WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 3, 916-936. Research Article ISSN 2277– 7105

SYSTEMATIC OPTIMIZATION OF FORMULATION OF INCLUSION COMPLEXES CONTAINING AN ANTI-DIABETIC DRUG

***[1]Nirmala P., [2]Marina Koland and [3]Narendra C.**

[1]Assistant Professor, Department of Pharmaceutics, Government College of Pharmacy, Bengaluru, Karnataka, India.

[2]Professor & Head of Department, Department of Pharmaceutics, NGSM Institute of Pharmaceutical Sciences, Mangalore, Karnataka, India.

[3]Assisstant Professor, Visveswarapura Institute of Pharmaceutical Sciences, Bengaluru, Karnataka, India.

Article Received on
29 Dec. 2016,

Revised on 19 Jan. 2017,
Accepted on 09 Feb. 2017

DOI: 10.20959/wjpr20173-7929

***Corresponding Author**
Nirmala P.
Assistant Professor,
Department of Pharmaceutics, Government College of Pharmacy, Bengaluru, Karnataka, India.

ABSTRACT

Glipizide (GZ), a BCS class II antidiabetic drug was formulated as inclusion complexes using HPβ-CD by physical mixture and solvent evaporation method. The effects of various excipients PEG 10000, HPβCD, PVP k30 and PEG 6000 on the solubility of Glipizide were investigated. The factorial design of experiment was applied to know the interaction effect of the excipients on the solubility of GZ. FTIR, DSC, H NMR, XRD, drug content and dissolution studies were performed. PEG 6000 was found to enhance the solubility of the drug only by 3.8 folds. The polynomial equation and experimented data concluded that HPβCD alone was sufficient to enhance the solubility of GZ. The formation of an inclusion complex of GZ with HPβCD was confirmed by FTIR, DSC and H NMR studies. The XRD results supported the finding of FTIR spectra and NMR spectras. The complex formed using solvent evaporation technique entrapped 100 percent of the drug within the cavity of the HPβCD carrier molecule. The inclusion complex shows a significant increase in the drug dissolution compared to physical mixture and pure drug. It was concluded that HPβCD is most useful for enhancing the solubility and thus drug release from its inclusion complex.

KEYWORDS: PEG 10000, HPβCD, PVP k30 and PEG 6000.

INTRODUCTION

Glipizide (GZ) is a second-generation sulfonylurea, hydrophobic which is poorly water-soluble (BCS class II) antidiabetic drug for the treatment of patients with type II diabetes.[1],[2] GZ acutely lowers the blood glucose level in humans by stimulating the release of insulin from the pancreas and is typically prescribed to treat non-insulin-dependent diabetes mellitus. The drug is insoluble in water, and its dissolution is considered to be a rate-determining step (*i.e.*, an effective factor) in its absorption from the gastrointestinal fluids.[3] This limits its bioavailability and may be the reason for its delayed absorption.[4] Glipizide is reported to have a short biological half-life (3.4±0.7 h) requiring it to be administered in 2 to 3 doses of 2.5 to 10 mg per day. A rapidly absorbed drug having faster elimination rate with short half-life make it a suitable candidate to be formulated for the sustained delivery.[5],[6]

Cyclodextrins (CDs) form a group of structurally related oligosaccharides with cylinder-shaped cavities that have the capacity to form inclusion complexes with many drugs by taking a whole drug molecule, or a part of it, into the cavity.[7],[8] Because of the large number of hydroxyl groups on CDs, they are water-soluble. They are known for their ability to molecularly encapsulate a wide variety of drugs into their hydrophobic cavity without the formation of any covalent bonds. CDs have widespread pharmaceutical applications mainly because of their effect on enhancing the solubility and bioavailability of many drug formulations. Complexation with cyclodextrins has been reported to enhance the solubility, dissolution rate, and bioavailability of poorly water-soluble drugs.[9],[10],[11],[12]

Several methods have been proposed to obtain solid drug-CD complexes, both in liquid and in solid medium, but there is still no general rule or universal method, probably because each drug to be entrapped is a special case and optimal conditions depend on the characteristic of both the host and guest molecules. Selection of the most suitable preparation method for a given drug requires careful evaluation because apart from consideration of the performance of the obtained product, such as the dissolution profile, other factors such as simplicity, low cost, high yield, swiftness and ease of scale-up also play a major role in the choice of a method.[13,14]

The objective of the present study was to prepare inclusion complexes of GZ with HPβ-CD using various methods such as physical mixture and solvent evaporation to improve its aqueous solubility and dissolution rate.

MATERIALS AND METHODS

Materials

Glipizide was a gift sample from Micro Labs, Bangalore. Polyethyleneglycol 10000 (PEG10000), polyethylene glycol 6000 (PEG 6000), polyvinylpyrrolidone K30 (PVP K30), Ethyl Cellulose, Magnesium stearate, Span 80 and Tween 80 were purchased from SD Fine Chem, India. Hydroxypropyl-β-cyclodextrin (HPβCD) was procured from M/s. Yarrow Chem Products., Mumbai, India. All other chemicals and reagents used were of analytical grade.

Methods

Preparation of standard calibration curve of glipizide

Preparation of standard solution: Primary stock solution: 100 mg of Glipizide was accurately weighed and dissolved in sufficient amount of 0.1N NaOH taken in 100 ml volumetric flask, finally the volume was made up to 100ml with the same. (1000 µg/ml). Secondary stock solution: 10 ml of the above solution was pipette out into second 100ml volumetric flask; volume was made up with 0.1N NaoH to get a concentration of 100µg/ml. Working standard solution: Aliquots of 0.4ml, 0.8ml, 1.2 ml, 1.4ml, 1.6ml, 2.0ml, 2.4ml, 2.8ml, 3ml and 4ml were pipette out from the working standard solution and transferred into 10 ml volumetric flask. Then the volume was made up with distilled water to get a concentration which falls within the Beer's range 2 – 50µg/ml. The absorbance of these solutions was measured at 276 nm against reagent blank.

Phase Solubility Study

Phase solubility studies were carried out according to the method reported by Higuchi and Connors.[15] An excess amount of Glipizide was added to 10ml aqueous solution containing increasing concentrations of various excipients (0 – 10% w/v). The suspensions were vigorously shaken at 25 ± 1^0C for 72 hours in order to reach equilibrium. After equilibrium was attained, the samples were filtered through a 0.45µm millipore membrane filter and Glipizide concentration was determined by UV spectroscopy (Shimadzu UV - 18000) at 276nm. Each experiment was carried out in triplicate. The apparent 1:1 stability constant Ks was calculated from the phase solubility diagrams using the equation:

$$Ks = \frac{slope}{S_0 \ (1 - slope)}$$

where the intercept corresponds to the intrinsic solubility (S_0) of Glipizide at 25^0C. The various excipients used were polyethyleneglycol 10000 (PEG10000), polyethylene glycol 6000 (PEG 6000), polyvinylpyrrolidone K30 (PVP K30) & hydroxypropyl-β-cyclodextrin (HPβCD).

