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Formulation and Characterization of Solid Dispersed, Hepatoprotective Agent Silymarin

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ABSTRACT

Silymarin, a hepatoprotective agent has poor aqueous solubility and high permeability [class II drug]. Its absorption is dissolution rate limited. In order to improve the dissolution profile of silymarin solubility enhancement techniques were employed. Among the various techniques, solid dispersions were employed due to its feasibility. Polymers used were HPMC and PEG. Silymarin was formulated by kneading method and solvent evaporation method, where HPMC and PEG were used in different ratios of drug: polymer. Polymer characterization for the prepared solid dispersion was done using FT-IR, XRPD, SEM and DSC. *In vitro* dissolution studies were carried out in 0.1M HCl and phosphate buffer mixed (pH 6.8). From the *in vitro* studies, dissolution efficiency and drug release kinetics was also calculated. FT-IR studies revealed that there was no interaction between the drug and the polymers used. DSC, XRPD and SEM supported the existence of silymarin in amorphous state, in the prepared solid dispersions. Drug content analysis showed maximum drug release of 101.03 for solid dispersion prepared using drug: HPMC in the ratio of 1:3 [KM_2] using polysorbate 80, employing kneading method. *In vitro* release of KM_2 was found to be 98.87 ± 0.32 in 0.1 M HCl buffer. Kneading method proved to be superior to solvent evaporation method. Compared to the pure drug silymarin, solid dispersions [KM_2] gave a 2.2 fold increase in the dissolution profile.

Keywords: Solubility enhancement, Solid dispersions, Kneading method.

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INTRODUCTION

Silymarin is a flavonolignan, commonly known as milk thistle. It is extracted from the seeds and fruits of *Silybum marianum* belonging to the family Asteraceae[1]. It is mainly composed of silybin, silidianin and silychristin which are responsible for the hepatoprotective activity of silymarin. Silymarin is used to treat different types of disease conditions like hepatitis, alcohol related liver diseases, cirrhosis and jaundice. Usual adult dose of silymarin is 140mg bid/tid. Silymarin, even though has no lipophilic structure in its molecule has low aqueous solubility[2]. Under BCS, silymarin is classified as Class II drug i.e. poorly soluble and highly permeable drug[4]. Only 20 – 50% of silymarin is being absorbed after oral administration from the GIT after which it undergoes enterohepatic circulation. So the drug release patterns of silymarin have to be augmented by means of various solubility enhancement techniques. Among them solid dispersion technique was one of the effective approaches employed to enhance bioavailability of silymarin. Kneading method and solvent evaporation method were employed using polymers HPMC and PEG.

MATERIALS AND METHODS

Materials used

Pure drug silymarin (Micro labs) was used for preparing the solid dispersions. HPMC, polysorbate 80, poly ethylene glycol and alcohol used were of analytical grade procured from Hi media laboratories and Sigma Aldrich.

Preparation of calibration graph

The UV absorbance of the working standard solution of silymarin in ethanol was obtained at 286 nm against 0.1N HCl as blank. Calibration graph was generated by plotting the absorbance values against corresponding concentrations of silymarin.

Preparation of solid dispersions of Silymarin

Kneading method:

Silymarin and HPMC where weighed in the ratio of 1:1, 1:3 and 1:5. Weighed amount of HPMC was transferred to the mortar and moistened with ethanol in order to ensure complete solubility of HPMC in water. Silymarin was then added to the above mixture and triturated well. Few drops of 2% w/v solution of polysorbate 80 were added into the resultant mixture to make a paste. It was then triturated for 30min in one direction. The paste formed was transferred to a china dish and desiccated at 80^0C for around 4-6 hrs until the paste has become a dried mass, which then was pulverized in a mortar and passed through 0.180 mm aperture sieve. The samples were collected and stored in a dessicator.

Silymarin, HPMC and PEG 6000 were weighed in the ratio 1:1, 1:3 and 1:5. Weighed amount of HPMC and silymarin was transferred into a mortar and wetted with ethanol. Weighed amount of PEG was dissolved in few ml of water and the solution was transferred to the above silymarin-HPMC mixture. It was then triturated for 30 min in one direction. The paste formed was then transferred to a china dish and desiccated at 80^0C for around 3-4 hrs. To ensure complete removal of moisture it was kept in a desiccator for 2 days (due to the presence of PEG). Then the dried mass was pulverized and passed through 0.180mm aperture sieve. The samples were collected and stored in a dessicator.

