was weighed and separately introduced into 25 ml stopper 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 287nm [15].

#### **Differential Scanning Calorimetry**

Thermal properties of formulations were analyzed by differential scanning calorimetric analysis using Toledo-DSC II. To characterize the changes in internal structure DSC analysis was carried out for pure drug, polymer and the lyophilized suspension. The 5mg of sample is taken in the aluminum vial and kept in the instrument. The sample was then heated from 20°C to 200°C at a heating rate of 10°C/ min under a stream of nitrogen at flow rate of 50 ml/min. Enthalpy changes ( $\Delta H$ ) were calculated peak to study the polymeric changes in the formulations [17].

#### **In-Vitro Drug Release Studies**

The *in-vitro* release of silymarin drug and its nanosuspension 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 7.4) 10 mg of pure drug and nanosuspension containing 10 mg of silymarin equivalent was taken and placed in dissolution medium. The volume and temperature of dissolution medium were 900 ml and  $37.0 \pm 0.2^\circ\text{C}$ , 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 batches of formulation were compared with the dissolution profile of unprocessed drug [15].

#### **Permeation Studies**

Permeation study was carried out for both unprocessed drug and different batches of nanosuspension using cellulose nitrate membrane. The membrane was attached to the diffusion cell and then it was dipped in a beaker containing phosphate buffer pH 7.4. The pure drug sample and equivalent quantity of lyophilized nanosuspension were weighed and placed in the different diffusion cell containing the specific quantity of buffer. The samples were withdrawn at specific time intervals for 1 hr and replaced with fresh buffer solution. Finally the samples were analyzed using UV spectrophotometer at 287 nm [16].

#### **Stability Study**

Stability studies were carried out for silymarin nanosuspension formulation as per ICH guidelines. The best silymarin nanosuspension formulation (SF3) was sealed in high density polyethylene bottles and stored at  $4 \pm 1^\circ\text{C}/\text{Ambient}$ ,  $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{RH}$  %,

$40 \pm 2$  °C/ $75 \pm 5$  % RH for 90 d. The samples were periodically evaluated for percentage silymarin nanosuspension. [18].

## RESULTS AND DISCUSSION:

### Particle Size, Poly Dispersity Index and Zeta Potential

The particle size distribution has most important characteristics affecting the *in-vivo* fate of nanosuspension. Polydispersity index gives degree of

particle size distribution and promotes the physical stability of nanosuspension. The determination of the zeta potential parameter (properly related to the double electric layer on the surface of colloidal particles) of a nanosuspension is an essential as it provides an indication about the physical stability of nanosuspension. The particle size, poly dispersity index and zeta potential of silymarin nanosuspension were shown in Table.2 and the graphs are presented in Fig. 1, 2 3 (particle size and PDI) 4, 5, 6 (zeta potential).

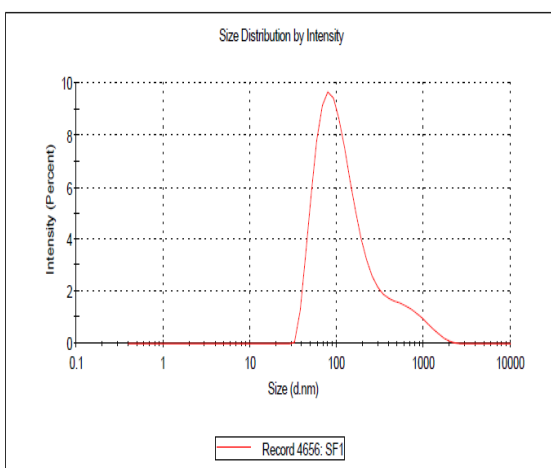
**Table.2: Particle size, Poly dispersity index, Zeta potential of silymarin nanosuspension**

S.No	Formulation code	Average Particle size (d.nm)	Poly dispersity index	Zeta potential
1.	SF1	$100.6 \pm 0.16$	$0.265 \pm 0.05$	-4.04
2.	SF2	$116.3 \pm 0.13$	$0.230 \pm 0.01$	-2.47
3.	SF3	$275.0 \pm 0.21$	$0.449 \pm 0.09$	-4.18

Mean of three observation  $\pm$  SD.