Design of experiment

A factorial design was adapted (Design expert version 6.05) to study the effect of 3 independent variables on the solubility of Glipizide.

Independent Variables

X_1 = Amount of HPβCD (0 to 10%)

X_2 = Amount of PVP K30 (0 to 10%)

X_3 = Amount of PEG 6000 (0 to 10%)

Response Variable

Y_1 = Glipizide solubility in water (mcg/ml)

Table 01. Design layout with response variable

Code	Type	X_1 (%)	X_2 (%)	X_3 (%)
1	Fact	0.00	0.00	0.00
2	Fact	10.00	0.00	0.00
3	Fact	0.00	10.00	0.00
4	Fact	10.00	10.00	0.00
5	Fact	0.00	0.00	10.00
6	Fact	10.00	0.00	10.00
7	Fact	0.00	10.00	10.00
8	Fact	10.00	10.00	10.00

Method

Phase solubility studies were carried out by taking an excess amount of Glipizide, which was added to 10ml aqueous solution containing concentrations of various excipients as mentioned above. The suspensions were vigorously shaken at 25 $\pm$ 1^0C for 72 hours in order to reach equilibrium. After equilibrium was attained, the samples were filtered through a 0.45µm millipore membrane filter and Glipizide concentration was determined by UV spectroscopy (Schimadzu UV-18000) at 276nm.

Preparation of Inclusion complex

Physical mixture method: The 1:1 molar ratio of physical mixture of drug: HPβCD was taken in a mortar and pestle and triturated for 30 minutes. The dry powder blend obtained was passed through sieve number 80 and stored in a desicator until further use.

Solvent evaporation method: The solid inclusion of Glipizide was prepared in 1:1 molar ratio by solvent evaporation method. Glipizide was dissolved in methanol and added to aqueous mixture containing HPβCD and the suspension was stirred for 4hr at 60^0C. The solvent was then evaporated under vacuum at 40^0C with a rotary evaporator. The powder obtained was passed through sieve number 80 and stored in a desicator until further use.

Characterization studies

Fourier-Transform Infrared (FTIR) Spectral studies

FTIR spectroscopy studies was carried out for pure Glipizide, inclusion complexes obtained by physical mixture and solvent evaporation method by using Shimadzu FTIR -8400 by KBr disc method using the scanning range from 500-4000 cm^{-1} and resolution of $4cm^{-1}$ and compared with pure Glipizide.

DSC studies

The DSC of pure Glipizide, inclusion complexes obtained by physical mixture and solvent evaporation method was carried out by using DSC apparatus Metler Toledo STAR SW model with a scan range from 30 to 300°C with a ramp (heating rate) of 10°C per minute. The sample holder was Standard 40µl Aluminium crucible (pin holed). About 3 to 6 mg of sample powder was taken into a standard aluminium crucible and crimped with a cap. Crucible was pin holed before putting into analysis. Data was collected from 30°C to 300°C with ramp of 10°C per minute. The position and intensities of thermograms were considered for the identification and comparison of drug or complex.

^{1}H NMR studies

The pure drug and inclusion complex were subjected to ^{1}H NMR studies spectroscopic experiments (Varian) at 500MHz with dual full band channels and Z axis gradients. The spectra obtained were measured at 298 K with an operating frequency of 499.742 MHz. The 90^0C pulse width for 1H was 10.8 at a transmitter power of 50. Solutions were purged prior to data collection under a stream of argon for 1hr to reduce the amount of dissolved oxygen. The obtained data of pure drug and inclusion complex was compared.

XRD studies

The X-ray diffractograms for the pure drug and the inclusion complex were obtained using on X-ray diffraction instrument (Bruker D8 Advance Powder XRD) with Ni-filtered, Cu radiation as anode target at $1.5406A^0$ at a voltage of 40 kV and current of 30 mA. The scan was performed in the range of 3^0 2Θ to 45^0 2Θ with 15 sec as time per step and step size is 0.01^0 2Θ.

Estimation of drug content

The inclusion complexes prepared by physical mixture and solvent evaporation were analyzed for the drug content. Mixtures containing 10mg equivalent of the drug were dissolved in 100ml of 0.1N NaOH and suitably diluted and absorbance was measured at 276nm using UV spectroscopy (Shimadzu UV- 18000). The drug content experiment was performed in triplicate.

Dissolution studies

The dissolution behaviour of the complex was compared with that of pure glipizide and physical mixture containing quantities of formulation equivalent to 10mg of Glipizide. The dissolution studies were carried out according to USP XXIII apparatus 2 (rotating paddle method -model TDT-08L, M/s.Electrolab). Distilled water was used as the dissolution medium, which was maintained at $37^0C \pm 0.5^0C$ and stirred at 50rpm. Powdered samples containing 10mg of Glipizide or its equivalent in complex or physically mixed with HPβCD were used in a capsule. At appropriate time intervals, 5ml samples were withdrawn and filtered rapidly through 0.8µm membrane filter. The concentration of Glipizide was determined by UV spectroscopy at 276nm. Similar experiments were repeated without using capsule as a dosage form. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Preparation of standard calibration curve of glipizide: The linear regression was done on absorbance data points. The results are presented as follows. For standard curve of Glipizide: The slope = 0.0216 and the correlation coefficient = 0.9981. A straight-line equation (Y = mx + c) was generated to facilitate the calculation of amount of drug. The equation is Absorbance = 0.0216 X Concentration (Table No. 01 and Fig.1).