Solvent evaporation method:

Silymarin and HPMC were weighed in the ratio 1:1, 1:3 and 1:5. Silymarin and HPMC were wetted with required amount of ethanol. Few drops of polysorbate 80 were added to the above mixture and it is then transferred into a conical flask. Suction method was employed for evaporating the solvent. The dried mass was then pulverised and passed through 0.180mm aperture sieve. The samples were collected and stored in a dessicator.

Silymarin, HPMC and PEG 6000 were weighed in the ratio 1:1, 1:3 and 1:5. Silymarin and HPMC were wetted with required amount of ethanol. Weighed amount of PEG was dissolved in few ml of water and then transferred to the above mixture into a conical flask. Suction method was employed for evaporating the solvent. The dried mass was then pulverised and passed through 0.180mm aperture sieve. The samples were collected and stored in a dessicator. The formulations of the prepared solid dispersions are shown in table 1

Table 1. The formulation code for the prepared solid dispersions

Formulation code	Carrier	Drug : Carrier Ratio	Method
KM1	HPMC:Polysorbate 80	1:1 ratio	Kneading method
KM2		1:3 ratio	
KM3		1:5 ratio	
KM4	HPMC: PEG 6000	1:1 ratio	Kneading method
KM5		1:3 ratio	
KM6		1:5 ratio	
SEM1	HPMC:Polysorbate 80	1:1 ratio	Solvent evaporation Method
SEM2		1:3 ratio	
SEM3		1:5 ratio	
SEM4	HPMC: PEG 6000	1:1 ratio	Solvent evaporation Method
SEM5		1:3 ratio	
SEM6		1:5 ratio	

Physical characterization of silymarin solid dispersions

FTIR spectroscopy[4]: IR-spectroscopic analysis was carried out using a FTIR spectrophotometer and the spectrum was recorded in the wavelength region of 4000–400 cm^{-1}. The procedure consisted of dispersing the samples in KBr and gentle grinding to prepare pellets.

Differential scanning calorimetry (DSC)[5]: DSC was performed using a differential scanning calorimeter to study the thermal behaviour of samples. The samples were heated in hermetically sealed aluminium pans at a scanning rate of 10 °C min^{-1} from 50 ± 0.2 to 550 ± 0.2 °C. An empty aluminium pan was used as a reference.

X-ray powder diffraction (XRPD)[6]: X-ray diffraction study was carried out to characterize the physical form of silymarin in selected samples. The sample was allowed to spread on the glass slide in approximately 0.5 mm thickness. The slide was then placed vertically at 0° angle in the X-ray diffractometer so as to allow the X-ray beam to fall on it. The results were recorded over a range of 0-90° (2q) using a Cu-target X-ray tube and Xe-filled detector.

Scanning electron microscopy (SEM)[6]: Surface characteristics of the samples were studied by SEM from 100 to 650x magnifications. A double sided carbon tape was affixed onto aluminium stubs. The powder sample was sprinkled onto the tape. The aluminium stubs were placed in the vacuum chamber of a scanning electron microscope. The samples were observed for morphological characteristics using a gaseous secondary electron detector with working pressure of 0.8 Pa and acceleration voltage of 30.00 Kv

Drug content analysis

Preparations of silymarin solid dispersions equivalent to 70 mg was weighed accurately and transferred to a 100 ml volumetric flask and dissolved in few ml of ethanol. Then the solution was made up to volume using 0.1M HCl. The solution was suitably diluted and absorbance was measured at 286 nm against 0.1N HCl as blank. The drug content was then estimated using the calibration graph.