#### Results

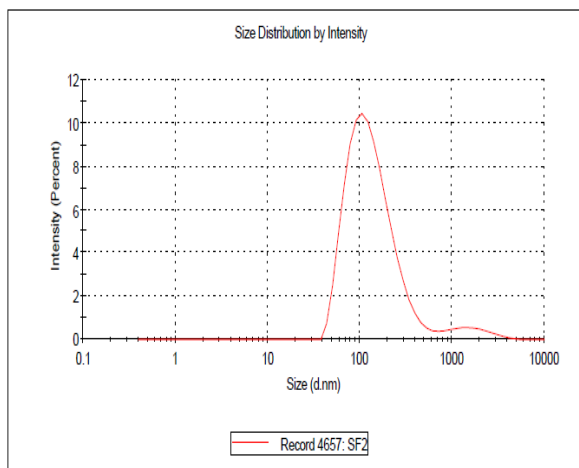
Size (d.nm): % Intensity: St Dev (d.nm...  
**Z-Average (d.nm): 100.6** Peak 1: 190.7 100.0 243.4  
**Pdi: 0.265** Peak 2: 0.000 0.0 0.000  
 Intercept: 0.675 Peak 3: 0.000 0.0 0.000  
 Result quality : Good



**Fig.1: SF1 particle size and PDI**

#### Results

Size (d.nm): % Intensity: St Dev (d.nm...  
**Z-Average (d.nm): 116.3** Peak 1: 145.8 94.8 95.40  
**Pdi: 0.230** Peak 2: 1686 5.2 842.1  
 Intercept: 0.669 Peak 3: 0.000 0.0 0.000  
 Result quality : Good