Table No. 1: Data for Standard curve of Glipizide in 0.1N NaoH

Sl. No	Concentration (µg/ml)	Average Absorbance	SEM (±) n=5
1	4	0.102	0.0030
2	8	0.184	0.0038
3	12	0.253	0.0128
4	16	0.352	0.0023
5	20	0.434	0.0020
6	24	0.527	0.0053
7	28	0.603	0.0012
8	30	0.655	0.0015
9	40	0.847	0.0120

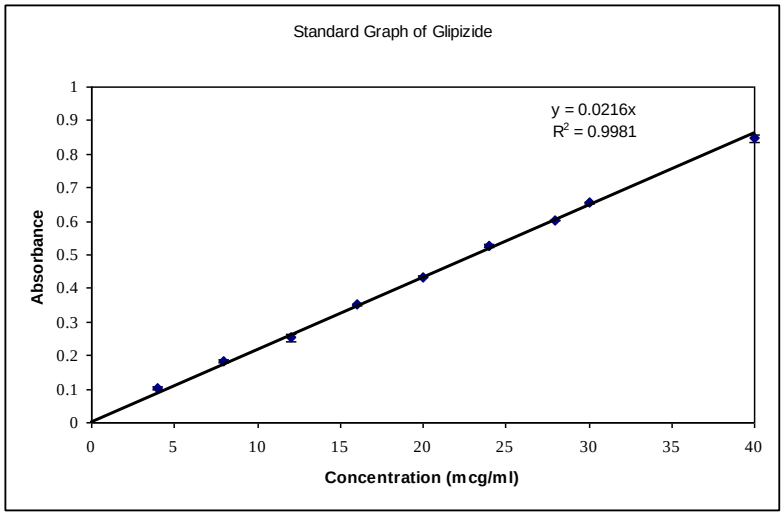

Fig. 1. Standard Calibration curve of Glipizide in 0.1NNaOH

Phase solubility studies

The effects of various excipients PEG 10000, HPβCD, PVP k30 and PEG 6000 on the solubility of Glipizide were investigated. Figure no's 01, 02, 03 and 04 shows the solubility profile of Glipizide with various excipients at different concentrations. From the solubility profile it was observed that the solubility of Glipizide increases linearly as the concentration of HPβCD and PVP k30 was increased. It was found that the phase solubility diagram of the HPßCD and PVP k30 solution showed a typical A_L-type, suggesting the formation of a soluble complex of 1: 1 molar ratio. PVP k30 showed a fourfold increase in solubility whereas HPβCD exhibited a 9.5 fold increase. In case of PEG 10000 the graph exhibited a nonlinear increase in solubility of Glipizide. Hence PEG 10000 was not considered for further studies. PEG 6000 enhanced the solubility of the drug only by 3.8 folds (Table No. 2 to 5 and Fig. 2 to 5).

Table No. 02: Phase solubility study of Glipizide using HPβCD

Conc of HPβCD	Conc of HPβCD	Average Absorbance*	Average Conc of Glipizide	SEM (±)
0	0	0.111	0.144	0.0032
1	7.18	0.215	0.305	0.0026
2	14.36	0.315	0.389	0.002
3	21.54	0.334	0.433	0.0032
4	28.72	0.387	0.48	0.0154
5	35.89	0.399	0.517	0.0054
6	43.07	0.434	0.568	0.0088
7	50.25	0.459	0.615	0.0085
8	57.43	0.546	0.7	0.0405
9	64.61	0.551	0.72	0.023
10	71.79	0.641	0.811	0.0047

*Average absorbance of three trials (n=3).

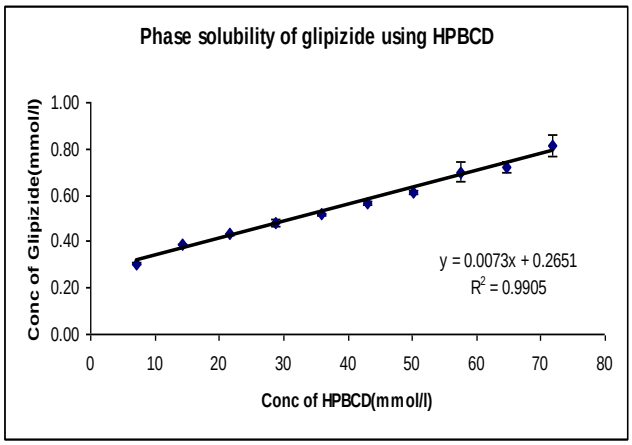

Fig. 2. Phase solubility of Glipizide using HPβCD

Table No. 3: Phase solubility study of Glipizide using PEG10000

Conc of PEG10000 (%w/v)	Conc of PEG10000 (mmol/liter)	Average Absorbance*	Average Conc of Glipizide (mmol/liter)	SEM (±) (n = 3)
0	0	0.06	0.078	0.0008
2	2	0.083	0.107	0.0012
4	4	0.113	0.146	0.002
6	6	0.083	0.107	0.0012
8	8	0.089	0.115	0.0008
10	10	0.043	0.056	0.0016

*Average absorbance of three trials (n=3).

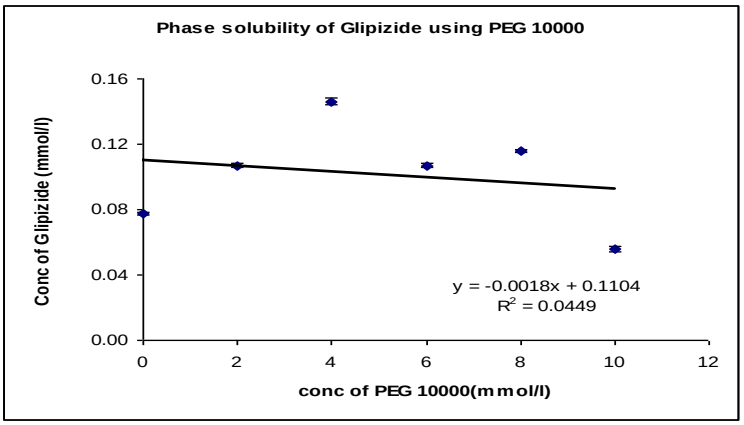

Fig. 3. Phase solubility of Glipizide using PEG10000

Table No. 4: Phase solubility study of Glipizide using PVPk30

Conc of PVP k30	Conc of PVP k30	Average Absorbance*	Average Conc of Glipizide	SEM (±)
(%w/v)	(mmol/liter)		(mmol/liter)	(n = 3)
0	0	0.091	0.118	0.0057
2	0.4	0.186	0.241	0.0037
4	0.8	0.218	0.283	0.0016
6	1.2	0.24	0.311	0.0012
8	1.6	0.263	0.341	0.0049
10	2	0.282	0.365	0.0029

*Average absorbance of three trials (n=3).

