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Inclusion parameters of pioglitazone hydrochloride and glipizide with β-cyclodextrin and its methyl derivative: calorimetric and spectroscopic studies

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ABSTRACT

Solution calorimetry, a direct method to determine the thermodynamic parameters was used to investigate the inclusion phenomenon (stability constant, K; complexation enthalpy, ΔH^o) of two poorly soluble oral hypoglycemic agents, pioglitazone hydrochloride (pioglitazone HCl) and glipizide with β-cyclodextrin (β-CD) and its methyl derivative (methyl-β-CD). The inclusion complexes prepared by kneading were characterized in the solid state by differential scanning calorimetry and X-ray powder diffraction. The host-guest geometry and stoichiometry for the complexes indicated by proton NMR studies was confirmed by solution calorimetry. In case of pioglitazone HCl, two types of 1:1 complexes co-existing in solution have been revealed as pyridine ring as well as thiazoledione ring can be included in the cavity of cyclodextrin. The value of K_1 and K_2 determined using two class binding model utilizing non-linear least square regression was found to be 1940 M^{-1} and 1478 M^{-1} at pH 8 for complex with β-CD. Glipizide, a bigger molecule showed 1:2 complex with β-CD with a stability constant of 1880 M^{-1} at pH 8 with β-CD. The magnitude of equilibrium constants decreased at pH 4 which is attributed to the presence of ionized species for both the drug molecules at lower pH. The inclusion of the drugs in the cyclodextrin cavity is an exothermic process accompanied by small negative value of ΔG^o and positive value of ΔS^o. The magnitude of equilibrium constant increased with the use of methyl-β-CD for both drugs indicating their better complexing ability. This supports the enhanced solubility and dissolution rates observed with methyl-β-CD.

Keywords: Pioglitazone, glipizide, encapsulation, solubility, calorimetry, equilibrium constant.

INTRODUCTION

Encapsulation of the drugs by β-CD and its derivative is well documented in literature and need not be reviewed (Esclusa-Diaz et al., 1996; Loftsson and Brewster, 1996; Irie and Uekama 1997; Szejtli, 1998; Giron, 1999; Ugwu et al., 1999; Ficarra et al., 2000; Iglesias, 2006). Incorporation of the drug molecule in the hydrophilic cyclodextrin will advantageously affect many of its physico-chemical properties. More than 30 marketed formulations of cyclodextrins with different drugs are available (Loftsson et al., 2005). The inclusion complexes of cyclodextrins on oral administration decompose allowing free active ingredient to

be released and absorbed by GIT mucosa (Rekarsky and Yoshihisa, 1998).

The parameter which most characteristically defines stoichiometric interactions of the complex is the binding constant, which is of much significance and eventually leads to dosage adjustment of the drugs. Very high value of equilibrium constant (>5000 kg/mol) leads to very slow release of the drug from complex while very low value (<200 kg/mol) reduces the effect that inclusion complexation has on the bioavailability of the drug (Szejtli, 1998). It is thus a parameter for evaluating the affinity and stability of the complex formed (Chadha et al., 2004).

Solution calorimetry is well established and the most reliable method of reasonable precision for directly measuring the equilibrium constant and other thermodynamic parameters accompanying inclusion process (Tong et al., 1991; Phipps and Mackin, 2000; Illapakurthy et al., 2005; Rodrigues-Perez et al., 2006). Thermodynamic studies of the drug-cyclodextrin complexes show that balance between van der Waal's contact and hydrophobic effects is responsible for the overall stability of the complex (Rekarsky and Yoshihisa, 2002). The geometry of the inclusion can be established by NMR spectroscopy which is an indirect method to derive three-dimensional structure of the complex without the use of realistic models (Marques et al., 1990; Moyano et al., 1997; Schneider et al., 1998; Hartell et al., 2004; Sinha et al., 2005).

The present study describes the combined use of spectroscopy and solution calorimetry to study the encapsulation behaviour of pioglitazone HCl and glipizide. These two poorly soluble oral hypoglycaemic agents are used to lower blood glucose level in type-2 diabetes and needs a suitable carrier. The literature survey has revealed that various approaches, such as microspheres (Chowdhary and Rao, 2003, Chowdhary et al., 2004, Patel et al., 2005), nanoparticles (Cartagena, 2007.), and inclusion complexes (Gan et al., 2002; Adol et al., 2003; Ali and Upadhyay, 2008, Elbary et al., 2008) have been used to overcome their solubility related problem. However, the thermodynamic parameters describing exact stoichiometry, precise binding constants and enthalpy accompanying the encapsulation by cyclodextrins using solution calorimetry is lacking.

MATERIALS AND METHODS

Pioglitazone HCl and glipizide were provided by Ind-swift Ltd. and Wallace Pharmaceuticals, India, respectively and were used as such without further purification. The buffers used were disodium hydrogen phosphate buffers (pH 4, 6.8 and 8) and HCl-0.3 M KCl buffer pH 2 (Christian, 2004). Triple distilled water was used for the preparation of buffers.

Preparation of inclusion complexes

The complexes of pioglitazone HCl and glipizide were prepared with β-CD and methyl-β-CD by kneading method. The cyclodextrin was wetted with water in a glass mortar until a paste was obtained. The drug was then added in divided portions and the slurry was kneaded for about 90 min. An appropriate amount of water was added in order to maintain suitable consistency. Further, the product was dried under vacuum at 40 ^{o}C for 48 h and sieved through 150 µm mesh.

Characterization of inclusion complexes

Phase solubility studies

The solubility studies were performed by introducing excess amounts of pioglitazone HCl to HCl-0.3 M KCl buffer (pH 2.0) and glipizide to phosphate buffer (pH 6.8) in the presence of cyclodextrins. The suspensions were shaken in a water-bath shaker MSW-275 (Macroscientific works, Delhi) at 37 °C. After 48 hours, the supernatant was filtered, suitably diluted and the absorbances were taken at 269 nm for pioglitazone HCl and at 275 nm for glipizide on a UV-spectrophotometer (Perkin-Elmer Lamda 15, USA).

Differential Scanning Calorimetry (DSC)

DSC thermograms of pioglitazone HCl and glipizide and their inclusion complexes were obtained on DSC, Q20, TA Instruments-Waters LLC, USA. The calorimeter was calibrated for temperature and heat flow accuracy using the melting of pure indium (mp 156.6 °C and ΔH of 25.45 Jg^{-1}). The temperature range was from 50-300 °C with a heating rate of 10 °C per minute.

