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European Journal of Pharmaceutics and Biopharmaceutics 59 (2005) 325–332
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Isothermal titration calorimetry method for determination of cyclodextrin complexation thermodynamics between artemisinin and naproxen under varying environmental conditions

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Received 5 April 2004; accepted in revised form 8 August 2004
Available online 7 October 2004

Abstract

A novel isothermal titration calorimetry method was used to determine the complexation thermodynamics for hydroxypropyl-β-cyclodextrin with artemisinin and naproxen at varying temperature and pH. The new method is very useful for studying complexation reactions between cyclodextrin and drugs with poor solubility and all the thermodynamic parameters of the cyclodextrin complexation were determined. The analysis of the thermodynamic data reveals involvement of hydrophobic bonding in the cyclodextrin complexes studied. The data also reveals the presence of enthalpy–entropy compensation in the system and provide information as to the orientation of the drug molecule inside the cyclodextrin cavity. From the thermodynamic parameters for dissociation of HPBCD complexes of artemisinin and naproxen at pH 2 it is concluded that the complexation is primarily driven by enthalpy with entropic assistance at all temperatures studied. From the dissociation studies of HPBCD complexes of naproxen at pH 10 it is concluded that the complexation is predominantly driven by entropy and moderately by enthalpy at lower temperatures and by enthalpy with entropic assistance at higher temperatures.
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Keywords: Isothermal titration calorimetry; Complexation thermodynamics; Enthalpy–entropy compensation; Hydroxypropyl-β-cyclodextrin; Artemisinin; Naproxen

1. Introduction

Isothermal titration calorimetry (ITC) is a thermodynamic technique for monitoring any chemical reaction initiated by a binding component. It has become the method of choice for characterizing biomolecular interactions. Recent developments in the instrumentation for calorimetry allow for detection of very weak interactions involving low heats of binding on the order of microcalories, thus making it suitable to evaluate weaker interactions associated with cyclodextrin complexation [1].

When substances bind, heat is either generated or absorbed. Measurement of this heat flow allows for accurate determination of binding constants, reaction stoichiometry (n), enthalpy and entropy thus providing a complete thermodynamic profile of the molecular interaction in a single experiment. Earlier studies using ITC have been shown to differentiate between the multi modal inclusion complexes for barbiturates and cyclodextrin [2–4].

Phase solubility analysis is the most common technique used to study cyclodextrin complexation. These techniques depend heavily on the accurate determination of the solubility of the drug. Such a determination can be difficult for poorly soluble drugs, in spite of the use of highly sensitive detectors. Isothermal titration calorimetry, however, does not depend on the accuracy of the drug solubility and is a true measurement of the binding interactions. With proper analysis of the data it might even be possible to interpret the complex binding equilibria of cyclodextrins in a greater detail than possible using other methods.

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doi:10.1016/j.ejpb.2004.08.006

The complexation of weak acids and bases with cyclodextrins is quite interesting, and earlier studies have shown the possibility of complexation of both the charged and uncharged species in the system [5]. The complexation of charged and uncharged species has been studied using phase solubility analysis of data obtained using various analytical techniques [5–7]. With the inherent limitations of these methods, the results are highly variable. ITC, provides an accurate method of measuring the interactions to study such systems. Moreover typical phase solubility techniques usually require about 1–7 days for equilibration. ITC experiments require only a few hours and provide extensive information about the complexes [1]. ITC also has the potential for high-throughput screening and can be used to evaluate the efficiency of cyclodextrins in solubility and stability enhancement. Additionally, a smaller sample is needed to compare most of the traditional methods, which could be an advantage in the initial development stages. The effect of various agents such as surfactants, and cosolvents on the complexation of drugs can be studied at a greater detail with the use of ITC and require a minimum number of experiments over a relatively short period of time [8].

Experiments utilizing ITC for complexation studies have been reported using various methods. The most widely used method is the 'classical' method, in which the receptor solution is placed in the cell and is titrated with the ligand solution from the syringe. These experiments need a relatively high concentration of the drug in solution to allow for saturation of the binding sites. To achieve these high concentrations various cosolvents are often employed [9].

Another approach for performing the ITC experiments is by placing the ligand solution in the cell and titrating with the receptor solution. This particular method is recommended for poorly soluble compounds. The mathematical treatment of the data obtained from such experiment can be performed using various software packages.

Studies that use a displacement method have been reported by Sigurskjold [10]. In this method, a saturated solution of ligand in a cyclodextrin solution was titrated with another ligand solution capable of displacing the earlier ligand from the complex. However, this method is limited by the need for a significant difference in association constants.

