



## COMMONSENSICAL PREDETERMINE DISSOLUTION TIME OF FUROSEMIDE ACHIEVE BY PREPARING INCLUSION COMPLEX

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### ABSTRACT

Furosemide, a weekly acidic, loop diuretic drug indicated for treatment of edema and hypertension having high permeability through stomach because it remain 99.8 % unionize in stomach (pKa of Furosemide 3.9, pH of gastric fluid - 1.2). Furosemide is practically insoluble in gastric fluid (0.006 mg/mL) and having highly permeability through stomach but due to its solubility limitation it can't enter in to systemic circulation. Gastric emptying time is ranging from 30 min to 2 hrs after this time it travel in to small intestine where it is soluble but can't permeate through its membrane due to its permeation limitation (furosemide having pH depended solubility and permeability). In present work, 40 mg furosemide was taken. Solubility depends on amount of solute, amount of solvent, temperature, stirring speed, time and other factors. Due to low dose of drug it can be dissolved within gastric emptying time if solubility enhancing excipients incorporated. It was logically decided to design experiments, so as to achieve the set objectives. Attempt was made to prepare furosemide inclusion complex with Hydroxypropyl  $\beta$ -cyclodextrin (HPBCD) by different method (Physical mixing, Kneading method and Solvent Evaporation) which would dissolve completely in less than 30 minutes (target selected by considering minimum gastric emptying time). These attempts improve bioavailability and consequently dose reduction would possible.

**Keywords:** Furosemide, Gastric emptying time, Inclusion complex, Hydroxyl propyl beta cyclodextrins, *In-vitro* dissolution

### INTRODUCTION

Recently more than 40% NCEs (new chemical entities) developed in Pharmaceutical Industry are practically insoluble in water. Formulation of poorly soluble compounds for oral delivery now presents one of the interesting challenges to formulation scientists in the pharmaceutical industry. In the case of poorly water-soluble drugs, dissolution is the rate-limiting step in the process of drug absorption. Potential bioavailability problems are prevalent with extremely hydrophobic drugs (aqueous solubility less than 0.1 mg/ml at 37°), due to erratic or incomplete absorption from GIT<sup>1</sup>.

In the Present Investigation, drug which is practically insoluble in gastric fluid and having high permeability through stomach was selected. The rationale for selecting such type is "Drug which having highly permeability through stomach but due to its solubility limitation in gastric fluid it can't enter in to systemic circulation. Gastric emptying time is ranging from 30 min to 2 hrs after this time drugs go in to small intestine where it is soluble but can't permeate through its membrane due to its permeation limitation."

To improve dissolution of such drug is challenging and rational. furosemide, a weekly acidic, non-steroidal anti inflammatory drug fall under above category having high permeability through stomach but due to its solubility limitation it can't enter in to systemic circulation and gastric emptying time is ranging from 30 min to 2 hr, after this time furosemide goes in to small intestine where it is solubilise but can't permeate through its membrane.

To improve dissolution of such drug is challenging and rational. In present investigation, dissolution of furosemide improves by preparing floating granules. Furosemide indicated for treatment of edema and hypertension having high permeability through stomach because it remain 99.8 % unionize in stomach<sup>2</sup> (pKa of Furosemide 3.9, pH of gastric fluid - 1.2). Furosemide is practically insoluble in stomach medium (0.006 mg/mL) and having highly permeability through stomach but due to its solubility limitation it can't enter in to systemic circulation. Gastric emptying time is ranging from 30 min - 2 hrs<sup>3</sup> after this time it goes in to small intestine where it is soluble but can't permeate through its membrane due to its permeation limitation (furosemide having pH depended solubility and permeability).

### MATERIALS

Furosemide Gifted by Torrent Research centre, Ahmedabad and Hydroxypropyl  $\beta$ -cyclodextrin (HPBCD) Gifted by Roquette Freres, France.

All other chemicals and reagents used are of analytical grade.

