Complexation Studies of Cyclodextrins with Tricyclic Antidepressants Using Ion-Selective Electrodes

Georgia N. Valsami,[1] Michael A. Koupparis,[2] and Panayotis E. Macheras[1,3]

Received April 22, 1991; accepted July 11, 1991

The complexation of six tricyclic antidepressant drugs [amitriptylin (AMN), nortriptylin (NRN), imipramin (IMN), doxepin (DXN), protriptylin (PTN), and maprotilin (MPN)] with α- and β-cyclodextrins (CDs) using ion-selective electrodes (ISEs) as drug ion sensors is described. Binding parameters were calculated by nonlinear fitting of the model described by the Scatchard equation, to the experimental data of a titration of a CD solution with the ion of interest. One binding site (the CD cavity) was found in all cases with both CDs. The calculated association constants at 25°C using CD concentrations in the range of 0.0100–0.0010 *M*, varied from 4.81×10^3 M^{-1} (MPN) to 23.9×10^3 M^{-1} (AMN) in the case of β-CD and from 50 M^{-1} (DXN) to 123 M^{-1} (MPN) in the case of α-CD. The precision for the estimation of the binding parameters was 0.1–5.0% (within-run RSD%) and 8–10% (between-run RSD%; $n = 3$). The complexation of the drugs with β-CD was also examined as a function of temperature in the range of 5–37°C; it was found to decrease by increasing temperature. Van't Hoff analysis gave good correlations ($r \geq 0.989$) for all drug ions studied. The estimates of the thermodynamic parameters indicate that the formation of inclusion complexes is enthalpy driven. A compensation plot based on the thermodynamic parameters ΔH and ΔS resulted in a linear relationship, which is indicative of a common type of force involved in the complexation of drugs to β-CD.

KEY WORDS: cyclodextrins; inclusion complexes; complex formation; tricyclic antidepressants; ion-selective electrodes.

INTRODUCTION

Cyclodextrins (CDs) are toroidally shaped oligosaccharides consisting of six to eight glucopyranose units connected with α(1→4) glycosidic bonds. The internal diameter of their relatively hydrophobic cavities, varying from 5.7 Å for α-CD to 7.8 Å for β-CD and 10 Å for γ-CD, allows inclusion-complex formation selectivity to a certain degree, based on the guest size and structure.

Interaction of drugs with cyclodextrins is of biopharmaceutical interest. When drugs are administered as cyclodextrin complexes, dissolution, absorption, and bioavailability may be altered (1,2).

Various experimental techniques have been developed for studying drug–cyclodextrin interactions (1–3). Recently, ion-selective electrodes (ISEs), which are electrochemical transducers responding selectively, directly, and continuously to the activity of the free ion in solution, have been applied to binding studies of drug ions with proteins (4,5) and cyclodextrins (6,7). The usefulness of ISEs in these studies is due to their ability to measure directly the activity (concentration when $C \leq 10^{-2}$ *M* or for constant ionic strength) of the free ion of interest in the presence of the macromolecule and the bound species. Hereafter when referring to CDs/drug interactions the terms "binding" and "inclusion-complex formation" are used synonymously.

In a previous work (7) the complexation of several ions (anions and cations) with α- and β-CD was studied using ISEs and was found to be driven by an exothermic enthalpy change, according to a "nonclassical hydrophobic interaction" (8). In this work the complexation of tricyclic antidepressants with CDs is studied in order (i) to explore the performance of ISEs in studying the complexation of a family of drugs to CDs, (ii) to elucidate the effect of the structural diversity of tricyclic antidepressants on the complexation to CDs, and (iii) to establish general relationships (if any), for the CDs/tricyclic antidepressants interaction. To this end the inclusion complex formation of six tricyclic antidepressants (Fig. 1), namely, amitriptylin (AMN), nortriptylin (NRN), doxepin (DXN), imipramin (IMN), protriptylin (PTN), and maprotilin (MPN), with α- and β-cyclodextrin was studied, using ISEs of either the PVC or the classic liquid ion-exchanger membrane type (9).

MATERIALS AND METHODS

Reagents. All reagents were of analytical reagent grade, and deionized water was used for aqueous solutions. Pure substances of the six drugs were obtained from local manufacturers. They were used without further purification for the preparation of standard solutions of the corresponding cations (0.0100–0.0500 *M*) containing either 0.020 *M* sodium sulfate or 0.060 *M* sodium chloride as ionic strength adjustor. Sodium tetraphenylborate (TPB), 2-nitrophenyloctyl ether (2-NPOE), and 1-isopropyl-4-nitrobenzene (*p*-nitrocumol) were from Fluka (Buchs, Switzerland). Polyvinyl chloride (PVC) of high molecular weight ($d = 1.385$) was from Janssen Chimica (Beerse, Belgium).

