Determination of Association Constants of β-Cyclodextrin and β-Cyclodextrin-N-succinamate with Drugs by High Performance Liquid Chromatography

ALAMDAR ASHNAGAR*, CHRISTOPHER J. EASTON† and NAHID GHARIB NASERI
Department of Medicinal Chemistry, School of Pharmacy
Ahwaz University of Medical Sciences, Iran.
E-mail: aashnagar2003@yahoo.com
Tel.: 0611-3340988; Fax.: 0611-3342197

Investigation of the Hummel and Dreyer method for the estimation of stability constants by high performance liquid chromatography (HPLC) is described. Comparison of the results obtained for binding of cimetidine (**2a**) and 2-(*p*-tolyl)ethylamine (**2b**) to β-cyclodextrin(1a) and β-cyclodextrin-N-succinamate (1b) by ^{1}H NMR (300 MHz) spectroscopy and the HPLC method were studied. The results indicated that the stability constants by HPLC method are higher and more accurate, and are 431 and 470 dm^3 mol^{-1} for (**2a**) and 138 and 111 dm^3 mol^{-1} for (**2b**) in binding to (**1a**) and (**1b**), respectively.

Key Words: Association constants, β-Cyclodextrin, β-Cyclodextrin-N-succinamate, High performance liquid chromatography.

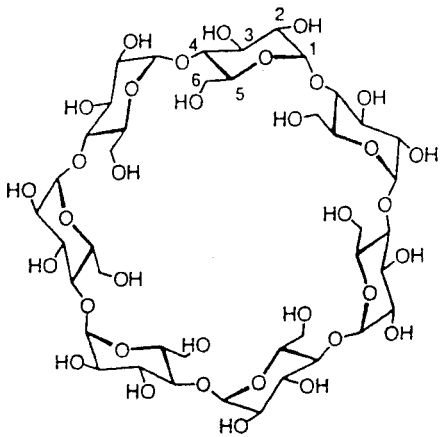
INTRODUCTION

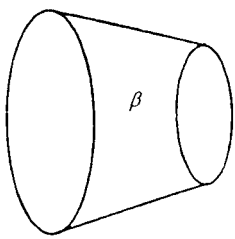
Monitoring the inclusion complexation of β-cyclodextrin and modified β-cyclodextrin with various types of guests using ^{1}H NMR spectroscopy is relatively slow and requires expensive equipment. On the other hand, high performance liquid chromatography (HPLC) has been reported as an alternative method for studying inclusion complexes[1-3]. This technique involves the injection of a small amount of the cyclodextrin onto an HPLC column which holds a mobile phase containing known concentration of the guest/wing studied. By measuring the amount of guest carried through the column by the cyclodextrin and repeating the experiment at various concentrations of guest, the stability constant of the complex can be obtained (**Scheme-1**). The procedure is relatively fast and the equipment is relatively inexpensive. The interactions of cimetidine (**2a**) and 2-(*p*-tolyl)ethylamine (**2b**) with β-cyclodextrin (**1a**) and β-cyclodextrin–N–succinamate (**1b**) were investigated using a modification of an HPLC method reported by Hummel and Dreyer[1].

†Research School of Chemistry (RSC), Australian National University (ANU), Canberra, ACT 200, Australia.

EXPERIMENTAL

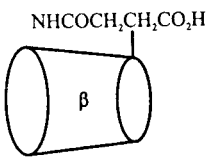
The columns (25 cm, L) were packed with commercial Lichrosorb dial (10 μm particle diameter and 100 Å pore diameter support, Merck). Water could be used as a mobile phase for the dial columns. This is particularly important because stability constants for cyclodextrin-drug complex must be measured in water.



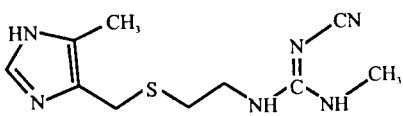


β-Cyclodextrin (**1a**)

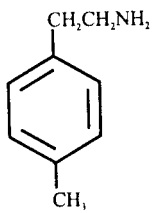
A truncated cone is often used to represent the torus of a cyclodextrin. A substituent drawn at the narrow end of the cone indicates that it replaces one of the C6 hydroxy groups in the cyclodextrin, while a substituent drawn at the wide end of the cone indicates that it replaces either a C2 or C3 hydroxy group.