A 'release protocol' for determining the partition coefficient of detergent between lipid bilayers and solvent has been reported in the literature [11]. In this study, equations are derived which are capable of calculating the association constants and thermodynamic parameters in such cases. A similar method was also used by Cooper et al. to study dissociation of dimers due to dilution [12]. These two methods, in contrast to other methods, measure the dissociation rather than association.

In the current study, modifications to the above mentioned release/dilution models are proposed to suit the cyclodextrin systems. Complexation thermodynamics of drug–cyclodextrin systems have been determined using the resulting model.

2. Theoretical development

Heerkoltz, et al. have shown that for ITC experiments the heat observed (q_{obs}) can be given by [11]

$$q_{obs} = \Delta h_d^{w\to b}\left[X^{syr}\frac{\partial D_b}{\partial D_t} + (1 - X^{syr})\frac{\partial D_b}{\partial L} - \frac{D_b^{syr}}{D_t^{syr} + L^{syr}}\right] + q_{dil} \quad (1)$$

which was derived for use in studying the partitioning of a non-ionic detergent, $C_{12}EO_7$ between water and palmitoyloleoylphosphatidylcholine (POPC) vesicles. In Eq. (1), the indices, L and D represent lipid and detergent and the superscripts, b and w represent bilayers and water. Heerkoltz also noted that this same derivation holds for the specific binding of ligands to receptors. To apply this derivation to the work presented here, Eq. (1) was rewritten to give:

$$q_{obs} = (h_D^b - h_D^f)\left[X^{syr}\frac{\partial D_b}{\partial D_t} + (1 - X^{syr})\frac{\partial D_b}{\partial M_t} - \frac{D_b^{syr}}{D_t^{syr} + M^{syr}}\right] + q_{dil} \quad (2)$$

Where M and D represents the receptor and ligand concentration respectively, the subscripts t, b, and f stand for total, bound and free concentrations respectively, the molar enthalpies (h) are denoted with the sub- and superscripts they are associated with. Superscript 'syr' corresponds to the concentrations of the respective species in the syringe. X^{syr} represents the mole fraction of the ligand concentration in the syringe and q_{dil} is the heat of dilution which is usually accounted for by performing blank titrations.

This heat observed is equal to the normalized ΔH (NDH) data in the ITC results and the data can be fitted by plotting the NDH on the y-axis and the receptor concentration in the cell on the x-axis.

The dissociation of a 1:1 complex is shown as

$$MD \overset{K_d}{\leftrightarrow} M + D \quad (3)$$

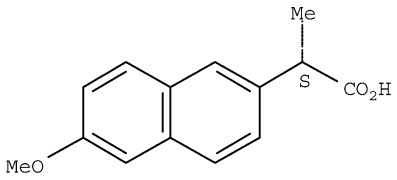
where K_d is the dissociation constant and is given in terms of concentrations of the various species as

$$K_d = \frac{[M_f][D_f]}{[MD]} = \frac{([M_t] - [MD])([D_t] - [MD])}{[MD]} \quad (4)$$

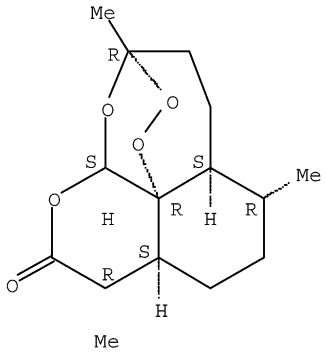
Solving the resulting quadratic equation for calculation of the concentration of the complex [MD], results in:

$$[MD] \text{ or } D_b = \frac{1}{2}\left\{(M_t + D_t + K_d) - \sqrt{(M_t + D_t + K_d)^2 - 4M_tD_t}\right\} \quad (5)$$

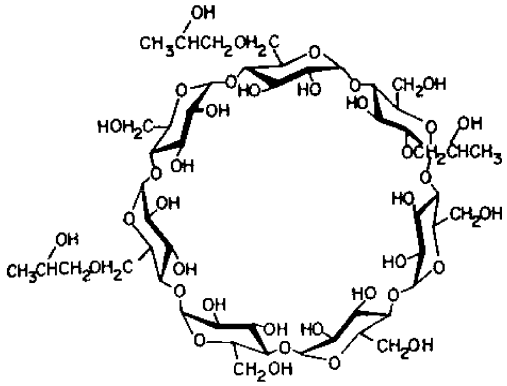
Taking the partial differential of the bound ligand concentration with respect to total ligand concentration



NAPROXEN



ARTEMISININ



Representative structure of HPBCD

Fig. 1. Chemical structures of naproxen, artemisinin and HPBCD.

and total receptor concentration results in:

$$\frac{\partial D_d}{\partial D_t} = \frac{1}{2}\left\{1 - \frac{(M_t + D_t + K_d) - 2M_t}{\sqrt{(M_t + D_t + K_d)^2 - 4M_t D_t}}\right\} \quad (6)$$

and,

$$\frac{\partial D_b}{\partial M_t} = \frac{1}{2}\left\{1 - \frac{(M_t + D_t + K_d) - 2D_t}{\sqrt{(M_t + D_t + K_d)^2 - 4M_t D_t}}\right\} \quad (7)$$

Incorporating these partial differentials (Eqs. (6) and (7)) into the equation for 'q_{obs}' (Eq. (2)) and fitting the equations to the data one can make estimates of enthalpy and dissociation constants.