### METHODS

#### Phase solubility study

The solubility measurements of furosemide with HPBCD were performed according to Higuchi Connors<sup>4</sup>. Excess amounts of furosemide (5 mg) and 2 - 14 milimole of HPBCD (2 MM of HPBCD = 27.5 mg HPBCD) taken in a series of 10 ml volumetric flasks containing 0.1 N HCl and the mixtures were shaken for 24 hours at room temperature. After 24 hours, solutions were filtered through Whatman filter paper (0.45 $\mu$ m). The filtered samples were diluted suitably and analyze for furosemide content by measuring absorbance at 239.2 nm. The apparent complexation constant  $K_{1:1}$  of furosemide - HPBCD were calculated from the slope and intercept of the straight lines of the phase-solubility diagrams, according to the equation;  $K_{1:1} = \text{slope}/\text{So}$  (1-slope)

Where  $K_{1:1}$  = apparent complexation constant,

So (intercept) = the intrinsic solubility of the compound in absence of complexing agent.

#### Preparation of inclusion complex

##### Kneading method

HPBCD and furosemide were mixed together in a mortar. Distilled water was then added slowly. The mixture was then ground for 1 hr. During this process, an appropriate quantity of water was added to the mixture in order to maintain a suitable consistency. The paste was dried in a Hot-air oven at 60° C until product is completely dried. Resultant powder was collected and pass through 60 # sieve.

##### Physical mixing

A physical mixing of HPBCD and furosemide were mixed together in a mortar for 15 min.

##### Solvent evaporation method

HPBCD and furosemide were dissolved in 100 ml of pure methanol (analytical grade). Methanol was evaporated by hot air oven at 60° C. Resultant powder was collected and passed through 60 # sieve. Amount of HPBCD and method of complexation in various formulations shown in following Table 1.

Table 1: Design data

Formulation code	Amount of HPBCD in MM	Method of complexation
H1	0.5	PM
H2	1.25	PM
H3	2	PM
H4	0.5	SE
H5	1.25	SE
H6	2	SE
H7	0.5	KM
H8	1.25	KM
H9	2	KM

#### Formulation of inclusion complex by applying factorial design

A 3-level 2-factor (one numerical and one categorical) design was used for the formulation of inclusion complex. This design is suitable for exploring quadratic response surface and constructing second order polynomial models. The design consists of replicated centre points and the set of points lying at the midpoint of the multidimensional cube that defines the region of interest. The non linear quadratic model generated by the design in the form

$$Y = X_0 + X_1A + X_2B + X_{11}A^2 + X_{22}B^2 + X_{12}AB.$$

Where, Y is the measure response associated with each factor level combination:  $X_0$  is an intercept:  $X_1$  and  $X_2$  are coefficients of main effects The coefficients with second order terms ( $X_{11}$  and  $X_{22}$ ) indicate the quadratic nature and  $X_{12}$  is the regression coefficient for the interaction term. This study investigated utility of a 2-factor, 3-level one factorial design and optimization process for inclusion complex of furosemide. Amount of HPBCD and method of complexation were selected as the independent variables whereas  $T_{100\%}$  (time require to dissolve 100% drug) were selected as dependent variables. The prepared inclusion complex of furosemide was evaluated for dissolution study. Polynomial equation was generated for each response using Multiple Linear Regression Analysis.

#### In-vitro dissolution of prepare inclusion complex

Dissolution of prepared formulations (equivalent to 40 mg of furosemide) was performed in 900 ml 0.1 N HCl (pH 1.2) in USP

type-II Dissolution apparatus at 50 RPM. Dissolution medium was kept at  $37 \pm 0.5^\circ\text{C}$ . 5 ml sample were collected at different time interval and filtered through a Whatman filter paper ( $0.45 \mu\text{m}$ ). The same amount of fresh dissolution medium was added to maintain sink condition. The absorbance was measured at 233 nm using UV-visible spectrophotometer. The concentration of furosemide was calculated by using standard curve equation.

