IJBPAS, October, 2014, 3(10): 2414-2423

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

INFLUENCE OF HYDROPHILIC POLYMERS ON COMPLEXATION AND SOLUBLIZING EFFICIENCIES OF BETA CYCLODEXTRIN OVER SILYMARIN

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ABSTRACT

Silymarin exhibits very low bioavailability (23 – 47%) due to its poor aqueous solubility and hence it needs improvement in the solubility and dissolution rate so as to enhance bioavailability and to derive its maximum therapeutic efficacy. Though cyclodextrin complexation and use of water-soluble polymers for enhancing the solubility of silymarin have been investigated separately, no reports are available on their combined use in enhancing the solubility. The objective of the present investigation is to study the complexation of silymarin with β-cyclodextrin alone and in the presence of hydrophilic polymers by phase solubility study and to evaluate the feasibility of enhancing the solubility of silymarin. The aqueous solubility of silymarin was linearly increased as a function of the concentration of β-cyclodextrin alone and in the presence of hydrophilic polymers, PVP, HPMC and PEG 6000. The increase in solubility was due to the formation of a 1: 1 M complexes in solutions. The complexes formed between β-cyclodextrin and silymarin were quite stable as indicated by apparent stability constants. Addition of hydrophilic polymers has markedly enhanced the complexation efficiency of β-cyclodextrin. Of the investigated polymers, PVP has given the highest enhancement in the

complexation efficiency of β-cyclodextrin. The order of hydrophilic polymers in enhancing the complexation efficiency was PVP > HPMC > PEG 6000. Hydrophilic polymers have markedly enhanced the solubilizing efficiency of β-cyclodextrin. PVP has given highest enhancement (12.67 fold) in the solublizing efficiency of β-cyclodextrin. Hence, a combination of β-cyclodextrin and hydrophilic polymers is recommended for enhancing solubility of silymarin.

Keywords: Silymarin, Cyclodextrin Complexation, Hydrophilic Polymers, Ternary Phase Solubility

INTRODUCTION

β-cyclodextrin (BCD) is the most widely used host molecule for inclusion complexes affecting physical and chemical properties, like solubility and stability of several guest molecules **[1]**. It comprises the most widely used pharmaceutical excipient to enhance solubility of several water insoluble or poorly soluble drugs in recent years due to low cost and their approval by various regulatory agencies **[2, 3]**. Unfortunately, the complexation efficiency of BCD is rather low and consequently a significant amount of BCD is required to make inclusion complexes. Moreover, commonly used excipients in drug formulation may further reduce complexation efficiency thus necessitating excessive use of BCD **[4]**, which increases formulation bulk making it unsuitable for oral administration especially of low potency herbal drugs. Thus, it is necessary to develop methods that can be applied to enhance the complexation efficiency of BCD. Addition of small amount of water-solublepolymer to aqueous CD solution has been shown to improve complexation efficiency of CDs **[5-8]**. The aim of this work was to examine whether water-soluble polymers improve complexation efficiency of BCD for silymarin (SLM), a poorly water-soluble drug with significant hepato-protective activity **[9]**. It is a mixture of three flavanolignans, namely, silybin, silydianin, and silicristine, with silybin being most active **[10]**. Due to poor water solubility its bioavailability is as low as 23 – 47% **[11]**. Although cyclodextrin complexation **[11]** and use of water-soluble polymers for enhancing the solubility and dissolution rate of silymarin have been investigated individually **[12-14]**, no report is available on their combined use in enhancing the solubility and dissolution rate. In the present study the individual and combined effect of BCD and water-soluble polymers on the solubility of SLM were investigated.

MATERIALS AND METHODS

Chemicals

Beta cyclodextrin (β-CD), Poly vinyl pyrrolidone K30 (PVP), PEG 6000 and HPMC 4000 cps were purchased from Sigma Aldrich Chemical Co. USA. Silymarin was purchased from Loba chemie Banglore, India. All other solvents and chemicals were of analytical grade and obtained from Sigma-Aldrich, USA.

Standard Plot of Silymarin

10 mg of standard Silymarin was accurately weighed, dissolved in methanol and diluted with distilled water (DW) to get a concentration of about100 µg/ml. From this solution, suitable aliquots were transferred into 10 ml volumetric flask and diluted with DW to get concentrations 1, 2, 4, 6, 8, and 10, µg/ml of SLM which were then analyzed by double beam UV spectrophotometer (Model V-630 Jasco, Japan) at 286 nm.

