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FORMULATION AND PHYSICOCHEMICAL CHARACTERIZATION OF SODIUM CARBOXY METHYL CELLULOSE AND β CYCLODEXTRIN MEDIATED TERNARY INCLUSION COMPLEXES OF SILYMARIN

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Keywords:

Silymarin, Cyclodextrin, Sodium carboxymethyl cellulose, Solubility, Dissolution.

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ABSTRACT: The objective of the present investigation was to study the complexation of silymarin, a poorly water soluble herbal drug, with β-cyclodextrin alone and in the presence of sodium carboxymethyl cellulose to evaluate the feasibility of enhancing the solubility and dissolution rate of silymarine. Phase solubility study and kneading method was employed to develop and formulate solid inclusion complexes. Solid inclusion complexes of silymarin –β-cyclodextrin in 1:1 molar ratio were prepared with and without carboxymethyl cellulose by kneading method. Obtained binary and ternary complexes were evaluated for solubility dissolution and physical nature by FTIR, XRD and NMR. The aqueous solubility of silymarin was linearly increased as a function of the concentration of β-cyclodextrin alone and in the presence of sodium carboxymethyl cellulose. Beta cyclodextrin formed binary molecular inclusion complexes with silymarin in1:1 molar ratio as revealed by phase solubility diagram. Sodium carboxymethyl cellulose in ternary complexes has resulted in 2.37 and 1.66 fold enhancements in complexation and solubilizing efficiencies of β-cyclodextrin respectively. Dissolution rate of silymarin in binary and ternary complexes was enhanced up to 1.3 and 1.6 folds respectively when compared with pure silymarin. It is evident that addition of sodium carboxymethyl cellulose affects the complexation and solubilizing efficiencies of β-cyclodextrin hence it may be used in solid dispersions to minimize the overall formulation bulk.

INTRODUCTION: Silymarin (SLM) is a natural biomaterial extracted from the seeds of *Silybum marianu* [1]. It is a mixture of isomeric flavonolignans such as silybin, isosilybin, silychristin and silydianin, of which, silybin is the most active pharmacologically [2-4].

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It has been investigated extensively owing to range pharmacological actions it possesses such as hepatoprotective [5-7], cardioprotective [8, 9], nephroprotective [10], neuroprotective [11], antibacterial [12], antiviral [13] and anticancer [14]. However, due to poor aqueous solubility, its bioavailability is low which should be enhanced to derive its maximum therapeutic efficacy [15].

Molecular inclusion complexes formation of poorly water soluble drugs with cyclodextrins is the most successful technique to improve solubility and dissolution behaviour of such drugs [16]. Beta-cyclodextrin (BCD) is most popular among natural

cyclodextrins due to economy, and appropriate cavity size. However, its complexation efficiency is relatively low owing to low water solubility; therefore a significant amount of BCD is required to make clinically useful inclusion complexes [17]. Furthermore, commonly used pharmaceutical excipients in drug formulation may further reduce complexation efficiency thus necessitating excessive use of BCD, which further increases formulation bulk making it unsuitable for oral administration especially low potency herbal drugs. Thus, it is necessary to develop methods that can be applied to enhance the complexation efficiency of BCD [18].

Addition of small amounts of water soluble polymers to the drug cyclodextrin binary systems are known to improve both the complexing and solubilizing efficiencies of cyclodextrins [19-22]. Ternary inclusion complexes of beta-cyclodextrin derivatives with sodium carboxymethyl cellulose (CMC) with have been reported for Rofecoxib [23], Nimuselide [24], cefpodoxime [25], Budesonide [26] and Tropicamide [27] in order to improve their solubility or therapeutic responses. There is no report on combined effect of beta-cyclodextrin and CMC over solubility and dissolution rate of SLM. In the present study the individual and combined effect of BCD and CMC on the solubility and dissolution rate of SLM were investigated.

MATERIALS AND METHODS:

Chemicals:

Beta cyclodextrin and sodium carboxymethyl cellulose were purchased from Sigma-Aldrich (St. Louis, MA, USA). Silymarine was purchased from Loba chemie (Banglore, India). All other solvents and chemicals were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MA, USA).