The fitting procedure was performed using MicroCal Origin software (MicroCal, LLC, Northampton, MA). Five variables were defined for the purpose of fitting and included, ΔH, D_t (syringe), M_t (syringe), D_b (syringe) and K_d. Among these variables D_t (syringe) and M_t (syringe) are fixed and the other three variables are iterated by a χ^2 minimization process until the χ^2 is not further affected. Initial values for D_b (syringe) were the same or slightly less than D_t (syringe).

3. Materials

Hydroxypropyl beta-cyclodextrin (HPBCD) (D.S. 0.6), naproxen along with the buffer salts were purchased from Sigma-Aldrich, MO. Artemisinin was provided courtesy of Dr Mitchell Avery, Department of Medicinal Chemistry, University of Mississippi. Nanopure water was used for preparation of the samples. The chemical structures for naproxen, artemisinin and hydroxypropyl beta-cyclodextrin are provided in Fig. 1.

ITC experiments were performed using a MicroCal VP-ITC microcalorimeter with a Thermovac-2 sample degasser and thermostat (MicroCal, LLC, Northampton, MA).

4. Methods

4.1. Sample preparation

About 5 g of HPBCD was exactly weighed and transferred to a 100 ml volumetric flask. An accurately weighed aliquot of the test drug, well below saturation, was transferred to the flask. Sufficient solvent was added to make 100 ml. The solvents were water or USP buffer solutions of pH 2 or 10 depending on the study design. The amounts of drug added for each experiment, and the solvent used are shown in Table 1.

4.2. Isothermal titration calorimetry

The drug–HPBCD solution and the solvent were degassed and thermostated by placing in the Themovac-2

Table 1
Experimental conditions maintained during ITC studies

Drug	Weight of drug added (mg)	Weight of HPBCD added (g)	HPBCD concentration (mM)	Temperature (°C)	Concentration of drug (mM)	Total volume (ml)	Solvent
Artemisinin	24	3.545	25.688	25, 30, 40	0.851	100	Water
Naproxen	23.4	4.132	29.941	25, 30, 35, 40	1.016	100	pH 2 buffer
Naproxen	31	4.965	35.980	25, 30, 35, 40	1.346	100	pH 10 buffer

sample degasser and thermostat. The degassed drug–HPBCD solution was loaded in a 250 μl syringe attached to the Microcal VP-ITC® microcalorimeter. The appropriate solvent used for preparation of the complex was filled in the cell of the microcalorimeter. The temperature for each study was adjusted according to the study design. The various temperatures studied for each system are also shown in the Table 1. The binding isotherm was obtained by monitoring the heat associated with injection of a predetermined volume of the complex solution into the solvent. Initially an injection of 1 μl was performed followed by a series of 34 injections of 5 μl each. The heat response was then acquired and used to generate binding isotherms which were then analyzed.

4.3. Data handling

The isotherms generated are stored as '.itc' files by the software. The isotherms obtained by titration are then fitted to the model developed for the dissociation of the complex due to dilution. The derivation of the model is shown earlier. A custom script, which fits the data using the model, was developed, which allows the enthalpy (ΔH) of dissociation, K_d (dissociation constant) and the amount of drug bound in the syringe (D_b) to be calculated as a result of the fitting process.

4.4. Curve fitting

The heat generated per mole of the titrant (complex) was plotted against the HPBCD concentration in the cell and the average of the heats generated, after HPBCD concentration in the cell reached ~1.0 mM, was calculated. The average heat value was then subtracted from the heats generated at each HPBCD concentration. This subtraction is similar to the baseline subtraction, as the heat generated after the HPBCD concentration reached 1 mM were almost constant suggesting no further dissociation. Microcal software is used to fit the curve to the data after subtraction using the custom script mentioned above. Chi-square minimization was performed iteratively to obtain the best fit parameters.

