

**A FACTORIAL STUDY ON THE EFFECTS OF HP $\beta$  CYCLODEXTRIN, PVP K30 AND SLS ON THE SOLUBILITY AND DISSOLUTION RATE OF EFAVIRENZ**

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**ABSTARCT :** The objective of the study is to evaluate the individual main effects and combined (or interaction) effects of Hydroxy propyl  $\beta$  cyclodextrin (HP $\beta$ CD), poly vinyl pyrrolidone (PVP K30) and sodium lauryl sulphate (SLS) on the solubility and dissolution rate of efavirenz in a series of 2<sup>3</sup> factorial experiments. The solubility of efavirenz in eight selected fluids containing HP $\beta$ CD, PVP K30 and SLS as per 2<sup>3</sup> factorial study was determined. The solubility of efavirenz was markedly enhanced by HP $\beta$ CD (2.95 fold), PVP K30 (2.49 fold) and SLS (226.96 fold) individually. Combination of HP $\beta$ CD with PVP K30 and SLS gave a markedly higher enhancement in the solubility of efavirenz than is possible with them individually. HP $\beta$ CD in combination with PVP K30 and SLS gave respectively 4.05 and 387.63 fold increase in the solubility of efavirenz. Solid inclusion complexes of efavirenz - HP $\beta$ CD were prepared with and without PVP K30 and SLS as per 2<sup>3</sup>-factorial design by kneading method and were evaluated. ANOVA indicated that the individual main effects of HP $\beta$ CD, PVP K30 and SLS and their combined effects in enhancing the solubility and dissolution rate ( $K_1$ ) and dissolution efficiency ( $DE_{30}$ ) were highly significant ( $P < 0.01$ ). HP $\beta$ CD alone gave a 16.74 fold increase in the dissolution rate of efavirenz. HP $\beta$ CD in combination with PVP K30 and SLS gave respectively 19.98 and 25.13 fold increase in the dissolution rate of efavirenz. HP $\beta$ CD in combination with both PVP K30 and SLS gave highest enhancement (41.61 fold) in the dissolution rate of efavirenz. Combination of HP $\beta$ CD with PVP K30 and SLS has markedly enhanced the solubility as well as dissolution rate of efavirenz than is possible with them individually. Hence a combination of HP $\beta$ CD with PVP K30 and / or SLS is recommended to enhance the solubility and dissolution rate of efavirenz, a BCS class II drug.

**Key words:** Efavirenz, HP $\beta$  Cyclodextrin, PVP K30, SLS, Solubility, Dissolution Rate, Factorial Study

**INTRODUCTION**

Efavirenz, a widely prescribed HIV-1 specific, non nucleoside reverse transcriptase inhibitor (NNRTI) drug belong to Class II under BCS and exhibit low and variable oral bioavailability due to its poor aqueous solubility. It is practically insoluble in water. Its oral absorption is dissolution rate limited and it requires enhancement in solubility and dissolution rate for increasing its oral bioavailability. Several techniques<sup>1</sup> such as micronization, cyclodextrin complexation, use of surfactants and solubilizers, solid dispersion in water soluble and dispersible carriers, use of salts, prodrugs and polymorphs which exhibit high solubility, microemulsions and self emulsifying micro and nanodisperse systems have been used to enhance the solubility, dissolution rate and bioavailability of poorly soluble drugs.

Among the various approaches complexation with cyclodextrins has gained good acceptance in recent years in industry for enhancing the solubility and dissolution rate of poorly soluble drugs. Cyclodextrins (CDs) are cyclic torus-shaped molecules with a hydrophilic outer surface and a lipophilic central cavity which can accommodate a variety of lipophilic drugs. As a consequence of inclusion process many physico-chemical properties such as solubility, dissolution rate, stability and bioavailability can be favourably affected<sup>2,3</sup>. Cyclodextrins have their approval by various regulatory agencies<sup>4,5</sup>. Use of poly vinyl pyrrolidone (PVP K30) and/or sodium lauryl sulphate (SLS) is also reported<sup>6-8</sup> for solubilization and to enhance dissolution rate of poorly soluble drugs.