**Fig.2: SF2 particle size and PDI**

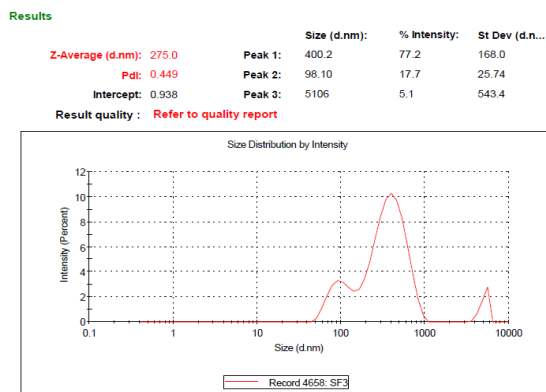


Fig.3: SF1 particle size and PDI

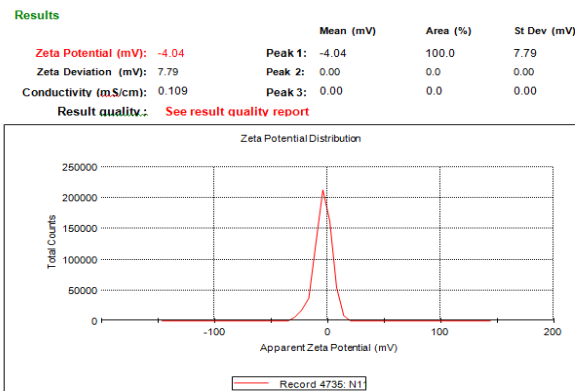


Fig.4: SF1 zeta potential

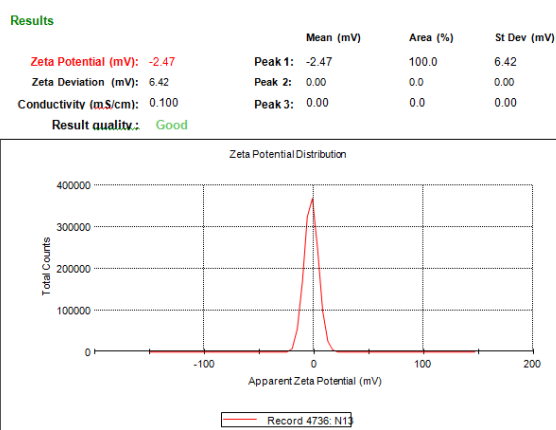


Fig.5: SF2 zeta potential

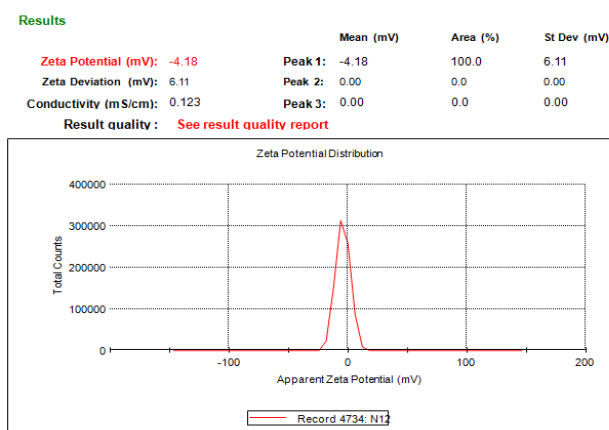


Fig.6: SF3 zeta potential

### Fourier Transform Infra Red Spectroscopy

The FT-IR analysis was used to evaluate the possible intermolecular interaction between silymarin and the excipients. Due to similarities in molecular structure of poloxamer 188 and poloxamer 407 showed similar absorption bands, in which the IR spectra of formulation showed all the characteristics peaks

without any makeable change in their position after successful lyophilized nanosuspension, indicate there is no chemical interaction between silymarin, poloxamer 188 and poloxamer 407, soya lecithin nanosuspension. The spectra are represented in Fig. 7, 8, 9.