5. Results

5.1. Dissociation of artemisinin–HPBCD complex in water

The heats of dissociation were measured by the VP-ITC software and the analysis was performed as described earlier and Fig. 2 is shown as a representative ITC plot obtained during the release/dilution protocol. The enthalpy, association constants and bound drug in syringe were determined by fitting the curve to the appropriate model with the parameters from the curve fits given in Table 2. Fig. 3 shows a representative curve fit of the binding isotherm for artemisinin–HPBCD dissociation at 25 °C. The resulting

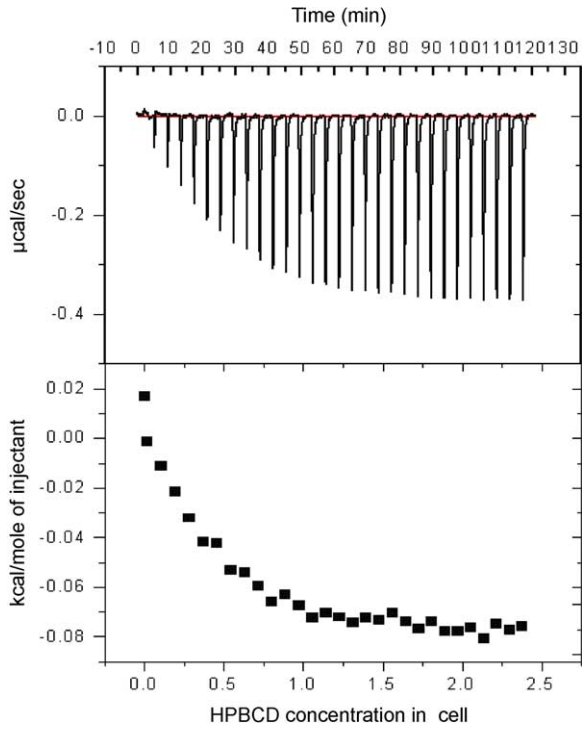


Fig. 2. Representative ITC plot artemisinin–HPBCD titration into water at 25 °C. A, signal of titration; B, binding isotherm.

temperature dependence of enthalpy of artemisinin–HPBCD dissociation is shown in Fig. 4.

5.2. Dissociation of naproxen–HPBCD complex in pH 2 buffer solution

The enthalpy, association constants and bound drug in syringe were determined by fitting the curve to the appropriate model. The results are shown in Table 2. The temperature dependence of naproxen–HPBCD dissociation in pH 2 buffer is given in Fig. 5.

Table 2
Fitting parameters as obtained by complex dissociation studies at varying temperatures and pH conditions

Drug/system	Temperature (°C)	Enthalpy (ΔH in cal/mole)	Association constant (K in M^{-1})	Bound drug (mM)
Artemisinin/water	25	−2870	1205	0.75
	30	−3487	948	0.71
	40	−3773	614	0.66
Naproxen/pH 2	25	−3185	6521	1.0
	30	−3521	4607	1.0
	35	−3680	3860	0.96
	40	−4177	3492	0.99
Naproxen/pH 10	25	−1498	1033	1.21
	30	−1837	774	1.15
	35	−2219	624	1.10
	40	−2397	536	1.06

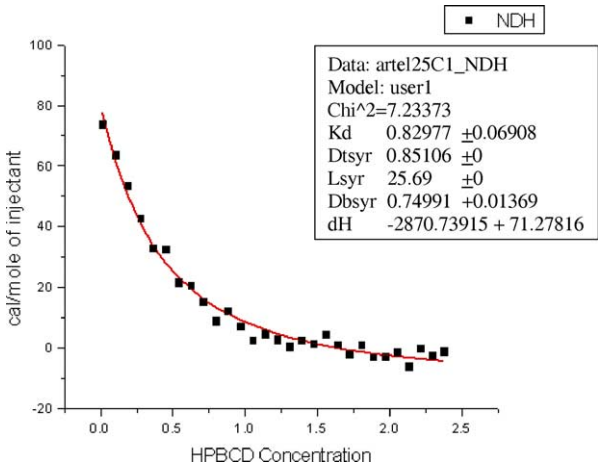


Fig. 3. Heat of dissociation vs. HPBCD concentration using artemisinin–HPBCD solution at 25 °C.

5.3. Dissociation of naproxen–HPBCD complex in pH 10 buffer solution

The enthalpy, association constant and amount of bound drug in syringe were determined by fitting the curve to the derived model using the custom script. The results are shown in Table 2. The temperature dependence of enthalpy of dissociation is plotted in Fig. 5.

6. Discussion

The thermodynamics of complexation of poorly soluble drugs with HPBCD were successfully estimated using the release protocol. The association constant (K_a) is estimated from the dissociation constant using Eq. (8), where K_d is the dissociation constant.

$$K_a = 1/K_d \tag{8}$$

All other thermodynamic parameters are calculated using Eq. (9).

$$\Delta G = -RT\ln K = \Delta H - T\Delta S \tag{9}$$

All the estimated thermodynamic parameters for complexation of artemisinin in water, and naproxen at pH 2 and 10 are listed in Table 3.