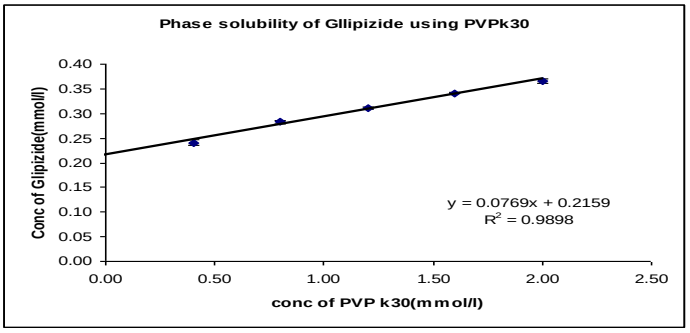

Fig. 4. Phase solubility of Glipizide using PVP k30

Table No. 5: Phase solubility study of Glipizide using PEG 6000

Conc of PEG 6000	Conc of PEG 6000	Average Absorbance*	Average Conc of Glipizide	SEM (±)
(%w/v)	(mmol/liter)		(mmol/liter)	(n = 3)
0	0	0.14	0.182	0.0008
2	3.33	0.204	0.265	0.0065
4	6.67	0.22	0.285	0.0008
6	10	0.239	0.309	0.0012
8	13.33	0.307	0.398	0.0069
10	16.67	0.241	0.312	0.0037

*Average absorbance of three trials (n=3).

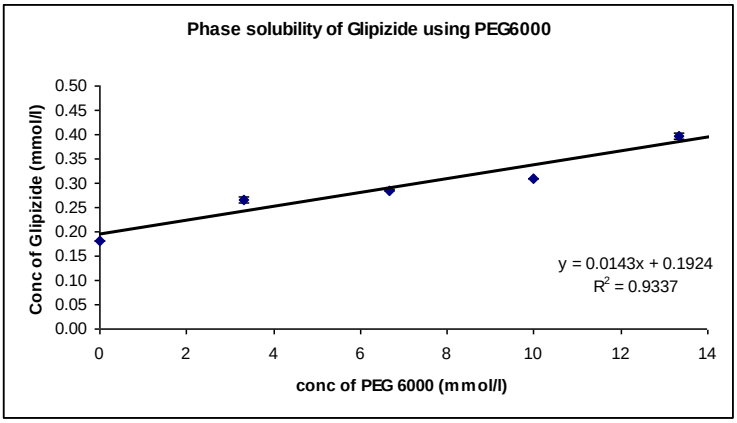

Fig. 5. Phase solubility of Glipizide using PEG 6000

Design of Experiment

The factorial design of experiment was applied to know the interaction effect of the excipients on the solubility of Glipizide. ANOVA report reveals that the combination of the excipients influenced the solubility of the drug.

Response Y_1: 229.08 + 106.50 X_1 - 40.81 X_1X_2 -15.57 X_1X_3 -24.83 X_2X_3 + 8.12 $X_1X_2X_3$

Based on the polynomial equation and the one factor plot (figure 05 & 05a) indicate that HPβCD alone was most significant among all the excipients used to achieve a maximum solubility.

The interaction effect between factor X_1 & X_2 can be studied with the help of figure no's 06 & 06a. In absence or presence of PEG 6000, increase in concentration of PVP k30 with HPβCD does not significantly increase the solubility of the drug.

Figure no.07 & 07a indicates the interaction effect of X_1 & X_3 in absence and presence of PVP k30. In absence of PVP and keeping HPβCD at high level, increasing the concentration of PEG 6000 from 0-10% decreased the solubility from 381.51 to 378.47mcg/ml.

In presence and absence of HPβCD, the interaction effect between X_2 & X_3 can be studied with the help of figure 8 & 8a. In absence of HPβCD, increase in concentration of PVP k30 with or without PEG 6000 enhances the solubility to a very minimal extent. But this effect was found to be opposite in presence of HPβCD.

Therefore, it was necessary to apply a multicriteria decision approach, like desirability function, to find the best compromise between the values of the variables, in order to

maximize solubility of the drug. Each response was associated with its own partial desirability function, which varied from 0 to 10, according to the closeness of the response to its target value. Based on the optimization studies, it was identified that presence of 10% of HPβCD and 9.3% of PEG was found to be optimal combination to enhance the solubility of Glipizide.

To validate the obtained solution further experiment was conducted and solubility was found to be 373 ± 3.58 mcg/ml, which was lesser as compared to the solubility exhibited by HPβCD 10% alone. Hence based on the polynomial equation & experimented data, it was concluded that HPβCD alone will be sufficient to enhance the solubility of Glipizide (Table No. 6 to 8 and Fig. 6 to 13).

Table No. 6: Design of experiment

X_1 (%)	X_2 (%)	X_3 (%)	Y_1 mcg/ml
0	0	0	39.5321
10	0	0	381.51
0	10	0	179.832
10	10	0	326.1
0	0	10	131.221
10	0	10	377.472
0	10	10	139.757
10	10	10	256.221

Table No. 7: ANOVA for 3 factor interaction model

Source	Sum of Squares	DF	Mean Square	F Value
Model	111451	5	22290.13	277.594
X_1	90729.7	1	90729.68	1129.921
X_1X_2	13325.1	1	13325.12	165.947
X_1X_3	1938.48	1	1938.478	24.141
X_2X_3	4930.47	1	4930.467	61.402
$X_1X_2X_3$	526.889	1	526.889	6.561
Residual	160.594	2	80.297	
Cor Total	111611	7		

* $p < 0.05$ significant.

Table No. 8: Statistical parameters

Std. Dev.	8.96	R-Squared	0.9985
Mean	229.08	Adj R-Squared	0.9949
C.V.	3.91	Pred R-Squared	0.9769
PRESS	2569.51	Adeq Precision	44.36

Response Y_1: 229.08 + 106.50 X_1 - 40.81 X_1X_2 -15.57 X_1X_3 -24.83 X_2X_3 + 8.12 $X_1X_2X_3$

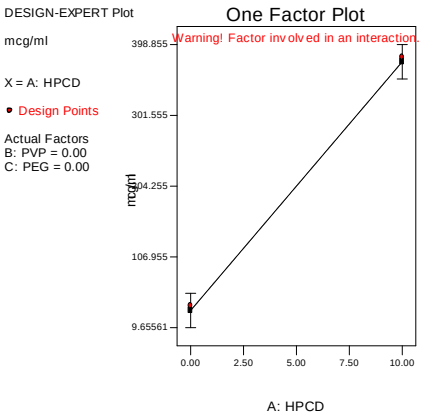

Fig. 6. Effect of factor X_1 on response Y_1 at low level of factor X_2 and X_3

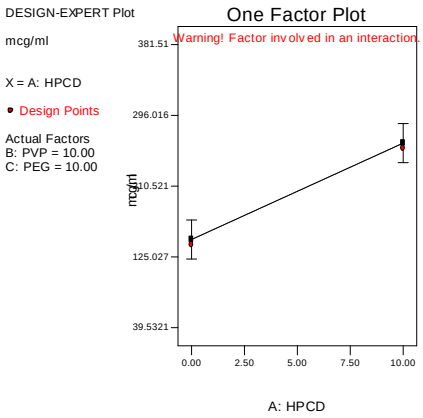

Fig. 7. Effect of factor X_1 on response Y_1 at high level of factor X_2 and

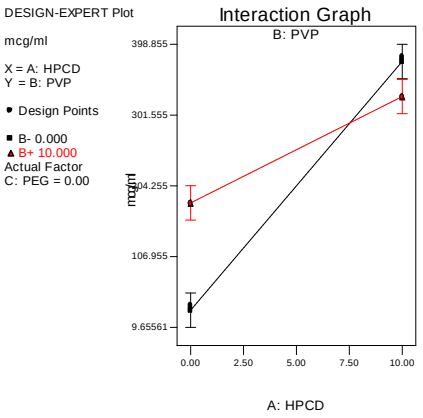

Fig.8. Effect of Interaction factor X_1X_2 on responseY_1 at low level of factor X_3

