Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, No. 4, pp. 399–406, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00
Pergamon Press plc

Microcalorimetric and chromatographic investigations of the binding of some pyridine derivatives to cyclodextrins

S. EL GEZAWI,* N. OMAR,* N. EL RABBAT,* H. UEDA† and J. H. PERRIN‡

* *Department of Pharmacy, University of Assiut, Assiut, Egypt*
† *Department of Pharmacy, Hoshi University, Tokyo, Japan*
‡ *College of Pharmacy, University of Florida, Gainesville, FL, USA*

Abstract: The binding of some pyridine derivatives to α-, β- and γ-cyclodextrins has been investigated by microcalorimetry. The strongest binding is to β-cyclodextrin, but the binding constants are of the order 10^2 M^{-1}. The binding to β-cyclodextrin was also investigated by high performance liquid chromatography. The addition of β-cyclodextrin to the mobile phase allowed separation of molecules with similar binding constants and of racemates in the case of tropicamide.

Keywords: *Microcalorimetry; cyclodextrins; HPLC; pyridine derivatives.*

Introduction

As a preliminary to investigating the use of a column having cyclodextrin as a stationary phase in the high performance chromatographic analysis (HPLC) of some pyridine derivatives it was decided to investigate the complex formation between some cycloamyloses and the drugs by microcalorimetry, and HPLC. The pyridine nucleus is included in drugs of widely different pharmacological action, including antihistamines, antiinflammatories, vitamins and drugs with psychotropic and cholinesterase–reactivator activity. Several pyridine derivatives are also potent antimicrobial agents. The advantage of a microcalorimetric investigation is that the data gives equilibrium constants, enthalpies and entropies from experiments conducted at a single temperature, with the only limitation that reaction occurs with a heat change [1]. No special spectroscopic properties of the guest molecule are required. If the drug binds to cyclodextrins, then addition of the cycloamyloses to the mobile phase should shorten the retention time which is dependent upon the binding constant for the drug–cyclodextrin complex in the prevalant conditions of the mobile phase. The three commonly available cyclodextrins α, β and γ with cavity diameters of 4.5, 7.5 and 9.5 A were used in the investigations.

Experimental

Materials

Pheniramine maleate, methapyrilene hydrochloride, niflumic acid from Sigma, St. Louis, MO, pyrilamine maleate and triprolidine hydrochloride from Chemical Dynamics, South Plainfield, NJ, nicotinic acid and methyl nicotinate from Kodak, Rochester, NY, thenyldiamine hydrochloride from Sterling Winthrop, Renselaar, NY, sulphapyridine and its sodium salt from Fisher Fair Lawn, NJ, piroxicam and niclamide from Pfizer Groton CT, pralidoxime chloride from Ayerst, Rouses Point, NY, iproniazide phosphate from Hoffman La Roche, Nutley, NJ, tropicamide from Alcon Fort Worth, TX, were all used as supplied by the manufacturers. The Zorbax 300 SCX, for cation exchange and Zorbax SAX, for anion exchange and chromatographic supports were obtained from Dupont Wilmington DE. HPLC grade methanol was obtained from Fisher, Fairlawn, NJ and deionized water was used throughout.

Methods

All microcalorimetric measurements were made in a LKB flow Microcalorimeter Model 2107–121 (LKB, Bromma, Sweden) equipped with LKB 10200 peristaltic pumps and a LKB 2550 chart recorder. Amplification of the microvolt signal was achieved by a Keithley 150 B microvolt ammeter (Keithley, Cleveland, OH). A Model 1005 water bath with a PTC 40 temperature controller (Tronac Inc. Orem, UT) capable of giving a short term temperature stability of ±0.002°C was used to enclose the calorimeter. The calorimeter was calibrated electrically. All measurements were made at 25°C and results calculated as previously described [1] using an iterative least squares technique to estimate the binding constant, K, from measurements of voltages at given reactant ratios, and the maximum voltage output for a given reaction, μV_{max}.