Phase solubility studies:

Solubility studies were performed by incubating excess amount of SLM at 25°C and 100 RPM in biological shaker with 0-20 mM of BCD solutions alone or BCD 0-20 mM containing 0.5% w/v of sodium CMC. Suspensions were filtered using 0.45 micron membrane filter after 72 hours and amount of SLM in solutions were analyzed by UV spectrophotometer after appropriate dilution.

Preparation of Inclusion complexes:

Solid binary complexes were prepared by kneading equimolar amounts of SLM and BCD cyclodextrin in 50:50 mixtures of water and ethanol. Sodium carboxymethyl cellulose (10% w/w) was added in binary mixtures in order to get a ternary mixture. Both binary and ternary mixtures were dried at 60°C in hot air oven, pulverized into fine state and stored for further evaluations.

Characterization of silymarine and inclusion complexes:

Pure silymarine and solid inclusion complexes were subjected to physicochemical evaluations and comparison using Erweka dissolution tester, X-Ray Differactrometry and spectroscopy such as FT-IR and NMR.

Dissolution studies of inclusion complex:

In vitro dissolution studies were carried out in USP dissolution apparatus II (Erweka, DT 720) using 900 ml of the dissolution medium constituting of phosphate buffer pH 6.8 and 1% sodium lauryl sulphate (SLS) at 37ºC). Speed was adjusted to 100 rpm. The samples were withdrawn periodically over a period of 2 hours and analyzed using Shimadzu UV spectrophotometer UV-1601 (Japan).

Powder X-ray diffractometry (X-RD):

Powder X-ray diffraction pattern of pure drug and inclusion complexes were recorded in X-ray diffractometer (Altima IV, Rigaku, Japan); Cu radiation, voltage of 40 kV and current of 40 mA. X ray patterns were obtained by scanning from 3º to 120º 2θ at a step size of 0.02º with step time of 0.5 s.

Nuclear Magnetic resonance spectroscopy:

Pure drug SLM, BCD, binary and ternary inclusion complexes were dissolved in dimethyl sulphoxide. ^{1}H-NMR spectra were recorded at 300 K on ultrashield plus 500 MHz NMR spectrometer (Bruker, Massachusetts, USA). Induced changes in the chemical shifts for BCD were calculated in free and bound states.

Fourier transform infra-red spectroscopy:

The Fourier transform infra-red spectroscopy (FT-IR) spectra of pure drug and inclusion complexes

were recorded on the FT-IR (Alpha, Germany) using the potassium bromide (KBr) disc technique.

RESULTS:

The Phase solubility diagram of SLM in aqueous BCD and sodium CMC solutions were shown in **Fig.1**. The apparent stability constant (Kc) and complexation efficiency (CE) were calculated from the slope of the corresponding linear plot of the phase solubility diagram using following equations:

$$Kc = Slope / So\,(1 - Slope) \quad \text{----------} \quad (1)$$

where So is the solubility of the drug in the absence of solubilizers.

$$CE = Slope / (1 - Slope) \quad \text{----------} \quad (2)$$

The estimated apparent stability constants and complexation efficiency values of complexes are given in **Table 1**.

TABLE 1: EFFECT OF CMC ON SOLUBILIZING AND COMPLEXATION EFFICIENCY OF BETA CYCLODEXTRIN.

PARAMETERS	BCD	BCD + CMC
Slope[a]	0.191	0.359
Stability constant (Kc)[b]	302	716
Complexation efficiency (CE)[c]	0.236	0.560
Solubilizing efficiency (SE)[d]	6.789	11.241

[a]Slope taken from linear equation of phase solubility diagrams; [b]Kc: Calculated as Kc = Slope/So (1-Slope), [c]CE: calculated as Slope / (1 −Slope) of respective phase solubility diagrams; [d]SE: Ratio between drug solubility in aqueous solution with or without solublizers in water.