The complex dissociation enthalpies in all the cases showed significant variation with temperature and generally become more exothermic (ΔH becoming more negative) with increasing temperature (Figs. 4 and 5). The temperature dependence of enthalpy is given by:

$$\Delta C_p = \frac{\Delta H^0_{T2} - \Delta H^0_{T1}}{T_2 - T_1} \tag{10}$$

where ΔC_P is the heat capacity, ΔH^0 is the enthalpy at varying temperatures corresponding to the superscripts, and T_1 and T_2 are temperatures.

Temperature dependence of the dissociation enthalpies shown in Figs. 4 and 5 all resulted in negative heat capacities (Table 4), which is typical of macromolecular associations in water [13,14].

From Figs. 6–8, it can also be seen that as a consequence of heat capacity [15], the data showed apparent enthalpy-entropy compensation. A linear relationship between ΔH and $T\Delta S$ with a relatively small temperature dependence of free energy of dissociation (ΔG) was observed. No explicit relationship between change in enthalpy and entropy has been derived from fundamental thermodynamics. However, the compensatory enthalpy–entropy relation was often observed in a wide variety of reactions and equilibria [16]. Indeed, much debate is devoted to the basis of this extrathermodynamic relationship.

In chemical equilibria, the association constant (K) and consequently the free energy (ΔG) vary critically with

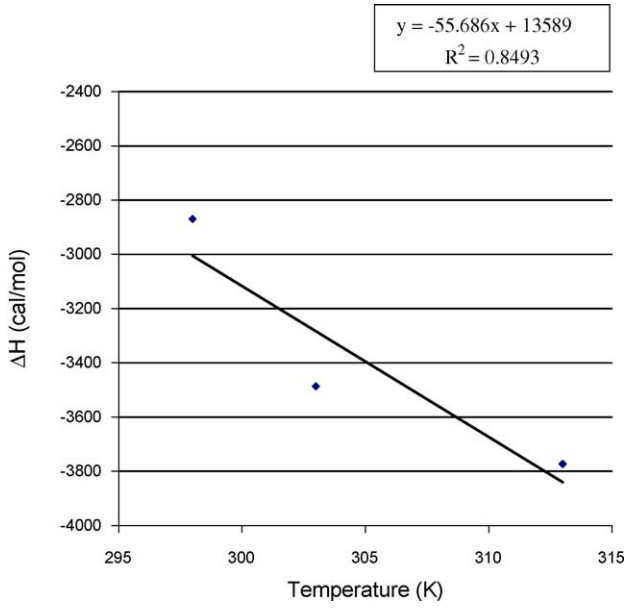


Fig. 4. Temperature dependence of enthalpy in artemisinin dissociation studies.

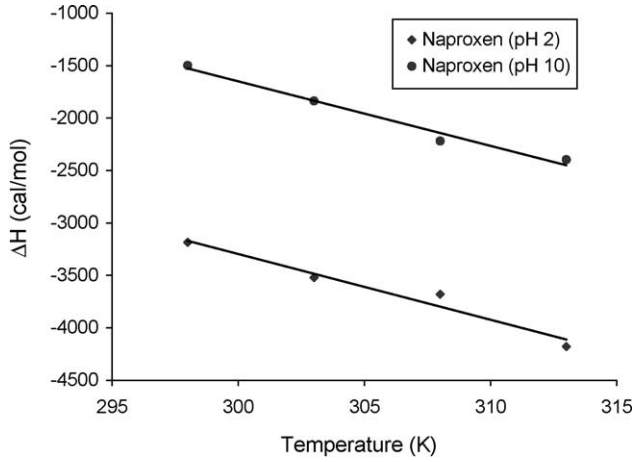


Fig. 5. Temperature dependence of enthalpy in Naproxen dissociation studies at pH 2 and 10.

Table 3
Estimated thermodynamic parameters for dissociation of drug–HPBCD complexes under various environmental conditions

