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Microcalorimetric investigation of the complexation between 2-hydroxypropyl-β-cyclodextrin and amine drugs with the diphenylmethyl functionality*

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Abstract. Solution calorimetry has been employed to evaluate the stability constants and standard-enthalpy changes ($\Delta H°$) associated with complex formation between 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and a group of amine compounds having the diphenylmethyl functionality in aqueous solution at 298.15 K. Data from microcalorimetric titrations of the compounds were analysed using a nonlinear least-squares method. Of the 12 compounds studied, only terfenadine·HCl formed a 1:2 (compound:HP-β-CD) complex. All the others formed 1:1 complexes. The standard free energy decrease accompanying the formation of inclusion complexes is generally due to a negative $\Delta H°$. This exothermic $\Delta H°$ can be interpreted as indicating that the binding forces for complexation include both the hydrophobic effect and strong van der Waals interactions. When a halogen substituent is in the aromatic ring, stability constants are higher and standard-entropy changes ($\Delta S°$) become positive, suggesting greater hydrophobic interaction. Both adiphenine·HCl and proadifen·HCl form more stable complexes, suggesting that hydrogen bonding to the carbonyl oxygen by the hydroxyl-group on the rim of the CD ring could be an important contributor to the complexation. Substitution on the aliphatic carbon of the diphenylmethyl group was also found to be important in determining the ability of compounds to bind with HP-β-CD. The independence of the thermodynamic constants on the degree of protonation in the case of bifunctional amines indicates that the amine functional groups do not penetrate into the HP-β-CD cavity.

Keywords: *Solution calorimetry; microcalorimetry; 2-hydroxypropyl-β-cyclodextrin; HP-β-CD; inclusion complex; amine drugs; diphenylmethyl derivatives.*

Introduction

Cyclodextrins (CDs) belong to a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic cavity in the center [1]. The most common natural CDs are α-CD, β-CD, and γ-CD, consisting of six, seven and eight (1-4)-linked glucose units, respectively. Many drug molecules are capable of residing within the central cavity of a CD molecule, thus forming an inclusion complex. Encapsulation of a molecule will affect many of its physico-chemical properties and can result in increased aqueous solubility and stability [2, 3].

Unfortunately, the cyclodextrin which is most useful for incorporating a number of drugs, i.e. β-CD, is poorly water soluble (1.85 g 100 ml^{-1} at 25°C). This low aqueous solubility of β-CD is associated with cytoplasmic crystals in rat renal tubules and severe nephrosis after parenteral administration [4]. Alkylation of some of the hydroxyl groups on the β-CD molecule results in derivatives with good aqueous solubility and excellent complexing capabilities [1–3, 5–8]. The hydroxyalkyl derivative of β-CD, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), is readily soluble in water (1 g ml^{-1}) and has been shown to be a useful drug solubilizer [6, 8]. Many *in vivo* studies also have shown that HP-β-CD has much lower acute toxicity when given *i.v.* to rats, rabbits or monkeys [5, 9].

Many amine compounds used medicinally possess the diphenylmethyl functionality. Poor aqueous solubility and stability of some of these diphenylmethyl derivatives make them good candidates for CD inclusion complexation.

Previously, the binding of some amine drugs bearing the diphenylmethyl functionality to α-, β- and γ-CDs has been studied [10, 11]. The objective of this investigation was to evaluate the binding of HP-β-CD with the same series of amine drugs. Also, based upon the calorimetric results, the structural effects on the stability constants, thermodynamics and inclusion complex geometry were explored.