Phase Solubility Studies

Solubility studies (with BCD and hydrophilic polymers separately and in combinations) were performed according to the method reported by Higuchi and Connors **[15]**. Excess amount of SLM was incubated at 25°C and 100 RPM in biological shaker with 0-20 mM of BCD solutions or hydrophilic polymers (0-2% w/v) or BCD 0-20 mM containing 0.5% w/v of hydrophilic polymers. Suspensions were filtered using 0.45 micron membrane filter after 72 hours and amount of SLM in solutions were analyzed by double beam UV spectrophotometer (Model V-630 Jasco, Japan) after appropriate dilution. To nullify the absorbance due to the presence of CD and polymers, the apparatus were calibrated with the corresponding blank in every measurement.

The apparent stability constants (Kc) were estimated from the straight line of the phase solubility diagrams according to the equation of Higuchi and Connors.

RESULTS

The influence of BCD concentration in the presence or absence of water-soluble polymer on the solubility of SLM was studied using the method of Higuchi and Connors. This is based on monitoring changes in the solubility of a SLM by the addition of BCD or polymers or both in combinations.

The Phase solubility diagram of SLM in aqueous βCD and polymer solutions were shown in **Figure 1** and **Figure 2** respectively. Both demonstrated A_L type equilibrium phase solubility diagram as SLM solubility increases linearly as a function of BCD and polymer concentrations.

The phase-solubility studies diagrams obtained with BCD alone and in presence of water-soluble polymers is shown in **Figure 3**.

They displayed A_L type equilibrium phase solubility diagrams for SLM: BCD binary and ternary systems, showing that SLM solubility increase linearly as a function of BCD concentration and that the soluble complexes were formed without occurrence of precipitation in the range of BCD concentration used.

The slope values were found to be less than one suggesting the formation of 1:1 stoichiometry complexes in solution and allowing to the calculation of the apparent stability constants (Kc) of the SLM: BCD complexes as per the Higuchi and Connors equation. The apparent stability constant (Kc) in each case was calculated from the slope of the corresponding linear plot of the phase solubility diagram according to the equation, Kc = Slope/So (1-Slope), where So is the solubility of the drug in the absence of solublizers (BCD or hydrophilic polymers). The estimated Kc values of various complexes are given in **Table 1**.

To evaluate the effect of polymers on the solublizing efficiency of BCD, the solublizing efficiency was calculated in each case as the ratio between drug solubility in aqueous solution of BCD (20 mM with or without hydrophilic polymers) and in water. The solublizing efficiency values are given in **Table 2**. BCD alone gave a 6.79 fold increase in the solubility of SLM, whereas 7.09, 9.12 and 12.77 fold increase was observed in the presence of PEG 6000, HPMC and PVP respectively. Thus the addition of polymers has markedly enhanced the solublizing efficiency of BCD.

Table 1: Apparent Stability Constants of Various SLM: BCD complex systems with and without polymers

Solvents	Kc (M^{-1})	*Complexation efficiency
BCD	**286.074**	**-**
BCD + PEG 6000	**337.812**	**1.18**
BCD+ HPMC	**492.281**	**1.72**
BCD +PVP	**881.138**	**3.08**

***(No of folds of increase in Kc): Ratio between Kc of BCD with and without polymers**

Table 2: Solubility of SLM in water, aqueous solutions of polymers (0.5 % W/V), aqueous solutions of BCD (20 mM), and ternary mixtures (0.5 % W/V polymers + 20 mM BCD)

Solvents	Solubility of SLM($\mu g.ml^{-1}$)	*Solubilizing efficiency
Water	377	-
BCD	2562	6.79
PEG 6000	639	1.69
BCD + PEG 6000	2675	7.09
HPMC	1017	2.70
HPMC + BCD	3444	9.12
PVP	1307	3.46
PVP + BCD	4821	12.77