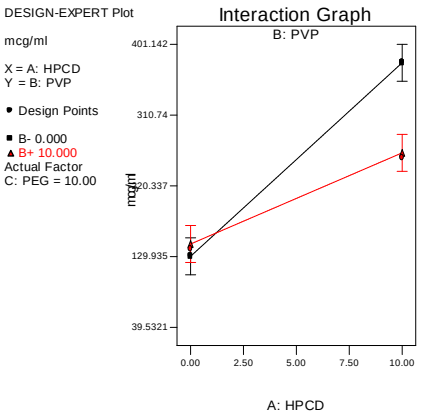

Fig.9. Effect of Interaction factor X_1X_2 on responseY_1 at high level of factor X_3

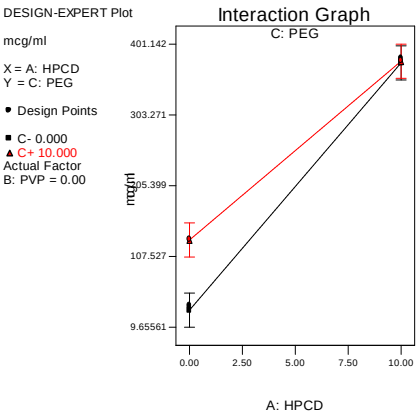

Fig. 10. Effect of Interaction factor X_1X_3

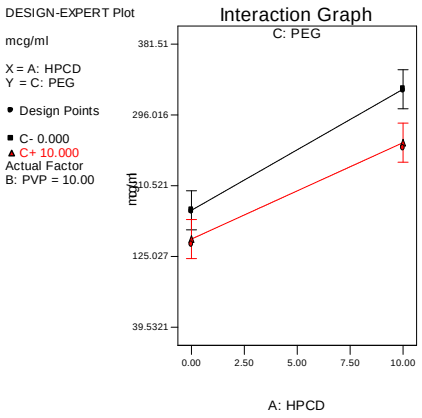

Fig. 11 Effect of Interaction factor X_1X_3

on responseY_1 at low level of factor X_2

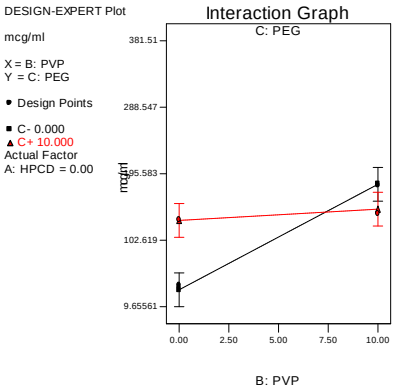

Fig. 12. Effect of Interaction factor X_2X_3

X_2X_3 on responseY_1 at low level of factor X_1

on responseY_1 at high level of factor X_2

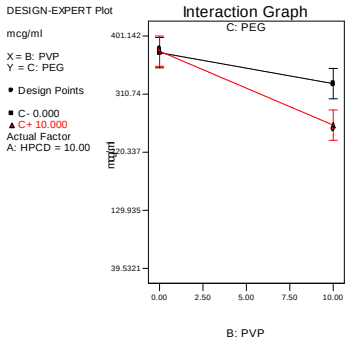

Fig. 13. Effect of Interaction factor

on responseY_1 at high level of factor X_1

Development of optimized formulae by using desirability function

Table No. 9: Constrain table

Independent variable	Goal	Lower Limit	Upper Limit
X_1	is in range	0	10
X_2	is in range	0	10
X_3	is in range	0	10
Y_1 mcg/ml	is in range	377.47	381.51

Table No. 10: Predicted solution

Independent variables			Response variable	Desirability
X_1	X_2	X_3	mcg/ml	
10	0	9.3	377.33	1

Table No. 11: Experimented data

Independent variables			Response variable
X_1	X_2	X_3	mcg/ml
10	0	9.3	373 ± 3.58

FTIR Spectral studies

The FTIR spectra of the inclusion complex, physical mixture, together with the pure drug are shown in Figure no. 09, 10 & 11 for comparison. Principal peaks of Glipizide were found at 3030, 1528, 1690, 1650, 1159, 1032, 900 cm^{-1}. As can be clearly seen from the spectra, the characteristic bands of Glipizide at 1528, 1690 and 1159 cm^{-1} were modified significantly in the physical mixture and inclusion complexes as a result of complex formation[16]. The C-H stretching peak due to aromatic ring at 3030 and the C-H aliphatic at 1528cm^{-1} are

completely absent in the complex. The C = O, C = C absorption band at 1689cm^{-1} shows a significant change in the complex when compared with pure drug. This indicates the formation of an inclusion complex (Fig .14 to 16).