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Experimental

Materials

Terfenadine, diphenidol·HCl, diphenhydramine·HCl, orphenadrine·HCl, cyclizine·HCl, chlorcyclizine·HCl, meclizine·2HCl, hydroxyzine·2HCl, and adiphenine·HCl were obtained from Sigma Chemical Co. (St. Louis, MO, USA); 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) from Pharmatec Inc. (Alachua, FL, USA). The amounts of hydrated water in HP-β-CD were measured by thermogravimetry (Perkin–Elmer, model TGA 7, Norwalk, CT, USA), prior to preparation of the solutions used for titration, and the apparent molecular weight of the HP-β-CD was calculated accordingly. Bromodiphenhydramine·HCl was donated by Parke–Davis Co. (Morris Plains, NJ, USA) and proadifen·HCl and diphenylpyraline·HCl by SmithKline & Beecham (Philadelphia, PA, USA). Diphenylpyraline·HCl was recrystallized from anhydrous ether. The purity of the recrystallized product was determined by DSC to be 99.65%. Terfenadine and cinnarizine hydrochlorides were prepared from their free bases using a hydrochloric acid solution in anhydrous ether. All other chemicals were used as received, and their purities, which were determined using either DSC or HPLC, were >99%. Solutions were prepared using doubly-distilled water. Figure 1 shows the structures of