#### Data analysis

The response surface methodology is a collection of mathematical and statistical techniques used for modelling and analysis of problems in which a response of interest is influenced by several variable and the objectives is to optimize this response. The run or formulation, which are designed based on factorial design were evaluated for the response. The response values are subjected to multiple regressions. The response values subjected for this analysis are  $T_{100\%}$ . The multiple regression analysis was done using DESIGN EXPERT 7.1.6 (STAT-EASE) demo version software, which specially meant for this optimization process.

#### Formulations optimization

The computation for optimized formulation was carried using software, DESIGN EXPERT 7.1.6 (STAT-EASE). The response variable considered for optimization was  $T_{100\%}$ . The optimized formulation was obtained by applying constraints (goals) on dependent (response) and independent variables (factors). Constraints for responses and factors are shown in Table 2.

Table 2: Constraints for optimization (HPBCD)

Name	Goal	Lower Limit	Upper Limit
Amt. of HPBCD in MM	In range	0.5	2
$T_{100\%}$	target = 30 minute	30	240

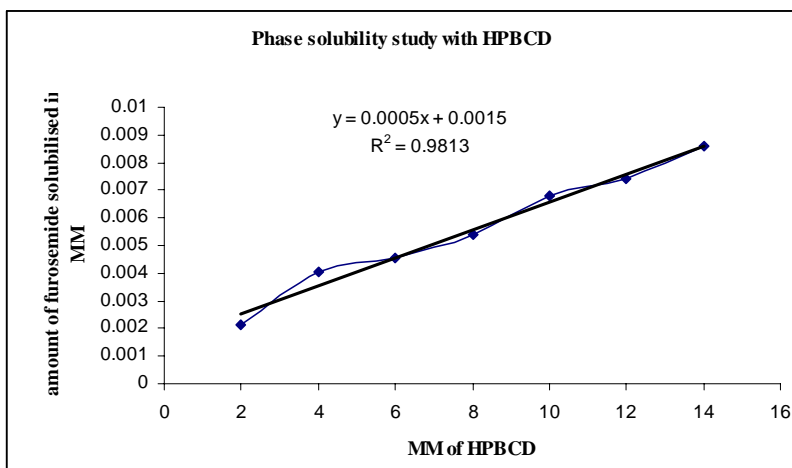


Fig. 1: Phase solubility diagram of furosemide - HPBCD

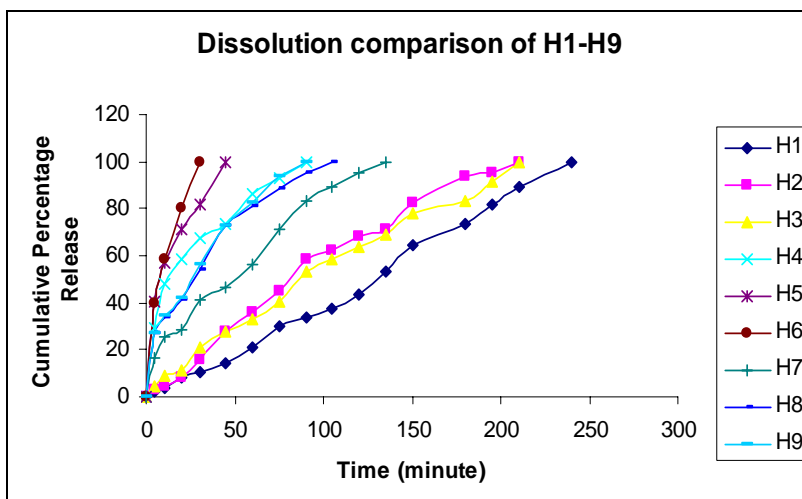


Fig. 2: Comparison of Dissolution Profile of Batch H1-H9

**RESULTS AND DISCUSSION**

Dissolution of Batch H1-H9 showed in figure 2. It was observed that as the amount of HPBCD increase T<sub>100</sub> % decrease (In all batches). Batch H3, H6 and H9 revealed T<sub>100%</sub> vary at the same amount of HPBCD (2 MM) with different method of complexation (Physical mixing, Solvent Evaporation and Kneading was the method of complexation in Batch H3, H6 and H9 respectively). It indicated lowest T<sub>100%</sub> achieved in Solvent Evaporation followed by kneading

method and physical mixing. That was due to, In kneading method, sticky complex formed because of sticky nature of HPBCD which decrease drug release while in solvent evaporation method completely free flow complex was achieved in which more drug was entrapped inside hydrophobic cavity of HPBCD molecules. In physical method very less amount of furosemide entrapped inside hydrophobic cavity of HPBCD molecules and more amount of drug remain in noncomplex form.