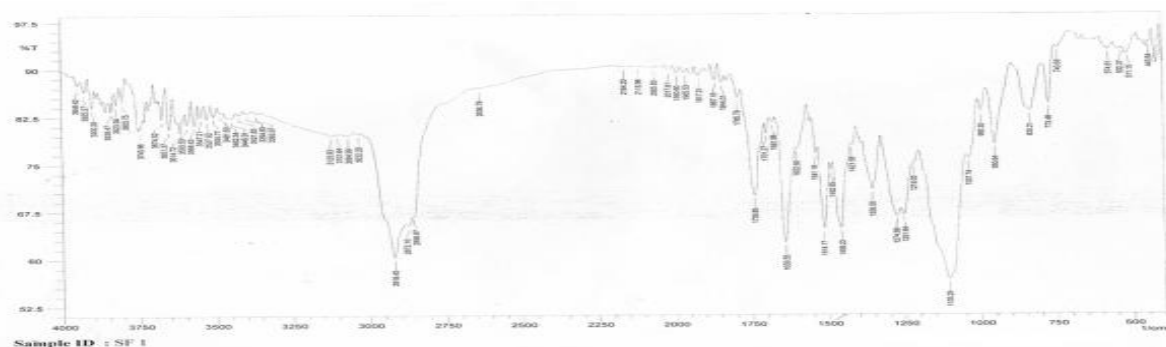


Fig.7: FT-IR of Silymarin

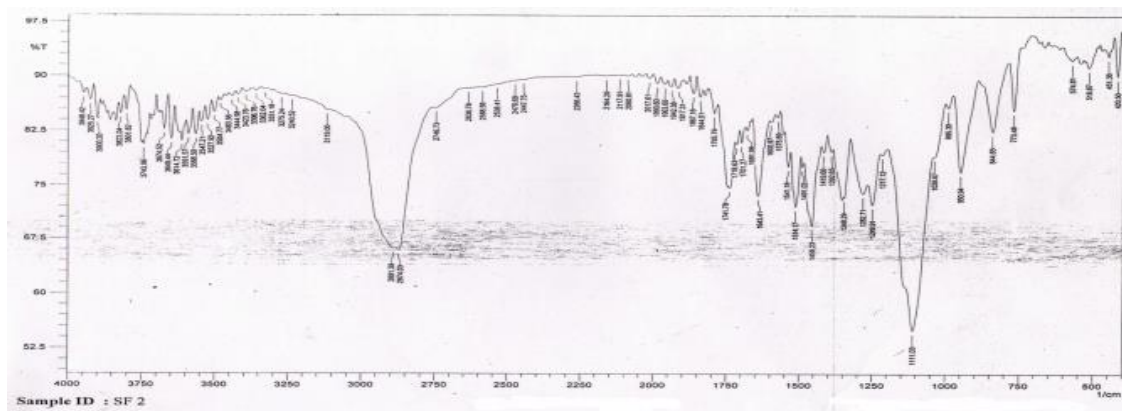


Fig.8: FT-IR of Poloxamer 407

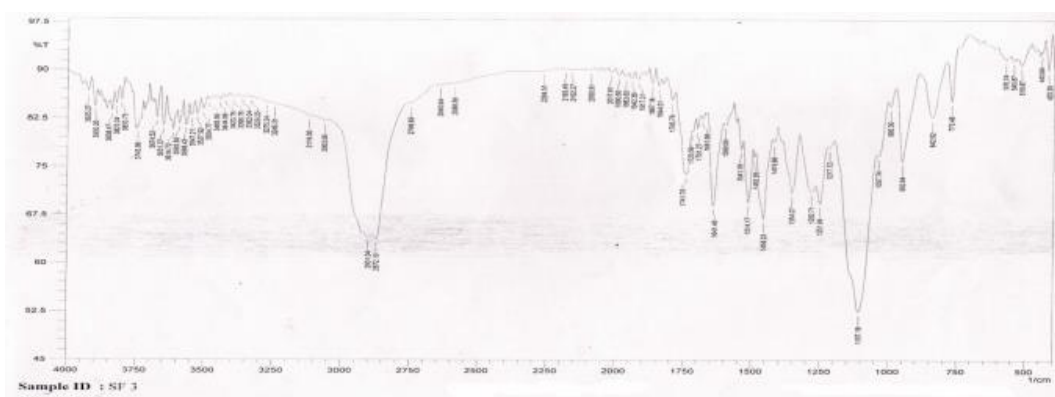


Fig.9: FT-IR of Soya lecithin nanosuspension

### Drug Content Determination

In nanosuspension formulation the drug particles were reduced to nano size. The results showed the all nanosuspension have shown the presence of high drug content low standard deviation and loss of drug

was lower during preparation process. It indicates that the drug is uniformly dispersed in the powder formulation. Therefore, the method used in the study appears to be reproducible for preparation of nanosuspension. The results are as given in Table 3.

Table.3: Percentage drug content of all lyophilized nanosuspension.

S.No	Formulation code	% drug content
1.	SF1	95.49 ± 0.63
2.	SF2	99.61 ± 0.32
3.	SF3	94.73 ± 0.12

Mean of three observations ± SD.

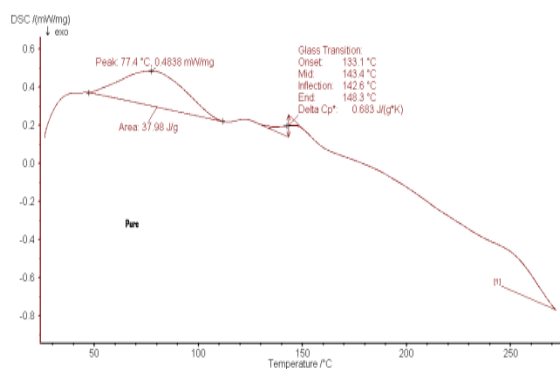


Fig.10: DSC thermogram of pure silymarin

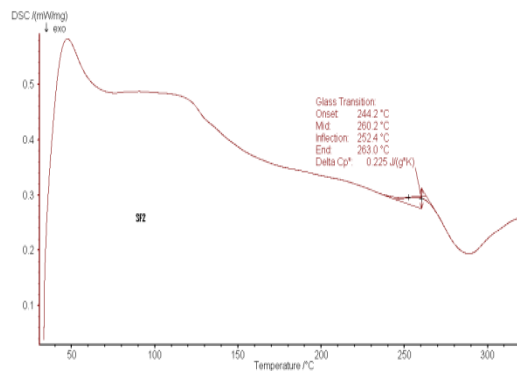


Fig.11: DSC curve of silymarin loaded poloxamer 407

### Thermal Analysis

Differential scanning calorimetry was used to elucidate the physical state of the drug within the system. In DSC thermogram of pure silymarin (Figure.10) a short melting endothermic peak was observed at 148.3°C due to melting point of the pure silymarin. At 263°C owing to the presence of amorphous form of the drug in nanosuspension or the dissolution of crystalline drug into the molten carriers. However, no characteristic melting peak of silymarin was observed in the DSC curve of silymarin loaded poloxamer 407. Lyophilized nanosuspensions were molecularly dispersed in an amorphous form. There was no important difference