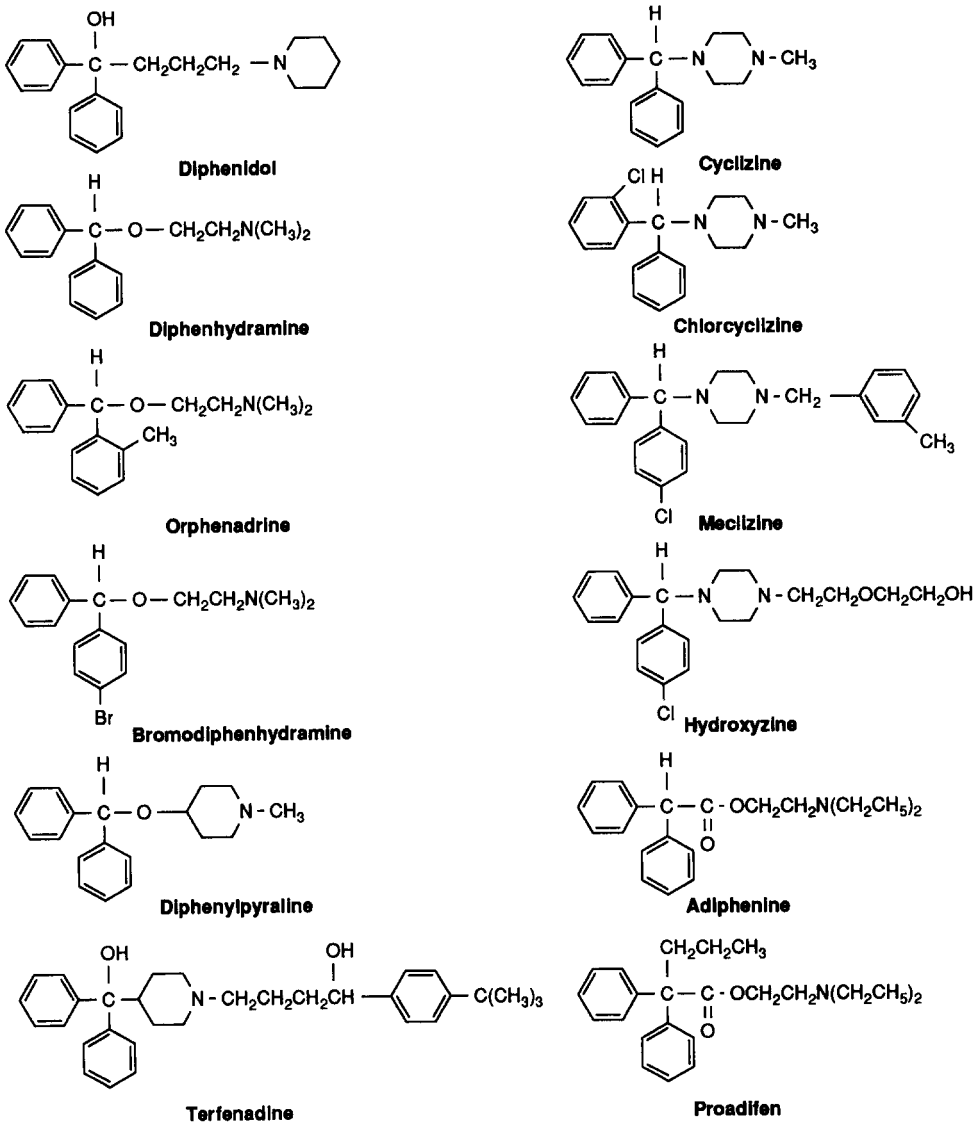

Figure 1
Chemical structures of amine compounds with the diphenylmethyl functionality.

the amine compounds used in this investigation.

Equipment and methods

Thermometric titrations were performed using the Tronac Model 450 isoperibol solution calorimeter (Tronac Inc., Orem, UT, USA). The calorimeter reaction vessel was submerged in a water bath maintained at 25° ± 0.0004°C by a Tronac PTC-40 temperature controller.

HP-β-CD solution with a concentration of 0.0243 M was delivered by means of a 10 ml Hamilton syringe, at a constant delivery rate of 0.424 ml min^{-1} into a 50 ml Dewar reaction vessel containing ligand solutions. The initial titrate volumes were *ca* 40 ml, and the total volume in the reaction vessel following titration was *ca* 49 ml. Two initial ligand concentrations, 1 and 0.5 mM were used in most cases. All the solutions were prepared immediately prior to use. A warm-up period of about 4 h was employed to permit the water bath temperature to stabilize. Prior to the run, the bridge was zeroed to the bath temperature. The temperature scale could then be read directly as the temperature difference between the reaction vessel and the constant temperature bath.

A titration calorimetry run basically consists of three parts: the initial heat capacity run, the heat of reaction run, and the final heat capacity run. The voltage change, which is directly proportional to the temperature change within the Dewar reaction vessel, was monitored at constant time intervals by means of an Apple II+ computer interfaced with the Tronac calorimeter through an analog–digital connector and voltage amplifier. The data acquisition program CALT was used for data collection. Data analysis consisted of four parts. The program WIN was used to calculate the initial and final heat capacities and also to correct the titration runs for extraneous heat effects. The program HK SEQFILE was then used for subtraction of the heat of dilution curve, which was measured separately, and to put the corrected data in a format for use with the modified NLLSQ program. Finally, the modified NLLSQ program was used to solve the thermograms for $\Delta H°$ and K associated with each step in the proposed series complexation model. All the programs used were developed in the authors' laboratory [12].

The solid complexes of adiphenine·HCl and proadifen·HCl with HP-β-CD were prepared by a freeze-drying method [13] using the freeze-drier Dura-Stop™ MP model TDS-2B-MP and Dura-Dry™ MP model FB-8-85B-MP (FTS Systems Inc., Stone Ridge, NY, USA). The calculated and accurately weighed (1:1 molar ratio) amounts of adiphenine·HCl or proadifen·HCl and HP-β-CD were dissolved in the appropriate volume of water, then sealed in a flask, and the solution was stirred for 2 days at 4°C and lyophilized. The physical mixtures were prepared by mixing exactly weighed (1:1 molar ratio) amounts of adiphenine·HCl or proadifen·HCl and HP-β-CD in an agate mortar in the Wig-L-Bug mixer.

Infrared spectra were obtained using the Nicolet Model 5DXB spectrometer, equipped with a DTGS detector and diffuse reflectance cell (Collector Cell, Spectra Tech, Inc., Stamford, CT, USA). Infrared spectra were the result of 300 co-added scans at a resolution of 4 cm^{-1} and a detector gain of 4. Spectra were ratioed to the background spectrum of potassium chloride powder (particle size <44 μm). Mixtures of each sample (2%) were prepared in KCl using the Wig-L-Bug mixer, filled into the macro sample cap (4 mm × 2 mm) of the Collector Cell, and leveled with a spatula. The Collector Cell was previously aligned on a removable baseplate to ensure optimal throughput. A 5 min purge was allowed prior to the collection of all spectra to equilibrate water and CO_2 vapours.

