Monatshefte für Chemie 133, 1239–1247 (2002)

Monatshefte für Chemie
Chemical Monthly

Cyclodextrins as Enhancers of the Aqueous Solubility of the Anthelmintic Drug Mebendazole: Thermodynamic Considerations

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Summary. Inclusion complexes of mebendazole with α-, β-, and γ-cyclodextrins, hydroxylpropyl-β-cyclodextrin (*HP-β-CD*), and methyl-β-cyclodextrin (*Me-β-CD*) were investigated employing the *Higuchi* and *Connors* solubility method. The solubility of mebendazole increased as a function of cyclodextrin concentration showing an A_L phase diagram indicating the formation of soluble complexes with 1:1 stoichiometry. The *Gibbs* free energies of transfer of the drug from aqueous solution to the cavity of cyclodextrin are negative and increase negatively with increasing cyclodextrin concentration. The solubility of mebendazole as well as the stability constant of its complex with *Me-β-CD* are found to be affected by the *pH* of the medium. The *Me-β-CD* cavity was found to have a greater affinity for the unionized mebendazole rather than the protonated one. Effects of methanol and temperature on these interactions were also investigated to gain further knowledge on the mechanism of the inclusion process. It was found that the interaction between the drug and the cyclodextrin is weakened as the medium becomes more apolar and the temperature increases. Moreover, the thermodynamic parameters for the binding were derived from the dependence of the stability constants on the temperature (*van't Hoff* analysis). The formation of the inclusion complexes between the drug and *β-CD* or its derivatives was found to be enthalpy controlled, with $|\Delta H^\circ| > T|\Delta S^\circ|$. This suggests that hydrophobic and *van der Waals* interactions as well as solvent reorganization are the main driving forces. Furthermore, the size of the cavity of cyclodextrins plays an important role in the association process.

Keywords. Cyclodextrin; Mebendazole; Inclusion complex; Solubility; Stability constant; Thermodynamics.

Introduction

Benzimidazole anthelminic drugs are widely used in the treatment of helminthe-parasities in animals and man [1]. Among these drugs, mebendazole (5-benzoyl-1*H*-benzimidazol-2-yl carbamic acid methyl ester) exhibits a broad spectrum of

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activity against echinococcosis and cysticercosis [2]. However, some difficulties are involved in preparing the pharmaceutical formulation of oral or injectable solutions due to the very poor aqueous solubility of the drug leading to a minimal bioavailability. In some cases high oral dosage is required, which could give rise to gastro-intestinal disturbances and liver impairment [3]. Furthermore, the correct intake of doses is critical especially in veterinary medicine [4]. In order to overcome this problem, efforts must be made to improve the aqueous solubility of anthelminitic drug mebendazole.

To turn a molecule into a usable drug with excellent therapeutic action, the drug should be not only water soluble for its adequate delivery to the cell but also somewhat hydrophobic in nature to cross the cellular membrane and reach its target site in a living organism. The aqueous solubility is of vital importance in pharmaceutical preparations. For insoluble drugs, the formulations involve the use of cosolvents, surfactants, microemulsion dosage forms, and/or *pH* adjustment for ionizable drugs. However, these formulations are often found to cause some adverse reactions and reduce the efficacy and safety of the drug. Therefore, another successful approach has been realized using cyclodextrin as a drug carrier. Cyclodextrin complexation improves the aqueous solubility, dissolution and release rates, chemical stability, and bioavailability of various drug molecules that are poorly soluble in water. In addition, the complexation may also suppress the volatility and unpleasant odours or tastes associated with the drug, avoid incompatibility problems with other drugs or excipients in a formulation, and reduce local irritance and haemolysis [5–7].

Cyclodextrins are cyclic oligosaccharides consisting of six or more *D*-(+)-glucopyranose units. They are linked to each other by $\alpha(1 \rightarrow 4)$ bonds to form macrocyclic rings exhibiting the shape of a truncated hollow cone. The interiors, which are the sites for the guest molecules, are largely hydrophobic; the hydroxyl groups are positioned on the outside of the molecule.

In continuation of earlier work [8–12], the present investigation was undertaken to improve the solubility of mebendazole and thereby its bioavailability characteristics by inclusion complex formation with cyclodextrin. For that purpose, five different types of cyclodextrins (α-, β-, and γ-cyclodextrins, methyl-β-*CD*, and hydroxypropyl-β-*CD*) were used. The thermodynamic parameters of mebendazole complexes with β-*CD* and its derivatives were calculated from the *van't Hoff* equation using the apparent stability constants at various temperatures. From these, it is possible to gain insight into the driving forces of complex formation.