Liquid Ion Exchangers. The liquid ion exchangers were, for all drugs, their TPB salts in the appropriate organic solvent (selected after preliminary experiments) at a concentration of 0.0100 *M*. The organic solvent was either 2-NPOE (AMN, NRN, IMN) or *p*-nitrocumol (PTN), while for DXN and MPN a 1:1 mixture of 2-NPOE and *p*-nitrocumol was utilized. For the preparation of the PVC membranes the method introduced by Graggs *et al.* (10) was used.

Internal Reference Solutions. These solutions were 0.010 *M* for the measured ion in 0.10 *M* sodium chloride, saturated with silver chloride.

α- and β-Cyclodextrin Stock Solutions. These solutions were 0.010 *M* with respect to the CD, in either 0.020 *M* sodium sulfate or 0.060 *M* sodium chloride as ionic strength adjustors. More dilute solutions were prepared from the stock solutions by dilution in the same medium.

All standard solutions of the drugs studied were kept in amber bottles in the refrigerator and renewed every week.

Apparatus. The system used for the measurements, the

[1] Department of Pharmacy, University of Athens, 104 Solonos Street, Athens 106 80, Greece.
[2] Department of Chemistry, University of Athens, Panepistimiopolis, Kouponia 15771, Greece.
[3] To whom correspondence should be addressed.

0724-8741/92/0100-0094$06.50/0

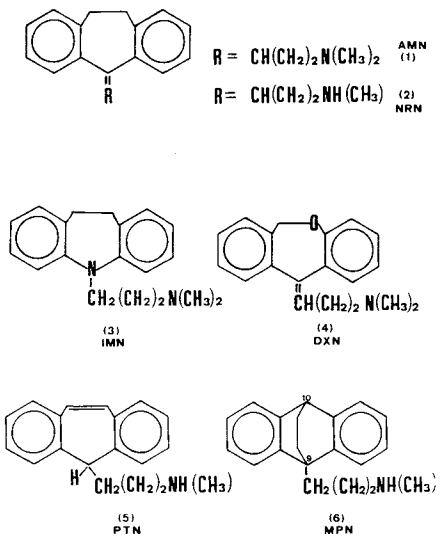

Fig. 1. Structures of the studied drugs. (1) Amitriptylin (AMN); (2) nortriptylin (NRN); (3) imipramin (IMN); (4) doxepin (DXN); (5) protriptylin (PTN); (6) maprotilin (MPN).

PVC (AMN, NRN, IMN, DXN, MPN), and the liquid ion-exchanger (PTN) membrane electrode assembly and external reference electrode were identical to those described previously (7).

Procedures. The procedures followed for the calibration curves and binding experiments were the same to those described previously (7).

Data Treatment. Scatchard model (11) for one class of binding sites (the CD cavity), described by Eq. (1), was fitted to the experimental data

$$B = \frac{nKF}{1 + KF} CD_t \qquad (1)$$

where B and F are the bound and free molar concentrations of the drug, respectively, n is the number of equal binding sites on the CD molecule (representing the stoichiometry of the complex), K is the intrinsic association constant, and CD_t is the total concentration of CD. After F was calculated from the calibration curve and B from the equation $B = T - F$, where T is the total drug concentration, the binding parameters were estimated by a computer program (4) which performs nonlinear least-squares fitting of the Scatchard model directly to the experimental data.

RESULTS

All ISEs used, except of that for the AMN cation (12), were constructed for the first time for the purpose of the present study. In all cases the effect of pH on the ISEs response was examined and the optimum working conditions were used in binding experiments. The potential was stable in the pH range where the drug exists entirely as a cation (Table I). The deviations noted in acid solutions are due to the interference of hydrogen ions with the electrode membrane causing alteration of its composition. The optimum working conditions along with the slopes of the ISEs used for all drugs studied are shown in Table II. As can be seen, the linear response range of all electrodes was 3 to 4 decades of the molar concentration scale, allowing the study of the complexation in a wide concentration range.