Results and Discussion

The thermodynamic parameters and binding constants for the complexation of HP-β-CD with amine drugs bearing the diphenylmethyl functionality are given in Tables 1 and 2. The standard deviations (SD) for $\Delta H°$ and K values were estimated by averaging the results of three separate titration runs. $\Delta G°$ and $\Delta S°$ were calculated from the averaged values of $\Delta H°$ and K, therefore, no standard deviation is reported.

Figure 2 shows a typical titration curve and fitted results for the 1:1 (drug:HP-β-CD) complexation reactions. Curve fitting was performed using a total of 238 points. The small residual is randomly distributed around the zero point line and shows no systematic error.

Two different concentrations of titrate were used in the titration for the compounds whenever solubility limitations and the sensitivity of the calorimeter permitted. The results showed

Table 1
Summary of the thermodynamic parameters and binding constants for the association of the diphenylmethyl derivatives with HP-β-CD in aqueous solution at 25°C (1:1 stoichiometry)

Compound	$\Delta H°$ (kJ mol^{-1})*	K (M^{-1})*	$\Delta G°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1}·K)
Diphenidol·HCl	−20.97 ± 0.74	157.7 ± 7.1	−12.54	−28.3
Diphenhydramine·HCl	−14.14 ± 0.92	242.9 ± 16.3	−13.62	−1.8
Orphenadrine·HCl	−14.65 ± 1.34	237.3 ± 4.0	−13.56	−3.7
Bromodiphenhydramine·HCl	−10.64 ± 0.62	551.8 ± 36.5	−15.65	16.8
Diphenylpyraline·HCl	−14.08 ± 0.52	354.4 ± 18.8	−14.55	1.6
Cyclizine·HCl	−14.23 ± 0.71	265.3 ± 14.9	−13.83	−1.3
Chlorcyclizine·HCl	−10.10 ± 1.10	430.6 ± 44.8	−15.03	16.6
Chlorcyclizine·2HCl	−10.61 ± 0.31	408.5 ± 68.0	−14.90	14.4
Meclizine·2HCl	−9.47 ± 0.26	350.0 ± 22.4	−14.52	16.9
Hydroxyzine·HCl	−10.06 ± 1.81	390.0 ± 15.2	−14.79	15.9
Hydroxyzine·2HCl	−8.88 ± 0.70	379.5 ± 34.7	−14.72	19.6
Proadifen·HCl	−16.16 ± 0.62	553.6 ± 2.3	−15.66	−1.7
Adiphenine·HCl	−23.04 ± 0.77	642.2 ± 42.5	−16.03	−23.5

*Average ±SD (n = 3).

Table 2
Summary of the thermodynamic parameters and binding constants for the association of terfenadine·HCl with HP-β-CD in aqueous solution at 25°C (1:2 model)

Compound	$\Delta H°$ (kJ mol^{-1})*	K (M^{-1})*	$\Delta G°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1}·K)
Terfenadine·HCl (1:1)	−7.33 ± 0.03	30660.0 ± 1949.0	−25.62	61.3
Terfenadine·HCl (1:2)	−9.40 ± 0.47	475.0 ± 38.0	−15.28	19.7

*Average ±SD (n = 3).

