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A FACTORIAL STUDY ON THE EFFECTS OF HYDROXYPROPYL β CYCLODEXTRIN AND POLOXAMER ON THE SOLUBILITY AND DISSOLUTION RATE OF NIMESULIDE

K.P.R. Chowdary*, K. Surya Prakasa Rao and Venu Sunkavalli

University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam- 530 003, Andhra Pradesh, India

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Correspondence to Author:

Prof. K.P.R. Chowdary

University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam- 530 003, Andhra Pradesh, India

ABSTRACT

The objective of the study is to evaluate the individual and combined effects of hydroxy propyl β cyclodextrin (HPβCD), surfactant (Poloxamer 407) on the solubility and dissolution rate of nimesulide, a BCS class II drug in a series of 2^2 factorial experiments. The solubility of nimesulide in four selected fluids containing HPβCD and Poloxamer 407 as per a 2^2 factorial study was determined. HPβCD alone gave a marginal increase (1.47 fold) in the solubility of nimesulide. Whereas Poloxamer 407 alone and combination of HPβCD and Poloxamer 407 gave a significantly higher enhancement in the solubility of nimesulide, 2.5 and 6.52 fold respectively. Solid inclusion complexes of nimesulide- HPβCD were prepared with and without Poloxamer 407 by kneading method as per a 2^2-factorial design. Both the individual and combined effects of HPβCD and Poloxamer 407 on the solubility, dissolution rate and dissolution efficiency (DE_{30}) of nimesulide were highly significant ($P < 0.01$). HPβCD and Poloxamer 407 alone gave 13.17 and 5.20 fold increase in the dissolution rate of nimesulide. Combination of HPβCD and Poloxamer 407 gave a markedly higher enhancement (21.79 fold) in the dissolution rate of nimesulide. HPβCD in combination with Poloxamer 407 gave much higher enhancement in the solubility and dissolution rate of nimesulide than is possible with them individually. Hence a combination of HPβCD and Poloxamer 407 is recommended to enhance the solubility and dissolution rate of nimesulide.

INTRODUCTION: About 95% of the newly developed organic drugs belong to class II under BCS and exhibit low and variable oral bioavailability due to their poor aqueous solubility. They are practically in soluble in water and aqueous fluids. As such, their oral absorption is dissolution rate limited and they require enhancement in solubility and dissolution rate for increasing their oral bioavailability. 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 physicochemical properties such as solubility, dissolution rate, stability and bioavailability can be favourably affected [1, 2]. Cyclodextrins have been receiving increasing application in pharmaceutical formulation in recent years due to their approval by various regulatory agencies [3, 4]. Poloxamer 407 is a polyethylene oxide- polypropylene oxide- polyethylene

oxide triblock co-polymer of non-ionic nature and is used as a solubilising agent [5-7].

Though cyclodextrin complexation and use of surfactants such as Poloxamer 407 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 of poorly soluble drugs. In the present investigation the individual main effects and combined (or interaction) effects of hydroxy propyl β- cyclodextrin (HPβCD) and surfactant (Poloxamer 407) on the solubility and dissolution rate of nimesulide from CD complexes were evaluated in a 2^2 factorial experiment.

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: Nimesulide was a gift sample from M/s. Natco Pharma Ltd., Hyderabad. Hydroxy propyl β-Cyclodextrin was a gift sample from M/s. Cerestar Inc., USA. Methanol (Qualigens) and Poloxamer 407 were procured from commercial sources.

Estimation of Nimesulide: An UV spectrophotometric method based on the measurement of absorbance at 397 nm in an alkaline borate buffer of pH 8.4 was used for the estimation of nimesulide. The method was validated for linearity, accuracy, precision and interference. The method obeyed Beer's law in the concentration range of 1-10 µg/ml. When a standard drug solution was repeatedly assayed (n=6), the relative error and coefficient of variance were found to be 0.8% and 1.1% respectively. No interference by the excipients used in the study was observed.

Solubility Determination: Excess drug (50 mg) was added to 15 ml of each fluid taken in a 25 ml stoppered conical flask and the mixtures were shaken for 24 h at room temperature (28±1°C) on Rotary Flask Shaker. After 24 h of shaking, 2 ml aliquots were withdrawn at 2 h interval and filtered immediately using a 0.45 µ disk filter. The filtered samples were diluted suitably and assayed for nimesulide by measuring absorbance at 397 nm. Shaking was continued until two consecutive estimations are the same. The solubility experiments were replicated four times each (n=4).

Preparation of Nimesulide-CD Complexes: Solid inclusion complexes of nimesulide- HPβCD were prepared in 1:2 ratio with and without Poloxamer 407 (2%) by kneading method. Nimesulide, HPβCD and Poloxamer 407 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°C until dry. The dried mass was powdered and sieved to mesh No. 120.