Table I. pK_a Values of the Drugs and Range of pH in Which the Potential of the Electrodes Remained Stable (Temp. = 25°C)

ISE	pK_a	pH range
AMN[a]	9.4	1–6
NRN	9.7	2–6
IMN	9.5	3–7
DXN	8.0	2–7
PTN	8.2	2–6
MPN	10.5	2–6

[a] Taken from Ref. 12.

The results of the binding experiments at 25°C are shown in Table III. Within-run precision of the binding parameters estimates ranged from 0.1 to 5.0%, whereas between-run precision was 8–10% (n = 3). The interaction of drugs with β-CD was further studied as a function of temperature and the results are summarized in Table IV. Typical Scatchard plots, for the AMN/β-CD interaction, at the various temperatures examined, are shown in Fig. 2 Typical saturation plots, for the saturation of β-CD with the PTN cation, as a function of temperature are presented in Fig. 3.

Thermodynamic analysis was performed according to the Van't Hoff equation utilizing the estimates for the association constants:

$$\log K = \frac{\Delta S}{2.303R} - \frac{\Delta H}{2.303R} \times \frac{1}{T} \qquad (2)$$

where ΔS and ΔH are the changes in entropy and enthalpy, respectively, T is the absolute temperature, R the gas constant, and K the association constant at T. Linear relationships ($0.989 \leq r \leq 0.9999$) were established in all cases and the associated thermodynamic parameters are listed in Table V.

DISCUSSION

Binding Parameters. Due to the size limitations, the binding of all drugs was much higher to β- than to α-cyclodextrin (Table III). In all cases linear Scatchard plots were obtained revealing one class of binding sites with $n \approx 1$. The location of the binding site is undoubtedly the cyclodextrin cavity, while the estimates of the association constant

Table II. Optimum Working Conditions and Characteristics of the ISEs Used (Temp. = 25°C)

ISE	Ionic strength adjustor[a]	Slope (mV/decade)	Linear response range (M)
AMN	Na_2SO_4, 0.020 M	55	6×10^{-5}–1×10^{-2}
NRN	NaCl, 0.060 M	56	2.6×10^{-5}–1×10^{-2}
IMN	NaCl, 0.060 M	55	8×10^{-5}–1×10^{-2}
DXN	NaCl, 0.060 M	54	4.5×10^{-5}–1×10^{-2}
PTN	Na_2SO_4, 0.020 M	54	8×10^{-6}–1×10^{-2}
MPN	NaCl, 0.060 M	55	1×10^{-5}–1×10^{-2}

[a] The pH of these solutions was 5–6.

Table III. Binding Parameters (±SD) for All Drugs Studied with α- and β-CD[a]

Drug	CD	n	$K \times 10^3$ (M^{-1})	Ns[b]	SD_{re}[c]
AMN	β	0.852 (±0.001)	23.90 (±0.05)	—	0.1
	α	0.81 (±0.01)	0.061 (±0.001)	—	0.1
NRN	β	0.911 (±0.001)	16.77 (±0.06)	—	0.2
	α	0.85 (±0.08)	0.087 (±0.009)	—	0.1
DXN	β	0.913 (±0.002)	13.21 (±0.07)	0.072 (±0.007)	0.3
	α	0.86 (±0.02)	0.050 (±0.002)	—	0.1
IMN	β	0.858 (±0.001)	8.70 (±0.06)	—	0.3
	α	0.86 (±0.02)	0.081 (±0.002)	—	0.1
PTN	β	0.920 (±0.002)	18.04 (±0.07)	—	0.2
	α	1.09 (±0.08)	0.107 (±0.009)	—	0.2
MPN	β	0.870 (±0.001)	4.81 (±0.02)	—	0.1
	α	0.73 (±0.04)	0.123 (±0.008)	—	0.3

[a] Temperature = 25°C; β-CD concentration, 0.0100 *M* (except for MPN and PTN, where it was 0.0020 and 0.0010 *M*, respectively); α-CD concentration, 0.0100 *M*.
[b] Nonspecific binding.
[c] Standard deviation of residuals.

ranged from 4.81×10^3 to 23.9×10^3 M^{-1}. Also, complexation was found to become stronger with decreasing temperatures, showing the exothermic character of the reaction (Table IV). In parallel, a 1:1 stoichiometry was justified for all complexes formed judging from the insignificant effect of temperature on the n values, $n \approx 1$ (Fig. 2).