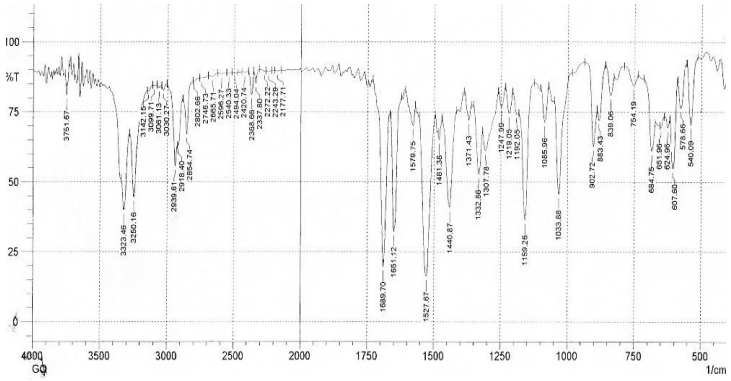

Fig. 14. FTIR of pure Glipizide

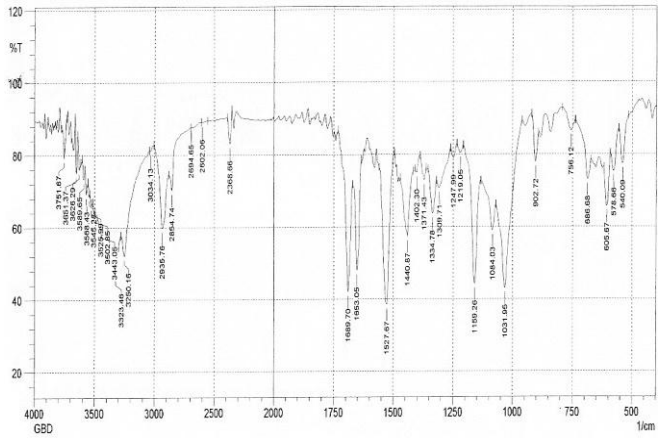

Fig.15. FTIR of Physical mixture containing Glipizide and HPβCD

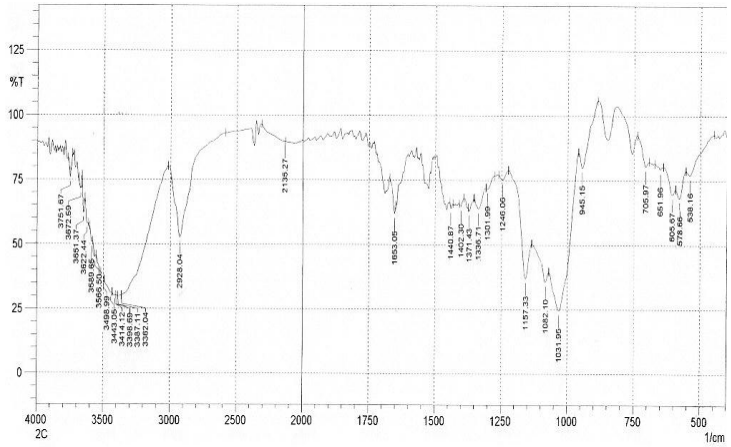

Fig. 16. FTIR of Inclusion complex containing (1:1) Glipizide and HPβCD

DSC studies

Differential Scanning Calorimetry enables the quantitative detection of all processes in which energy is required or produced (i.e. endothermic and exothermic phase transformations). The DSC thermogram of Glipizide is shown in Fig. 17 to 19. A single endothermic peak corresponding to its melting point was observed at 214.28^{0}C. The onset of melting point was observed at 210.44ºC. The DSC thermogram of Glipizide/HPβCD physical mixture shows a broad peak at 47.31^0 representing the HPβCD melting and an endothermic peak of glipizide at 205^{0}C with less intensity than that of the pure drug, which may be a result of decrease in crystallinity from physical mixing. The latter peak disappears completely in a complex state, thereby confirming the formation of a inclusion complex with HPßCD.

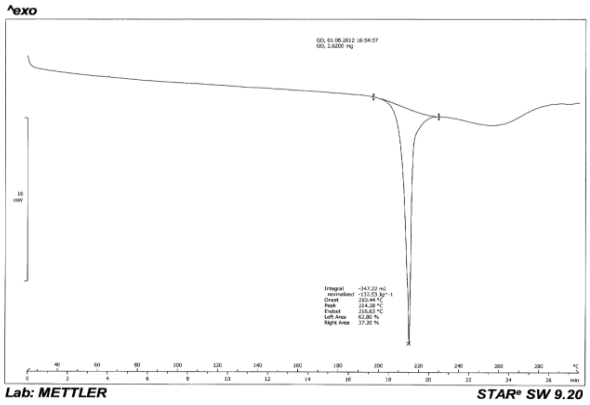

Fig.17. DSC of pure Glipizide

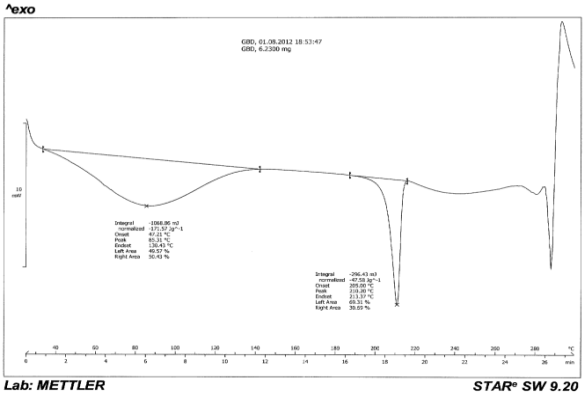

Fig.18. DSC of Physical mixture containing Glipizide and HPβCD

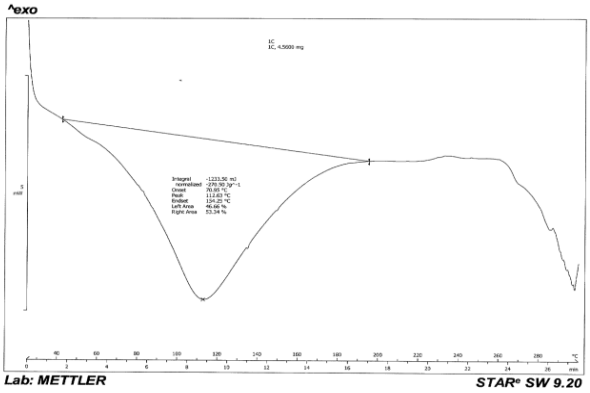

Fig. 19. DSC of Inclusion complex of (1:1) Glipizide with HPβCD

^{1}H NMR studies

^{1}H NMR studies of Glipizide and the inclusion complex are presented in figure no.15 & 16 for comparison. A singlet (2H) at δ 8.35 corresponds to heterocyclic hydrogens, a singlet (2H) at δ 9.26-9.27 for CONH, appearance of a multiplet (5H) at δ 7.26 -7.91 corresponds to aromatic ring hydrogen & heterocyclic hydrogen. A doublet (1H) corresponds to SO_2NH is present at δ 6.45-6.49. Methyl (3H) is present at δ 2.65 as a singlet. δ 3.01 –3.82 represent the ethylene group (triplet) and δ 1.1-1.86 represents 10H of cyclohexane. The inclusion complex possesses the SO_2NH and CONH groups. However the intensity of the aliphatic hydrogen is significantly reduced as observed from δ 3.5-3.8. The aromatic groups have completely disappeared. Thus, the formation of an inclusion complex of Glipizide with HPβCD is confirmed (Fig. 20 to 21).