The heat of reaction per mole of complex, H joules mole^{-1}, is then given by

$$H = \mu V_{max} \times \frac{1}{0.0632} \times \frac{1}{\text{total flow rate}}$$

0.0632 is the calibration constant (W V^{-1}) and the flow rate is in moles s^{-1}. The free energy change, ΔG, and the entropy change, ΔS, are calculated by the classical relationship.

$$\Delta G = -RT\ln K \text{ and } \Delta S = \frac{\Delta H - \Delta G}{T}$$

ΔS is expressed on the unitary scale. The effect of the addition of β-cyclodextrin to the mobile phase was observed using a model 5000 (Varian, Monrovia, CA) liquid chromatograph with a 757 spectrophotometric detector (Kratos, Ramsey, NJ) set at 254 nm, and a 3392A integrator, Hewlett–Packard, Avondale, PA. A stainless steel column (15 cm × 4.6 mm) was packed with the appropriate ion exchange pellicular support. The mobile phase of pH 6.0 and ionic strength 0.2 contained $Na_2H_2PO_4$. Concentrations of β-cyclodextrin of 0.881–8.81 × 10^{-3} M were used in this buffer. 20 μl of solutions in methanol or phosphate buffers containing 2.0 × 10^{-5} M drug were injected onto the column at 25°C. Flow rates, of 1.0–1.8 ml min^{-1} were used from measurements of the effect of cyclodextrin on the retention time. The 1:1 binding

constants for the drug cyclodextrin complexes were calculated from the retention times as outlined below by the method of Uekama *et al.* [2].

It can be shown that

$$T_{obs} = \frac{T_o + T_c K\,(CyD)}{1 + K\,(CyD)} \tag{1}$$

where T_{obs} = retention time of the drug at a given concentration of cyclodextrin (CyD) in the mobile phase. T_o is the retention time of the drug. T_c is the retention time of the complex. K is the binding constant. This equation is satisfied by any concentration of cyclodextrin, if two concentrations of cyclodextrin $(CyD)_1$ and $(CyD)_2$ are considered the equation 1 can be rearranged to give

$$\frac{(CyD)}{T_{obs}} = \frac{K\,(CyD)_1 + 1}{T_o - T_c} \cdot (CyD)_2 + \frac{K\,(CyD) + 1}{K\,(T_o - T_c)} \tag{2}$$

where T_{obs} is the difference in the retention times at the two cyclodextrin concentrations of difference (CyD).

If $(CyD)_1$ is held constant, then a graph of $(CyD)/T_{obs}$ against $(CyD)_2$ is linear and K can be obtained from the slope to intercept ratio.

Results and Discussion

More of the drugs react with β- rather than α-cyclodextrin, and only two, niflumic acid and piroxicam gave measurable heats of reaction with γ-cyclodextrin. Figures 1, 2 and 3 show heat fluxes, measured as μV as function of α- and β-cyclodextrin concentrations for the reactions with the drug concentrations fixed. Solubilities and extent of reaction were such that sufficient concentrations of cyclodextrins could be added to complex all of the drugs involved. Tables 1 and 2 give the thermodynamic parameters derived from the data of Figs 1, 2 and 3. All the data are consistent with 1:1 complex formation, as is confirmed by the Jobs Plot [3] for the pyrilamine maleate–β-CyD interaction shown in Fig. 4. In general the pyridine derivatives seem to be weakly bound to the cyclodextrins, the highest affinity constants observed being less than 10^3.