Though cyclodextrin complexation and use of PVP K30 and surfactant, SLS for enhancing the solubility and dissolution rate of poorly soluble drugs have been investigated individually, no reports are available on their combined use in enhancing the solubility and dissolution rate. In the present investigation the individual main effects and combined (or interaction) effect of HP $\beta$ CD, PVP K30 and SLS on the solubility and dissolution rate of efavirenz, a BCS class II drug were evaluated in a series of 2<sup>3</sup> factorial experiments.

In factorial experiments the effects of several factors of variation are studied and investigated simultaneously, the treatments being all the combinations of different factors under study. In these experiments an attempt is made to estimate the effects of each of the factors and also the interaction (or combined) effects, i.e., the variation in the effect of one factor as a result to different levels of other factors.

## **MATERIALS AND METHODS**

### **Materials**

Efavirenz was a gift sample from M/s. Hetero Drugs Ltd., Hyderabad. Hydroxy propyl  $\beta$ -Cyclodextrin was a gift sample from M/s. Cerestar Inc., USA. Methanol (Qualigens), poly vinyl pyrrolidone, K30 (PVP K30) and sodium lauryl sulphate (SLS) were procured from commercial sources. All other materials used were of Pharmacopoeial grade.

### **Estimation of Efavirenz:**

An UV Spectrophotometric method based on the measurement of absorbance at 246 nm in a phosphate buffer of pH 7.4 was used for the estimation of efavirenz. The method was validated for linearity, accuracy, precision and interference. The method obeyed Beer's law in the concentration range of 1-10 $\mu$ g/ml. When a standard drug solution was repeatedly assayed (n=6), the relative error and coefficient of variation were found to be 0.8% and 1.2% respectively. No interference by the excipients used in the study was observed.

### **Solubility Determination:**

Excess drug (50mg) was added to 15ml of each fluid taken in a 25ml stopped conical flask and the mixtures were shaken for 24 h at room temperature (28 $\pm$ 1 $^{\circ}$ C) on Rotary Flask Shaker. After 24 h of shaking, 2ml aliquots were withdrawn at 2 h interval and filtered immediately using a 0.45 $\mu$  disk filter. The filtered samples were diluted suitably and assayed for efavirenz by measuring absorbance at 246 nm. Shaking was continued until two consecutive estimations are the same. The solubility experiments were replicated for four times each (n=4).

### **Preparation of Efavirenz-HP $\beta$ CD Complexes:**

Solid inclusion complexes of efavirenz - HP $\beta$ CD in 1:2 ratio with and without PVP K30 (2%) and SLS (2%) as per 2<sup>3</sup> factorial design were prepared by kneading method. Efavirenz, HP $\beta$ CD, PVP K30 and SLS were triturated in a mortar with a small volume of solvent consisting of a blend of water: methanol (1:1). The thick slurry formed was kneaded for 45 min and then dried at 55 $^{\circ}$ C until dry. The dried mass was powdered and sieved to mesh No. 120.

### **Dissolution Rate Study:**

The dissolution rate of efavirenz as such and from HP $\beta$ CD complexes prepared was studied in 1000 ml of phosphate buffer of pH 7.4 using Disso 2000 (Labindia) 8-station dissolution test apparatus with a paddle stirrer at 50 rpm. A temperature 37 $\pm$ 0.5 $^{\circ}$ C was maintained throughout the study. Efavirenz or Efavirenz-HP $\beta$ CD complex equivalent to 50 mg of efavirenz was used in each test. Samples of dissolution media (5ml) were withdrawn through a filter (0.45 $\mu$ ) at different intervals of time, suitable diluted and assayed for efavirenz at 246 nm. The sample of dissolution fluid withdrawn at each time was replaced with fresh fluid. The dissolution experiments were replicated four times each (n=4).

**Analysis of Data:**

Dissolution rate data were analysed as per zero order and first order kinetic models to assess the kinetics of dissolution of drug from the HP $\beta$ CD complexes prepared. Dissolution efficiency (DE<sub>30</sub>) values were calculated as suggested by Khan<sup>9</sup>. Solubility and dissolution data were subjected to Analysis of Variance (ANOVA) to find out the significance of the main individual and combined effects of the factors involved.