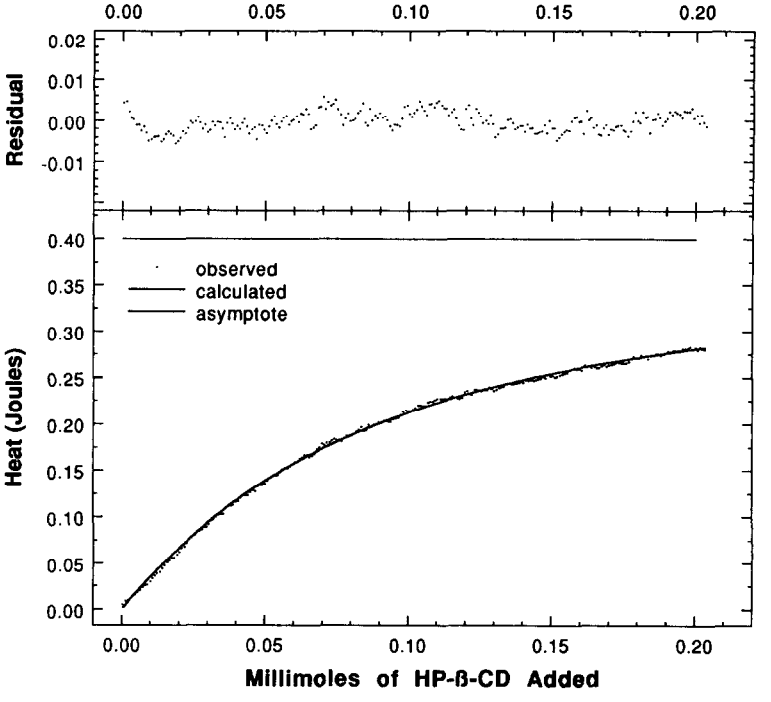

Figure 2
Calorimetric titration curve and fitted results for the interaction between bromodiphenhydramine·HCl and HP-β-CD in aqueous solution at 25°C.

no significant dependence of the parameters on the substrate concentrations, suggesting that other effects such as dimerization of the substrate and ionic strength changes during the titration were negligible in the determination of binding parameters.

All the compounds were studied in their HCl salt form. For bifunctional amine compounds such as chlorcyclizine and hydroxyzine, both HCl and 2·HCl salts were studied. The results show independence of the thermodynamic parameters on the degree of protonation, indicating that amine functional groups do not penetrate into the cavity of HP-β-CD. This is also supported by the observation that the pH of the solution in the reaction vessel does not change as titration progresses. Furthermore, the close similarity of the thermodynamic parameters for the binding of HP-β-CD with two amine compounds bearing markedly different amine functional groups; diphenhydramine-HCl and cyclizine-HCl, suggests that the diphenylmethyl groups of these compounds are the structural features which interact with the CD cavity.

HP-β-CD used in this investigation is a very complex mixture of literally billions of possible positional and optical isomers with an average degree of substitution equal to 7.6. When the hydrophilic edges of cyclodextrin are substituted, the channel of the cyclodextrin cavity is extended. Therefore, the stability of the complex formed with an otherwise suitable guest molecule is expected to increase. However, the hydroxyl groups of the hydroxypropyl substituents may make the extended part of the cyclodextrin cavity partially hydrophilic. Therefore, the binding ability of HP-β-CD is largely dependent on the structures of guest molecules.

Figure 3 shows the comparison of the binding constants for the association of β-CD and HP-β-CD with the amine drugs [10]. It is clear that the diphenylmethyl derivatives bind to β-CD much more strongly than to HP-β-CD. The enthalpy decreases accompanying the complexation are larger in the case of complexes formed with β-CD, while the entropy gains are smaller. Actually, in most of the cases, complexation with HP-β-CD is accompanied by a positive entropy change ($\Delta S°$).

When $\Delta H°$ is plotted against $\Delta S°$, a linear relationship is observed ($R^2 = 0.96$). The slope of such a graph is often referred to as a 'compensation temperature' or 'isoequilibrium temperature', and is denoted by T_c. The value of T_c determined for the amine complexes with hydroxypropyl-β-cyclodextrin is 276 K. The corresponding value determined for complexes with β-cyclodextrin is 245 K ($R^2 = 0.98$). Such correlations have been taken to imply a common interaction mechanism [14]. Because the compensation effect is found even when substrates have widely differing structures, it is considered more reasonable to relate the common mechanism to common