Drug/Solvent	Temperature		K_d (mM)	K_a (M^{-1})	ΔG (cal/mole)	ΔH (cal/mole)	ΔS (cal/mole)	$T\Delta S$
	°C	K						
Artemisinin/water	25	298	0.830 (0.069)	1205 (100)	−4201 (49)	−2870 (71)	4.466 (0.290)	1439
	30	303	1.055 (0.060)	948 (54)	−4127 (34)	−3487 (53)	2.111 (0.208)	1253
	40	313	1.628 (0.226)	614 (85)	−3993 (86)	−3773 (117)	0.703 (0.465)	1262
Naproxen/pH 2	25	298	0.153 (0.022)	6521 (949)	−5201 (86)	−3185 (210)	6.763 (0.762)	1914
	30	303	0.217 (0.019)	4607 (411)	−5079 (54)	−3521 (141)	5.141 (0.498)	1271
	35	308	0.259 (0.019)	3860 (286)	−5054 (45)	−3680 (121)	4.461 (0.420)	1212
	40	313	0.286 (0.018)	3492 (224)	−5074 (40)	−4177 (118)	2.866 (0.398)	1352
Naproxen/pH 10	25	298	0.968 (0.110)	1033 (118)	−4110 (67)	−1498 (59)	8.763 (0.301)	2306
	30	303	1.291 (0.078)	775 (47)	−4005 (37)	−1837 (34)	7.156 (0.165)	2577
	35	308	1.601 (0.116)	624 (45)	−3939 (44)	−2219 (44)	5.586 (0.203)	2066
	40	313	1.864 (0.172)	536 (50)	−3909 (57)	−2397 (58)	4.830 (0.261)	2075

Error in estimation is given in parentheses. Error in K_d and ΔH are calculated by the fitting function, the error for the rest of the parameters is calculated by error propagation.

changes in solvent, substituent, temperature, etc. However, the changes in K and ΔG are generally much smaller than that expected for the induced enthalpic change alone since the entropy term often compensates to cancel out a substantial part of enthalpic change. Qualitatively, this is the source of 'enthalpy–entropy compensation' effect [16].

A finite heat capacity (ΔC_p) gives rise to temperature dependence of enthalpy and entropy changes that cancel each other. Cooper [15] have derived a relationship describing changes in enthalpy and entropy (Eq. (11)) over a limited temperature range.

$$\Delta H(T) \approx \Delta H(T_{ref}) + T_{ref}[\Delta S - \Delta S(T_{ref})] \quad (11)$$

From the above equation a plot of enthalpy as a function of entropy should be linear with a slope equal to a reference temperature (T_{ref}). The T_{ref} arising from such a correlation would simply be the temperature over which the approximation (ΔT small) is most appropriate. The above theory leads to the much broader phenomenon of 'enthalpy–entropy compensation'.

Though a contribution from other interactions and changes in macromolecular dynamics cannot be ruled out, negative heat capacity values are usually considered to be evidence of significant hydrophobic interaction in the binding process. It may also be correlated with changes in exposed non-polar surface area during complexation.

The association of naproxen at pH 2 and pH 10 does not show a significant change in the heat capacity (Table 4) suggesting that no significant difference in exposed non-polar surface area is seen. It suggests that most of the non-polar surface area of the molecule is inside the HPBCD cavity at either pH as expected.

The heat capacity values for artemisinin are slightly lower than those for naproxen and this could be due to relatively smaller surface area of the tricyclic artemisinin molecule as compared to linear naproxen molecule.

From the thermodynamic parameters for dissociation of HPBCD complexes of artemisinin and naproxen at pH 2 (Table 3), it can be concluded that: enthalpy (ΔH) is less than zero, product of temperature and entropy ($T\Delta S$)

Table 4
Heat capacity (ΔC_p) calculated from slope of ΔH vs. temperature plots

Drug/HPBCD complex	ΔC_p (Heat capacity)
Artemisinin	−55.6
Naproxen (pH 2)	−62.7
Naproxenate ion (pH 10)	−61.6

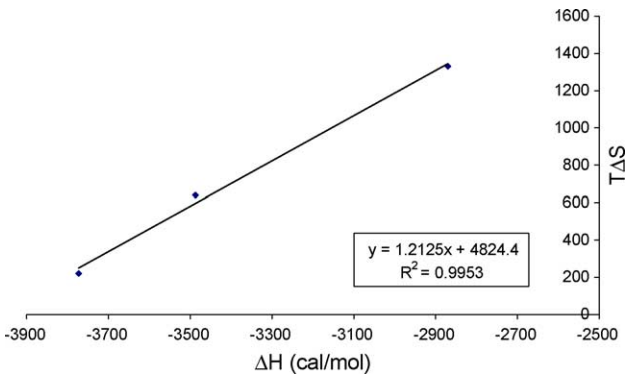


Fig. 6. Enthalpy–entropy diagram for artemisinin complexation.