X-ray powder diffraction

Powder diffraction patterns of pioglitazone HCl and glipizide and their complexes were recorded on an X-ray diffractometer (XPERT-PRO, PANalytical, Netherlands, Holand) with Cu as tube anode. The diffractograms were recorded under the following conditions: voltage 40 kV, 35 mA, angular range 5, fixed divergence slit.

Proton Nuclear Magnetic Resonance (^1H NMR)

^1H NMR spectra were recorded on Brucker AC 300C (300 MHz) spectrophotometer using tetramethylsilane (TMS) as an internal standard and D_2O as solvent.

Solution calorimetry

Isoperibol solution calorimeter (ISC) (Calorimetry Science Corporation, UTAH, USA) Model 4300 was used to determine the heat of solution of the drugs and their complexes in phosphate buffers (P^H 4 and 8). The apparatus consists of constant temperature bath held at 37 °C (± 0.005 °C) and heater assembly. The drug was filled into batch adaptor of 0.9 ml, sealed on both sides with 'O' rings and was inserted into the reaction vessel (Dewar flask) containing buffer. The speed of glass stirrer was 100 revolutions min^{-1} and the system was allowed to equilibrate for 90 min. after which electrical calibration was performed which imparted a known heating signal to contents of the Dewar flask. The ampoule was shattered automatically by means of a plunger and temperature change noted. The performance of the system was checked using KCl, which has known enthalpy of solution and a good agreement (± 0.03 kJ mol^{-1}) was found with literature value (Balk and Benson, 1959).

Dissolution studies

The dissolution studies of inclusion complexes were performed using USP (12) apparatus equipped with paddle type tribune (USP, 2004) at 50 rpm in 900 ml of HCl-0.3 M KCl buffer pH2 in the case of pioglitazone HCl and at 75 rpm in 900 ml of phosphate buffer pH 6.8 in the case of glipizide at 37±0.5 °C. Each study was performed in duplicate.

RESULTS AND DISCUSSION

Phase solubility study

The solubility profiles of inclusion complexes of pioglitazone HCl show that solubility increases up to a certain concentration of cyclodextrins after which a plateau is attained resulting in Bs type Higuchi phase solubility diagram (Figure 1). This may be due to the increased tendency of drugs to form electrostatic bonds between themselves and decreases their ability to form complexes at higher concentration. The inclusion complexes of glipizide with both the cyclodextrins resulted in a A_p type Higuchi phase solubility diagram suggesting formation of a higher than one order stoichiometric ratio of glipzide:CD (Figure 2). The comparison to the literature revealed that at this point our results do not agree fully with the results reported by Gan et al. (2002) who suggested 1:1 stoichiometry up to 0.025 M concentration of cyclodextrin. The difference may be due to the higher concentration of cyclodextrin used in our study. The phase solubility studies also suggest that both drugs form significantly stronger inclusion complexes with methyl-β-CD than with β-CD.

DSC

The DSC profiles of pure components and binary systems in the melting region of the drugs are shown in Figures 3 and 4. The DSC curve of pioglitazone HCl and glipizide showed characteristic fusion peaks at 201.9 °C and 216.3 °C respectively. The thermograms of pioglitazone HCl (1:1) and glipizide (1:2)

complexes show that the peak corresponding to melting has broadened and shifted to a lower temperature. However, the magnitude of shift is more in case of methyl-β-CD complex suggesting the better interaction of both drugs with the methyl-derivative as compared to β-CD. Besides this, the melting enthalpy corresponding to the drug decreases from 113.56 Jg^{-1} to 34.89 Jg^{-1} in β-CD and 8.66 Jg^{-1} in methyl-β-CD complex of pioglitazone HCl. In case of glipizide the melting enthalpy changes from 290.13 Jg^{-1} to 43.03 Jg^{-1} in β-CD and 12.80 Jg^{-1} in methyl-β-CD complex. These are in agreement with results reported by Gan et al (Gan et al, 2002).

XRPD

The XRPD pattern of pioglitazone HCl and glipizide showed intense and sharp peaks indicating their crystalline nature (Figures 5 and 6). The characteristic peaks for pioglitazone HCl appeared at 2θ values of 12.75, 18.78, 20.03, 20.63, 22.71 and 26.18 while glpizide showed major peaks at 2θ values of 17.87, 22.46, 23.37, 28.29 and 29.53. However, a reduced number of signals, with remarkably low intensity of peaks were observed in diffractograms of β-CD complexes of both drugs indicating their inclusion into the cyclodextrin cavity. Interestingly, the inclusion complex of pioglitazone HCl with methyl-β-CD showed a complete amorphous hallow while in the case of glipizide complex with methyl-β-CD the peaks were almost smoothened and were of diminished intensity suggesting complete complexation.

NMR

NMR studies have revealed changes in the chemical shifts of protons attached to pyridine and thiazoledione rings of pioglitazone HCl (Figure 7a) indicating their potential to enter the cyclodextrin cavity (Table 1). Looking at the length of pioglitazone HCl (~ 17Å), it is unlikely for one molecule to be simultaneously introduced into two molecules of β-CD due to repulsive forces. Thus, two types of inclusion complexes with 1:1 stoichiometry are expected which can co-exist. This observation is in partial agreement with the results reported by Ali and Upadhyay who have mentioned the NMR results of complexation of pioglitazone HCl with β-CD. The authors have mentioned a 1:1 stoichiometry and suggested that pioglitazone HCl can enter either through pyridine or thiazolidione rings. However, according to them the pyridine ring protrudes from the other end and the only part which interacts with the cavity is the aromatic moiety (Ali and Upadhyay, 2008). But according to our study, the protons H-c, H-d and H-e of the pyridine ring and protons H-m and H-n of thiazoledione ring show significant downfield shifts indicating their interaction with the oxygen atoms of the CD ring. Whereas, both H-a and H-b protons of the ethyl group and H-l proton observed an upfield shift which may be due to interaction with hydrogen atoms inside the cavity. In our study, the change in the chemical shift is less in aromatic protons and this small shift maybe due to the steric perturbation and dislocation of the charges of the aromatic ring outside the aromatic cavity as a result of inclusion (Veiga et al., 2001). Even Ali and Upadhyay have reported more downfield shift in pyridine ring as compared to phenyl ring. Thus the proposed geometry of the complex differs from that proposed by Ali and Upadhyay though the stochiometry is same. The most probable structure of inclusion complex according to our study was drawn using ligand Fit module of Accerlys (Discovery studio 2.0 version) and is given in Figure 8a. In glipizide (Figure 7b), pyrazine ring proton (H-q), cyclohexyl protons (H-a, H-b, H-e and H-f) as well as amide proton (H-g) show downfield shifts suggesting that their penetration is deep into the cavity (Table 1). Glipizide is a big molecule (length ~ 22-24 Å) which suggests that there is a much likelihood for one molecule to be simultaneously introduced into two molecules of cyclodextrins in solution (Figure 8b). There is a possibility that the rings facing each other of the two cyclodextrin molecules may interact attractively in a way

similar to substrate promoted ligand dimerization. As disucussed above, H-c, H-d of cyclohexyl and H-p of pyrazine ring are close to the hydrogen atom of the cyclodextrin molecule and are closer to oxygen atom (Schneider et al., 1998).