Results and Discussion

The solubility diagrams of mebendazole in different cyclodextrins were constructed by plotting the evaluated equilibrium concentrations of mebendazole against the concentration of the respective *CD*s. As it appears (Fig. 1), all studied cyclodextrins enhanced the aqueous solubility of mebendazole. Also, this figure shows that the aqueous solubility of the drug increases linearly as function of the cyclodextrin concentration, suggesting the formation of inclusion complexes of the A_L type following the *Higuchi* and *Connors* classification [13]. In aqueous solutions of mebendazole-*CD* complexes, the free drug molecules are in equilibrium

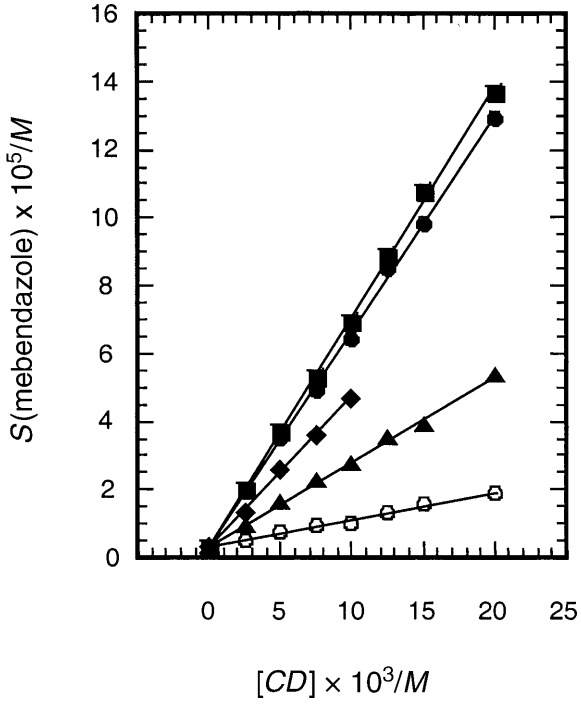

Fig. 1. Solubility phase diagrams of mebendazole in *BR* buffer ($pH = 7.2$) in presence of different concentrations of (◯) *α-CD*, (◆) *β-CD*, (▲) *γ-CD*, (●) *HP-β-CD*, and (■) *Me-β-CD* at 298.15 K

with the drug molecules entrapped within the cavity. Thus, upon increasing the concentration of *CD*, more mebendazole molecules will transfer from the aqueous solution to the hydrophobic cavity of the *CD*. Therefore, one can expect more mebendazole being dissolved in water in the presence of *CD* than in its absence and, consequently, the solubility of mebendazole increases with increasing concentration of *CD*.

The *Gibbs* free energies of transfer of mebendazole from aqueous solution to the cavity of the *CD* (Table 1) have been calculated from Eq. (1) where S and S_o are the solubilities of mebendazole in presence and absence of *CD*, respectively.

$$\Delta G^{\circ}_{\text{transf}} = -RT\ln(S/S_o) \tag{1}$$

As can be seen from Table 1, the $\Delta G^{\circ}_{\text{transf}}$ values are negative and increase negatively with increasing *CD* concentration. This indicates that *CD* is a more favourable environment for mebendazole than water. Moreover, the interaction between mebendazole and *CD* increases with increasing *CD* content. With regard to the linear solubility diagrams, the formation of 1:1 mebendazole-*CD* complexes is suggested in aqueous solutions of all studied cyclodextrins (the slope is smaller than 1 for each *CD*). The apparent stability constants (K, Eq. (2)) were calculated according to *Higuchi* and *Connors* [13] from the slope of the solubility diagram of mebendazole in the buffered solution of the respective cyclodextrin obtained by the least squares method and the buffered solubility of mebendazole (S_o).

$$K = \text{slope}/S_o(1 - \text{slope}) \tag{2}$$

Table 1. Values of *Gibbs* energy of transfer of mebendazole from aqueous solution to the hydrophobic cavity of various cyclodextrins at 298.15 K

$[CD]/M$	$-\Delta G^{\circ}_{transf}/kJ \cdot mol^{-1}$				
	α-CD	*β-CD*	*γ-CD*	*HP-β-CD*	*Me-β-CD*
0.0025	1.16	3.54	2.68	4.52	4.56
0.0050	2.21	5.18	4.02	5.96	6.09
0.0075	2.65	6.01	4.86	6.78	6.97
0.0100	2.87	6.66	5.34	7.44	7.62
0.0125	3.53	–	5.96	8.14	8.24
0.0150	3.94	–	6.22	8.48	8.72
0.0200	4.45	–	6.98	9.17	9.31

Table 2. Stability constant (M^{-1}) and thermodynamic parameters ($kJ \cdot mol^{-1}$) of the association of mebendazole with various cyclodextrins in *BR* buffer ($pH = 7.2$)

Parameter	T/K	*α-CD*	*β-CD*	*γ-CD*	*HP-β-CD*	*Me-β-CD*
K	288	–	1950	–	2884	3090
	293	–	1622	–	2399	2570
	298	251	1386	778	1990	2131
		150[a]	1500[a]	780[a]	1800[a]	
	303	–	1175	–	1698	1820
	313	–	891	–	1259	1380
$-\Delta G^{\circ}$	298	13.70	17.93	16.50	18.84	19.01
$-\Delta H^{\circ}$	298	–	23.45	–	24.90	24.26
$-T\Delta S^{\circ}$	298	–	5.52	–	6.06	5.25