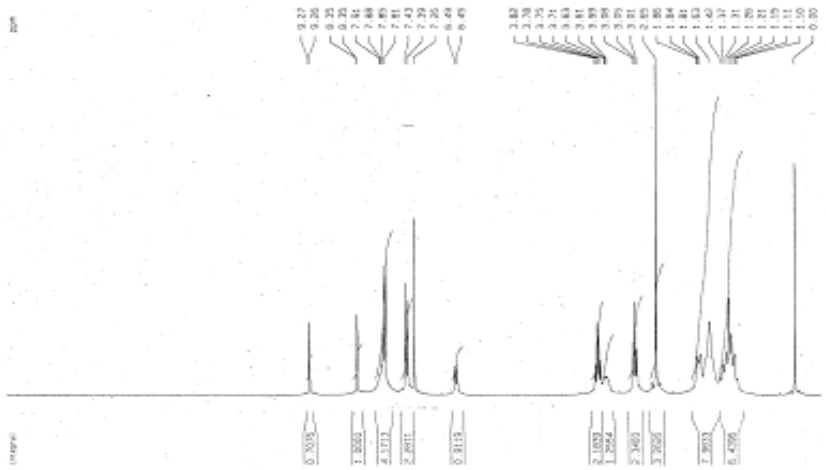

Fig. 20. ^{1}HNMR of Pure glipizide

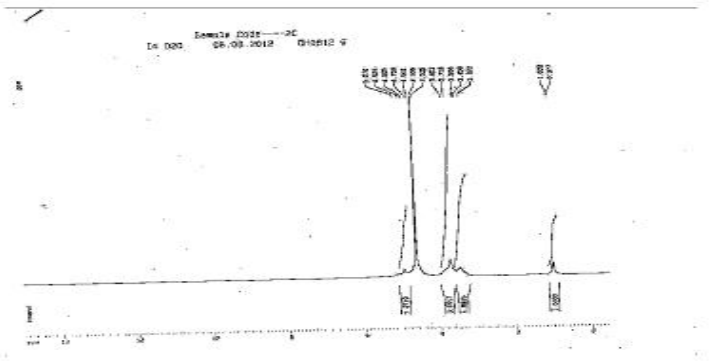

Fig.21. ^{1}HNMR of Inclusion complex (1:1) Glipizide with HPBCD

XRD studies

The diffractogram of pure drug reveals its highly crystalline nature, as indicated by the numerous distinctive peaks. The peak position (angle of diffraction) is an indication of crystal structure in which, peak height is the measure of sample crystallinity. A lack of numerous intense peaks signifies that the drug is distributed homogenously in an amorphous state within the inclusion complex without any interaction. The complete crystallinity of the drug is lost. This result supports the finding of FTIR spectra and NMR spectras (Fig. 22 to 23).

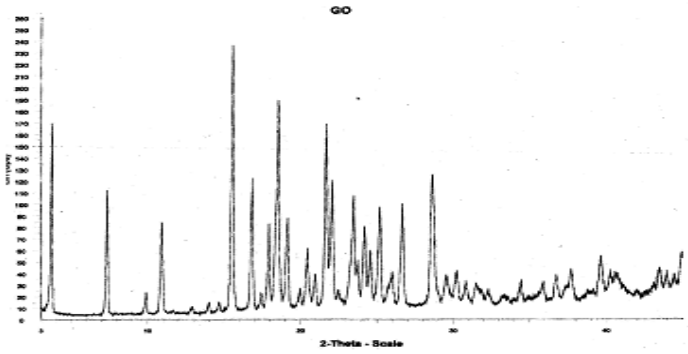

Fig. 22. XRD of Pure Glipizide

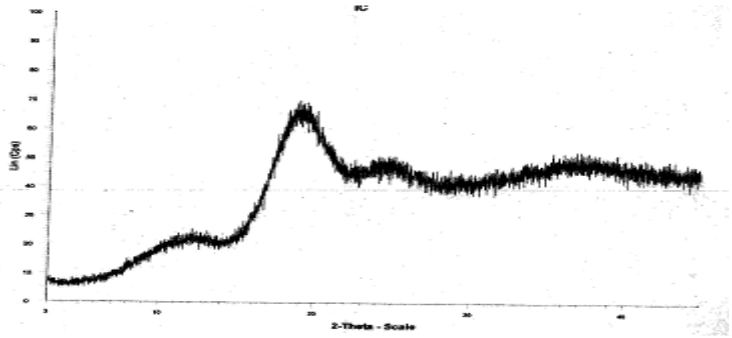

Fig. 23. XRD of Inclusion complex(1:1) Glipizide with HPßCD

Analysis of drug content

The drug content analysis from the inclusion complex analysed is shown in table 11. Using solvent evaporation technique for the complex formation 100 percent of the drug was entrapped within the cavity of the HPβCD carrier molecule. The driving force for the complex formation may be release of enthalpy rich water molecules from the cavity, hydrogen bonding, Vanderwaal's interaction, charge transfer interaction etc.

Dissolution studies

The dissolution studies of the pure drug along with the physical mixture and complex using capsule as a dosage form are shown in figure 19. The inclusion complex shows a significant increase in the drug dissolution compared to physical mixture and pure drug. It means that HPβCD is most useful for enhancing the solubility and thus drug release from its inclusion complex. Similar studies were repeated without using capsule as a dosage form. This was to understand the effect of capsule on the dissolution rate. Results reveal that there was a slight delay in the release of the drug, which may be due to delay in erosion of the capsule (Table No. 12 to 13 and Fig. 24 to 25).

Table No. 12: Dissolution studies using capsule as dosage form

Time	% Drug release		
Minutes	F1	F2	F3
2.5	5.10 ± 0.35	4.55 ± 0.37	11.25 ± 0.28
5.0	6.65 ± 0.477	7.61 ± 0.43	15.82 ± 0.20
10	8.29 ± 0.58	9.36 ± 0.62	31.48 ± 2.11
15	11.32 ± 0.65	16.61 ± 0.64	56.31 ± 1.78
20	13.73 ± 0.56	25.45 ± 0.81	93.46 ± 3.69
30	15.70 ± 0.55	44.53 ± 0.57	104.29 ± 2.08
45	18.45 ± 0.58	78.96 ± 0.68	---
60	21.41 ± 0.53	91.27 ± 0.67	---
90	23.32 ± 0.60	97.00 ± 1.01	---

*n=3 F1= pure drug, F2 = Physical mixture containing 1:1 molar ratio of drug and HPßCD, F3 = Inclusion complex