Calorimetric study

The interaction between the drug and cyclodextrins was determined calorimetrically by determining the enthalpy of solution of drugs in pure buffers (Table 2) and in the buffered aqueous solutions of cyclodextrins over the wide range of concentrations (Table 3). The enthalpy of solution of pioglitazone HCl has been found to be smaller in magnitude in presence of β-cyclodextrin than enthalpy of solution in pure buffer at pH 4. The same trend was followed for pioglitazone HCl with methyl derivative and glipizide with both the cyclodextrins at pH 4 and 8. The complete results for pioglitazone HCl with β-CD at pH4 are tabulated here (Table 3).

The thermodynamic parameters associated with the inclusion phenomenon are determined by calculating the enthalpy of interaction per mole of drug and cyclodextrin.

$$\Delta_{sol}H_{int(M)} = \frac{(\Delta_{sol}H_{(CD)} - \Delta_{sol}H)/v}{M_{drug} + M_{CD}}$$

$$= \frac{\Delta_{sol}H_{int}(l)}{M_{drug} + M_{CD}} = \frac{\Delta_{sol}H_{(M)\,(CD)} - \Delta_{sol}H_{(M)}}{1+(x_2/x_1)} \quad ...(1)$$

Where, $\Delta_{sol}H$, $\Delta H_{sol(CD)}$= enthalpy of solution of drug in buffer and in buffered aqueous solution of cyclodextrins

$\Delta_{sol}H_{(M)}$= molar enthalpy of solution of drug in buffer

v (l) = volume of reaction vessel in litre

$\Delta_{sol}H_{int}(l)$ = enthalpy of interaction between drug and cyclodextrins per litre of solution

M_{CD} and M_{drug} = concentration of cyclodextrin and drug

$\Delta_{sol}Hint_{(M)}$ = enthalpy of interaction per mole of drug and cyclodextrin

$\Delta_{sol}H_{(M)\,(CD)}$ = molar enthalpy of solution of drug in buffered aqueous solution of cyclodextrins

x_1 and x_2 are the apparent mole fractions of the drug and cyclodextrin ignoring the concentration of buffers.

The stoichiometry was determined by plotting molar enthalpy of interaction ($\Delta_{sol}H_{int(M)}$) versus mole fraction of drug (x_2) for both the drugs with β-CD and its methyl derivative. The plots between the $\Delta_{sol}H_{int(M)}$ and x_2 for pioglitazone HCl with β-CD and its methyl derivative (Figure 9) show minima at x_2 = 0.5 indicating 1:1 stoichiometry. As discussed in NMR results both pyridine and thiazoledione are favourable as inclusion groups in void volume of cyclodextrin cavity, two types of 1:1 complexes co-existing in solution are expected. However, in case of glipizide the plot of ($\Delta_{sol}H_{int(M)}$) vs x_2 is asymmetric bell-shaped curve indicating a non 1:1 stoichiometry. The position of minima (x_2 = 0.67) indicates it to be a 1:2 glipizide:CD complex (Figure 10). NMR studies also indicate the existence of 1:2 glipizide:CD complex based on the magnitude of the chemical shifts observed and further supported by the large size of the molecule.

After the stoichiometry was established the thermodynamic constants were calculated assuming the following equilibria

CD + drug ↔ CD : drug ..……(2)

The interaction enthalpy is proportional to the amount of drug:CD complex formed

$$\Delta_{sol}H_{int} = \Delta H^o \times CD{:}drug \times v \quad .....(3)$$

$$\Delta_{sol}H_{int}(l) = \frac{\Delta_{sol}H_{int}}{V} = \Delta H^o \times CD{:}drug \quad ...(4)$$

Where, ΔH^o = inclusion enthalpy per mole of the drug

In case of pioglitazone HCl a two class binding model was utilized to determine the concentration of pioglitazone HCl:CD complex.

$$M_{CD}{:}drug = M_{CD}(T) - M_{CD}(f) \quad .....(5)$$

$M_{CD}(T)$ is the total concentration of cyclodextrin

$M_{CD}(f)$ is concentration of free cyclodextrin

$$\frac{M_{CD}(T)-M_{CD}(f)}{M_D} = \frac{n_1K_1\,M_{CD}(f)}{1+K_1\,M_{CD}(f)} + \frac{n_2\,K_2\,M_{CD}}{1+K_2\,M_{CD}(f)} \quad ...(6)$$

which gives

$M_{CD}(f)^3+AM_{CD}(f)^2+BM_{CD}(f)+C=0$ ...(7)

where

$A=M_D(n_1+n_2)+(1/K_1+1/K_2)-M_{CD}(T)$...(8)

$B=M_D(n_1/K_1+n_2/K_2)-$

$M_{CD}(T)(1/K_1+1/K_2)+1/K_1K_2$ ...(9)

$C=-M_{CD}(T)/K_1K_2$ ...(10)

K_1 is the equilibrium constant for one type of complex where pyridine ring enters the cyclodextrin cavity

K_2 is the second type of complex where thiazole dione ring enters the cavity.

$n_1 = n_2 = 1$, in the présent study

In case of glipizide, the equilibrium constants have been calculated assuming the following equilibria

2CD+drug↔CD:drug:CD ...(11)

and the concentration of drug cyclodextrin complex can be written as

$$\mathbf{M_{CD}:drug=[(M_{CD}+M_{drug}+1/K)-\sqrt{(M_{CD}+M_{drug}+1/K)2-4\times2M_{drug}M_{CD}}]/2} \quad ..(12)$$

The results for glipizide have shown to be fitting well in the above equation.

The best fitted values of equilibrium constant (K) and inclusion enthalpy (ΔH^o) for pioglitazone HCl and glipizide were calculated from equation 6 and 12 respectively using non-linear least square regression method prepared in our laboratory (Tables 4 and 5). The magnitude of calorimetrically determined equilibrium constants (K_1 and K_2) for pioglitazone with β-CD and its derivatives is between 1478 to 2960 M^{-1} which lies between the optimum values required for complexation, reflecting a favourable position of the guest molecule inside the cyclodextrin cavity. Similarly, the value of equilibrium constant (K) for glipizide with all the three cyclodextrins lies between 1200 to 2820 M^{-1} which is most suitable for complexation. Although reports are available on the complexes of cyclodextrins with these two drugs but no literature is available for direct comparison of equilibrium constant as well as for equilibrium constant for one type of complex (K) of these complexes.