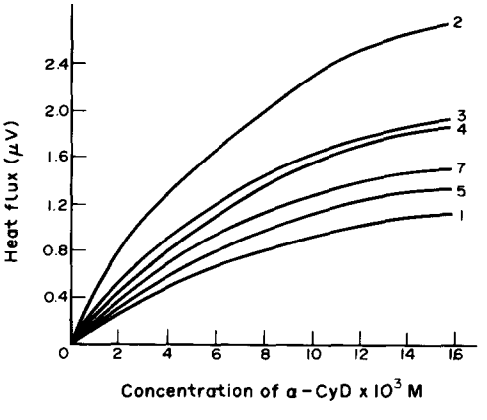

Figure 1
Heat flux as a function of α-cyclodextrin concentration with the drug concentration fixed. Computer generated curves. (1), Pheniramine maleate; (2), pyrilamine maleate; (3), thenyldiamine HCl; (4), methapyrilene HCl; (5), triprolidine HCl and (7), piroxicam.

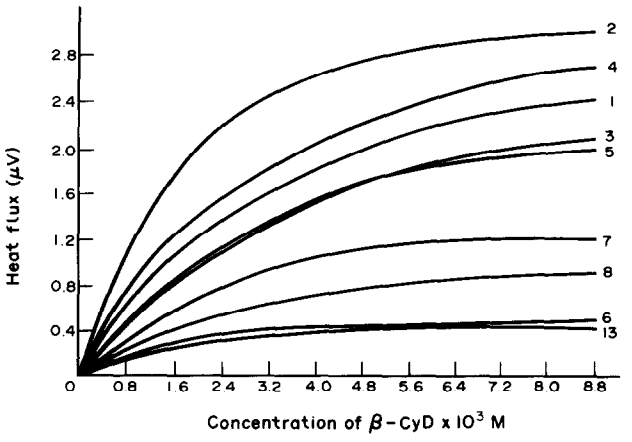

Figure 2
Heat flux as a function of β-cyclodextrin concentration with the drug concentration fixed. Computer generated curves. (1), Pheniramine maleate; (2), pyrilamine maleate; (3), thenyldiamine HCl; (4), methapyrilene HCl; (5), triprolidine HCl; (6), sulphapyridine; (7), piroxicam; (8), niflumic acid and (13), tropicamide.

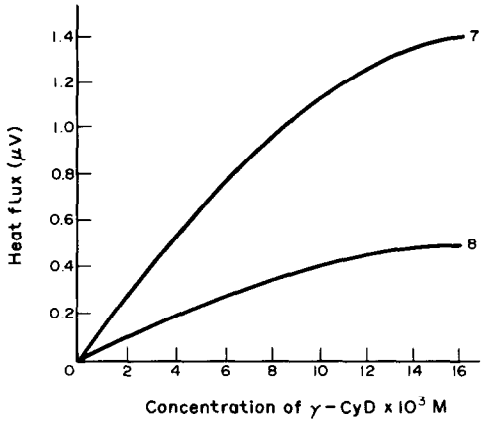

Figure 3
Heat flux as a function of γ-cyclodextrin concentration with the drug concentration fixed. Computer generated curves. (7), Piroxicam and (8), niflumic acid.

Table 1
Derived thermodynamic parameters for the interaction between pyridine derivatives and β-cyclodextrin at 25°C and pH 7.0 from microcalorimetry. Ks are from HPLC at pH 6.0

Compounds	K mol l^{-1}	ΔH J mol l^{-1}	ΔG J mol l^{-1}	ΔS e.u.	K^1
Pyrilamine maleate	731	−15300	−26200	+37	223
Niflumic acid	520	−19000	−25400	+49	1131
Sulphapyridine	460	−33900	−25100	−29	619
Tropicamide	430	−25000	−25000	−0.19	384,360
Methapyriline HCl	353	−15500	−24500	+30	323
Pheniramine maleate	310	−14600	−24200	+32	161
Triprolidine HCl	265	−13800	−23800	+34	347
Thenyldiamine HCl	202	−14800	−23100	+28	324
Piroxicam	94	−10500	−21200	+36	173
Niamide	—	—	—	—	162

Table 2
Derived thermodynamic parameters from the interaction between some pyridine derivatives and α- and γ-cyclodextrins at 25°C and pH 7.0