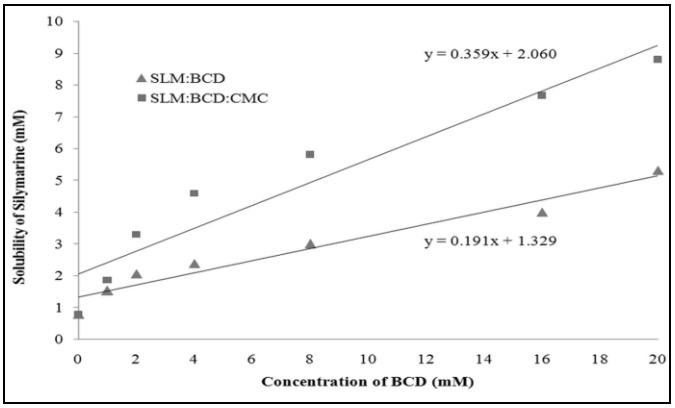

FIG. 1: PHASE SOLUBILITY DIAGRAM OF SILYMARINE IN BINARY AND TERNARY MIXTURES.

Dissolution Profiles:

The dissolution study of pure SLM and its inclusion complexes was carried out in USP dissolution apparatus II. Dissolution profiles of all tested samples are shown in **Fig. 2**. The dissolution of SLM was rapid and higher from both binary and ternary cyclodextrin inclusion complexes prepared when compared to pure drug. The dissolution studies revealed that about 55% and 73% SLM were dissolved from pure silymarin and binary inclusion complexes respectively, whereas ternary inclusion complex exhibited around 90% release in the same time period.

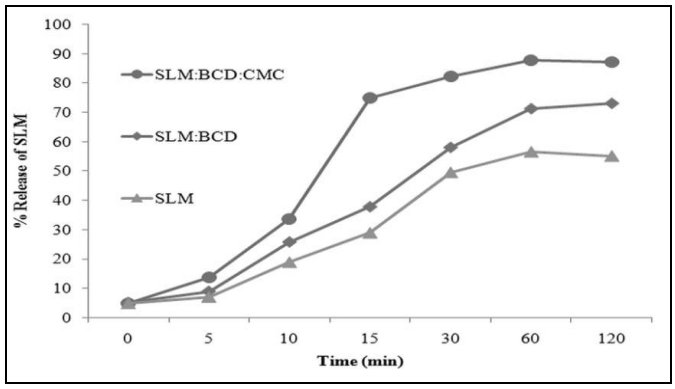

FIG.2: *IN VITRO* RELEASE PROFILES OF SILYMARIN AND INCLUSION COMPLEXES. SLM (▲), SLM: BCD BINARY COMPLEXES (■), SLM: BCD: CMC TERNARY COMPLEXES (●).

Powder X-ray diffractometry (X-RD):

X ray diffractrograms of pure SLM, BCD, binary and ternary inclusion complex prepared by kneading method are shown in **Fig.3.** These curves were indicative of formation of inclusion complex in solid state. The disappearance of sharp peaks of SLM might be attributed to an amorphous state and/or to an inclusion complexation.

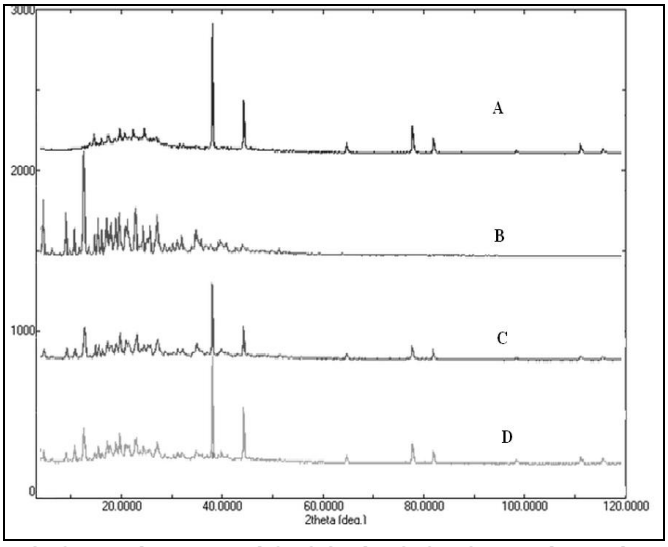

FIG. 3: X-RAY DIFFRACTOGRAMS OF SILYMARIN AND INCLUSION COMPLEXES.
A-SLM, B-BCD, C- SLM: BCD binary complex, D- SLM: BCD: CMC ternary complexes.