Table 4 shows that in case of pioglitazone HCl both K_1 and K_2 are found to be affected by the P^H of solution. Pyridine and thiazoledione ring of pioglitazone HCl are ionizable with ionization constant pK_1 = 5.9 and pK_2= 6.4. At pH 4 nitrogen of pyridine as well as thiazole ring are protonated which decreases the possibility of guest molecule to penetrate the cyclodextrin cavity. Simlarly, in glipizide (Table 5) the higher value of equilibrium constant K at pH 8 is attributed to unprotonated form of the drug. Besides this, the complexation ability increases in methyl-β-CD for both the drugs as methylation enlarges the cavity of cyclodextrin making the environment around it more hydrophobic allowing for increased adaptability of cyclodextrin towards the guest through enhanced flexibility.

The values of free energy of inclusion (ΔG^o) and entropy of inclusion (ΔS^o) for pioglitazone HCl and glipizide have been calculated and are given in Tables 4 and 5. This suggests that the inclusion phenomenon is both enthalpically ($\Delta H^o<0$) and entropically ($\Delta S^o>0$) driven. As a result of the negative enthalpy change, a decrease in the standard free energy is observed. When a solute is transferred from a polar to non-polar bulk phase, release of water molecule results in an entropy increase associated with moderate negative value of enthalpy. It is clear from Tables 4 and 5 that at pH 4 there is not much difference in entropy of inclusion (ΔS^o) with β-CD and methyl-β-CD complex, however, the ΔS^o is less positive for the complexes with methyl-β-CD at pH 8. This behaviour appears to be reasonable since facilitation of guest into more hydrophobic cavity induces much stronger van der Waal interactions giving higher negative ΔH^o but greatly reduces the guest freedom giving less positive entropy.

Dissolution study

The dissolution profiles of inclusion complexes of both drugs show that the rate of dissolution is faster for inclusion complexes as compared to drugs alone (Figure 11). The complexes dissolve more rapidly than the pure drug as they form a hydrodynamic layer surrounding the particles of drug, resulting in the *in situ* inclusion process, giving an

increased amount of drug dissolved. Figure 11 shows that the dissolution rate of the complexes decrease in the order M-β-CD>HP-β-CD >β-CD and these results are very well co related with the magnitude of equilibrium constant and other thermodynamic parameters determined calorimetrically. The numerical value of equilibrium constants indicate that M-β-CD has best complexation ability of all the three cyclodextrins (Tables 4 and 5) which is reflected in the best dissolution profile of inclusion complexes of both the drugs with M-β-CD. Thus, the results meet the proposed objectives of the study.

The inclusion complexes of these hypoglycaemic agents with M-β-CD have the potential to be further explored by preparing a formulation containing pioglitazone/glipizide and M-β-CD. This formulation can be commercialized, once its improved bioavailability is established in *in vivo* studies.

Table 1: Changes in chemical shifts (ppm) for protons of pioglitazone HCl and glipizide upon complexation with β-cyclodextrin and methyl-β-cyclodextrin.

Pioglitazone HCl	**Pioglitazone HCl + β-CD Δ δ= $\delta_{complex}$- $\delta_{pioglitazone\ HCl}$ (ppm)**	**Pioglitazone HCl + methyl-β-CD Δ δ= $\delta_{complex}$- $\delta_{pioglitazone\ HCl}$ (ppm)**	**Glipizide**	**Glipizide + β-CD Δ δ= $\delta_{complex}$- $\delta_{glipizide}$ (ppm)**	**Glipizide + methyl-β-CD Δ δ $\delta_{complex}$- $\delta_{glipizide}$ (ppm)**
H-a	-0.0119	-0.0005	H-a	+0.0074	+0.0102
H-b	-0.0288	-0.0045	H-b	+0.0110	+0.0170
H-c	+0.0804	+0.0032	H-c	-0.0004	-0.0140
H-d	+0.0628	+0.036	H-d	-0.0049	-0.5294
H-h	+ 0.0093	+0.0001	H-e	+0.0027	+0.0207
H-i	+0.0076	+0.0069	H-f	+0.0046	+0.0335
H-j	+0.0013	+0.0026	H-g	+0.0018	+0.013
H-k	+0.0094	+0.0004	H-h	+0.1068	-
H-e	+0.0565	+0.0242	H-o	+0.1873	+0.1467
H-l	-0.0432	-0.2076	H-p	-0.0307	-0.0277
H-m	+0.0989	+0.1589	H-q	+0.0270	+0.0223
H-n	+0.0826	-			

ppm = parts per million; Δ δ = change in chemical shift; $\delta_{complex}$ = chemical shift of complex formed by pioglitazone and glipizide with cyclodextrins; $\delta_{pioglitazone\ HCl}$ = chemical shifts of pioglitazone; $\delta_{glipizide}$ = chemical shift of glipizide

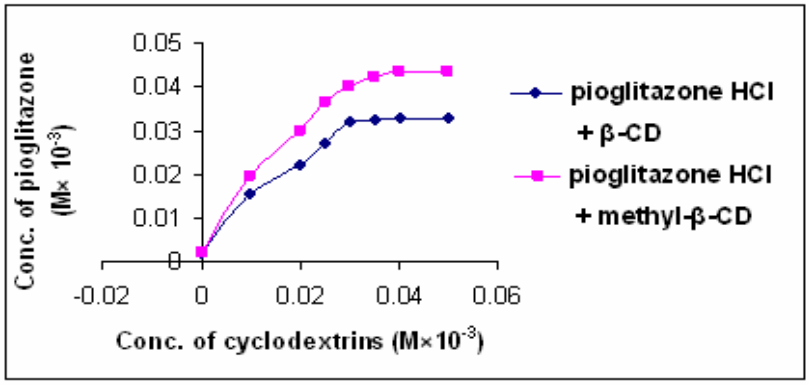


Figure 1: Solubility diagram of pioglitazone HCl with β-CD and methyl-β-CD in HCl-0.3 M KCl buffer pH2 at 37 ºC.

Table 2: Enthalpy of solution of pioglitazone HCl and glipizide in pure buffered aqueous solutions.