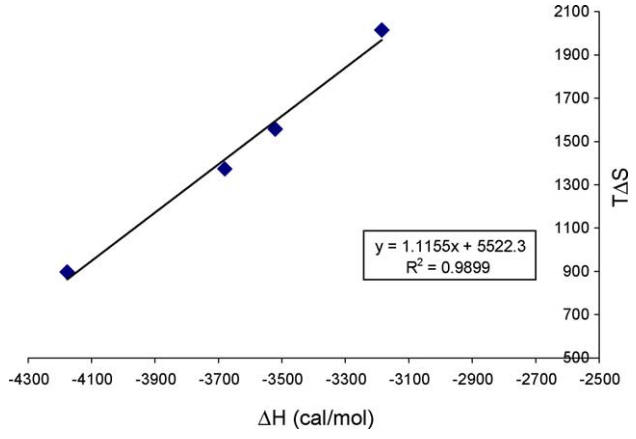


Fig. 7. Enthalpy–entropy diagrams for naproxen complexation at pH 2.

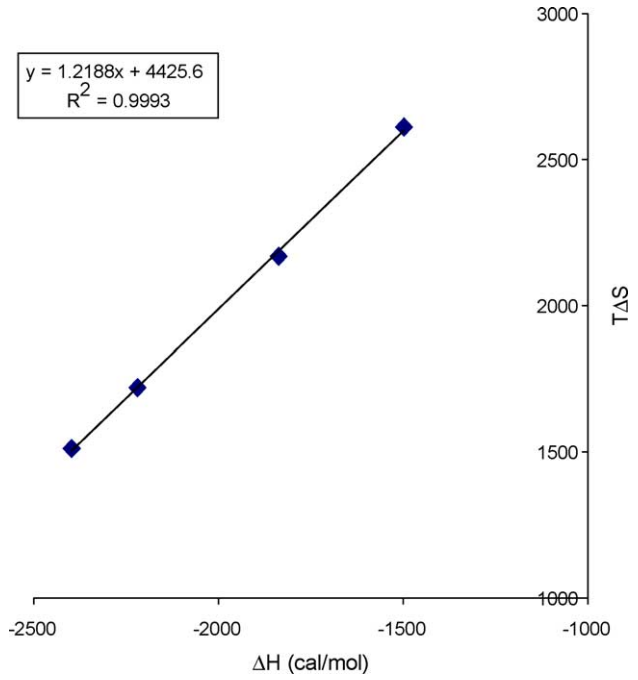


Fig. 8. Enthalpy–entropy diagram for Naproxen complexation at pH 10.

is greater than zero, and the absolute value of enthalpy is greater than the absolute value of the product of temperature and entropy ($|\Delta H| > |T\Delta S|$). Such host-guest combinations are classified as complexation primarily driven by enthalpy with entropic assistance [16].

From the dissociation studies of HPBCD complexes of naproxen at pH 10 (Table 3), it can be concluded that: enthalpy (ΔH) is less than zero, product of temperature and entropy ($T\Delta S$) is greater than zero, but the absolute value of enthalpy is smaller than the product of temperature and entropy ($|\Delta H| < |T\Delta S|$) at lower temperatures (25 and 30 °C), and is the contrary ($|\Delta H| > |T\Delta S|$) at higher temperatures (35 and 40 °C). From these findings, it can be concluded that the complexation is predominantly driven by entropy and moderately by enthalpy at lower temperatures (25 and 30 °C), and by enthalpy with entropic assistance at higher temperatures (35 and 40 °C).

The higher entropic contribution at lower temperatures could be due to the more extensive host/guest desolvation with the ion-pairing interaction [16]. Such results were observed earlier in another study with guest molecules containing charged or neutral hydrophilic groups similar to the ionized form of naproxen at pH 10 in the current study. It can also be seen from the data that the enthalpy values for naproxen at pH 2 are significantly higher than the values at pH 10 while the entropy values are significantly higher at pH 10 than at pH 2.

7. Conclusions

Previously, several authors have presented evidence of the formation of complexes between artemisinin and naproxen with cyclodextrin analogues, including HPBCD [17–19]. However, the ability to determine the thermodynamics of complexation has been limited by the poor aqueous solubility of the compounds. The ITC method described here is suitable for poorly soluble drugs and was shown to be successful in determining the thermodynamics of cyclodextrin complexation under varying environmental conditions. In this method, sufficient concentrations of the drug can be achieved easily by taking advantage of the cyclodextrin's ability to enhance the drug solubility. The drug concentration was usually a limiting consideration for performing earlier analysis. Also the drug concentrations in the system are well below saturation, thus precluding any saturation effects seen commonly in phase solubility analysis.

ITC studies in general suffer from the disadvantage of not being able to predict the solubility of the drug in a cyclodextrin solution. These studies provide no information about the type of phase solubility diagram for the drug–cyclodextrin system. However, once a strong interaction between the drug and cyclodextrin is established, it is quite feasible to evaluate the complexation using saturated solubility methods.