***Ratio between drug solubility in aqueous solutions (BCD 20 mM with OR without hydrophilic polymers) and in water.**

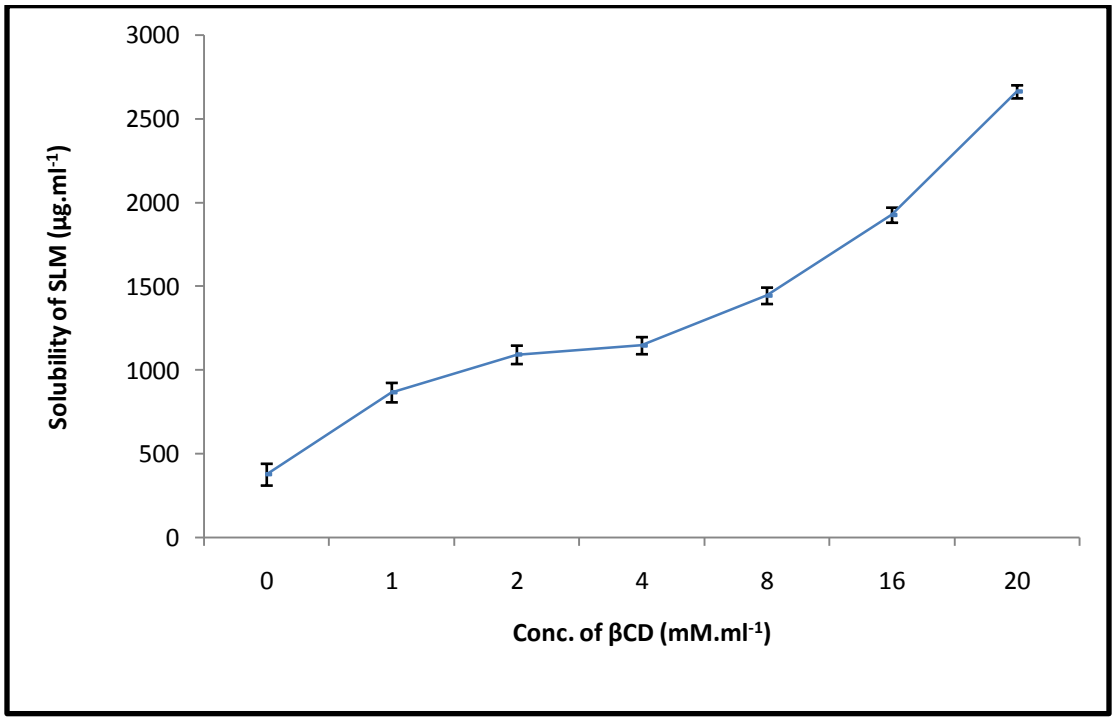

Figure 1: Phase solubility diagram of SLM in BCD

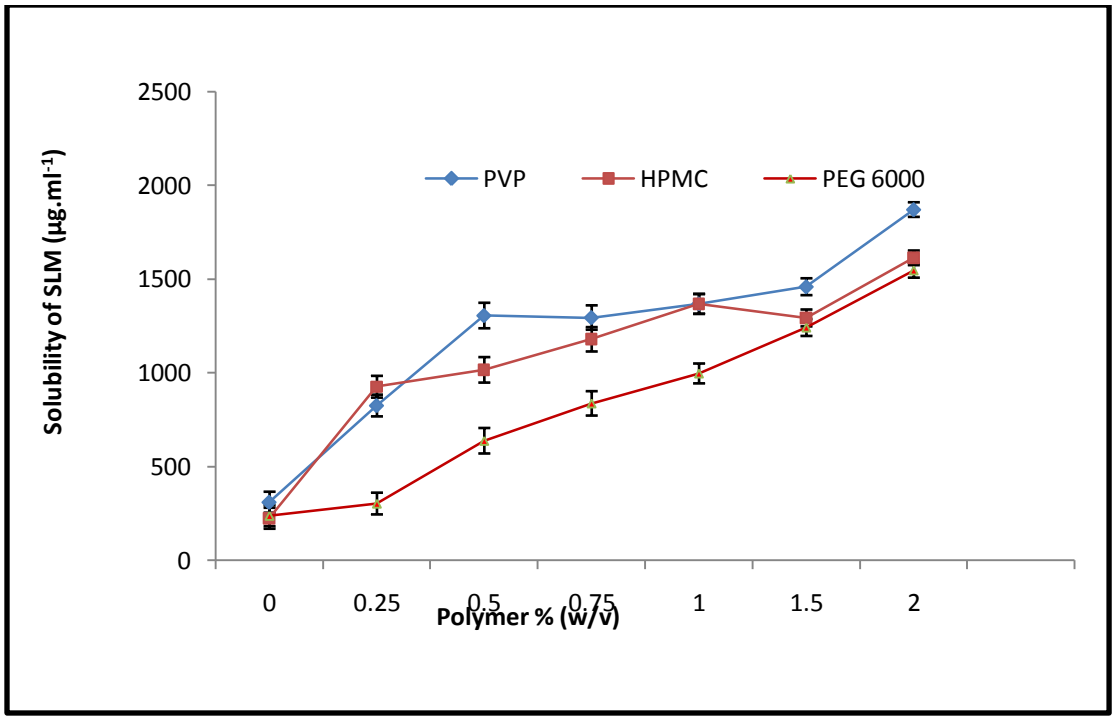

Figure 2: Phase Solubility Diagram of SLM in Different Polymer Solutions

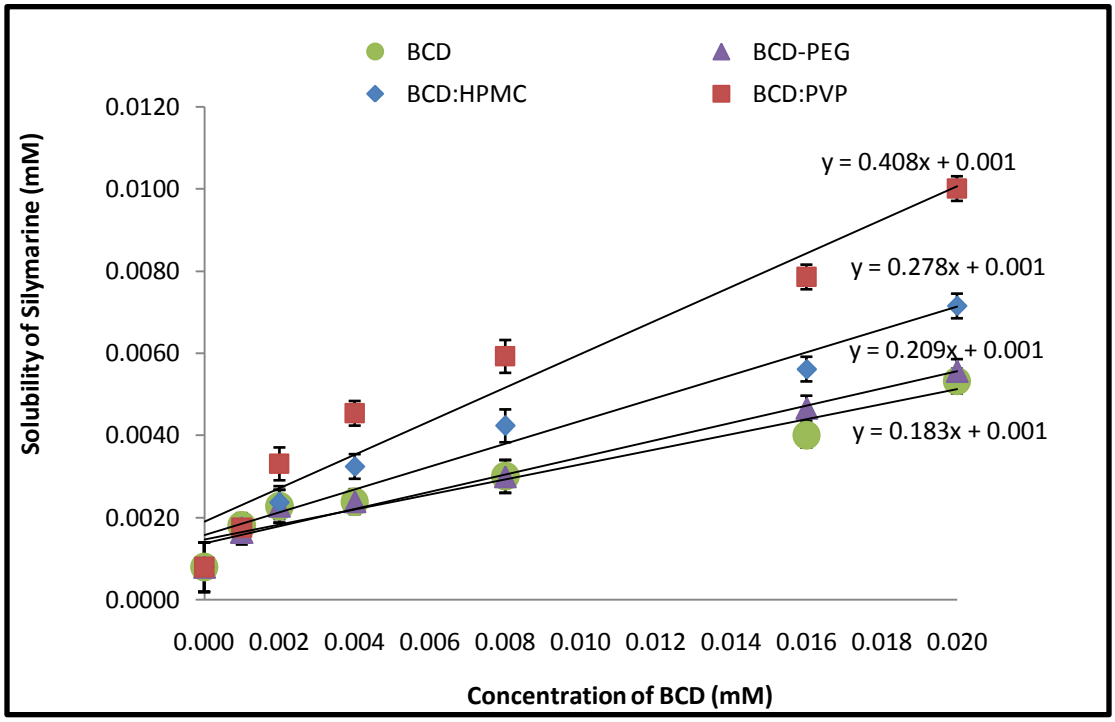

Figure 3: Phase solubility diagram of SLM in binary and ternary mixtures

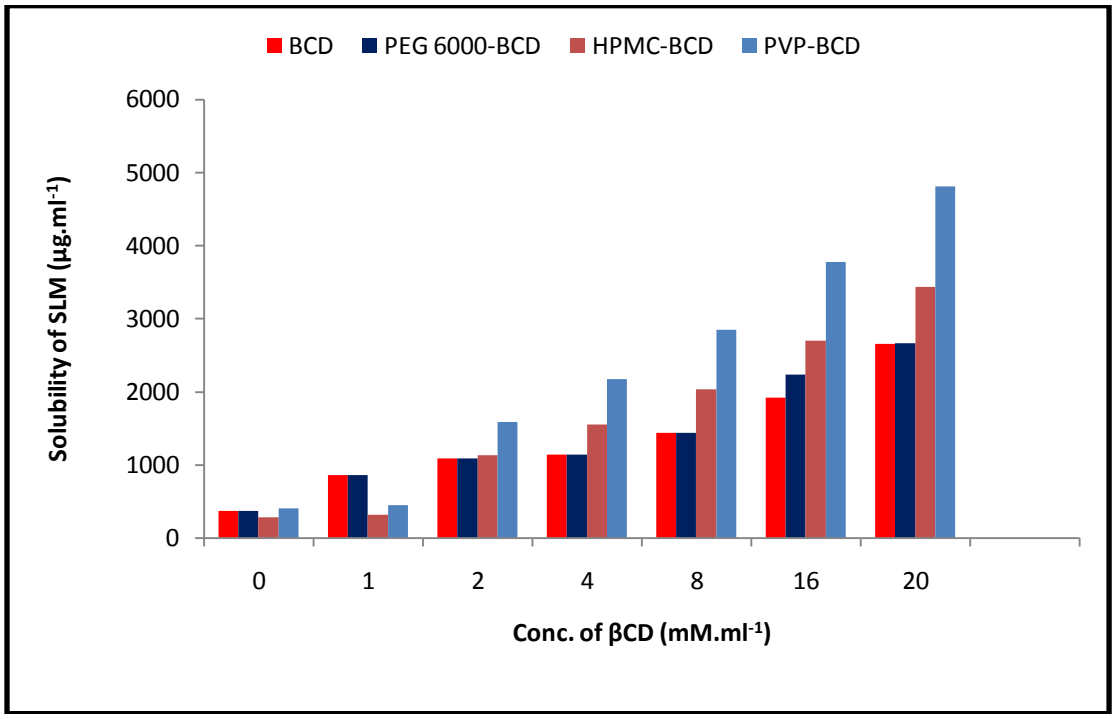

Figure 4: Phase Solubility Diagrams of SLM: BCD with or without polymers

DISCUSSION

It has been observed that addition of water-soluble polymers to the BCD solution did not change the type of phase-solubility diagrams obtained for binary systems and resulted in increase in stability constant **(Figure 3)**. The observed enhancement of stability constant with addition of the polymers shows that all investigated polymers were able to interact with SLM-BCD binary complexes. The solublizing effect of CDs was increased in the presence of 0.5% (W/V) polymers in following order PVP> HPMC >PEG 6000, consequently, a synergistic