between the components during heating. From thermogram it was concluded that the drug and the surfactant do not interact with each other. The thermogram was represented in Fig.10 and 11.

### Saturation Solubility Studies

The solubility profile of nanosuspension increases dissolution velocity and saturation solubility, size reduction leads to increase in dissolution rate. Enhancement in saturation solubility was found to in order of poloxamer 407 > poloxamer 188 > soya lecithin. The solubility of prepared nanosuspension in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.2) are represented in Fig.12.

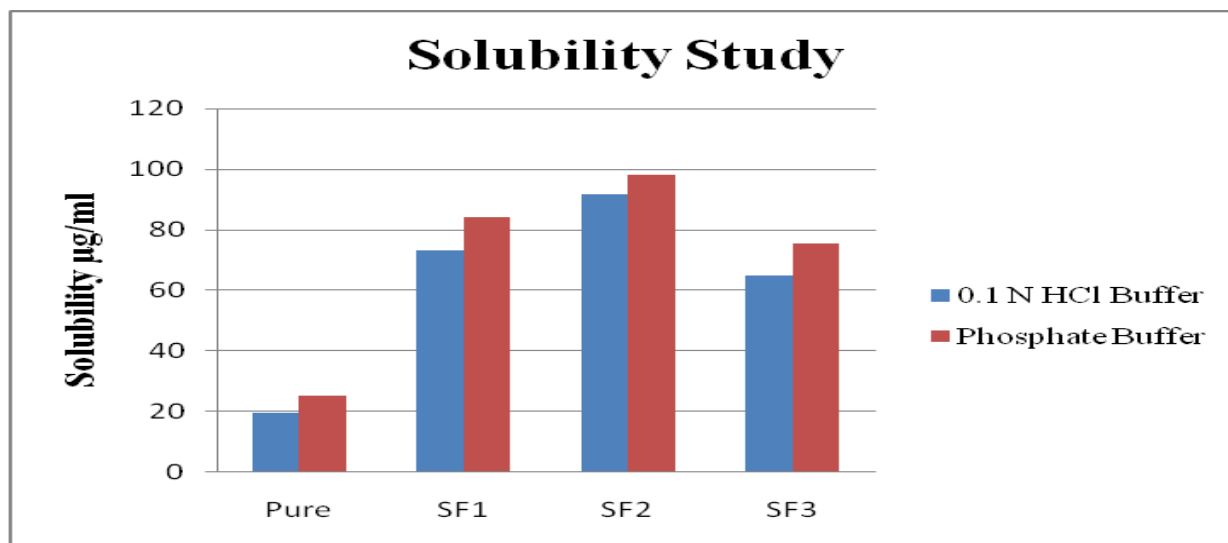


Fig.12: Saturation solubility studies of pure drug and nanosuspension.

### ***In – Vitro* Dissolution Studies**

The most important feature of nanosuspension is the increase in the dissolution velocity and saturation solubility. Silymarin is a poorly soluble drug. Its solubility is pH dependant increasing with 0.1 N HCl (pH1.2) and phosphate buffer (pH 7.4) was selected for dissolution studies to stimulate gastric condition and allows the greater discrimination of our

processing effects. *In-vitro* dissolution profile of the percentage release versus time profile of pure silymarin and different silymarin nanosuspension samples were determined in 0.1 N HCl and phosphate buffer under sink condition. *In-vitro* drug release data from the nanosuspension were carried out for 12 hours and graphically represented as % drug release versus time profile (Fig. 13 and 14).