Dissolution Rate Study: The dissolution rate of nimesulide as such and from HPβCD complexes prepared was studied in 900 ml of alkaline borate buffer of pH 8.4 using Disso 2000 (Labindia) 8-station dissolution test apparatus with a paddle stirrer at 50 rpm. A temperature 37±1°C was maintained throughout the study. Nimesulide or nimesulide-CD complex equivalent to 50 mg of nimesulide was used in each test. Samples of dissolution media (5ml) were withdrawn through a filter (0.45µ) at different intervals of time, suitably diluted and assayed for nimesulide at 397 nm. The sample of dissolution fluid withdrawn at each time was replaced with fresh fluid. The dissolution experiments were replicated three times each (n=3).

RESULTS AND DISCUSSION: The individual main effects and combined (interaction) effects of HPβCD (Factor A) and Poloxamer 407 (Factor B) on the aqueous solubility of nimesulide were evaluated in a 2^2-factorial experiment. For this purpose, two levels of HPβCD (0, 5mM) and two levels of Poloxamer 407 (0, 2%) were selected in each case and the corresponding four treatments involved in the 2^2-factorial study are purified water (1), water containing 5 mM HPβCD (a); water containing 2% Poloxamer 407 (b); water containing 5 mM HPβCD and 2% Poloxamer 407 (ab).

The solubility of nimesulide in the above mentioned four fluids was determined (n=4) and the results are given in **Table 1**. The aqueous solubility of nimesulide

was markedly enhanced by HPβCD and Poloxamer 407 alone and in combination. The solubility data were subjected to Analysis of variance (ANOVA) to find out the significance of main and combined effects of HPβCD and Poloxamer 407 on the solubility of nimesulide. The results of ANOVA are shown in **Table 2**. The individual and combined effects of HPβCD and Poloxamer 407 in enhancing the solubility of nimesulide were highly significant ($P < 0.01$). HPβCD alone gave a marginal increase (1.47 fold) in the solubility of nimesulide. Whereas Poloxamer 407 alone and combination of HPβCD and Poloxamer 407 gave a significantly higher enhancement in the solubility of nimesulide, 2.5 and 6.52 fold respectively.

TABLE 1: SOLUBILITY OF NIMESULIDE IN VARIOUS FLUIDS AS PER 2^2 – FACTORIAL STUDY

Fluids (Code as per 2^2 – Factorial Experiment)	Solubility (mg/ml) (n=4) $\bar{x}$± sd)	Increase in Solubility (Number of Folds)
Distilled water (1)	0.040±0.004	-
Water containing 5 mM HPβCD (a)	0.059±0.003	1.47
Water containing 2% Poloxamer (b)	0.100±0.003	2.5
Water containing 5mM HPβCD and 2% Poloxamer (ab)	0.261±0.005	6.52

TABLE 2: ANOVA OF SOLUBILITY DATA OF NIMESULIDE IN VARIOUS FLUIDS AS PER 2^2 – FACTORIAL STUDY (HPβCD – POLOXAMER 407)

Source of Variation	D.F	S.S	M.S.S	F-Ratio	Significance
Total	15	0.1215	0.0081	-	-
Treatment	3	0.1213	0.0404	1997.5	$P < 0.01$
a	1	0.0315	0.0315	1579.5	$P < 0.01$
b	1	0.0693	0.0693	3465.0	$P < 0.01$
ab	1	0.0203	0.0203	1018.5	$P < 0.01$
Error	12	0.000243	0.000020	-	-

$F_{0.01\ (1,\ 12)} = 9.33$; $F_{0.05\ (1,\ 12)} = 4.75$; $F_{0.01\ (3,\ 12)} = 5.95$; $F_{0.05\ (3,12)} = 3.49$

To evaluate the individual and combined effects of HPβCD and Poloxamer 407 on the dissolution rate of nimesulide, solid inclusion complexes of nimesulide - HPβCD were prepared with and without Poloxamer 407 as per a 2^2-factorial design. For this purpose two levels of HPβCD (0 and 1 : 2 ratio of drug : CD) and two levels of Poloxamer 407 (0 and 2%) were selected and the corresponding four treatments involved in the 2^2-factorial study were nimesulide pure drug (1); nimesulide- HPβCD (1:2) inclusion binary complex (a); nimesulide - Poloxamer 407 (2%) binary mixture (b); nimesulide- HPβCD (1:2) – Poloxamer 407 (2%) ternary complex (ab).

The CD complexes were prepared by kneading method. All the solid inclusion complexes of nimesulide-CD - Poloxamer 407 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 nimesulide alone and from HPβCD complexes prepared was studied in alkaline borate buffer of pH 8.4 The dissolution of nimesulide followed first order kinetics with r (correlation coefficient) above 0.88. Dissolution efficiency (DE_{30}) values were calculated as suggested by Khan [8]. The dissolution parameters are given in **Table 3**. The dissolution of nimesulide was rapid and higher in the case of all nimesulide- HPβCD complexes prepared when compared to nimesulide as such.