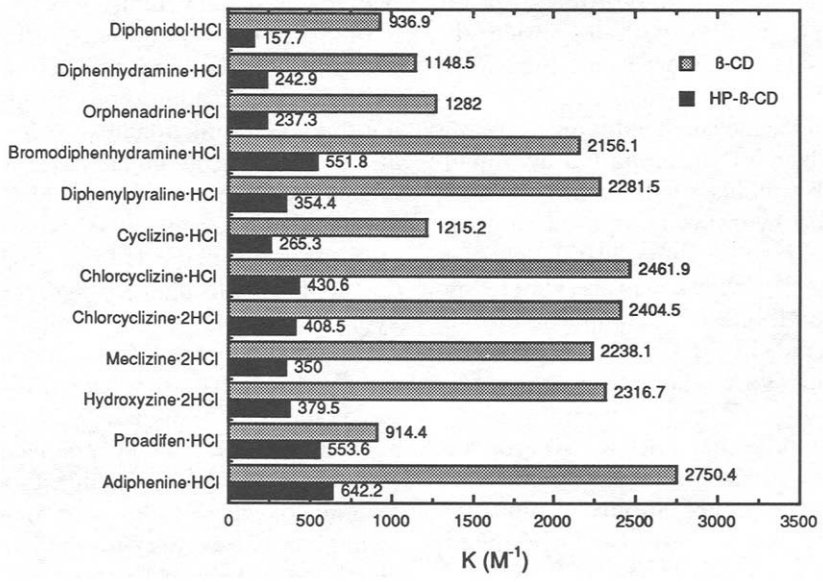

Figure 3
Comparison of the binding constants of amine compounds with β-CD and HP-β-CD from calorimetric titrations at 25°C.

attributes of these systems, i.e. the solvent and the host, rather than to the structure of the substrate.

The values of T_c determined are, within experimental error, consistent with the 250–320 K range [14] characteristic of processes dominated by solvation phenomena. Similar correlations in cyclodextrin complexes have been pointed out by several workers [15–18]. Some authors have stated that this effect is limited to highly ordered and hydrogen-bonded solvents, such as water. However, recently, it has been demonstrated [19] that a similar compensation effect is found in *N*,*N*-dimethylformamide, a solvent which can neither be regarded as a hydrogen bonding nor a highly ordered solvent.

Considering the structural features of the diphenylmethyl derivatives, these molecules can only partially fit into the CD cavity. The steric hindrance from another aromatic ring prohibits the phenyl group from penetrating into the cyclodextrin cavity very deeply. In the case of complexes formed with β-CD, the phenyl ring resides inside the cavity, in good contact with the wall of the cavity, as is indicated by the large binding constants and negative ΔH° values. When 2-hydroxypropyl substituents are introduced, as in the HP-β-CD molecule, the cavity is extended on both ends. The phenyl ring would most probably not be able to make contact with the cavity as well as in the case of β-CD because the guest molecule simply cannot penetrate the cavity as deeply owing to the presence of the substituents. If the aromatic group resides partially inside the cavity of the CD ring and partially inside the extended part of the cavity, relatively low binding constants and small enthalpy decreases are expected because of the partial hydrophilicity of the substituents. On the other hand, the polarity of the hydroxyl groups on the substituents should make the solvation changes accompanying the inclusion process more significant. Therefore, the complexation reactions between HP-β-CD and the diphenylmethyl derivatives are relatively more entropy-driven.

Substituents on either one of two aromatic rings of the diphenylmethyl functionality greatly affect the binding affinity of diphenylmethyl derivatives for HP-β-CD. Diphenhydramine·HCl and cyclizine·HCl are two basic compounds having no substituents on their aromatic rings. Orphenadrine·HCl and bromodiphenhydramine·HCl are two derivatives of diphenhydramine·HCl, whereas chlorcyclizine·2HCl, meclizine·2HCl and hydroxyzine·2HCl can be considered to be derivatives of cyclizine·HCl.