Earlier reported results (6) concerning the study of the complexation of dicyclomine and chlorpromazine with α- and β-CD using ISEs should also be mentioned here in relation to this work and recent results (7). Scatchard model for one class of binding sites was also used (6) to describe the binding phenomenon; however, the value of n was adjusted

Table IV. Estimates (±SD) of Binding Parameters for the Interactions of Drugs with β-CD[a] at Various Temperatures

Drug	Temp (°C)	n	$K \times 10^3$ (M^{-1})	NS[b]	SD_{re}[c]
AMN	5	0.876 (±0.004)	62.0 (±0.3)	0.127 (±0.003)	0.2
NRN		0.910 (±0.001)	36.9 (±0.3)	—	0.5
IMN		0.849 (±0.001)	19.3 (±0.1)	—	0.2
DXN		0.948 (±0.001)	41.4 (±0.3)	0.058 (±0.007)	0.2
PTN		0.964 (±0.002)	40.3 (±0.4)	—	0.3
MPN		0.823 (±0.002)	9.39 (±0.03)	—	0.1
AMN	15	0.840 (±0.001)	38.0 (±0.1)	0.180 (±0.004)	0.1
NRN		0.932 (±0.001)	24.0 (±0.2)	—	0.3
IMN		0.850 (±0.001)	15.0 (±0.2)	—	0.3
DXN		0.917 (±0.001)	22.5 (±0.1)	0.136 (±0.004)	0.2
PTN		0.931 (±0.002)	26.9 (±0.2)	—	0.2
MPN		0.797 (±0.003)	7.41 (±0.07)	—	0.3
AMN	25	0.852 (±0.001)	23.90 (±0.05)	—	0.1
NRN		0.911 (±0.001)	16.77 (±0.06)	—	0.2
IMN		0.858 (±0.001)	8.70 (±0.06)	—	0.3
DXN		0.913 (±0.002)	13.21 (±0.07)	0.072 (±0.007)	0.3
PTN		0.920 (±0.002)	18.04 (±0.07)	—	0.2
MPN		0.870 (±0.001)	4.81 (±0.02)	—	0.1
AMN	37	0.821 (±0.001)	14.70 (±0.06)	—	0.2
NRN		0.887 (±0.002)	8.40 (±0.05)	—	0.3
IMN		0.852 (±0.001)	5.32 (±0.03)	—	0.2
DXN		0.912 (±0.002)	7.66 (±0.03)	0.058 (±0.002)	0.1
PTN		0.910 (±0.006)	11.0 (±0.1)	—	0.2
MPN		0.765 (±0.003)	3.24 (±0.02)	—	0.1

[a] β-CD concentration was 0.0100 *M* except for the studies with PTN and MPN, where it was 0.0020 and 0.0010 *M*, respectively.
[b] Nonspecific binding.
[c] Standard deviation of residuals.

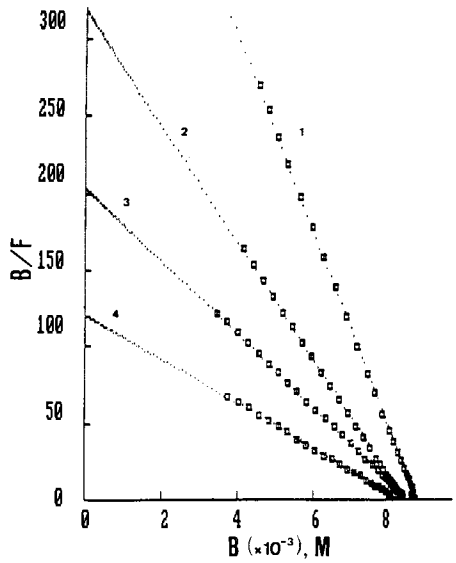

Fig. 2. Temperature effect on the complexation of AMN with β-CD; the symbols of axes are defined in the text [Eq. (1)]. Scatchard curves at (1) 5, (2) 15, (3) 25, and (4) 37°C. The theoretical Scatchard curves (dotted lines), based on the calculated binding parameters, are also shown. The concentration of β-CD was 0.0100 *M* and was kept constant during the experiment.