TABLE 3: DISSOLUTION RATE AND DE_{30} OF NIMESULIDE- HPβCD – POLOXAMER 407 COMPLEX SYSTEMS PREPARED AS PER 2^2 – FACTORIAL STUDY

Treatment*	Average K_1 (n=3) ($\bar{x}$± sd)	Increase in K_1 (Number of Folds)	Average DE_{30} (n=3) ($\bar{x}$± sd)	Increase in DE_{30} (Number of Folds)
1	0.012±0.009	-	6.49±0.04	-
a	0.162±0.17	13.17	36.92±0.007	5.69
b	0.064±0.004	5.20	34.9±0.09	5.38
ab	0.268±0.48	21.79	44.77±0.041	6.88

*1: nimesulide pure drug; a: nimesulide- HPβCD (1:2) inclusion binary complex; b: nimesulide - Poloxamer 407 (2%) binary mixture; ab: nimesulide- HPβCD (1:2) – Poloxamer 407 (2%) ternary complex

The dissolution rate (K_1) and DE_{30} values were subjected to ANOVA to find out the significance of the main and combined effects of HPβCD and Poloxamer 407 on the dissolution rate and efficiency (DE_{30}) of nimesulide. The results of ANOVA are shown in **Tables 4-5**. ANOVA indicated that all the individual main effects of HPβCD and Poloxamer 407 and their combined effects in enhancing the dissolution rate (K_1) and DE_{30} of nimesulide were highly significant ($P < 0.01$).

HPβCD and Poloxamer 407 alone gave 13.17 and 5.20 fold increase in the dissolution rate of nimesulide. Combination of HPβCD and Poloxamer 407 gave a markedly higher enhancement (21.79 fold) in the dissolution rate of nimesulide. Similarly HPβCD and Poloxamer 407 alone gave 5.69 and 5.38 fold increase in the dissolution efficiency (DE_{30}) of nimesulide. Combination of HPβCD and Poloxamer 407 gave a 6.88 fold increase in the DE_{30} of nimesulide.

TABLE 4: ANOVA OF DISSOLUTION RATE (K_1) OF NIMESULIDE- HPβCD – POLOXAMER 407 COMPLEX SYSTEMS PREPARED AS PER 2^2 – FACTORIAL STUDY

Source of Variation	D.F	S.S	M.S.S	F-Ratio	Significance
Total	11	0.116	0.011	-	-
Treatment	3	0.0115	0.038	208.50	P < 0.01
a	1	0.548	0.00525	2993.93	P < 0.01
b	1	14.520	0.00504	79289.38	P < 0.01
ab	1	101.53	0.000855	554424	P < 0.01
Error	8	0.0015	0.00018	-	-

$F_{0.01\ (1,\ 8)} = 11.26$; $F_{0.05\ (1,\ 8)} = 5.32$; $F_{0.01\ (3,8)} = 7.59$; $F_{0.05\ (3,8)} = 4.07$

TABLE 5: ANOVA OF DE_{30} DATA OF NIMESULIDE- HPβCD – POLOXAMER 407 COMPLEX SYSTEMS PREPARED AS PER 2^2 – FACTORIAL STUDY

Source of Variation	D.F	S.S	M.S.S	F-Ratio	Significance
Total	11	2542.69	231.15	-	-
Treatment	3	2521.11	840.37	311.55	P < 0.01
a	1	1217.86	1217.86	451.48	P < 0.01
b	1	985.90	985.90	365.51	P < 0.01
ab	1	317.34	317.34	117.65	P < 0.01
Error	8	21.57	2.69	-	-

$F_{0.01\ (1,\ 8)} = 11.26$; $F_{0.05\ (1,\ 8)} = 5.32$; $F_{0.01\ (3,8)} = 7.59$; $F_{0.05\ (3,8)} = 4.07$

Thus, the results of the study indicated that combination of HPβCD with Poloxamer 407 has markedly enhanced both the solubility and dissolution rate of nimesulide, a BCS class II drug. Hence a combination of HPβCD and Poloxamer 407 is recommended to enhance the solubility and dissolution rate of nimesulide.

CONCLUSION: Both the individual and combined effects of HPβCD and Poloxamer 407 on the solubility, dissolution rate and dissolution efficiency (DE_{30}) of nimesulide were highly significant ($P < 0.01$). HPβCD alone gave a marginal increase (1.47 fold) in the solubility of nimesulide. Whereas Poloxamer 407 alone and combination of HPβCD and Poloxamer 407 gave a significantly higher enhancement in the solubility of nimesulide, 2.5 and 6.52 fold respectively. HPβCD and Poloxamer 407 alone gave 13.17 and 5.20 fold increase in the dissolution rate of nimesulide. Combination of HPβCD and Poloxamer 407 gave a markedly higher enhancement (21.79 fold) in the dissolution rate of nimesulide. HPβCD in combination with Poloxamer 407 gave much higher enhancement in the solubility and dissolution rate of nimesulide than is possible with them individually. Hence a combination of HPβCD and Poloxamer 407 is recommended to enhance the solubility and dissolution rate of nimesulide.

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