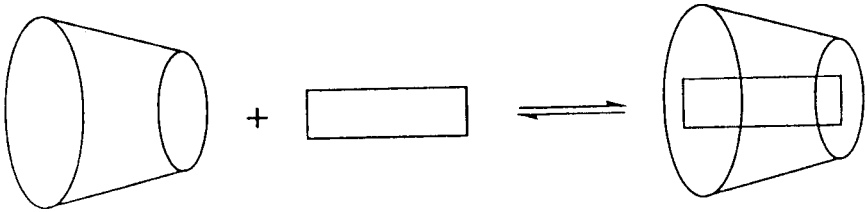
β-Cyclodextrin -N-succinamate (**1b**)



Cimetidine (**2a**)



2-(*p*-tolyl)ethylamine (**2b**)



Cyclodextrin Host Guest Inclusion Complex

Scheme-1

$$\text{Inclusion complex association constant} = K = \frac{[\text{Inclusion complex}]}{[\text{Cyclodextrin host}]\cdot[\text{Guest}]}$$

The diol columns also allow adequate separation of the guests (**2a** and **2b**) and the host cyclodextrins (**1a** and **1b**). Binding of the cyclodextrins to the column is quite low and it passes out almost within the void volume of the column, unlike the guests (**2a** and **2b**).

A model 410 (Waters Association) difference refractometer was used for detecting β-cyclodextrin and its derivative. A model 486 variable wavelength UV detector (Waters Association) was used for drug monitoring. A model 717 plus autosampler (Waters Association) was used for injection. Model 510 pumps (Waters Association) were used for delivering the running solvents.

A. A stock solution of the guest (**2a/2b**) in 0.1 molar phosphate buffer (pD 6.8) was prepared and diluted with D_2O (Table-1), enabling solution with concentrations ranging from 10^{-6}–10^{-4} molar to be available for use as the running solvents.

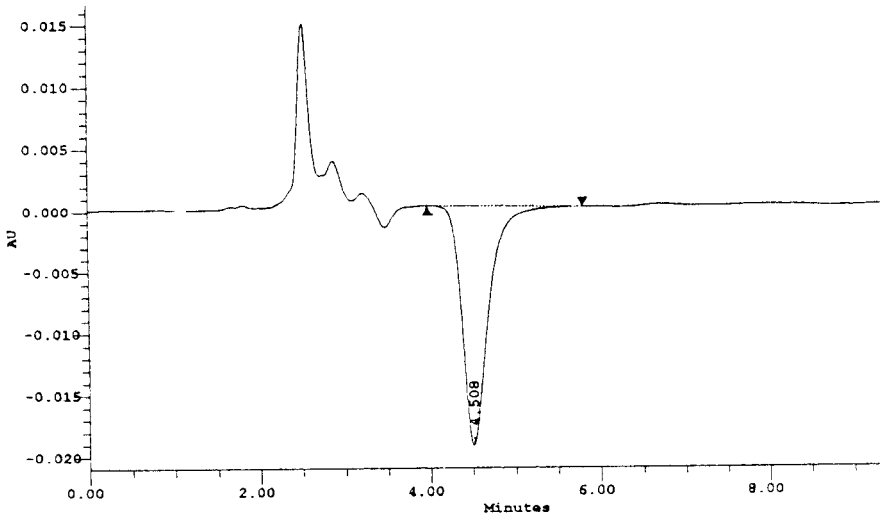
TABLE-1
SOLUTIONS OF THE GUEST

No.	Volume of stock solution taken (mL)	Final volume of diluted stock solution (mL)	Molarity of the diluted stock solution (L)
1.	2	250	8×10^{-6} M
2.	5	250	2×10^{-5} M
3.	10	250	4×10^{-5} M
4.	25	250	1×10^{-4} M
5.	50	250	2×10^{-4} M
6.	100	250	4×10^{-4} M
7.	50	1000	5×10^{-5} M = 50×10^{-6} M