In vitro release studies

The release studies of silymarin preparations were carried out in USP XXIII apparatus 2 (basket type). The dissolution medium used was 900 ml of 0.1 M HCl and mixed phosphate buffer (pH 6.8). The system was thermostatically maintained at 37±0.5°C at 50 rpm. Solid dispersions, containing 70 mg equivalent weight of silymarin was transferred into a capsule and placed inside the vessel. At predetermined time intervals (15 min), 10 ml of the aliquots were withdrawn from the dissolution media, filtered through whatmann filter paper. 10 ml of the fresh dissolution media was replaced into the vessel in order to maintain sink condition. The collected samples were analysed spectrophotometrically at 286 nm.

DRUG RELEASE KINETICS [7]

The drug release kinetics of silymarin solid dispersions were determined by plotting various kinetic models, using the data collected from *in-vitro* release studies.

Zero order kinetics:

Cumulative amount of drug released was plotted against time. This kinetics describes concentration independent drug release from the formulations.

$$C = k_0t,$$

Where zero order rate constant is expressed in units of concentration and time, t in hours.

Log percent drug:

Log percent drug remaining vs. time was plotted. This kinetics describes concentration dependent drug release from the formulations.

$$\text{Log } C = \text{Log } C_0 - {kt}/{2.303}$$

where C_0 is the initial concentration of drug and k is the first order rate constant.

Higuchi model:

This was described by plotting against cumulative percentage of drug released vs. square root of time. This model describes the release of drug on the basis of fickian diffusion as a square root of time dependent process from swellable matrix.

$$Q = kt_{1/2}$$

Where k is the constant reflecting the design variables of the system.

Hixson-Crowell cube root law:

This was obtained by plotting cube root of percentage drug remaining against time. This kinetics correlates the release from the systems with polymer erosion/ dissolution.

$$Q_0^{1/3}-Q_t^{1/3} = k_{HC}\, t,$$

Where Q_t is the amount of drug released in time t, Q_0 is the initial amount of drug in the formulation and k_{HC} is the rate constant for the Hixson-Crowell rate equation.

RESULTS AND DISCUSSION

Physical characterization of silymarin solid dispersions

FT-IR spectroscopy

The FT-IR spectrum of the prepared solid dispersion (KM_2) is given in fig 1. From the spectra it was observed that there were no significant changes observed in the spectra of the solid dispersions to that of the corresponding pure drug and the polymers. The characteristic peak of silymarin was observed at 3426.89 (-OH stretching vibration), 2937 (O-H stretching), 1639.2 (C=O stretching), 996 (O-H out of plane stretching), 1032-1163 (in plane =C-H bending) and 645-823 cm^{-1}.

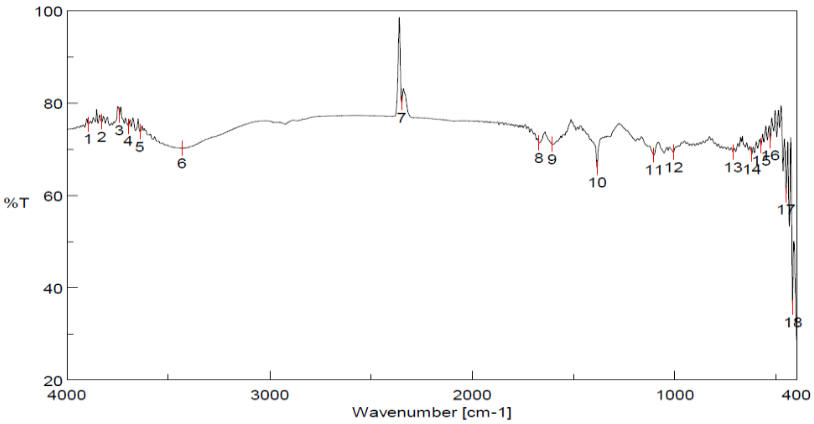

Figure 1: FT-IR spectrum of KM_2

KM2 indicates the formulation code for the one with the best release characteristics.