Table 3: The design and response summary data

Formulation code	Factors		Response
	amt. of HPBCD in MM	method of complexation	
H1	0.5	PM	T <sub>100%</sub> 240
H2	1.25	PM	210
H3	2	PM	195
H4	0.5	SE	90
H5	1.25	SE	45
H6	2	SE	30
H7	0.5	KM	135
H8	1.25	KM	105
H9	2	KM	90

Equation in terms of actual factors:  $PM-T_{100} = +273.61111 - 74.44444 * \text{amount of HPBCD in MM} + 17.77778 * \text{amount of HPBCD in MM}^2$

$SE-T_{100} = +126.11111 - 84.44444 * \text{amount of HPBCD in MM} + 17.77778 * \text{amount of HPBCD in MM}^2$

$KM-T_{100} = +168.61111 - 74.44444 * \text{amount of HPBCD in MM} + 17.77778 * \text{amount of HPBCD in MM}^2$

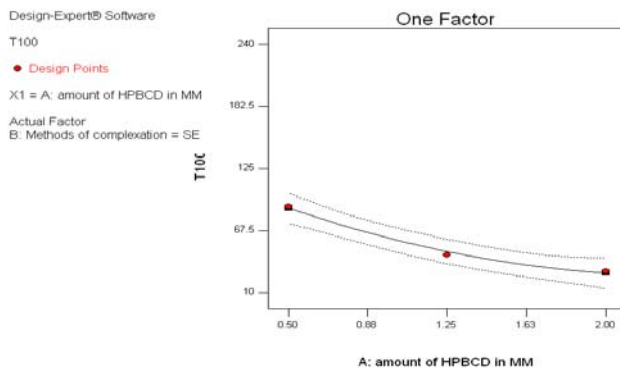


Fig. 3: Effect of amount of HPBCD on T<sub>100%</sub>

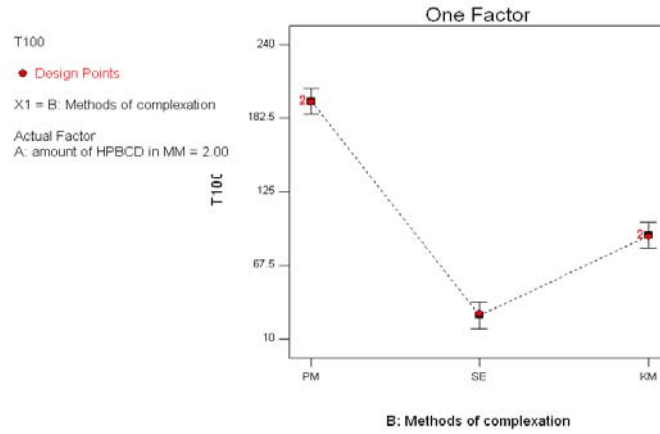


Fig. 4: Effect of method of complexation on T<sub>100%</sub>

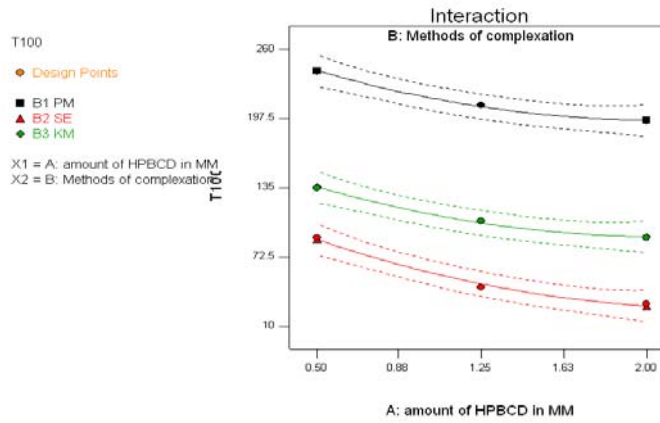


Fig. 5: Effect of HPBCD and method of complexation on T<sub>100%</sub>

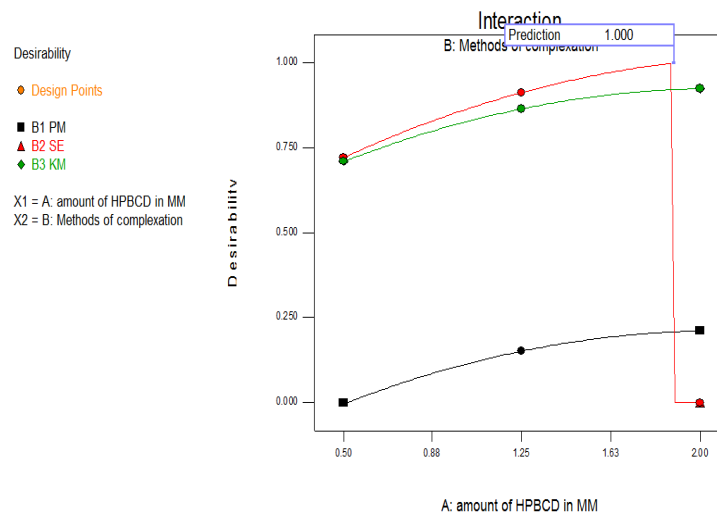


Fig. 6: Optimisation plot for HPBCD

A 3<sup>2</sup> one factorial design was adopted, using the amount of HPBCD and method of complexation as independent variables for formulation optimization. The response values subjected for this analysis was T<sub>100%</sub>. It was logically decided to obtain the values of T<sub>100%</sub> 30 minutes or less from the formulated products. The results for dependent variable T<sub>100%</sub> of the prepared batches are shown in Table 3. One factor plot (figure 3) shows that as the amount of HPBCD increased, T<sub>100%</sub> decreased at the fix method of categorical factor (method of complexation: solvent evaporation) due to hydrophobic furosemide drug was entrapped in to the inner hydrophobic cavity of HPBCD and outer hydrophilic cavity easily dissolved in gastric fluid. One factor plot (figure 4) shows that