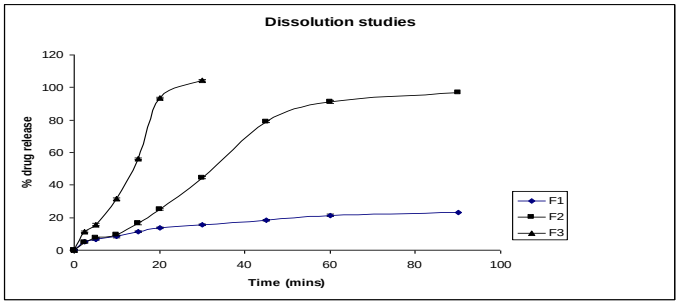

Fig. 24. Dissolution studies using capsule as dosage form

Table No. 13: Dissolution studies without using capsule as dosage form

Time	% Drug release		
Minutes	**F1**	**F2**	**F3**
2.5	10.57 ± 1.54	12.77 ± 0.58	69.46 ± 0.76
5.0	12.43 ± 2.47	30.87 ± 1.33	75.72 ± 0.44
10	15.80 ± 1.86	39.69 ± 1.12	80.68 ± 0.53
15	21.37 ± 0.50	53.45 ± 0.85	85.61 ± 0.59
20	23.44 ± 0.20	78.26 ± 1.69	89.97 ± 0.26
30	25.74 ± 0.99	94.91 ± 0.96	100.70 ± 0.62
45	31.49 ± 2.68	97.38 ± 0.70	- - -
60	33.21 ± 2.87	103.37 ± 1.68	- - -
90	37.44 ± 1.86		- - -

*n=3

F1= pure drug, F2 = Physical mixture containing 1:1 molar ratio of drug and HPßCD

F3 = Inclusion complex

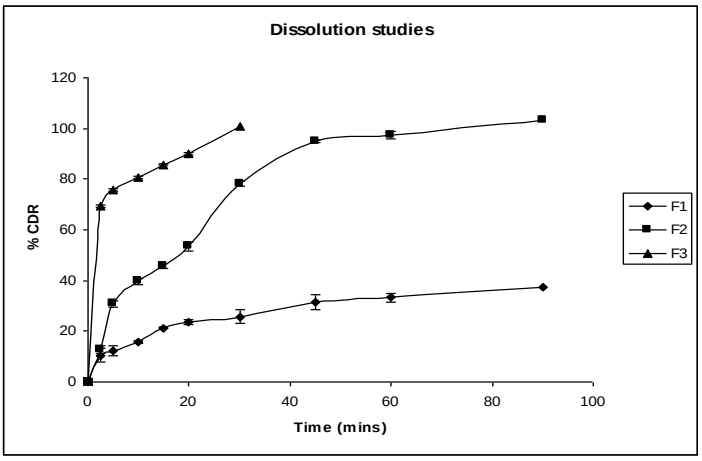

Fig. 25. Dissolution studies without using capsule as dosage form

CONCLUSION

The inclusion complexes of GZ with HPβ-CD using various methods such as physical mixture and solvent evaporation were prepared.

The phase solubility diagram of the HPßCD and PVP k30 solution showed a typical A_L-type, suggesting the formation of a soluble complex of 1: 1 molar ratio. PVP k30 showed a four fold increase in solubility whereas HPβCD exhibited a 9.5 fold increase. In case of PEG 10000 the graph exhibited a nonlinear increase in solubility of Glipizide. Hence PEG 10000 was not considered for further studies. PEG 6000 enhanced the solubility of the drug only by 3.8 folds. The factorial design of experiment was applied to know the interaction effect of the excipients on the solubility of Glipizide. ANOVA report reveals that the combination of the

excipients influenced the solubility of the drug. Using solvent evaporation technique for the complex formation 100 percent of the drug was entrapped within the cavity of the HPβCD carrier molecule. The inclusion complex showed a significant increase in the drug dissolution compared to physical mixture and pure drug. It meant that HPβCD was most useful for enhancing the solubility and thus drug release from its inclusion complex.

ACKNOWLEDGEMENT

I extend my sincere gratitude to Government college of Pharmacy, Bengaluru for providing the required facilities to carry out my work. I also acknowledge Microlabs, Bengaluru for providing the gift sample for the work.

Conflict of interest

None.

REFERENCE

1. Serajuddin AT. Solid dispersion of poorly water soluble drugs: Early promises, subsequent problems and recent breakthrough. J Pharm Sci, 1999; 88: 1058-66.
2. Lobenberg R, Amidon GL. Modern bioavailability bioequivalence and biopharmaceutics classification system: New scientific approaches to international regulatory standards. Eur J Pharm Biopharm, 2000; 50: 3-12.
3. Dollery SC. Therapeutic drugs. London: Churchill Livingstone, 1991.
4. Parfitt K. Martindale, the complete drug reference. London: Pharmaceutical Press, 1999.
5. Jamzad S, Fassihi R. Development of controlled release low dose class II drug-glipizide. Int J Pharm, 2006; 312: 24-32.
6. Sweetman SC. Martindale the complete drug reference. 33rd ed. London: Pharmaceutical press, 2002.
7. Pitha J, Milecki J, Fales H, Pannell L, Uekama K. Hydroxypropyl-β-cyclodextrin: preparation and characterization; effects on solubility of drugs. Int J Pharm. 1986; 29: 73–82.
8. Duchêne D. Cyclodextrins and their industrial uses. Editions de Santé Paris, 1987; SS. 447–460.
9. Baboota S, Agarwal SP. Rofecoxib complexation with β-cyclodextrin: influence on the anti-inflammatory and ulcerogenic activity. Die Pharmazie. 2003; 58: 73–74.

10. Fernandes CM, Teresa VM, Veiga FJ. Physicochemical characterization and *in vitro* dissolution behavior of nicardipine-cyclodextrins inclusion compounds. Eur J Pharm Sci. 2002; 15: 79–88.
11. Kamada M, Hirayama F, Udo K, Yano H, Arima H, Uekama K. Cyclodextrin conjugate-based controlled release system: repeated- and prolonged-releases of ketoprofen after oral administration in rats. J Control Release. 2002; 82: 407–416.
12. Mukne AP, Nagarsenker MS. Triamterene-β-cyclodextrin systems: preparation, characterization and *in- vivo* evaluation. AAPS PharmSci Tech. 2004; 5(1): E19.
13. Winters CS, York P, Timmins P. Solid state examination of a glipizide-β- cyclodextrin complex. Eur J Pharm Sci1997; 5: 209-15.
14. Al-Marzouqi AH, Shehatta I, Jobe B, Dowaidar A. Phase solubility and inclusion complex of itraconazole with beta-cyclodextrin using supercritical carbon dioxide. J Pharm Sci. 2006; 95(2): 292-304.
15. Higuchi T, Connors KA. Phase solubility techniques. Adv Anal Chem Instrum 1965; 4: 117- 212.
16. Kapil K, Manisha G, Pankaj N, Singh RP, Jain DA. Solubility enhancement of rifapentine by inclusion complex. Int J drug delivery 2011; 3(3): 413-420.