B. Following equilibration of the column with each of the running solvents, samples of pure phosphate buffer were injected (3×50 μL) onto the column and changes in the steady state concentration of the solution were monitored by a UV detector. On injection of pure buffer solution a negative peak was observed at the retention time of the guest [cimetidine (**2a**) and 2-(*p*-tolyl)ethylamine (**2b**)]. Based on the above results, the number of (**2a/2b**) displaced or bound per unit area were calculated.

C. On injection of a sample of known concentration of the host [β-cyclodexthrin or β-CD (**1a**) and β-cyclodextrin-N-succinamate or β-CDNSC (**1b**)] in phosphate buffer (Table-1) at each concentration of the guests in the running solvents, an increase in the size of the negative peak was observed. This was consistent with the guests (**2a/2b**) having been removed from the solution (in the column) by complex formation. In addition a positive peak near the void volume of the column was noted.

This was attributed to the guest (**2a/2b**) forming a UV active complex with the hosts (**1a/1b**) that did not interact with the column to a great extent. A typical HPLC trace is shown in Fig. 1. The area of the negative peaks increased regularly as the concentration of the guests (**2a/2b**) in the running solvents was increased.



PEAK RESULTS

S.No.	Ret. Time (min)	Area (uV * sec)	Height (uV)
1	4.058	390876	-19462

Fig. 1. Hummel and Dreyer chromatogram obtained from inclusion complexation measurement of cimetidine (**2a**) eith β-cyclodextrin (**1a**).

By calculating the increase in the size of the negative peak over the size of the peak when the pure buffer was injected, the amount of the guest (**2a/2b**) removed in the form of complex was determined. Further manipulation of the results gave r, the molar ratio of guest bound to the host present. The process was repeated with increasing concentrations of the guest in the running solvent (Table-2). The resulting values of r were plotted against r/[*l*] , where [*l*] is the initial concentration of the guest, to obtain a Scatchard plot[4]. The slope and intercept, gave the stability constant of cimetidine (**2a**) in β-CD (**1a**) and β-CDNSC (**1b**) 431 and 470 dm^3 mol^{-1} and 2-(*p*-tolyl)ethylamine (**2b**) in β-CD (**1a**) and β-CDNSC (**1b**) 138 and 111 dm^3 mol^{-1}, respectively (Table-3).

TABLE-2
CALCULATION OF HPLC RESULTS

1	2	3	4	5	6	7	8	9	10
Sample	Average of Integrations (from HPLC trace)	Difference between the average integrations of each host from the pure buffer	No. of moles of the host $\times 10^{-3}$	Concentration of the host (Molar)	Volume injected	No. of moles of guest/host injected n = M.V. = C.V.	Moles of guest bound = No. of moles of guest displaced	Moles bound r = Moles of the host injected r = Binding ratio	r/[L]
Pure buffer	207766	–	–	–	50 μL = 50×10^{-6} L	[L] = M = C 4×10^{-10}	–	–	–
β-CD (1a)	387222	179456	22.70/1135 $= 2 \times 10^{-5}$	2×10^{-3} M	50 μL = 50×10^{-6} L	1×10^{-7}	3.45×10^{-10}	$3.45 \times 10^{-10}/1 \times 10^{-7}$ $= 3.45 \times 10^{-3}$	$3.45 \times 10^{-3}/8 \times 10^{-6}$ = 431.25
β-CD NSC (1b)	403601	195835	24.68/1234 $= 2 \times 10^{-5}$	2×10^{-3} M	50 μL = 50×10^{-6} L	1×10^{-7}	3.76×10^{-10}	3.76×10^{-3}	$3.76 \times 10^{-3}/8 \times 10^{-6}$ = 460

[L] = 8×10^{-6} M — Initial concentration of the running solvent.