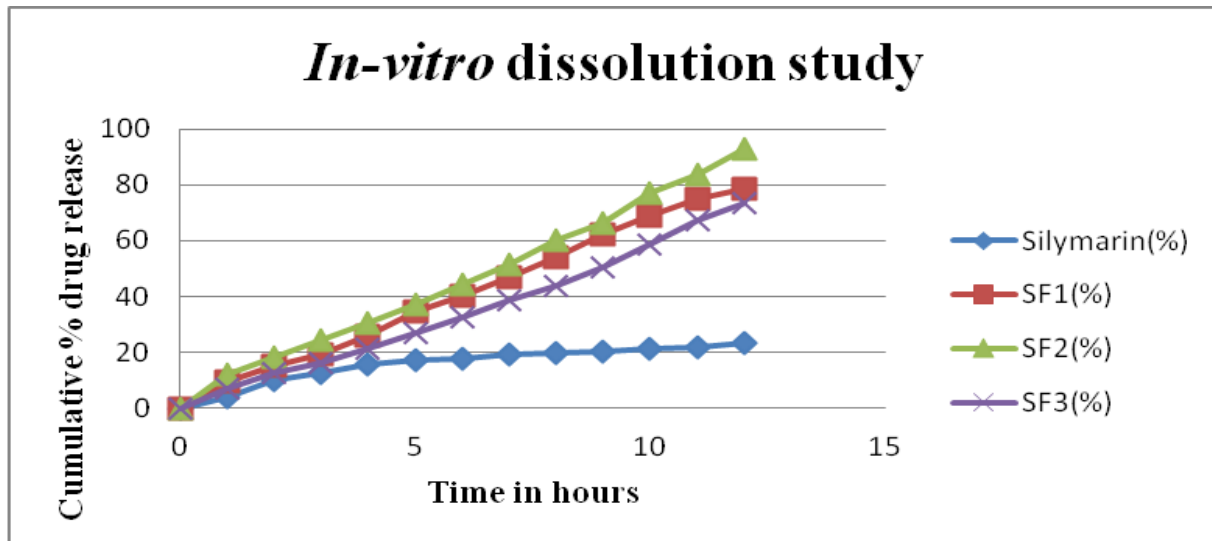


Fig.13: Comparative dissolution study of different silymarin formulations in acid buffer (pH 1.2)

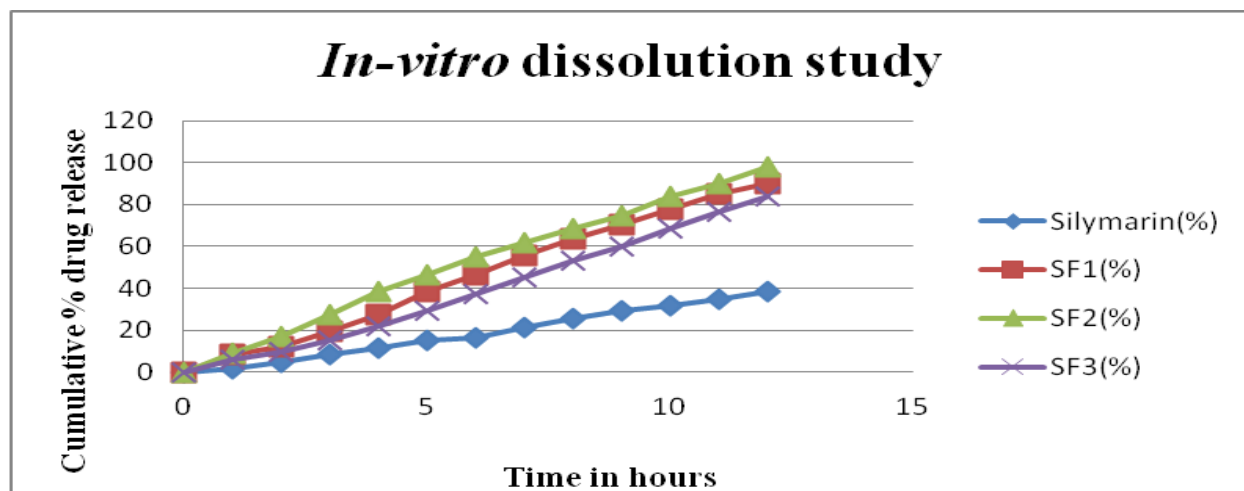


Fig.14: Comparative dissolution study of different silymarin formulations in phosphate buffer (pH 7.2)