	$\Delta_{sol}H$ (kJ mol^{-1})	
	pH 4	**pH8**
Pioglitazone HCl	**31.25**	**52.00**
Glipizide	**75.87**	**102.47**

$\Delta_{sol}H$ = enthalpy of solution of drug in buffer.

Table 3: Thermodynamic parameters of inclusion complexes of pioglitazone HCl with β-cyclodextrin at pH 4.

x_2	$M_{pio} \times 10^4$ (a)	$M_{\beta\text{-CD}} \times 10^4$ (b)	$\Delta_{sol}H_{(CD)} \times 10^2$ (J)	$\Delta_{sol}H_{int(l)}$ (J/l)	$\Delta_{sol}H_{int(M)}$ (kJ/mol)
0.8617	1.83	11.40	11.72	-1.03	-0.78
0.8488	2.03	11.40	13.02	-1.14	-0.846
0.8178	2.54	11.40	16.34	-14.01	-10.05
0.7813	3.19	11.40	20.58	-1.73	-1.19
0.7519	3.76	11.40	24.33	-2.02	-1.33
0.7369	4.07	11.40	26.38	-2.16	-1.40
0.5572	4.53	11.40	29.44	-0.054	-0.12
0.6913	5.09	11.40	33.17	-2.64	-1.59
0.6789	5.39	11.40	35.17	-2.77	-1.65
0.6647	5.75	11.40	37.59	-2.93	-1.71
0.6466	6.23	11.40	40.83	-3.13	-1.78
0.5772	8.35	11.40	55.28	-3.98	-2.01
0.5282	10.18	11.40	67.97	-4.63	-2.14
0.4768	12.51	11.40	96.51	-5.33	-2.23

M_{pio}= concentration of pioglitazone; $M_{\beta\text{-CD}}$ = concentration of β-cyclodextrin; $\Delta H_{sol(CD)}$= enthalpy of solution of drug in buffered aqueous solution of cyclodextrins; $\Delta_{sol}H_{int}(l)$ = enthalpy of interaction between drug and cyclodextrins per litre of solution; $\Delta_{sol}Hint_{(M)}$ = enthalpy of interaction per mole of drug and cyclodextrin

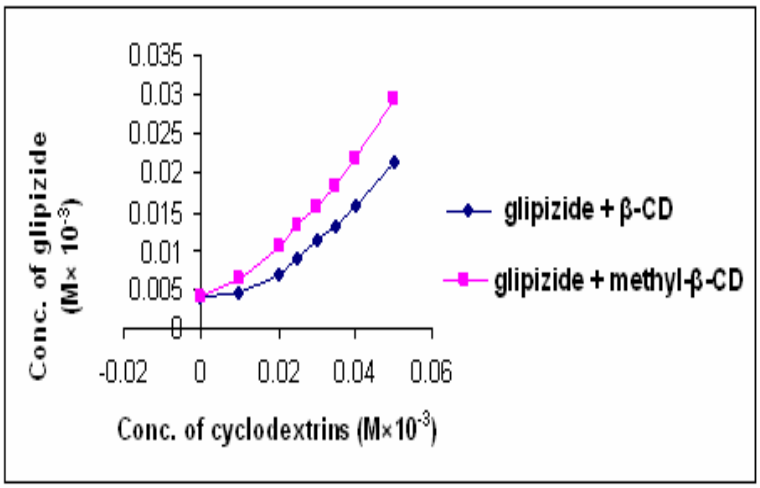


Figure 2: Solubility diagram of glipizide with β-CD and methyl-β-CD in phosphate buffer pH 6.8 at 37 ºC.

Table 4: Thermodynamic parameters of pioglitazone HCl with β-cyclodextrin and methyl-β-cyclodextrin at pH 4 and pH 8.

System	K_1 (M^{-1})	K_2 (M^{-1})	ΔH^o ($kJmol^{-1}$)	ΔG^o_1 ($kJmol^{-1}$)	ΔG^o_2 ($kJmol^{-1}$)	ΔS^o_1 ($Jmol^{-1}K^{-1}$)	ΔS^o_2 ($Jmol^{-1}K^{-1}$)
Pioglitazone HCl+β-CD (pH4)	1478	1130	-9.80	-18.81	-18.12	29.08	26.84
Pioglitazone HCl+methyl-β-CD (pH4)	2160	1570	-10.60	-19.79	-18.97	29.65	26.99
Pioglitazone HCl+β-CD (pH8)	1940	1240	-11.60	-19.51	-18.36	25.53	21.85
Pioglitazone HCl+methyl-β-CD (pH8)	2960	1800	-13.20	-20.57	-19.32	23.77	19.75

K_1 = equilibrium constant for complex of pioglitazone where pyridine ring enters the cyclodextrin cavity; K_2 = equilibrium constant for complex of pioglitazone where thiazole dione ring enters the cavity; ΔH^o = molar enthalpy of inclusion of pioglitazone with cyclodextrin cavity; ΔG^o_1 = free energy of inclusion of pioglitazone where pyridine ring enters the cyclodextrin cavity; ΔG^o_2 = free energy of inclusion of pioglitazone where thiazole dione ring enters the cavity ; ΔS^o_1 = entropy of inclusion of pioglitazone where pyridine ring enters the cyclodextrin cavity; ΔS^o_2 = entropy of inclusion of pioglitazone where thiazole dione ring enters the cavity

Table 5: Thermodynamic parameters of inclusion complexes of glipizide with β-cyclodextrin and methyl- β-cyclodextrin at pH 4 and pH 8.

System	K (M^{-1})	ΔH^o (kJ mol^{-1})	ΔG^o (kJ mol^{-1})	ΔS^o (J $mol^{-1}K^{-1}$)
Glipizide+β-CD (pH4)	1200	-9.60	-18.27	27.96
Glipizide+methyl-β-CD (pH4)	1650	-10.20	-19.09	28.68
Glipizide+β-CD (pH8)	1880	-11.20	-19.43	26.55
Glipizide+methyl-β-CD (pH8)	2820	-13.20	-20.48	23.48

K = equilibrium constant for complex of glipizide; ΔH^o = molar enthalpy of inclusion of glipizide with cyclodextrin cavity; ΔG^o = free energy of inclusion of glipizide with cyclodextrin cavity; ΔS^o = entropy of inclusion of glipizide with cyclodextrin cavity

Conclusion

In conclusion, the present study demonstrates the potential of calorimetric technique for the determination of thermodynamic parameters associated with encapsulation. Phase solubility, NMR spectroscopy and solution calorimetry studies revealed two types of 1:1 complexes of pioglitazone HCl with β-CD and methyl-β-CD whereas glipizide was found to form 1:2 complex with both the cyclodextrins. The complexation ability increases with the methyl derivative as revealed by the higher magnitude of equilibrium constant of both pioglitazone HCl and glipizide with methyl-β-CD. This was further reflected in the better dissolution profile of methylated complexes. Thus, the encapsulation of pioglitazone and glipizide with β-CD and its methyl and hydroxypropyl derivatives is a useful strategy to improve the solubility and dissolution of these poorly soluble therapeutic agents.