Bromodiphenhydramine·HCl forms more stable complexes with HP-β-CD than diphenhydramine·HCl. The bromo-substituent on the para-position permits the bromodiphenhydramine·HCl molecule to participate in stronger van der Waals interaction with the HP-β-CD, and also may increase the hydrophobicity of the molecule. Both of these effects result in enhanced binding. This result also implies that the substituted aromatic ring resides inside the cyclodextrin ring. When a methyl group is introduced at the ortho-position of one aromatic ring, as in the case of orphenadrine·HCl, no significant difference in the binding ability is found. It is possible that the substituent may not be in contact with the cavity because the extended part of the cavity may not necessarily be continuous due to partial substitution. Another possibility could be that the non-substituted aromatic ring resides inside the cyclodextrin cavity; therefore, substitution does not affect the binding. Similarly, when a chloro-substituent is on one of the aromatic rings, either on the ortho-position (as in chlorcyclizine) or the para-position (as in the other two compounds), stability constants are higher. An improved fit between the substituted aromatic ring and the HP-β-CD cavity may be responsible for these higher stability constants.

In most of the compounds studied, a hydrogen is on the aliphatic carbon of the diphenylmethyl group in addition to the group containing the amine functionality. A hydroxyl group at this position, as in the case of diphenidol, makes the complexes formed less stable (compared to the complexes of diphenhydramine·HCl and cyclizine·HCl). This might largely be due to the hydrophilicity and polarity of the hydroxyl groups.

Both adiphenine·HCl and proadifen·HCl form more stable complexes than the rest of the compounds studied. Hydrogen bonding between the guest and host molecules might be responsible for this enhanced binding. The carbonyl oxygens on the guest molecules might form hydrogen bonds with the hydroxyl group of the hydroxypropyl substituents instead of the hydroxyl group on the rim because of the steric hindrance discussed in the case of β-CD

[10]. The relatively stronger binding for adiphenine·HCl indicates that the presence of the propyl group may have a negative steric effect on the binding, even if it apparently does not entirely prevent the formation of a hydrogen bond.

Further evidence of hydrogen bonding comes from the FTIR spectroscopic study. The strong absorption peak at 1738.5 cm^{-1} for adiphenine·HCl (Fig. 4, A), and at 1730.4 cm^{-1} for proadifen·HCl (Fig. 5, A) is the characteristic peak for carbonyl stretching. HP-β-CD alone does not exhibit absorption in this carbonyl stretching region. The FTIR spectra of HP-β-CD complexes with adiphenine·HCl and proadifen·HCl prepared using a freeze-drying method exhibit a shift in the carbonyl stretching region to higher frequencies (1784 and 1736.1 cm^{-1}, respectively) as illustrated in Figs 4 and 5. These shifts are not found in the spectra of the corresponding physical mixtures (Figs 4 and 5, B).

Of the 12 compounds studied, only the terfenadine·HCl complex with HP-β-CD has 1:2 (compound:CD) stoichiometry. For each substrate, $\Delta H°$ is more favourable and $\Delta S°$ is less favourable for the 1:2 complex than for the 1:1 complex. The $\Delta S°$ effect (an unfavourable entropy change) arises from ternary versus

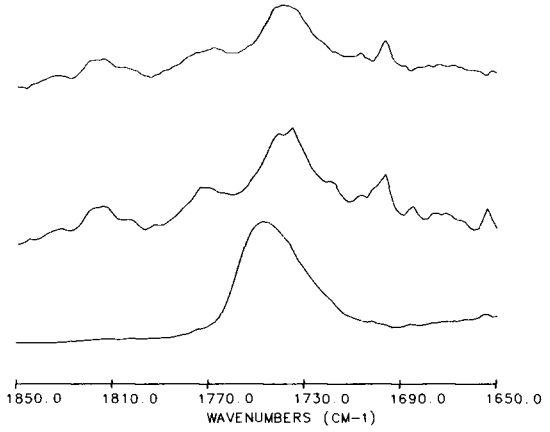

Figure 4
FTIR spectra for the adiphenine·HCl–HP-β-CD system. (A) adiphenine·HCl alone, (B) 1:1 physical mixture, (C) 1:1 complex.