effect in SLM solubility was observed in the presence of these polymers **Table 2 and Figure 4**. Water-soluble polymers increase the solublizing effect of cyclodextrin by increasing Kc of the drug-CD complexes, as reported by several authors **[5, 16]**. This increase enhances the complexation efficiency and thus less cyclodextrin is needed for solublizing a given amount of drug when hydrophilic polymers are present in the aqueous media. In the present work addition of small amount of polymers to cyclodextrin solutions resulted in higher Ks values for ternary complexes in comparison to corresponding binary ones suggesting a significant improvement in the complexation efficiency between SLM-BCD thereby markedly enhancing the solublizing

efficiency of BCD and this is in agreement with previous reports **[17-19]**.

CONCLUSION

The complexation of SLM with BCD was investigated by phase solubility studies. The phase solubility diagrams for the complex formation between SLM and BCD in the presence and absence of hydrophilic were evaluated. The aqueous solubility of SLM was increased linearly as a function of the concentration of BCD. The phase solubility diagrams of SLM-BCD complexes can be classified as type $A_L$according to Higuchi and Connors. Because the straight line had a slope < 1 in each case, the increase in solubility was due to the formation of a 1: 1 M complex in solution with BCD both; in the presence and absence of hydrophilic polymers. The values of Kc indicated that all the complexes formed between SLM and BCDare quite stable. The values of stability constant (Kc) were found to be higher in the presence of hydrophilic polymers indicating higher complexation efficiency. We observed a 2.33, 1.93 and 1.26 fold increase in the Kc values, in the presence of PVP, HPMC and PEG 6000 respectively. PVP has given higher enhancement in complexation efficiency. The order of hydrophilic polymers in enhancing the complexation efficiency was PVP > HPMC >PEG 6000.

ACKNOWLEDGEMENTS

Authors are grateful to Dean, College of Pharmacy, for providing opportunity, resourses, instrumentation and facility to carryout and complete this research project.

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