Compounds	K mol l^{-1}	ΔH J mol l^{-1}	ΔG J mol l^{-1}	ΔS e.u.
Pyrilamine maleate (α)	118	−19100	−21800	+9
Thenyldiamine HCl (α)	105	−13900	−21500	+25
Methapyrilene HCl (α)	71	−16000	−20500	+15
Pheniramine maleate (α)	68	−9720	−20400	+36
Triprolidine HCl (α)	56	−14300	−19900	+19
Piroxicam (α)	48	−15700	−19500	+13
Piroxicam (γ)	52	14300	−19700	+18
Niflumic acid (γ)	273	6360	−23800	+59

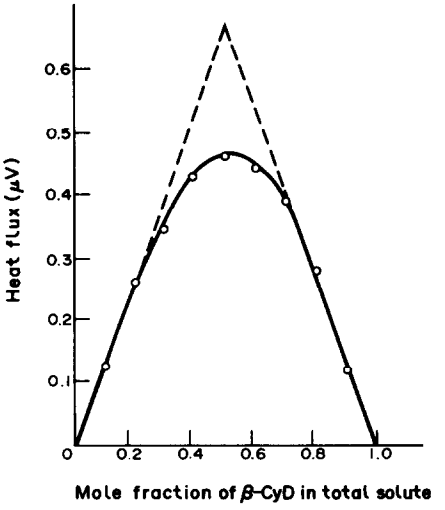

Figure 4
Job's plot for pyrilamine maleate β-cyclodextrin interaction.

The cavity of the β-cyclodextrin molecule seems more suited to complexing the pyridine derivatives than the larger cavity of the γ-cyclodextrin. This phenomenon has frequently been observed for molecules having aromatic rings. It is of interest to note the lower affinity of niflumic acid for β- and α-cyclodextrin than that of flufenamic acid. The replacement of an aromatic ring by the pyridine ring greatly lowers the affinity of the drug for the cyclodextrins. Nicotinic acid and methyl nicotinate, with the small substituents in the 3 position did not give a measurable heat of reaction with any of the cyclodextrins. Nicotinic acid is fully ionised at pH 7.0 and the affinity of benzoic acid for β-cyclodextrin is very low when ionised. Iproniazide phosphate and nialamide did not give any heat of reaction with the three cyclodextrins, but another drug tropicamide also substituted in the 4 position reacted with cyclodextrin. The substituents on the amide group apparently give enough bulk to stop the molecule passing through the cavity of the β-cyclodextrin. All of the pyridines substituted in the 2 position reacted with β-cyclodextrin, except pralidoxime chloride. Pralidoxime has the smallest substituent in the 2 position, whilst piroxicam has the largest substituent, but a low binding constant. As has been the case in previous studies heat is evolved on reaction between the drugs and the cyclodextrins and most of the complexes are formed with significant gains in entropy

[1] with the exceptions of tropicamide and to a larger extent sulphapyridine. In these cases the interaction is dominated by enthalpic contributions. In general β-cyclodextrin is a better host for the pyridine derivatives investigated than the α- or γ-cyclodextrins, apparently the cavity has more suitable dimensions for formation of the inclusion complexes. There is no evidence, in the current investigations, showing the pyridine ring to be enclosed within the cyclodextrin cavity. Indeed all the molecules shown to react contain a second ring structure, usually aromatic in nature which could also enter the cavity. Although the side chains alter the detail of the interactions, it is likely that changes in the behaviour of water following complex formation can explain the observed thermodynamic parameters. These changes include breakdown of water structure within the cavity, removal of water from the cavity, restructuring of water around the guest molecule following reaction and release of water into the bulk following reaction. Other contributions to the overall energies of reaction include that due to the restriction in rotation around the glycosidal linkages of the cyclodextrin when the guest molecule penetrates the cavity [4]. Although the positive entropies suggest significant contributions from the redistribution of water, the large enthalpies are consistent with dipole interactions and hydrogen bond formation. As with other thermodynamic investigations of cyclodextrin complexes the free energies change very little for the series of related compounds [1]. The changes in ΔH are almost compensated for by changes in the corresponding ΔS values. The data fits the following equations