to 1 and the analysis of data was performed according to Eq. (3), which is the linearized form of Eqn. 1, with $n = 1$,

$$r/F = K - K \cdot r \qquad (3)$$

and $r = B/CD_t$. The good linearity of the Scatchard curves

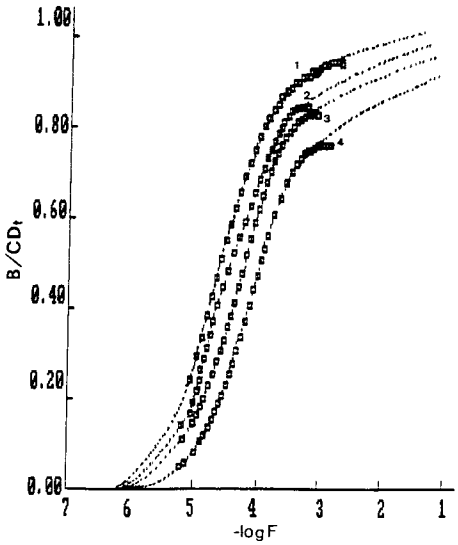

Fig. 3. Saturation plots of β-CD with PTN at (1) 5, (2) 15, (3) 25, and (4) 37°C. The symbols on ordinate are defined in the text [Eq. (1)]; the abscissa represents the negative logarithm of the free measured ion concentration in the solution. The theoretical curves (dotted lines), based on the calculated binding parameters, are also shown. The concentration of β-CD was 0.0010 *M* and was kept constant during the experiment. The limiting values of isotherms correspond to the estimates for *n* (Table IV). The deviations of the data points at the far end of the binding isotherms are most likely due to the reduction of the cyclodextrin and drug-ion activities.

and the *x*-intercepts confirmed the 1:1 stoichiometry of the complexes (6). In this work *n* was considered a nonadjustable parameter. The estimates derived for *n* were, in all cases, very close to 1, again supporting the 1:1 stoichiometry for the formed complexes. It is interesting to note, however, that all estimates for *n* were slightly lower than unity, which is the theoretically anticipated value for *n*. This should be attributed to a weak interaction between the bulky counter anion of the ion exchanger and β-CD, causing a slight "dissolution" of the ISE liquid membrane and resulting, therefore, in a transfer of a small amount of the measured drug-cation in the sample solution. The lack of the slight interference in the study by Takisawa *et al.* (6) could be attributed to the use of a modified PVC with anion end groups as ion-exchange sites (without bulky counter ion), which probably does not cause the "dissolution" of the ion exchanger. Also, it is interesting to note that the association constant for the β-CD/CPM complexation obtained from a spectroscopic study (13) was found to be $1.2 \times 10^3\ M^{-1}$. This value agrees fairly well with the statistically equivalent association constants found independently utilizing ISEs (6,7). Besides, spectroscopic studies of PTN, prochlorperazin, and levopromazin with β-CD have resulted in association constants of a similar magnitude (2,14,15).

Some remarks concerning the parameter nonspecific binding, Ns, quoted in Tables III and IV, are required at this point. The reasons for its adoption to explain the constancy of the term $B/CD_t/F$ at the far end of the Scatchard plot have been given previously (7). By analogy to protein binding studies (4,16,17), a physical interpretation was also attempted (7). It was shown, using chlorpromazine as model drug, that the extent of Ns was dependent on the β-CD concentration (Table VIII in Ref. 7). It is worthy to note that Takisawa *et al.* (6), utilizing another type of ISE and a $2 \times 10^{-3}\ M$ β-CD concentration, did not observe irregular Scatchard curves for the drugs dicyclomine and chlorpromazine. It seems likely therefore that the finding of nonspecific binding is dependent on the experimental conditions. Since its magnitude becomes more appreciable (i) with higher concentrations of β-CD and lower temperatures (Table IV and Ref. 7) and (ii) for drugs which exhibit strong binding to β-CD as CPM, AMN, and DXN, it can be postulated that its origin is related to the high concentration of the complex formed at the last experimental measurements. Probably, the buildup of the bulky drug/β-CD complex (with some ionic character) in the sample solution reduces the activity of the measured free drug cation at the neighborhood of the ISE membrane, resulting in an apparent lower free drug concentration. The immediate impact of this interference is an apparent increase in the drug–CD binding affinity, which is manifested as a curvature at the end of the Scatchard curve leading to an apparent constancy of the term $B/CD_t/F$. However, the accuracy of the estimates for the binding parameters was not affected by the presence of Ns since results from independent studies for the association constant of chlorpromazine/β-CD (6,7,13) and naproxen/β-CD (7,18) interactions are identical.