Nuclear Magnetic resonance spectroscopy

The significant distinction between the 1H NMR spectra of SLM and the inclusion complex of beta-cyclodextrin with SLM in DMSO confirms that the inclusion complex was formed and the value of chemical shifts for different protons in SLM, BCD and SLM: BCD inclusion complex as shown in **Table 2** and **Fig.4**.

TABLE 2: CHEMICAL SHIFTS OF PROTONS OF BETA CYCLODEXTRIN IN FREE AND BOUND STATE

BCD PROTONS	Chemical shift of BCD in pure and complexed states (δ)				
	Free BCD	Bound binary complex	Δ δ	Bound ternary complex	Δ δ
H-1	5.734	5.759	0.025	5.759	0.025
H-2	3.342	3.359	0.017	3.358	0.016
H-3	5.692	5.709	0.017	5.709	0.017
H-4	3.324	3.339	0.015	3.326	0.002
H-5	4.516	4.522	0.006	4.512	-0.004
H-6	4.824	4.841	0.017	4.839	0.015

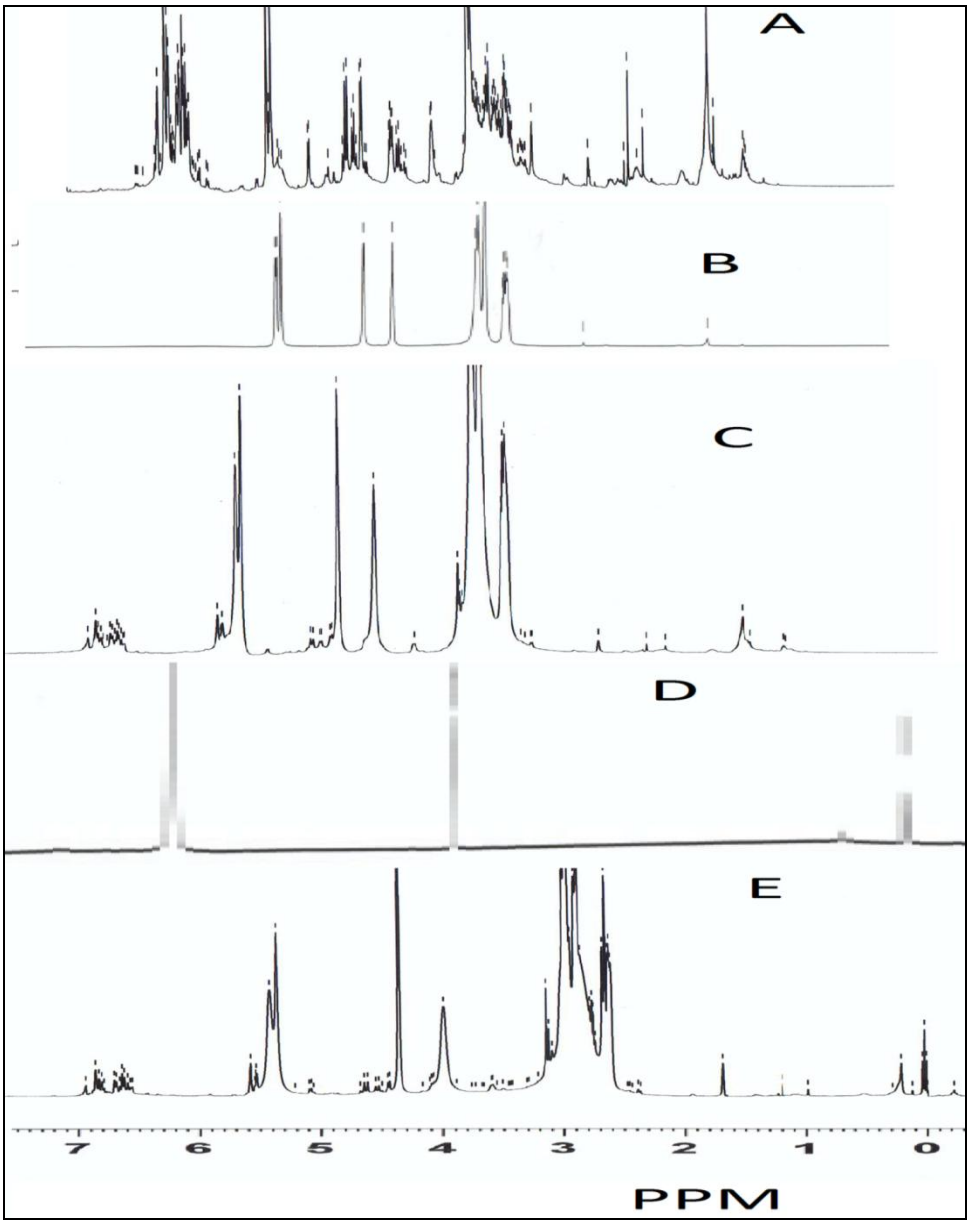

FIG.4: NMR SPECTROGRAMS OF SILYMARIN AND INCLUSION COMPLEXES

A-SLM, B-BCD, C- SLM: BCD binary complex, D- CMC, E- SLM: BCD: CMC ternary complexes.

Fourier transform infra-red spectroscopy:
FT-IR curves of pure components and ternary inclusion complex prepared by kneading method are shown in **Fig.5.** These curves were indicative of formation of inclusion complex in solid state. The disappearance of sharp peaks of SLM is attributed to an amorphous state and/or to formation of inclusion complexes.