**RESULTS AND DISCUSSION**

The individual main effects and combined (interaction) effects of HP $\beta$ CD (Factor A), PVP K30 (Factor B) and SLS (Factor C) on the aqueous solubility of efavirenz were evaluated in a 2<sup>3</sup>-factorial experiment. For this purpose, two levels of HP $\beta$ CD (0, 5 mM), two levels of PVP K30 (0, 2%) and two levels of SLS (0, 2%) were selected in each case and the corresponding eight treatments involved in the 2<sup>3</sup>-factorial study were purified water (1), water containing 5mM HP $\beta$ CD (a); water containing 2% PVP K30 (b); water containing 5mM HP $\beta$ CD and 2% PVP K30 (ab); water containing 2% SLS (c); water containing 5mM HP $\beta$ CD and 2% SLS (ac); water containing 2% PVP K30 and 2% SLS (bc) and water containing 5mM HP $\beta$ CD and 2% of each of PVP K30 and SLS (abc).

The solubility of efavirenz in the above mentioned eight fluids was determined (n=4) and the results are given in Table 1. The aqueous solubility of efavirenz was markedly enhanced by HP $\beta$ CD alone and in combination with PVP K30 and SLS. The solubility data were subjected to Analysis of Variance (ANOVA) to find out the significance of main and combined effects of HP $\beta$ CD, PVP K30 and SLS on the solubility of efavirenz. The results of ANOVA are shown in Table 2. The individual and combined effects of HP $\beta$ CD, PVP K30 and SLS in enhancing the solubility of efavirenz were highly significant (P < 0.01). Among the three factors, SLS (factor C) gave highest enhancement (226.96 fold) in the solubility of efavirenz. HP $\beta$ CD alone gave a 2.95 fold increase in the solubility of efavirenz. Whereas HP $\beta$ CD in combination with PVP and SLS respectively gave 4.05 and 387.63 fold increase in the solubility of efavirenz. HP $\beta$ CD in combination with both PVP K30 and SLS gave highest enhancement (496.89 fold) in the solubility of efavirenz. Combination of HP $\beta$ CD with PVP K30 and SLS has given a markedly higher enhancement in the solubility of efavirenz than is possible with them individually.

**Table 1: Solubility of Efavirenz in Various Fluids as per 2<sup>3</sup> – Factorial Study**

Fluids (Code as per 2 <sup>3</sup> – Factorial Experiment)	Solubility (mg/ml) (n=4) ( $\bar{x}\pm sd$ )	Increase in Solubility (Number of Folds)
Distilled water (1)	0.02 $\pm$ 0	-
Water containing 5 mM HP $\beta$ CD (a)	0.05 $\pm$ 0.001	2.95
Water containing 2% PVP K30 (b)	0.04 $\pm$ 0	2.49
Water containing 5mM HP $\beta$ CD and 2% PVP K30 (ab)	0.07 $\pm$ 0.002	4.05
Water containing 2% SLS (c)	3.90 $\pm$ 0.097	226.96
Water containing 5mM HP $\beta$ CD and 2% SLS (ac)	6.66 $\pm$ 0.161	387.63
Water containing 2% PVP K30 and 2% SLS (bc)	5.03 $\pm$ 0.128	292.82
Water containing 5mM HP $\beta$ CD, 2% PVP K30 and 2% SLS (abc)	8.54 $\pm$ 0.083	496.89

To evaluate the individual and combined effects of HP $\beta$ CD, PVP K30 and SLS on the dissolution rate of efavirenz, solid inclusion complexes of efavirenz - HP $\beta$ CD were prepared with and without PVP K30 and SLS as per 2<sup>3</sup>-factorial design. For this purpose two levels of HP $\beta$ CD (0 and 1: 2 ratio of drug : HP $\beta$ CD) and two levels of each of PVP K30 and SLS ( 0 and 2%) were selected and the corresponding eight treatments involved in the 2<sup>3</sup>-factorial study were efavirenz pure drug (1); efavirenz - HP $\beta$ CD (1:2) inclusion binary complex (a); efavirenz - PVP K30 (2%) binary complex (b); efavirenz - HP $\beta$ CD (1:2) – PVP K30 (2%) ternary complex (ab); efavirenz – SLS (2%) binary complex (c); efavirenz - HP $\beta$ CD (1:2) – SLS (2%) ternary complex (ac); efavirenz - PVP K30 (2%) – SLS (2%) ternary complex (bc) and efavirenz - HP $\beta$ CD (1: 2) – PVP K30 (2%) SLS (2%) complex (abc).