In the field of protein binding studies using ISEs, values for Ns have been reported (4) for the picrate–albumin interaction. The characteristics of nonspecific binding have been found (4) to be identical to these of the present and previous

Table V. Theoretical Structural Data (R) and Experimental Estimates of Physicochemical Parameters, for All Drugs Studied

Drug	R^a	log P^b	ΔH (±SD) (kcal/mol)	ΔS (±SD) (cal/mol/deg)	$K \times 10^3$ $(M^{-1})^c$
AMN	102.4	1.31	−7.74 (±0.07)	−5.89 (±0.25)	23.90
NRN	102.1	1.31	−7.79 (±0.70)	−6.99 (±2.45)	16.77
IMN	100.7	0.98	−7.14 (±0.75)	−5.88 (±2.55)	8.70
DXN	101.4	0.64	−9.06 (±0.22)	−11.46 (±0.77)	13.21
PTN	100.9	1.18	−6.95 (±0.19)	−3.89 (±0.64)	18.04
MPN	103.1	1.32	−5.87 (±0.50)	−2.79 (±1.70)	4.81
CPM[d]	101.8	1.51	−9.82 (±0.15)	−14.20 (±0.50)	12.70

[a] Defined in the text; it corresponds to an axial approach (22) of the guest to β-CD.
[b] Taken from Ref. 21.
[c] Estimates are quoted for comparative purposes and correspond to 25°C.
[d] Data for CPM are taken from Ref. 7 and correspond to the same experimental conditions.

(7) study. The similarity of the observations for Ns allows one to generalize the postulated interfering mechanism for protein binding studies utilizing ISEs. Simply, the drug–protein complex plays the role of the drug–CD complex. This suggestion is also supported by the findings of another study (19) dealing with the potentiometric determination of albumin in human serum and whole blood, utilizing a bromocresol purple (BCP) and a picrate ISE. The potentiometric BCP assay exhibited increasing positive bias (Table 2 in Ref. 19) with increasing albumin concentration. Since a considerable Ns participation to the overall binding was observed (20) in the potentiometric study of the BCP–albumin interaction, the positive bias (19) could be explained by the above proposed interfering mechanism.

Attempts were made to fit the Scatchard equation for a model with 1:1 followed by 1:2 complexation (Drug:CD) to the data of AMN and DXN. In both cases the fit was worse than using the 1:1 model.

Structural Features and Thermodynamic Analysis. The importance of the central ring structure for the binding is apparent from the data for AMN and DXN. The only structural difference in these two molecules is the presence of an oxygen atom in DXN instead of a $-CH_2-$ group in AMN, at the same position of the central ring (Fig. 1). This structural difference results in a significant decrease in the association constant for DXN (Table V). At first glance, this behavior could be correlated with the lower lipophilicity (21) of the DXN molecule (Table V). This consideration seems to be valid also for PTN and, to some extent, for IMN, since both exhibit a parallel decrease in the values of log P and the

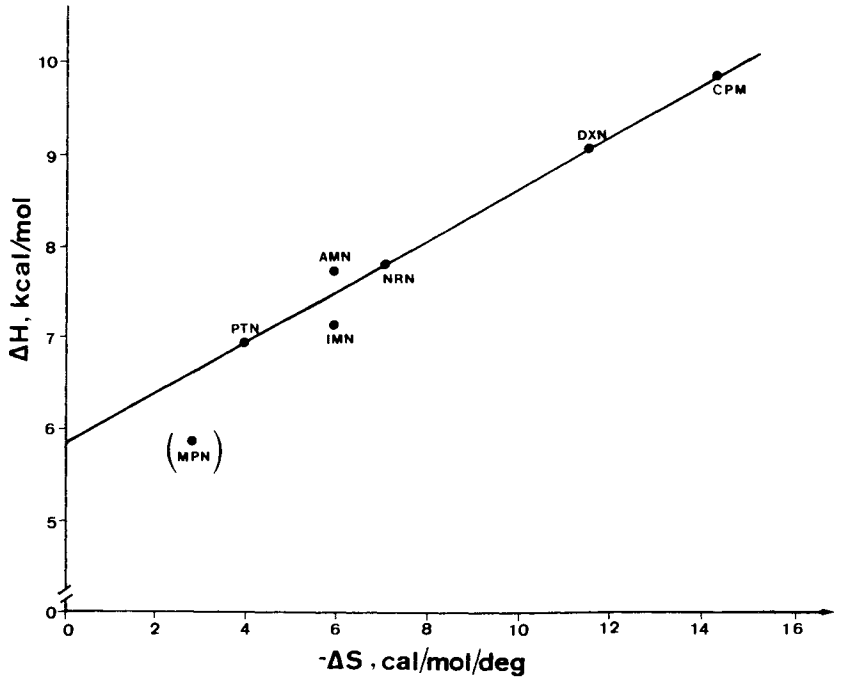

Fig. 4. Compensation plot based on linear regression analysis of $-\Delta H$ versus $-\Delta S$. The datum point for MPN is not incorporated into analysis and it is shown only for illustrative purposes.