References

[1] G. Buckton, A. Beezer, The application of microcalorimetry in the field of physical pharmacy, Int. J. Pharm. 72 (1988) 181–191.
[2] H. Aki, T. Niiya, Y. Iwase, M. Yamamoto, Calorimetry to evaluate inclusion mechanism in the complexation between 2-hydroxyproyl-β-cyclodextrin and barbiturates in aqueous solution, J. Therm. Anal. Calorim. 64 (2001) 713–719.
[3] H. Aki, T. Niiya, Y. Iwase, M. Yamamoto, Multimodal inclusion complexes between barbiturates and 2-hydroxypropyl-β-cyclodextrin in aqueous solution: isothermal titration calorimetry ^{13}C NMR spectrometry and molecular dynamics simulation, J. Pharm. Sci. 90 (8) (2001) 1186–1197.
[4] H. Aki, T. Niiya, Y. Iwase, M. Yamamoto, Two types of inclusion realized in the complexation between phenobarbital and 2-hydroxypropyl-β-cyclodextrin in aqueous solution, Thermochim. Acta 308 (1998) 115–121.
[5] E. Junquera, E. Aicart, A fluorimetric, potentiometric, and conductimetric study of the aqueous solutions of naproxen and its association with hydroxypropyl-β-cyclodextrin, Int. J. Pharm. 176 (1999) 169–178.
[6] E. Junquera, E. Aicart, Effect of pH on the encapsulation of the salicylic acid/salicylate system by hydroxypropyl-β-cyclodextrin at 25 °C. A fluorescence enhancement study in aqueous solution, J. Inclusion Phenom. Mol. Recog. Chem. 29 (2) (1997) 119–136.
[7] P. Mura, M.T. Faucci, G. Bettinetti, The influence of polyvinylpyrollidone on naproxen complexation with hydroxypropyl-β-cyclodextrin, Eur. J. Pharm. 13 (2) (2001) 187–194.
[8] P. Fini, M. Castagnolo, Determination of enthalpic interaction coefficients by ITC measurements 2-Hydroxypropyl-β-cyclodextrin in aqueous solution of NaCl, J. Therm. Anal. Calorim. 66 (2001) 91–102.
[9] R. Chadha, N. Kashid, A. Kumar, D.V.S. Jain, Calorimetric studies of diclofenac sodium in aqueous solution of cyclodextrin and water–ethanol mixtures, J. Pharm. Pharmacol. 54 (2002) 481–486.

[10] B.W. Sigurskjold, Exact analysis of competition ligand binding by displacement isothermal titration calorimetry, Anal. Biochem. 277 (2000) 260–266.
[11] H.H. Heerklotz, H. Binder, R.M. Epand. A Release protocol for isothermal titration calorimetry, Biophys. J. 76 (1999) 2606–2613.
[12] D. McPhail, A. Cooper, Thermodynamics and kinetics of dissociation of ligand-induced dimers of vancomycin antibiotics, J. Chem. Soc., Faraday Trans. 93 (13) (1997) 2283–2289.
[13] M.M. Lopez, G.I. Makhatadze, Solvent isotope effect on thermodynamics of hydration, Biophys. Chem. 74 (1998) 117–125.
[14] T.K. Dam, S. Oscarson, C.F. Brewer, Thermodynamics of binding of the core trimannoside of aspargine-linked carbohydrates and deoxy analogs to *Dioclea grandiflora* lectin, J.Biol. Chem. 273 (1998) 32812–32817.
[15] A. Cooper, Thermodynamic analysis of biomolecular interactions, Curr. Opin. Chem. Biol. 3 (5) (1999) 557–563.
[16] M.V. Rekharsky, Y. Inoue, Complexation of chiral recognition thermodynamics of 6-amino-6-deoxy-β-cyclodextrin with anionic, cationic and neutral chiral guests: counterbalance between van der Waals and coulombic interactions, J. Am. Chem. Soc. 124 (5) (2002) 813–826.
[17] F.J. Otero-Espinar, S. Anguiano-Igea, N. Garcia-Gonzalez, J.L. Vila-Jato, J. Blanco-Mendez, Interaction of naproxen with β-cyclodextrin in solution and in the solid state, Int. J. Pharm. 79 (2–3) (1992) 149–157.
[18] N. Erden, N. Celebi, A study of the inclusion complex of naproxen with β-cyclodextrin, Int. J. Pharm. 48 (1-3) (1988) 83–89.
[19] A.C. Illapakurthy, A.S. Yogesh, B.A. Avery, M.A. Avery, C.M. Wyandt, Interaction of artemisinin and its related compounds with hydroxypropyl-β-cyclodextrin in solution state: experimental and molecular-modeling studies, J. Pharm. Sci. 3 (92) (2003) 649–655.