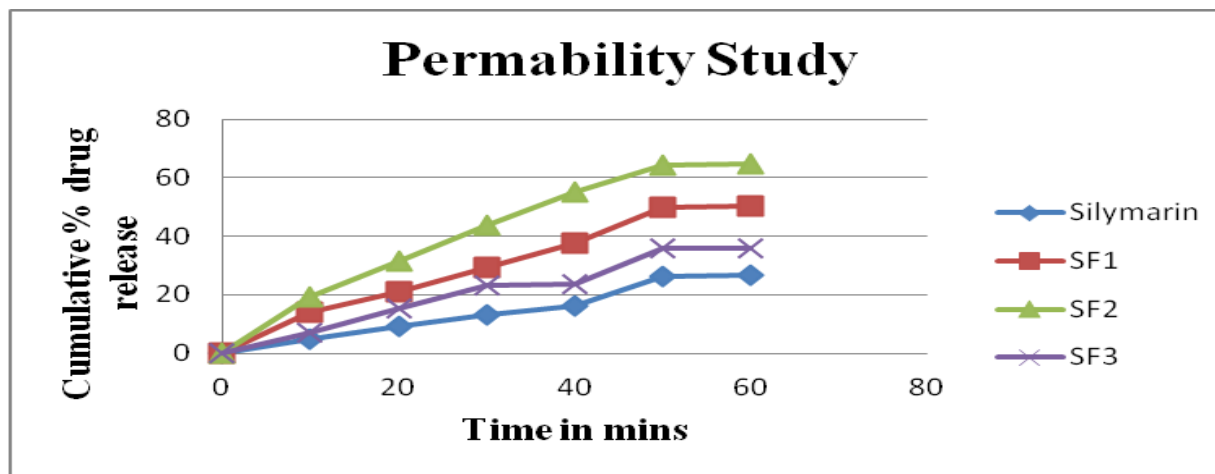


Fig.15: Comparative permeability studies of lyophilized nanosuspension and pure drug in phosphate buffer (pH 7.2).

#### **In-Vitro Permeability Studies:**

The *in-vitro* permeability study was carried out using Franz Diffusion Cell. After 1 hour of diffusion, 50.33% (SF1), 64.83% (SF2), and 35.95% (SF3) of the drug was diffused from the lyophilized nanosuspension respectively, while from pure drug, the diffusion was found to be 26.61% respectively. Thus, the amount of the drug diffused through the nitro cellulose membrane has doubled when it is given in the form of a nanosuspension. It can be clearly seen that the permeation of the drug from lyophilized nanosuspension is much faster than the pure drug. The results are shown in Fig.15.

#### **Stability Study**

After 3 months, storage of SF3 formulation at  $4\pm 1$  °C/Ambient,  $25\pm 2$  °C/ $60\pm 5$  % RH,  $40\pm 2$  °C/ $75\pm 5$  % RH, percentage of drug content and *in-vitro* dissolution were checked and found to be almost similar to the initial values. There was no substantial alteration in any value and also the physical appearance. So it can be said that silymarin nanosuspension prepared with poloxamer 407 is stable.

#### **CONCLUSION:**

Silymarin nanosuspension was successfully prepared by high pressure homogenization technique. This method of manufacturing was found to be simple, did not require specialized equipments and has scale – up feasibility. From the reports, the particle size and zeta potential values were measured immediately after

preparation of nanosuspension. The particle size of the nanosuspension is homogenous in size and size distribution. All the formulation showed lower particle sizes. The zeta potential of best formulation (SF2) indicating good quality. *In-vitro* dissolution studies indicated that the dissolution rate of the drug from the lyophilized nanosuspension is significantly higher than that of the pure drug. These observations lead us to the conclusion that nanosuspension 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 and poor systemic bioavailability.

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