The HP $\beta$ CD complexes were prepared by kneading method. All the solid inclusion complexes of efavirenz -HP $\beta$ CD - PVP K30 - SLS prepared were found to be fine and free flowing powders. Low coefficient of variation (c.v.) values (< 1%) in the percent drug content indicated uniformity of drug content in each batch of solid inclusion complexes prepared. The dissolution rate of efavirenz alone and from HP $\beta$ CD complexes was studied in phosphate buffer of pH 7.4. The dissolution of efavirenz followed first order kinetics with r (correlation coefficient) above 0.91. The dissolution parameters are summarized in Table 3. The dissolution of efavirenz was rapid and higher in the case of all efavirenz – HP $\beta$ CD binary and ternary complex systems prepared when compared to efavirenz pure drug as such.

**Table 2: ANOVA of Solubility Data of Efavirenz in Various Fluids Prepared as per 2<sup>3</sup> – Factorial Study**

Source of variation	D.F	S.S	M.S.S	F-Ratio	Significance
Total	31	336.29	10.85	6563.78	P<0.01
Treatments	7	336.11	48.02		
a	1	20.04	20.04	2740.03	P<0.01
b	1	4.67	4.67	638.12	P<0.01
ab	1	0.27	0.27	37.35	P<0.01
c	1	287.15	287.15	39254.09	P<0.01
ac	1	19.29	19.29	2636.72	P<0.01
bc	1	4.40	4.40	601.41	P<0.01
abc	1	0.28	0.28	38.73	P<0.01
Error	24	0.18	0.01		

$$F_{0.01(7, 24)} = 3.50; F_{0.05(7, 24)} = 2.43; F_{0.01(1, 24)} = 7.82; F_{0.05(1, 24)} = 4.26$$

The dissolution parameters ( $K_1$  and  $DE_{30}$ ) were subjected to ANOVA to find out the significance of the main and combined effects of HP $\beta$ CD, PVP K30 and SLS on the dissolution rate of efavirenz. The results of ANOVA are shown in Tables 4 and 5. ANOVA indicated that the individual main effects of HP $\beta$ CD, PVP K30 and SLS and their combined effects in enhancing the dissolution rate ( $K_1$ ) and  $DE_{30}$  were highly significant ( $P < 0.01$ ). HP $\beta$ CD, PVP K30 and SLS alone gave respectively 16.74, 11.01 and 16.41 fold increase in the dissolution rate of efavirenz. HP $\beta$ CD in combination with PVP K30 and SLS gave respectively 19.98 and 25.13 fold increase in the dissolution rate of efavirenz. HP $\beta$ CD in combination with both PVP K30 and SLS gave highest enhancement (41.61 fold) in the dissolution rate of efavirenz.

**Table 3: Dissolution Parameters of Efavirenz- HP $\beta$ CD Complex Systems Prepared as per 2<sup>3</sup> Factorial Study**

EFA-HP $\beta$ CD Complexes	Composition	K <sub>1</sub> ×10 <sup>3</sup> (min <sup>-1</sup> )	Increase in K <sub>1</sub> (no.of folds)	DE <sub>30</sub> (%)	Increase in DE <sub>30</sub> (no.of folds)
F <sub>1</sub>	EFA	0.23	--	1.04	--
F <sub>a</sub>	EFA - HP $\beta$ CD (1:2)	3.88	16.74	9.17	8.80
F <sub>b</sub>	EFA - PVP K-30 (2%)	2.44	11.01	4.52	4.34
F <sub>ab</sub>	EFA - HP $\beta$ CD (1:2) - PVP K-30 (2%)	4.63	19.98	11.87	11.39
F <sub>c</sub>	EFA - SLS (2%)	3.80	16.41	8.63	8.28
F <sub>ac</sub>	EFA - HP $\beta$ CD (1:2) - SLS (2%)	5.83	25.13	19.28	18.50
F <sub>bc</sub>	EFA - PVP K-30 (2%) - SLS (2%)	4.67	20.15	12.36	11.86
F <sub>abc</sub>	EFA - HP $\beta$ CD (1:2) - PVP K-30(2%) – SLS (2%)	9.65	41.61	23.15	22.21