Differential scanning calorimetry (DSC)

The DSC thermogram of silymarin solid dispersion (KM_2) is shown in fig 2. Thermogram of silymarin solid dispersions (KM_2) depicted considerable shift in the endothermic peaks for kneaded solid dispersions from 120^0 C to 180^0 C. This indicates that there is a change in the crystalline structure of the drug. DSC technique had limitations to characterize the solid dispersions of silymarin due to low enthalpy [ΔH=24.280Jg^{-1}] of silymarin

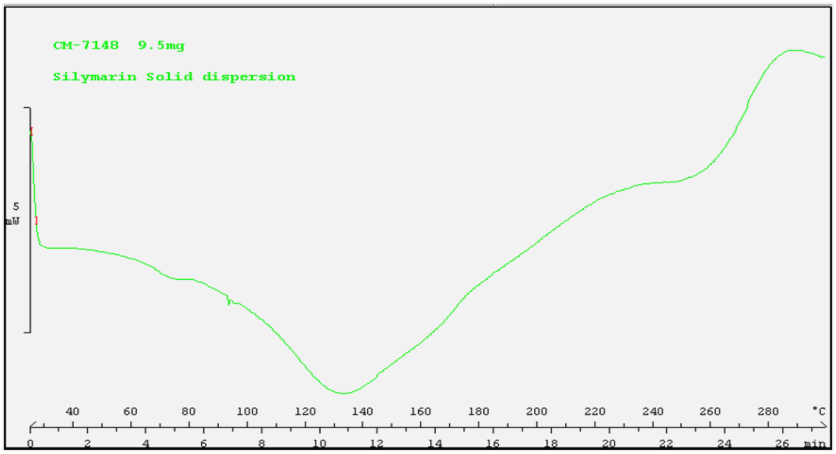

Figure 2: DSC of KM_2

X-ray powder diffraction (XRPD)

The presence of silymarin in amorphous state in the solid dispersion was evidenced by the less intense peaks obtained from X-ray diffraction studies when compared with that of standard silymarin.

Scanning Electron Microscopy

The SEM images of silymarin solid dispersions (KM_2) are shown in fig 4.The image depicts particles with increased surface area. The images also shows drug particles adsorbed onto the surface of the carrier. Porous formation is also seen in the particles along with cervices and fissures. The surface image of solid dispersions also supports the existence of silymarin in an amorphous state in the prepared solid dispersions.

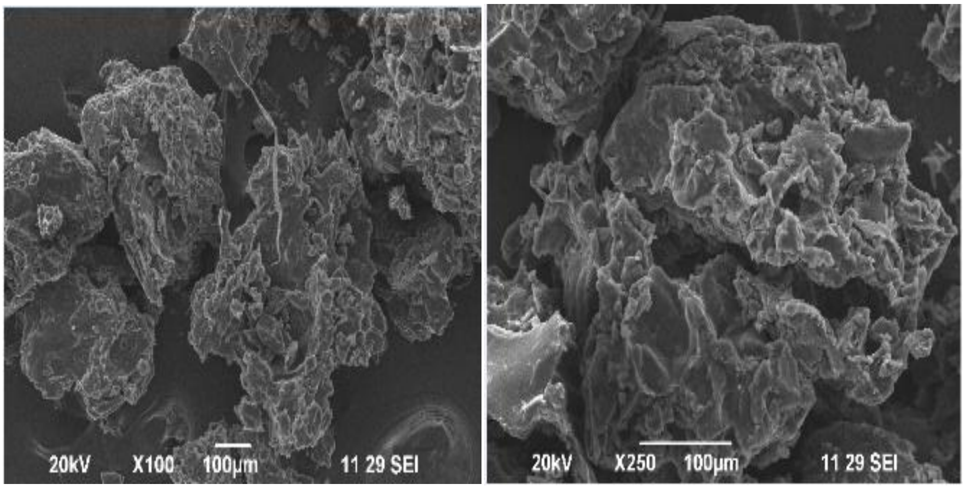

Figure 3: SEM images of KM_2

Drug content analysis

The drug content in solid dispersions prepared by kneading method (KM) and solvent evaporation method (SEM) ranged from 97.24- 101.03%. The data of drug content analysis is summarized in table 2.