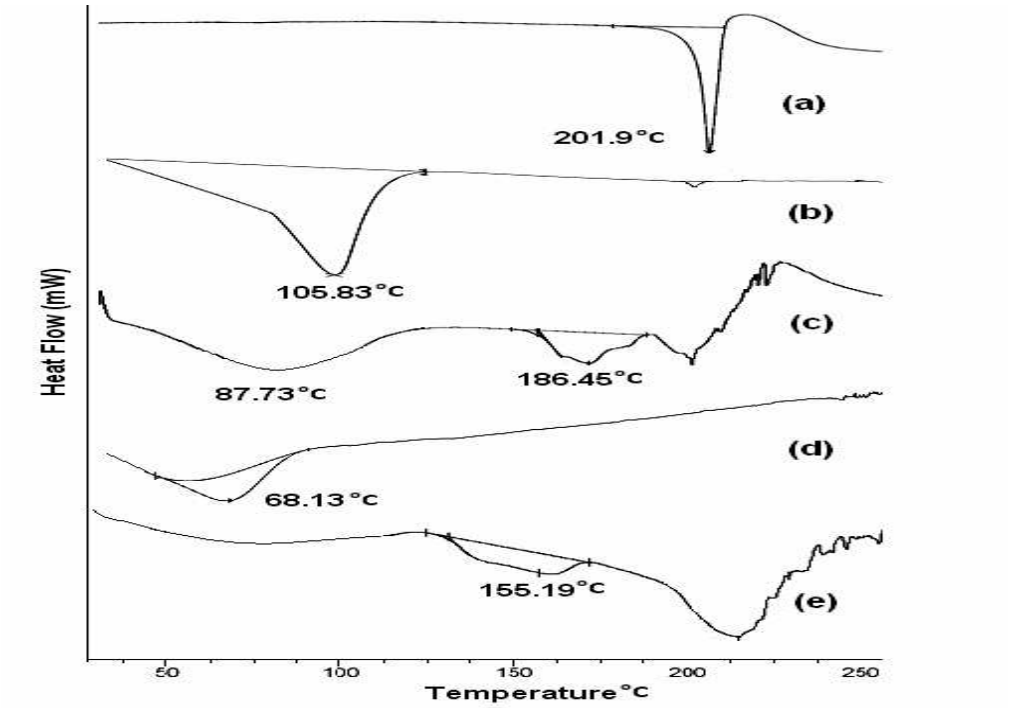


Figure 3: DSC thermograms of (a) pioglitazone HCl (b) β-CD (c) pioglitazone HCl-β-CD (d) M-β-CD (e) pioglitazone HCl-M-β-CD complex.

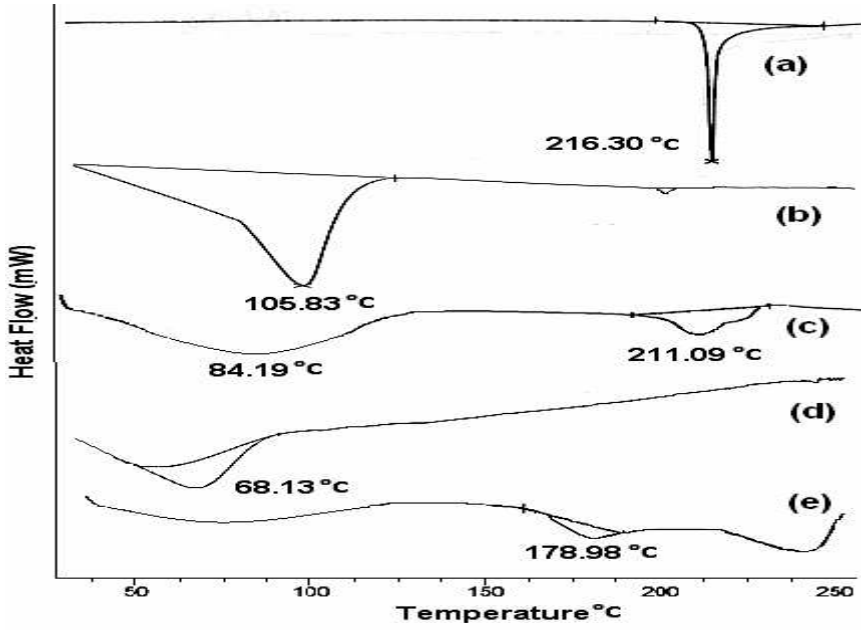


Figure 4: DSC thermograms of (a) glipizide (b) β-CD (c) glipizide-β-CD (d) M-β-CD (e) glipizide M-β-CD complex.

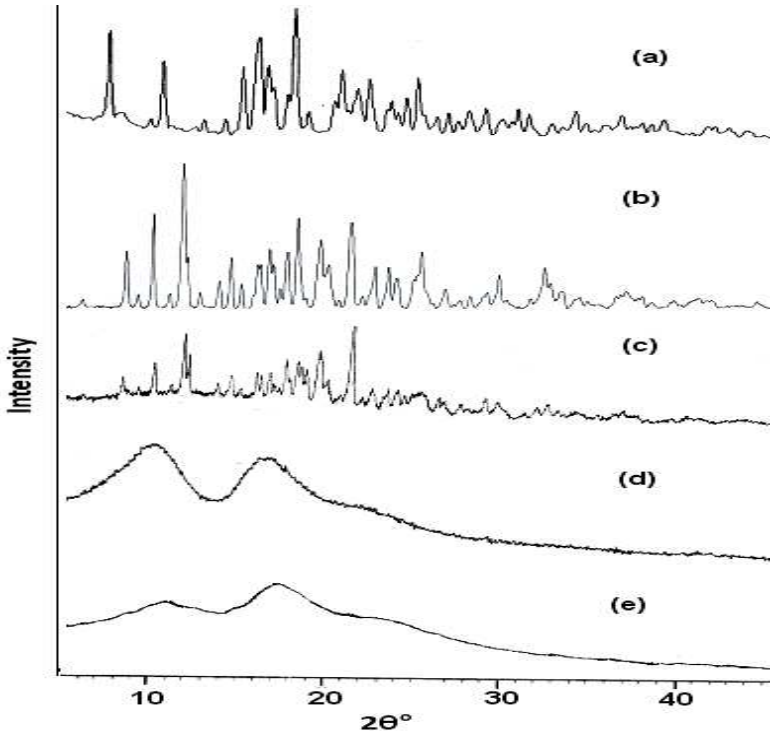


Figure 5: XRPD patterns of (a) pioglitazone HCl (b) β-CD (c) pioglitazone HCl-β-CD (d) M-β-CD (e) pioglitazone HCl-M-β-CD complex.