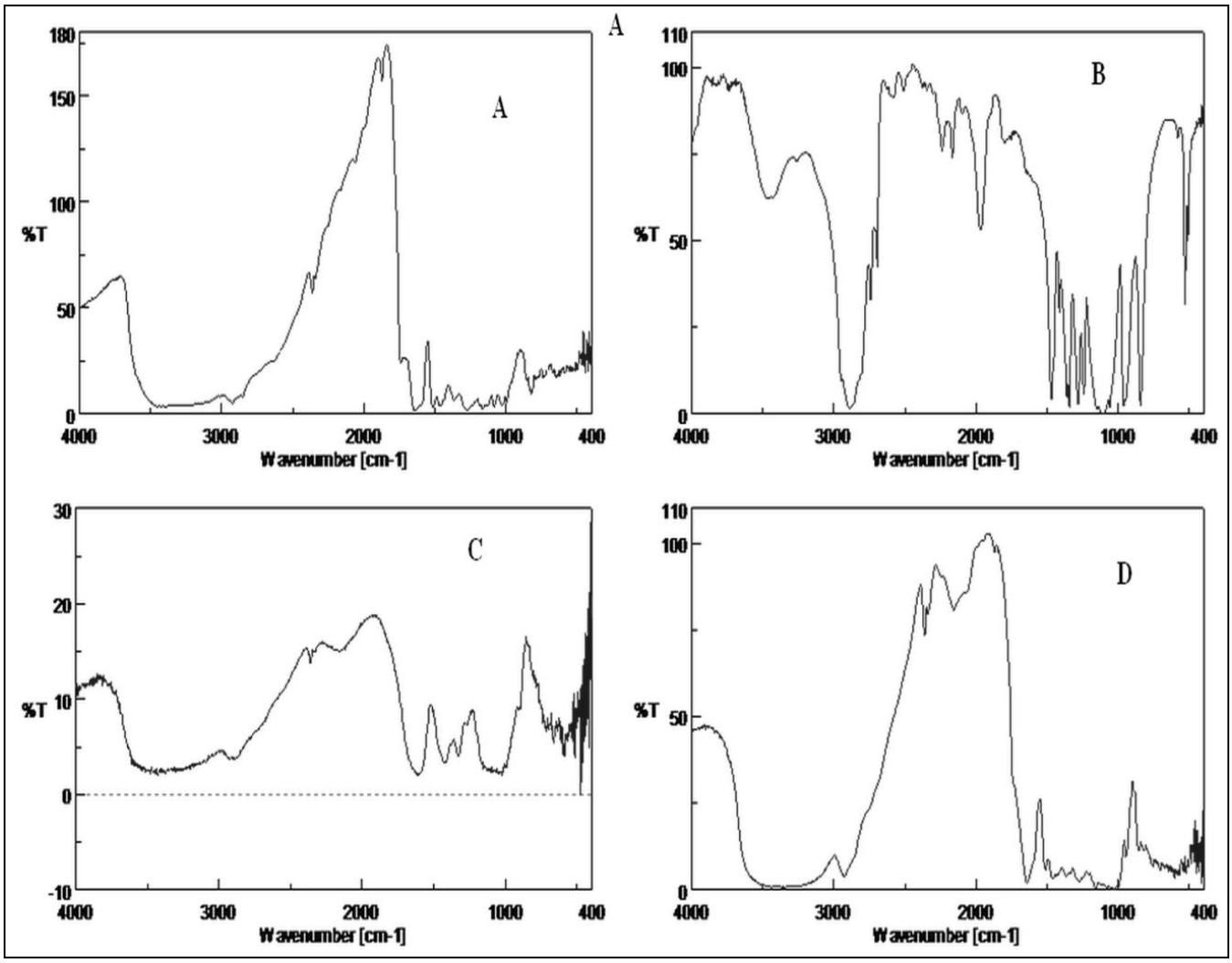

FIG.5: FT-IR SPECTROGRAMS OF SILYMARIN AND INCLUSION COMPLEXES
A-SLM, B-BCD, C- CMC D- SLM: BCD: CMC ternary complexes.

DISCUSSION: Both binary and ternary systems demonstrated A_L type equilibrium phase solubility diagram as SLM solubility increases linearly as a function of BCD and CMC concentrations. The slope values were found to be less than one suggesting the formation of 1:1 stoichiometry complexes. Complexation efficiency is the concentration ratio between cyclodextrin in a complex and free cyclodextrin. It is considered more accurate method for determination of the solubilizing effect of cyclodextrins as it is independent of both so and the intercept. It is more reliable when the influences of various pharmaceutical excipients such as polymers on the solubilization of drug are to be investigated [28]. It has been observed that addition of CMC to the BCD solution did not change the type of phase-solubility diagrams obtained for binary systems and resulted in increase in stability constant.

The observed enhancement of stability constant with addition of the CMC shows that the CMC is able to interact with SLM-BCD binary complexes. The solubilizing effect of BCD was increased in the presence of CMC; consequently, a synergistic effect in SLM solubility was observed in the presence of BCD and CMC. In the present work addition of small amount of hydrophilic polymer

such as CMC to cyclodextrin solution resulted in significant improvement in the complexation efficiency between SLM-BCD thereby markedly enhancing the solubilizing efficiency of BCD and this is in agreement with previous reports [19-27]. *In vitro* studies in distilled water for inclusion complexes of BCD with CMC showed higher rate of dissolution than those of SLM alone and its binary complexes with BCD. These findings confirm that the addition of small amount of CMC improves solubilizing and complexing ability of cyclodextrin which further related to increased release of drug in dissolution medium.

CONCLUSION: The complexation of SLM with BCD in absence or presence of CMC was investigated by phase solubility studies. Solubility and dissolution of SLM by BCD complexation was synergised due to formation of ternary complexes with higher complexation and solubility efficiencies in the presence of CMC. Study signifies the use of CMC in combination with BCD for the formation of inclusion complex of SLM.

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