association constants (Table V). However, the results for NRN and MPN are ruling out such a possibility. In fact, the reported log P values for AMN, NRN, and MPN are almost identical, while the estimates for the association constants differ remarkably (Table V). The unique structure of the central ring of MPN seems to be responsible for the fivefold reduction of the association constant estimate in respect to that of AMN. It seems likely that the hydrocarbon bridge between carbon 9 and carbon 10 sterically hinders the binding of MPN to β-CD. Finally, a comparative evaluation of the results for AMN and NRN clearly indicates that the detailed structure of the side carbon chain also plays an important role in the intensity of the inclusion-complex formation since AMN is simply the N-methyl derivative of NRN. An additional indirect proof of the importance of the detailed structure in the β-CD/drug complexation is derived from the insignificant differences in the values of the parameter $R = (l_g/d_c) \times 100$, which has been used (7) as a measure of "snugness of fit" (Table V). lg is the maximum length of the guest molecule, associated with the specific mode of complexation involved (22) (axial or equatorial), and d_c is the β-CD diameter (7.8 Å). l_g was calculated with the Desktop Molecular Modeller program (23). All drugs possess an ideal value of $R \approx 100\%$ for an axial approach of the quest to the cavity, which indicates that the size characteristics are favourable to the interaction. In reality, however, the diversity in the affinity of drugs to β-CD is correlated with the type(s) of the specific forces developed in various degrees between the guests and β-CD.

The negative estimates for enthalpy and entropy changes, calculated from the thermodynamic analysis of the data (Table V), show that the formation of inclusion complexes is enthalpy driven. This nonclassical hydrophobic effect reveals that both hydrophobic interactions and van der Waals forces participate in the drug/CD complexation; the $-\Delta H$ value is an indicator of the contribution of the specific noncovalent interactions involved to the inclusion complex formation (8). It is interesting to note that the higher $-\Delta H$ value was calculated for the interaction of DXN with β-CD, and not for AMN, which showed the strongest affinity to β-CD (Table V). The presence of an oxygen atom in the DXN molecule seems to be responsible for this observation. The nonbonding electrons of this oxygen atom lend properties of a dipole inducer to the DXN molecule; besides, hydrogen bonds between DXN and the β-CD hydroxyl groups, and/or the water molecules that preexist in the CD cavity and are replaced, partly or totally, by the bound DXN, can be developed. It is also worthy to note that previous reported (7) results concerning chlorpromazine (CPM) are in agreement with these observations. Although CPM is not a tricyclic antidepressant drug, it shows great structural similarity to these molecules and, especially, to DXN. The presence of a sulfur atom in the CPM molecule, which has the same electron conformation as the oxygen atom of the DXN molecule, results in a similar type of interaction with β-CD. The similarity in the estimates for the association constants and thermodynamic parameters is conclusive evidence (Table V).

When ΔH was plotted against ΔS for all drugs examined a linear plot was obtained ($r = 0.955$), showing that the loss in free energy, reflected in the negative ΔH values, is compensated by the negative ΔS values. However, the data point of MPN deviates significantly, lying outside the 95% confidence range of the regression line. By considering MPN as an outlier and reanalyzing the data, an improvement was achieved ($r = 0.966$). A further improvement was observed when chlorpromazine was added in the analysis ($r = 0.986$) (Fig. 4). This behavior is possibly related to a different contribution of the specific forces involved in the complexation of MPN, implying higher participation of the hydrophobic interactions in the complexation of MPN with β-CD, which is in agreement with the lower $-\Delta H$ and $-\Delta S$ values calculated (Table V). These observations, along with the significant lower association constant determined for MPN, support the suggestion that its tetracyclic structure sterically hinders its complexation with β-CD.

ACKNOWLEDGMENT

The authors wish to thank the Greek State Scholarship Foundation for financial support.