Calculation of column (8), *i.e.*, [Complex]: Moles of the guest bond = (mole/unit area) × difference in areas (column 3) (*i.e.*, the no. of moles of the guest displaced)

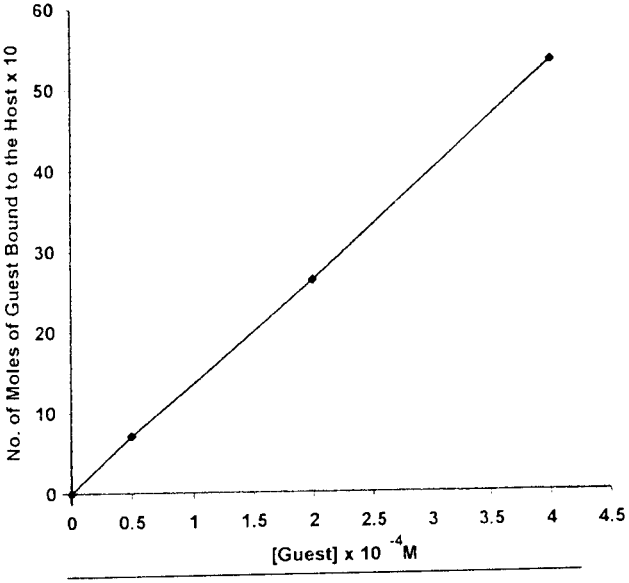
Mole/Unit area (for the buffer solution) = No. of moles of pure buffer (Column 7)/Area of the negative peak (average) (Column 2)

Caculation of column (7) for the buffer: No. of moles of pure buffer = [L] × Volume injected = [Initial concn. of the running solvent] × 50 μL

TABLE-3
ASSOCIATION CONSTANTS

Host \ Guest	K (dm^3 mol^{-1})	
	Cimeditine	2-(*p*-tolyl) ethylamine
β-Cyclodextrin (β-CD) (**1a**)	431	138
β-Cyclodextrin-N-succinamate (β-CDNSC) (**1b**)	470	111

In this experiment the number of moles of the guest bound after each injection of the host was found to increase linearly with the concentration of the guest present in the running solvent (Figs. 2 and 3).

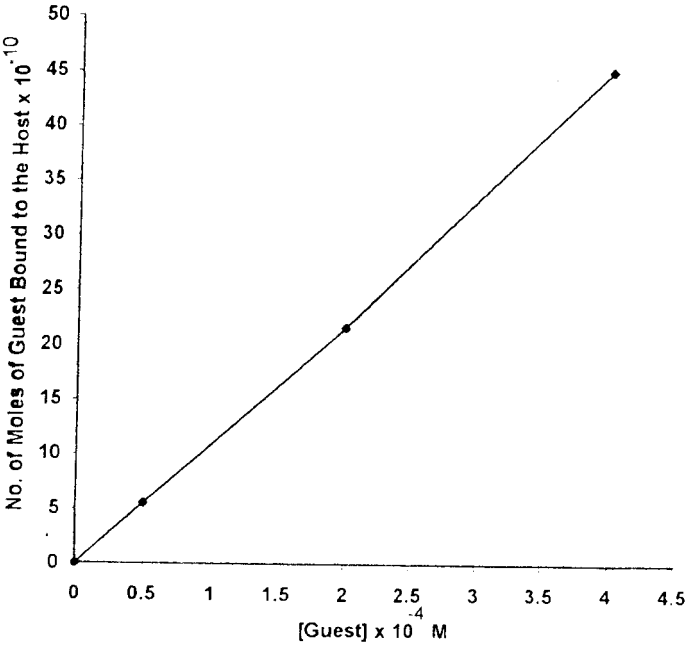


Conc.^{n} of guest × 10^{-10} M	No. of moles of guest bound to the host × 10^{-10}
0.5	7.07
2.0	26.20
4.0	53.20

Fig. 2. Moles of guest bound to the host *vs.* concentration of guest.