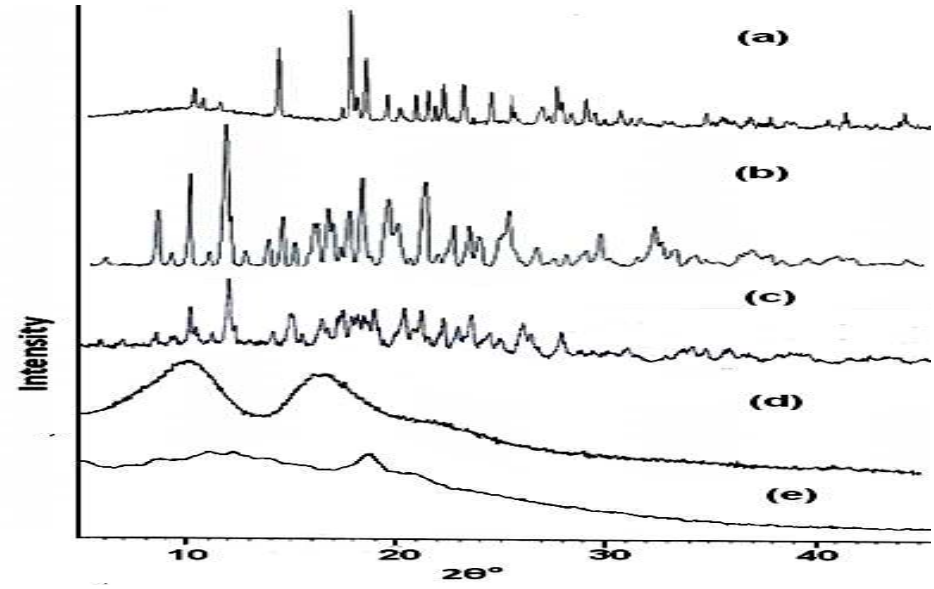


Figure 6: XRPD patterns of (a) glipizide (b) β-CD (c) glipizide-β-CD (d) M-β-CD (e) glipizide-M β-CD complex.

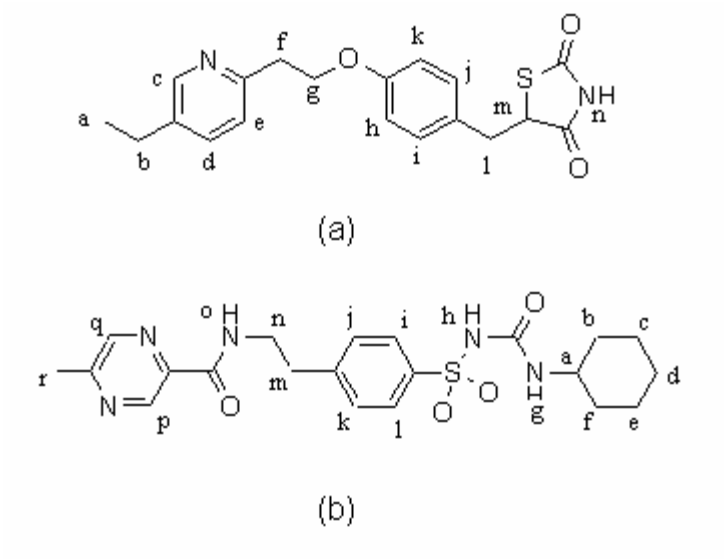


Figure 7: (a) Chemical structure of (a) pioglitazone HCl (b) glipizide.

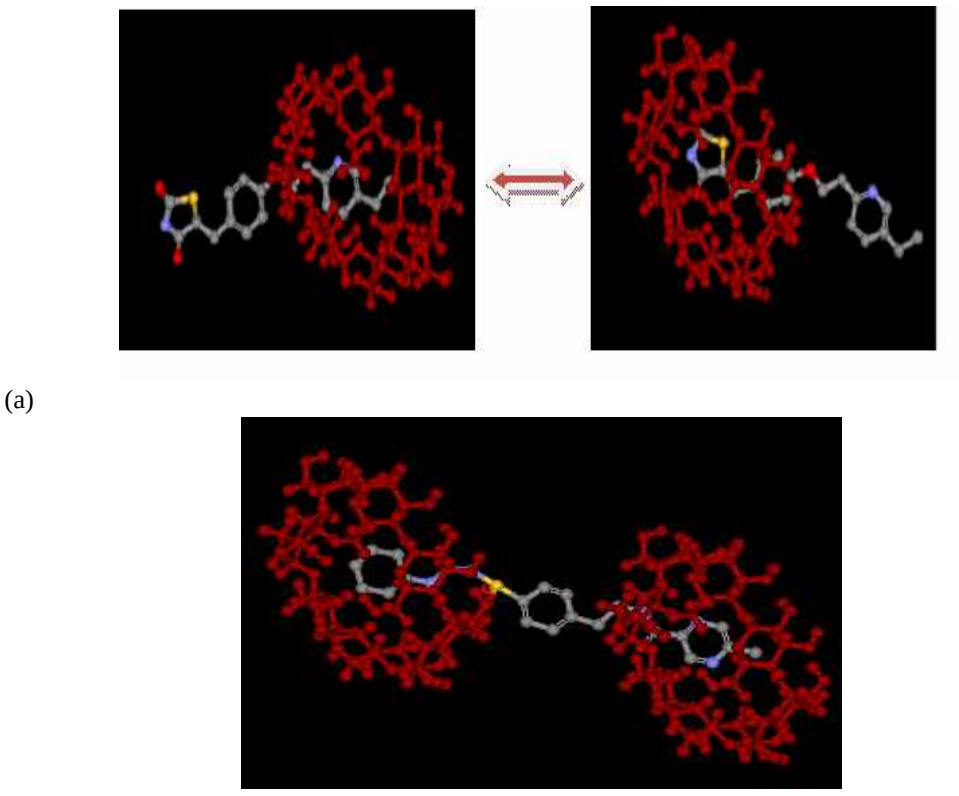
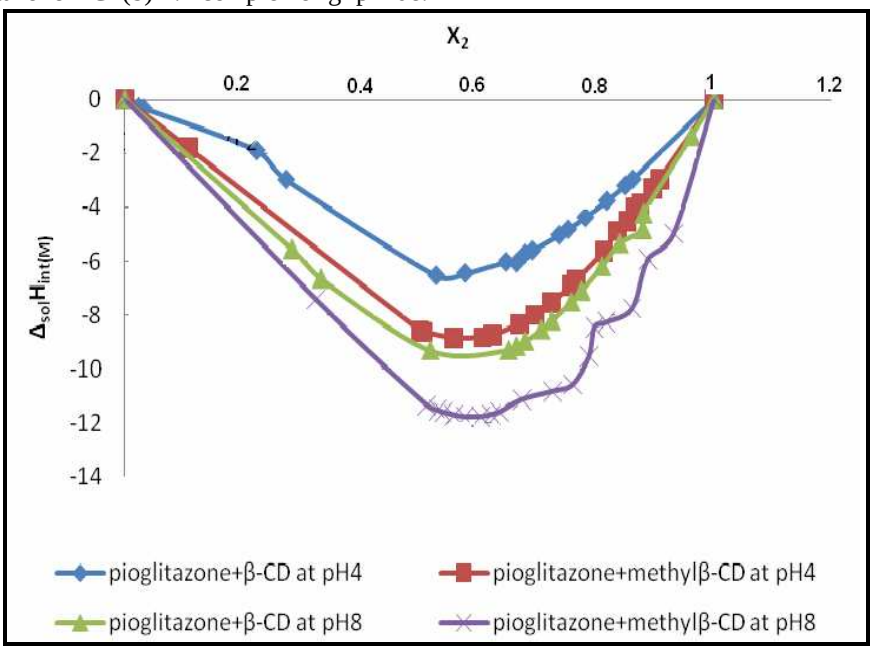