$$\Delta G = \Delta H - 303.6\ (\Delta S)\ r = 0.9630;\ n = 6 \text{ for } \alpha\text{-cyclodextrin}$$

$$\Delta G = \Delta H - 309.8\ (\Delta S)\ r = 0.9805;\ n = 9 \text{ for } \beta\text{-cyclodextrin.}$$

These compensatory temperatures are consistent with the strong influence water has on the thermodynamics of the reaction. Table 3 shows the effect of pH on the 1:1 binding constant for the pyrilamine maleate–β-cyclodextrin complex. The binding constant increases as the pH increases, due to the decreased ionisation of the pyrilamine. Drugs are usually bound more strongly to the cyclodextrins when unionised and when the water solubility is a minimum. For the chromatographic experiments, only the β-cyclodextrin was used in the mobile phase because it had the highest affinity for the drugs.

Table 3
Relationship between binding constants and pH for the pyrilamine maleate–β-cyclodextrin complex at 25°C

pH	K mol l^{-1}
3.0	*
3.5	*
4.0	177
5.0	223
5.5	283
6.0	564
6.5	617
7.0	731
8.0	508

* There is no observed interaction.

Preliminary experiments showed that pH 6.0 was a suitable pH for removal of the drugs from the ion exchange columns. Increasing the concentration of cyclodextrin, in the mobile phase shortened the retention time of all the drugs, and allowed 1:1 binding constants to be calculated as outlined in the method section.

Typical data is shown for sulphapyridine in Fig. 5. The binding constants, for sulphapyridine, tropicamide and niamide, shown in Table 1, were obtained using the cation exchange column, the other drugs using the anionic packing. The phosphate buffer alone, gave extremely long retention times, but the addition of low concentrations of β-cyclodextrin significantly shortened the retention time. Figure 6 shows the effect of cyclodextrin concentration on the retention of piroxicam (a) and tropicamide (b). Tropicamide is a racemic mixture, the addition of cyclodextrin clearly separates the two epimers. The binding constants obtained from this chromatographic data are 360 M^{-1}

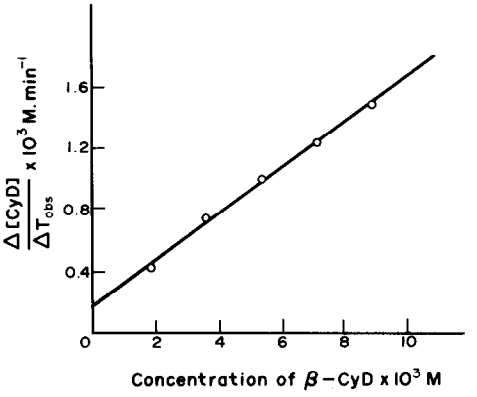

Figure 5
Graphical representation of the effect of cyclodextrin on the retention time of sulphapyridine according to equation 2.