as the effect of categorical factor on T<sub>100%</sub> at the fix amount of numerical factor (amount of BCD: 2 MM). Lowest T<sub>100%</sub> achieved in solvent evaporation method followed by kneading and physical mixing due to HPBCD is slightly sticky material. In kneading method, sticky complex formed which decrease drug release while in solvent evaporation method completely free flow complex was achieved in which more drug was entrapped inside hydrophobic cavity of HPBCD molecules. In physical method very less amount of furosemide entrapped inside hydrophobic cavity of HPBCD molecules and more amount of drug remain in noncomplex form.

The relationship between the dependent and independent variables was further elucidated using contour plots. In contour plot only formulation H6 showed T<sub>100%</sub> near to desired T<sub>100%</sub> (figure 5,

Indicated by red line). For the optimization of inclusion complex of furosemide, constraint was fixed for all factors and response (Table 2). Constraints were set according to formulation of inclusion complex using minimum amount of excipients, which would give desired response values. In the present study our aim was T<sub>100%</sub> should be achieved in 30 minutes or less. In optimization plot (figure 6), desirability 1.0 indicated optimum formulation was same to formulation H6 (T<sub>100%</sub> achieved in 30 minutes by inclusion complex with 2.00 MM of HPBCD prepared by solvent evaporation method).

#### CONCLUSION

From above research work it was concluded that for improving dissolution of weakly acidic and those drugs which are mostly absorb through stomach, inclusion complex is the gold standard approach to get desire release profile due to inherent solubilization effect of hydroxyl propyl beta cyclodextrins.

#### REFERENCES

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