EFA - Efavirenz; HP $\beta$ CD – Hydroxy propyl  $\beta$  Cyclodextrin; PVP K30 - Poly vinyl pyrrolidone K30; SLS – Sodium lauryl sulphate

**Table 4: ANOVA of K<sub>1</sub> Values of Efavirenz - HP $\beta$ CD – PVP K30 – SLS Complexes Prepared as per 2<sup>3</sup> – Factorial Study**

Source of Variation	D.F	S.S	M.S.S	F-Ratio	Significance
Total	23	0.0002	0.00001		
Treatments	7	0.0002	0.00002	1472.12	P<0.01
a	1	0.00006	0.00006	4120.50	P<0.01
b	1	0.00002	0.00002	1459.76	P<0.01
ab	1	0.00000	0.00000	56.12	P<0.01
c	1	0.00006	0.00006	4073.91	P<0.01
ac	1	0.00000	0.00000	33.38	P<0.01
bc	1	0.00000	0.00000	74.79	P<0.01
abc	1	0.00001	0.00001	486.40	P<0.01
Error	16	0	0		

F<sub>7,16</sub> (0.01) = 4.03; F<sub>7,16</sub> (0.05) = 2.66; F<sub>1,16</sub> (0.01) = 8.53; F<sub>1,16</sub> (0.05) = 4.49

**Table 5: ANOVA of DE<sub>30</sub> values of Efavirenz - HPβCD – PVP K30 – SLS Complexes Prepared as per 2<sup>3</sup> – Factorial Study**

Source of Variation	D.F	S.S	M.S.S	F-Ratio	Significance
Total	23	1105.27	48.06		
Treatments	7	1105.03	157.86	10763.02	P<0.01
a	1	510.96	510.96	34837.31	P<0.01
b	1	71.10	71.10	4847.76	P<0.01
ab	1	0.16	0.16	10.69	P<0.01
c	1	508.41	508.41	34663.69	P<0.01
ac	1	13.32	13.32	908.19	P<0.01
bc	1	0.76	0.76	51.80	P<0.01
abc	1	0.32	0.32	21.68	P<0.01
Error	16	0.23	0.01		

$$F_{7,16} (0.01) = 4.03; \quad F_{7,16} (0.05) = 2.66; \quad F_{1,16} (0.01) = 8.53; \quad F_{1,16} (0.05) = 4.49$$

Thus the results of the study indicated that combination of HPβCD with PVP K30 and SLS has markedly enhanced the solubility as well as dissolution rate of efavirenz than is possible with them individually. Hence a combination of HPβCD with PVP K30 and / or SLS is recommended to enhance the solubility and dissolution rate of efavirenz, a BCS class II drug.

## CONCLUSIONS

The solubility of efavirenz was markedly enhanced by HPβCD (2.95 fold), PVP K30 (2.49 fold) and SLS (226.96 fold) individually. Combination of HPβCD with PVP K30 and SLS gave a markedly higher enhancement in the solubility of efavirenz than is possible with them individually. HPβCD in combination with PVP K30 and SLS gave respectively 4.05 and 387.63 fold increase in the solubility of efavirenz. ANOVA indicated that the individual main effects of HPβCD, PVP K30 and SLS and their combined effects in enhancing the solubility and dissolution rate ( $K_1$ ) and dissolution efficiency (DE<sub>30</sub>) were highly significant ( $P < 0.01$ ). HPβCD alone gave a 16.74 fold increase in the dissolution rate of efavirenz. HPβCD in combination with PVP K30 and SLS gave respectively 19.98 and 25.13 fold increase in the dissolution rate of efavirenz. HPβCD in combination with both PVP K30 and SLS gave highest enhancement (41.61 fold) in the dissolution rate of efavirenz. Combination of HPβCD with PVP K30 and SLS has markedly enhanced the solubility as well as dissolution rate of efavirenz than is possible with them individually. Hence a combination of HPβCD with PVP K30 and / or SLS is recommended to enhance the solubility and dissolution rate of efavirenz, a BCS class II drug.

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