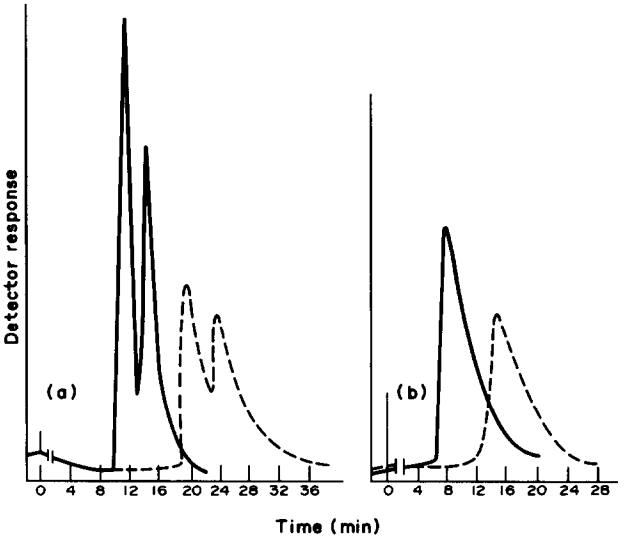

Figure 6
(a) Liquid chromatograms of 2 × 10^{-5}M tropicamide solution on cation exchange column. See Experimental for conditions. (b) Liquid chromatograms of 2 × 10^{-5}M piroxicam solution on anion exchange column. See Experimental for conditions. β-cyclodextrin [10^{-3}M]: (----------) 0.881 (———) 8.81.

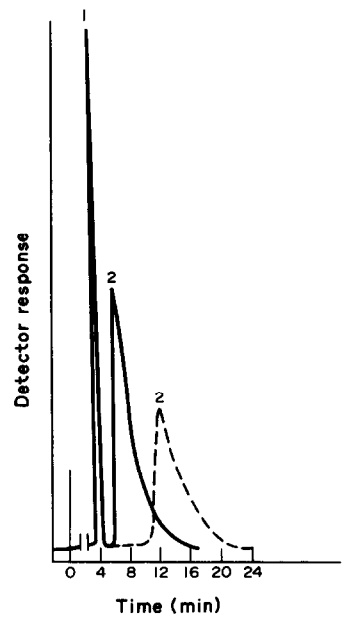

Figure 7
Liquid chromatograms of 2×10^{-5}M pyrilamine maleate solution on anion exchange column. See Experimental for conditions. β-cyclodextrin [10^{-3}M]: (----------) 0.881 (———) 8.81. Peak 1: maleate anion. Peak 2: pyrilamine base.

and 384 M^{-1}, i.e. a small difference, yet the addition of β-cyclodextrin to the mobile phase clearly allows separation, especially at high concentrations of cyclodextrin. Other separations of racemates using cyclodextrins have been reported in the literature [5]. Elution of the drugs from the anion column was practically impossible in the phosphate buffer, except in the case of the maleate salts of pheniramine and pyrilamine. Both of these drugs gave a single peak of retention time about 3.5 min, this peak had the same retention time as maleic acid. The position of this peak did not change on the addition of β-cyclodextrin to the mobile phase, however other peaks appeared on the addition of β-cyclodextrin. These peaks were dependent upon the concentration of cyclodextrin (Fig. 7), so allowing the binding constants of pheniramine and pyrilamine to be determined. The binding constant for the niamide–β-cyclodextrin interaction was determined chromatographically to be 162 M^{-1}, but little or no heat was detected in the microcalorimetric investigations and hence no constant could be determined by microcalorimetry. Although the binding constants of these pyridine derivatives to β-cyclodextrin are only of the order of 10^2 M^{-1}, separation and quantitative analysis of the pyridine derivatives can be effected readily under the given chromatographic conditions.

Acknowledgement — This work is taken from the Ph.D. thesis (Assiut Egypt 1986) of S.E.G. She would like to thank Amideast, Washington, USA for financial support.

References

[1] G. E. Hardee, M. Otagiri and J. H. Perrin, *Acta Pharm. Suec.* **15**, 188–199 (1978).
[2] K. Uekama, F. Hirayama, S. Nasu, M. Matsuo and T. Irie, *Chem. Pharm. Bull.* **26**, 3477–3484 (1978).
[3] P. Job, *Ann Chim.* **9**, 113 (1928).
[4] R. J. Bergeron, D. M. Pillor, G. Gibeily and W. P. Roberts, *Bioorg. Chem.* **7**, 263–271 (1978).
[5] J. Debowski, D. Sybilska and J. Jurczak, *J. Chromat.* **237**, 303–306 (1982).

[*Received for review 16 June 1987*]