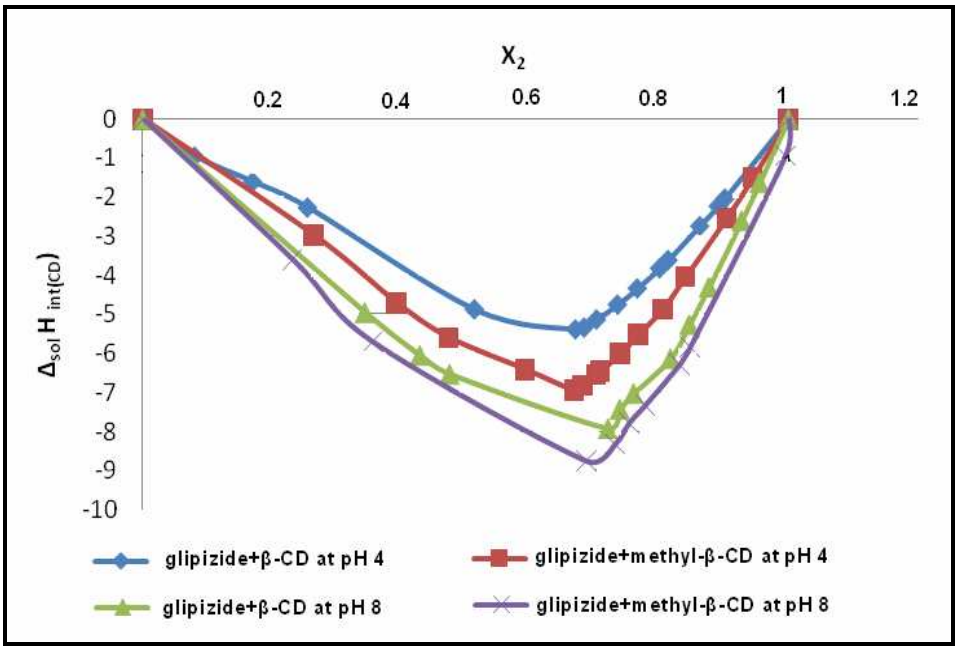
Figure 8: Molecular representation of inclusion complex of (a) two types of 1:1 complexes of

pioglitazone HCl (b) 1:2 complex of glipizide.



$\Delta_{sol}H_{(M)}$= molar enthalpy of solution of drug in buffer

Figure 9: Plot of $\Delta_{sol}H_{int(M)}$ (kJ/mole) vs mole fraction of pioglitazone HCl with (a) β-CD at pH 4 (b) methyl-β-CD at pH 4 (c) β-CD at pH 8 (d) methyl-β-CD at pH 8.



$\Delta_{sol}H_{(M)}$= molar enthalpy of solution of drug in buffer

Figure 10: Plot of $\Delta_{sol}H_{int(M)}$ (kJ/mole) vs mole fraction of glipizide with (a) β-CD at pH 4 (b) methyl-β-CD at pH 4 (c) β-CD at pH 7 (d) methyl-β-CD at pH 7.

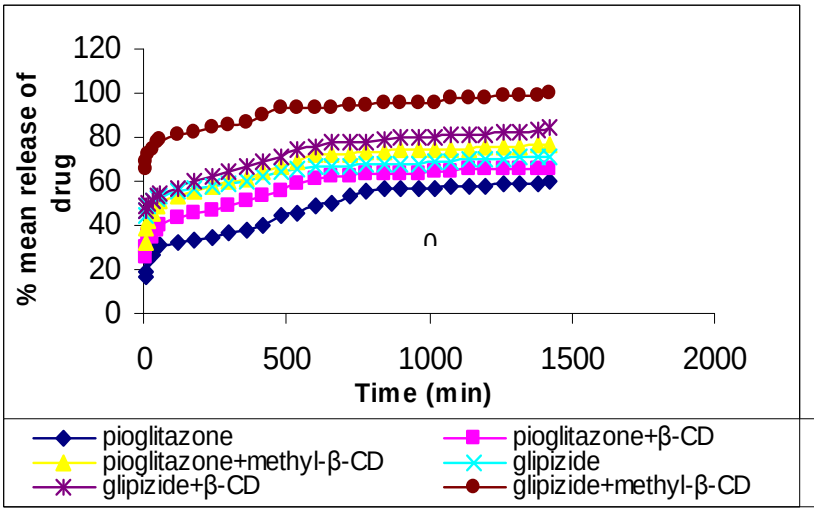


Figure 11: Dissolution profile of (a) pioglitazone HCl (b) with β-CD and (c) methyl- β-CD in HCl 0.3 M KCl buffer pH 2 and (c) glipizide (d) with β-CD and (e) methyl-β-CD in phosphate buffer pH 6.8 at 37 ºC.

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