REFERENCES

1. J. Szejtli. *Cyclodextrins and Their Inclusion Complexes,* Akademiai Kiado, Budapest, 1982.
2. D. Duchene (ed.). *Cyclodextrins and Their Industrial Uses,* Les Edition de Sante, Paris, 1987.
3. K. Connors. *Binding Constants—The Measurement of the Molecular Complex Stability,* J. Wiley & Sons, New York, 1987.
4. T. Christopoulos and E. Diamandis. Binding studies using ion-selective electrodes. Examination of the picrate-albumin interaction as a model system. *Anal. Chem.* 62:360–367 (1990).
5. G. Valsami, P. Macheras, and M. Koupparis. Binding study of the fluorescense probe 1-anilino-8-naphthalene-sulfonate to human plasma and human, bovine serum albumin using potentiometric titration. *Pharm. Res.* 8:888–892 (1991).
6. N. Takisawa, D. Hall, E. Wyn-Jones, and P. Brown. The construction and characteristics of drug-selective electrodes. Applications for the determination of complexation constants of inclusion complexes with α- and β-cyclodextrins including a kinetic study. *J. Chem. Soc. Faraday Trans.* 84:3059–3070 (1988).
7. G. Valsami, P. Macheras, and M. Koupparis. Binding studies of ions with cyclodextrins using ion-selective electrodes. *J. Pharm. Sci.* 79:1087–1094 (1990).
8. M. Eftink, M. Andy, K. Bystrom, H. Perlmutter, and D. Kristol. Cyclodextrin inclusion complexes: Studies of the variation in the size of alicyclic guests. *J. Am. Chem. Soc.* 111:6765–6772 (1989).
9. J. Coryta. *Ion-Selective Electrodes,* Cambridge University Press, Cambridge, 1975.
10. A. Graggs, G. Moody, and J. Thomas. PVC matrix membrane ion-selective electrodes. *J. Chem. Educ.* 51:541–544 (1974).
11. G. Scatchard. The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* 51:660–672 (1949).
12. A. Mitsana-Papazoglou, T. Christopoulos, E. Diamandis, and T. Hadjiioannou. Construction of ion-selective electrodes for chlorpromazine, amitriptyline, propantheline and meperidine: Analytical study and application to pharmaceutical analysis. *Analyst* 110:1091–1094 (1985).
13. K. Uekama, T. Irie, M. Sunada, M. Otagiri, K. Iwasaki, Y. Okano, T. Miyata, and Y. Kase. Effects of cyclodextrins on chlorpromazine-induced haemolysis and central nervous system responses. *J. Pharm. Pharmacol.* 33:707–710 (1981).
14. T. Hoshino, F. Hirayama, K. Uekama, and M. Yamasaki. Reduction of protriptyline-photosensitized haemolysis by β-cyclodextrin complexations. *Int. J. Pharm.* 50:45–52 (1989).
15. K. Uekama, T. Irie, M. Sunada, M. Otagiri, Y. Arimatsu, and

S. Nomura. Alleviation of prochlorperazine-induced primary irritation of skin by Cyclodextrin complexation. *Chem. Pharm. Bull.* **30**:3860–3862 (1982).
16. P. Munson and D. Rodbard. Ligand: A versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107**:220–239 (1980).
17. Y.-C. Tsang and J. Thiessen. Competitive binding of sulfamethazine and its N-acetylated metabolite. *J. Biopharm. Drug Disp.* **10**:465–479 (1989).
18. N. Erden and N. Celebi. A study of the inclusion complex of naproxen with β-cyclodextrin. *Int. J. Pharm.* **48**:83–89 (1988).
19. T. Christopoulos and E. Diamandis. A general method for the assay of binders. Application to the potentiometric determination of albumin in human serum, plasma and whole blood. *Clin. Biochem.* **19**:151–160 (1986).
20. T. Christopoulos. *Application of Ion Selective Electrodes to Binding Studies of Several Ions with Albumin,* Ph.D. thesis, Athens, Greece, 1986.
21. K. Thoma and K. Albert. Kolloidasozition von Tri- und Tetracyclischen Antidepressiva und Neuroleptika. *Pharm. Acta Helv.* **56**:69–77 (1981).
22. G. Catena and F. Bright. Thermodynamic study on the effects of β-cyclodextrin inclusion with anilinonaphthalenesulfonates. *Anal. Chem.* **61**:905–909 (1989).
23. M. James, C. Crable, and J. R. Appleyard. *Desktop Molecular Modeller,* Oxford University, Oxford, 1989.