RESULTS AND DISCUSSION

β-CD (**1a**) and β-CDNSC (**1b**) are eluted at about the void volume of the column, as monitored by refractive index (RI) detection. It is easy to compare the binding capacity of the various host molecules to one drug from observation of the negative peak in Fig. 1. A known molar amount of injected β-CDNSC binds more molecules of cimetidine (**2a**) than β-CD, whereas β-CD binds more molecules of 2-(*p*-tolyl)ethylamine (**2b**) than β-CDNSC.



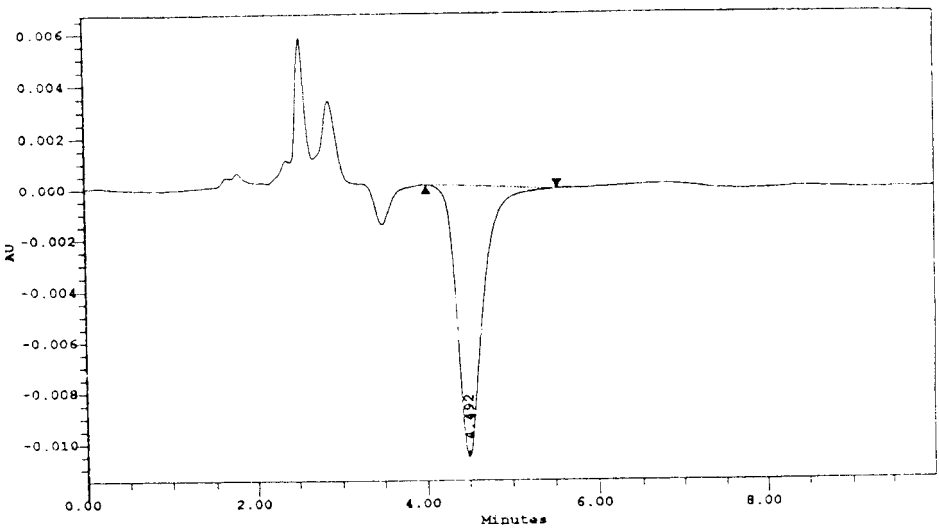
Conc.^{n} of guest $\times 10^{-10}$ M	No. of moles of guest bound to the host $\times 10^{-10}$
0.5	5.57
2.0	21.60
4.0	45.00

Fig. 3. Moles of guest bound to the host *vs.* concentration of guest.

The negative peak areas in Fig. 1 allow one to calculate according to a prior calibration obtained by injecting pure buffer (Fig. 4), the amount of the drug that has been complexed by the host molecule. Obviously, the area of the positive peak, where the host molecule and its inclusion complex emerge is not identical with that of the negative peak, due to the difference in UV molar absorption for the bound and unbound drug.

Cimetidine (**2a**) and 2-(*p*-tolyl)ethylamine (**2b**) are basic drugs with pK_b of 7.2 and $pK_b < 4$, respectively. They are positively charged under the experimental pH conditions. β-CDNSC has a greater binding capacity than β-CD for the guest (cimetidine).

The results indicate that there is attractive electrostatic interaction between oppositely charged hosts and guests and repulsive effects between identically charged couples. ^{1}HNMR results were not satisfactory and were much lower than the corresponding results obtained from HPLC.



PEAK RESULTS

S.No.	Ret. Time (min)	Area (uV * sec)	Height (uV)
1	4.492	211831	–10665

Fig. 4. HLPC trace of pure buffer.

REFERENCES

1. J.P. Hummel and W.J. Dreyer, *Biochem. Biophys. Acta*, **63**, 530 (1962).
2. B. Sebille, N. Thuaud, J. Piquion and N. Behar, *J. Chromatogr.*, **409**, 61 (1987).
3. N. Thuaud, B. Sebille, A. Derantani and G. Leliever, *J. Chromatogr.*, **503**, 453 (1990).
4. A. Croft, B.Sc. Thesis, University of Adelaide, Australia (1994).

(*Received*: 1 March 2004; *Accepted*: 27 July 2004) AJC-3510