Table 2. Drug content of silymarin in solid dispersions

S. No.	Drug : HPMC	% Drug content
1	KM 1	100.61
2	KM 2	101.03
3	KM 3	100.22
4	SEM 1	98.34
5	SEM 2	97.80
6	SEM 3	98.67
7	KM 4	97.45
8	KM 5	99.94
9	KM 6	98.32
10	SEM 4	97.24
11	SEM 5	97.86
12	SEM 6	98.90

In vitro release studies

The *in vitro* dissolution data of standard silymarin, Silymarin solid dispersions prepared by kneading method, and marketed product in 0.1M HCl is shown in table 3. The drug release studies were carried out for 120min. The dissolution rate of silymarin from the solid dispersions prepared using HPMC or combination of HPMC and PEG were found to be faster than the dissolution rate of standard silymarin. Enhanced dissolution could be attributed to many factors such as decreased particle size of the drug, in addition to the increase in drug wettability, prevention of drug aggregation by the polymers and possibility due to reduction in drug crystallinity. Generally, the release of silymarin in 0.1M HCl was found to be higher than that achieved in phosphate buffer of pH 6.8.

Table 3. Comparative dissolution profile of silymarin, its solid dispersions and marketed product in 0.1 M HCl by kneading method.

Time (hrs)	**Silymarin**	**KNEADING METHOD**						**Marketed product**
		Drug : HPMC			**Drug : HPMC : PEG**			
		1:1	**1:3**	**1:5**	**1:1**	**1:3**	**1:5**	
0	0	0	0	0	0	0	0	0
15	23.3 ± 0.21	27. 35 ± 0.43	35.67 ± 0.5	32.43 ± 0.23	22. 67 ± 0.21	29.64 ± 0.32	22.8 ± 0.64	31.65 ± 0.54
30	24.8 ± 0.43	37.94 ± 0.25	42.37 ± 0.4	39.6 ± 0.54	26.1 ± 0.54	32.82 ± 0.25	28.04 ± 0.11	33.05 ± 0..43
45	25.6 ± 0.51	49.25 ± 0.64	47.41 ± 0.11	51.35 ±0.8	28.32 ± 0.47	40.55 ± 0.11	41.4 ± 0.21	34.02 ± 0.11
60	26.8 ± 0.43	58.84 ± 0.36	57.42 ± 0.9	63.38 ± 0.4	34.21 ± 0.11	47. 60 ± 0.5	42.9 ± 0.34	35.202 ± 0.62
75	38.8 ± 0.2	69.36 ± 0.7	69.43 ± 0.3	70.69 ± 0.2	40.09 ± 0.56	52.09 ± 0.21	43.3 ± 0.4	41.181 ± 0.2
90	42.6 ± 0.32	74.41 ± 0.21	79.66 ± 0.3	81.41 ± 0.44	45.7 ± 0.41	54.89 ± 0.53	46.3 ± 0.45	45.9777 ± 0.7
105	43.9 ± 0.11	76.43 ± 0.81	84.48 ± 0.39	85.75 ± 0.91	52.06 ± 0.33	57.98 ± 0.47	47.32 ± 0.23	48.175 ± 0.11
120	45.29 ± 0.34	77.11 ± 0.11	98. 87 ± 0.32	88.42 ± 0.35	55.47 ± 0.2	60.21 ± 0.55	48.57 ± 0.43	51.107 ± 0.24

The cumulative percentage release were found to be in the order of kneading method > solvent evaporation method > pure drug. This suggests that preparation method has an influence on dissolution profile of silymarin. Solid dispersions of silymarin prepared using HPMC showed greater dissolution profile than that of a combination of HPMC and PEG polymers. Among the various solid dispersions, silymarin: HPMC in the ratio of 1:3 resulted in maximum cumulative release than the other two ratios, 1:1 and 1:5. On comparison with standard silymarin, this formulation resulted in 2.2 fold increase in dissolution of silymarin at the end of 2 hrs in 0.1 M HCl. Dissolution of silymarin was found to decrease with further increase in polymer ratio. This may be due to the complete dispersion of silymarin with HPMC at 1:3 ratio.

The current study also revealed that the pH of dissolution media had a significant role on the release of silymarin from solid dispersions prepared using various polymer ratios. The percentage amount of standard silymarin released after two hours in 0.1M HCl and mixed phosphate buffer of pH 6.8 was found to be 45.29 ± 0.34 and 1.812 ± 0.45 respectively. The solid dispersion formulation of silymarin and HPMC (1:3 ratio) yielded a maximum drug release of 98.87 ± 0.32 in 0.1M HCl while the cumulative release obtained in mixed phosphate buffer of pH 6.8 was only 13.854 ± 0.56.