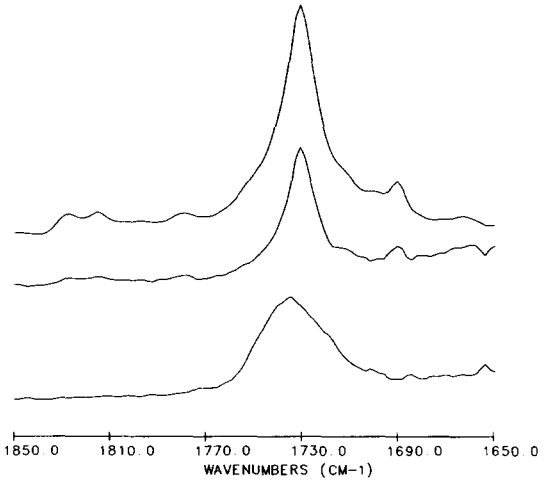

Figure 5
FTIR spectra for the proadifen·HCl–HP-β-CD system. (A) proadifen·HCl alone, (B) 1:1 physical mixture, (C) 1:1 complex.

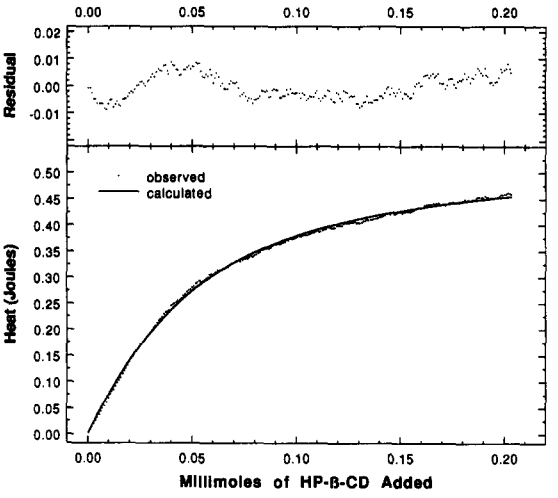

Figure 6
Calorimetric titration curve and fitted results from fitting the 1:1 model to the data for the titration of HP-β-CD into terfenadine·HCl solution at 25°C. (Initial concentration of terfenadine·HCl = 0.90 mM.)

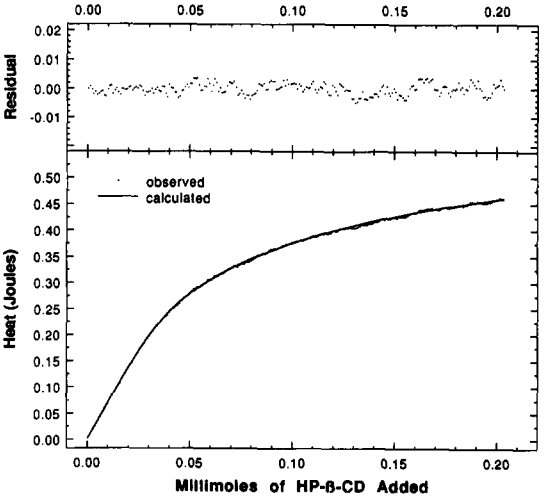

Figure 7
Calorimetric titration curve and fitted results from fitting the 1:2 stepwise-association model to the same set of data as in Fig. 6 for the titration of HP-β-CD into terfenadine·HCl solution at 25°C. (Initial concentration of terfenadine·HCl = 0.90 mM.)

binary complex formation. The evidence for this comes from a comparison of the calorimetric titration results from fitting different models to the same set of data. When the 1:1 model is fitted to the data, a systematic deviation as shown in the residual plot (Fig. 6) is obvious. Only a more complex stepwise-association model; the formation of both 1:1 and 1:2 complexes, can account for the calorimetric titration results. In these calculations, all four quantities ($\Delta H°$s and Ks) were allowed to vary simultaneously. An example of the fit between the calculated values and experimental values is shown in Fig. 7.

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