Significant increase in dissolution obtained with the various solid dispersions when compared with standard silymarin is also evident from the values of DP_{10} (the percentage of drug dissolved in 10 min), T_{50} (Time taken for 50% dissolution) and DE_{30} (dissolution efficiency at t= 30 min). From table 4 it was clear that higher value of DP_{10}, DE_{30} and lowest value of T_{50} are shown by solid dispersion of silymarin prepared using HPMC in the ratio of 1:3 by kneading method.

From this study it was evident that in acidic media dissolution of standard silymarin, marketed product and solid dispersions prepared by kneading method employing HPMC: PEG and solvent evaporation technique were less than 61 % after 120 minutes.

Table 4. DE_{30}, T_{50} and DP_{15} of silymarin and its solid dispersions in 0.1M HCl.

SD	DE	T_{50}	DP_{15}
KM1	32.645	18	27.35
KM2	39.02	16	35.67
KM3	36.105	17	32.43
KM4	24.384	17	22.67
KM5	35.73	24	33.64
KM6	36.56	18	35.08
SEM1	27.77	31.75	22.94
SEM2	29.78	48.57	24.11
SEM3	28.71	37.5	26.34
SEM4	25	32	22.51
SEM5	24.74	19	20.242
SEM6	26.96	18	23.12

DE: Dissolution efficiency at 30 minutes, T_{50}: Time taken for 50% dissolution

DP_{15}: Percentage drug released at 15 minutes, SD: Solid dispersion

Drug release kinetics

The regression coefficient [r^2] value obtained for all the formulations are shown in table 5. The regression coefficient obtained for first order kinetics was found to be higher [r^2 = 0.725 to 0.9883] than that compared to zero order kinetics [r^2= 0.7608 to 0.9456]. These values indicate that the drug release from the formulations was dependent upon the concentration. All the formulations showed good linearity with Hixson –Crowell cube root law [r^2 = 0.8312 to 0.9882] signifying that the release of silymarin from the solid dispersions were erosion based.

Table 5: Drug release kinetics.

BATCH	**Zero Order Kinetics**		**First Order Kinetics**		**Higuchi Kinetics**		**Hixson- Crowell Kinetics**	
	r^2	**k**	**r2**	**k**	**r2**	**k**	**r2**	**k**
KMI	0.8971	0.548	0.9717	18.56	0.9877	0.141	0.9554	31.98
KM2	0.9456	0.42	0.90	19.106	0.9728	0.108	0.8896	23.65
KM3	0.9284	0.462	0.9883	18.88	0.9919	0.119	0.9882	26.408
KM4	0.9283	0.661	0.966	18.29	0.9748	0.17	0.9577	39.37
KM5	0.8398	0.506	0.9289	18.7	0.9812	0.13	0.9029	29.239
KM6	0.7608	0.657	0.8216	18.3	0.7608	0.169	0.8023	39.11
SEM1	0.7789	0.653	0.8557	18.308	0.9612	0.168	0.8312	38.86
SEM2	0.7881	0.622	0.8712	18.374	0.9605	0.16	0.8453	36.827
SEM3	0.7918	0.642	0.8705	18.331	0.9617	0.165	0.8459	38.167
SEM4	0.8559	0.665	0.9183	18.504	0.9774	0.172	0.8999	39.71
SEM5	0.8784	0.741	0.943	18.374	0.9947	0.1913	0.9242	44.576
SEM6	0.8527	0.648	0.9232	18.331	0.9842	0.1675	0.9024	38.571

CONCLUSION

Rate of dissolution of silymarin was enhanced by kneading method and solvent evaporation method. *In vitro* dissolution studies and DP_{10}, T_{50} and DE_{30} values showed increased dissolution patterns for KM_2. Release patterns of silymarin from solid dispersions were dependent on first order kinetics. It also showed good linearity with Hixson- Crowell cube root law. From the results we can conclude that solid dispersions prepared by kneading method [KM_2] showed best release compared to Solvent evaporation method. A 2.2 fold increase in the solubility profile of silymarin was seen after formulating it into solid dispersions using kneading method.

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