[a] Ref. [25], phosphate buffer ($pH = 7.5$)

It is important to note that the carefully measured S_o value in absence of *CD* is used for the calculation instead of using the intercept obtained from the least squares analysis. This is necessary because a correct S_o value is a critically important factor for the reliable calculation of mutually consistent values of the apparent stability constants. Table 2 summarizes the apparent stability constants of the complexes formed in *Britton-Robinson* buffer ($pH = 7.2$). As can be seen from Fig. 1 and Table 2, there were marked mutual differences between the *CD*s with respect to both the solubilization efficiency and the stability of their complexes with mebendazole. Since the optimum value of K to get good bioavailability should be between 200 and $5000\,M^{-1}$ [14], the studied cyclodextrins are suitable candidates for the improvement of the bioavailability of mebendazole.

Effect of pH

The influence of *pH* on the apparent stability constant of the mebendazole–*Me-β-CD* inclusion complex, as a representative example, and on the efficacy of the solubility enhancing effect was studied. The decrease in *pH* values leads to a decrease in the apparent stability constant ($K = 2131\,M^{-1}$ at *pH* 7.2, whereas at

pH 2 the *K* value amounts to 408 M^{-1}), indicating that *K* of mebendazole–*Me-β-CD* is larger in the less protonated form ($pK_a = 3.5$). Thus, the inclusion process with mebendazole is more favourable than that with mebendazole–H^+. At $pH = 2$, the slope and the S_o value (the solubility of mebendazole in absence of *CD*) are larger than the corresponding values for $pH = 7.2$. At this lower *pH*, mebendazole, which is a basic drug, is present in the protonated form; it is more soluble, that is, it interacts with *CD* more weakly than the unionized form [15, 16]. This is in accordance with the fact that the *K* values decrease as a result of the decreasing hydrophobicity of the drug when the drug solubility increases [17].

Temperature effect

In principle, evaluation of *K* over a significant temperature range can lead to the enthalpy (ΔH°) and entropy (ΔS°) of association. These terms can provide insight into the driving forces for the binding interaction. Therefore, the *K* values were calculated from solubility measurements at 288, 293, 298, 303, and 313 K. The corresponding thermodynamic parameters of binding (ΔG°, ΔH°, ΔS°) were calculated using the standard relations $\Delta G^\circ = -RT\ln K = \Delta H^\circ - T\Delta S^\circ$ where the ΔH° and ΔS° can be deduced from the slope and the intercept of a *van't Hoff* plot ($\ln K$ *vs.* $1/T$). All these parameters are shown in Table 2. By careful inspection, the following conclusions can be made:

i) The K values decrease with rising in temperature, *i.e.*, as the temperature increases, the affinity of the cyclodextrin for the drug decreases.
ii) The negative value of ΔG° suggests that the complexation is thermodynamically favoured.
iii) The drug binds to *CD* with a favourable enthalpic term ($\Delta H^\circ < 0$) and an unfavourable entropic term ($\Delta S^\circ < 0$).
iv) A linear relationship of the $\ln K$ *vs.* $1/T$ data is obtained, indicating the independence of ΔH° and ΔS° on *T* and, thus, $\Delta C_p = 0$.

Effect of solvent

In order to advance the individual molecular level description of solvent effects on apolar host-guest associations, the *K* values of mebendazole-*β-CD* were determined at 298 K in the presence of several percent of methanol. Methanol is the solvent of choice due to its low affinity for binding with *CD* [18], which renders it a poor competitor with mebendazole. Therefore, all effects observed in the presence of methanol can be attributed to a change of the solvophobic characteristics of the medium, which obviously affects the affinity of an apolar drug with respect to its binding to cyclodextrin.

The values of ΔG° for mebendazole-*β-CD*, reported in Table 3, are plotted as a function of the volume fraction ϕ of methanol (Fig. 2). As can be seen, the *Gibbs* free energy decreases negatively as long as the hydrophobicity of the medium increases due to increasing methanol content. Also, a strongly linear free energy relationship ($R = 0.9953$) exists between the free energy of formation of the mebendazole-*β-CD* complex and the solvent polarity parameter $E_T(30)$ [19] as indicated from Fig. 3. All these observations reveal a clear contribution of the

Table 3. Stability constant and *Gibbs* free energy of association of mebendazole-*β-CD* at various methanol contents at 298.15 K

ϕ (MeOH)	K/M^{-1}	$-\Delta G^{\circ}/\text{kJ} \cdot \text{mol}^{-1}$
0.00	1386	17.93
0.05	1202	17.58
0.10	891	16.84
0.20	617	15.93
0.30	479	15.30
0.40	302	14.16

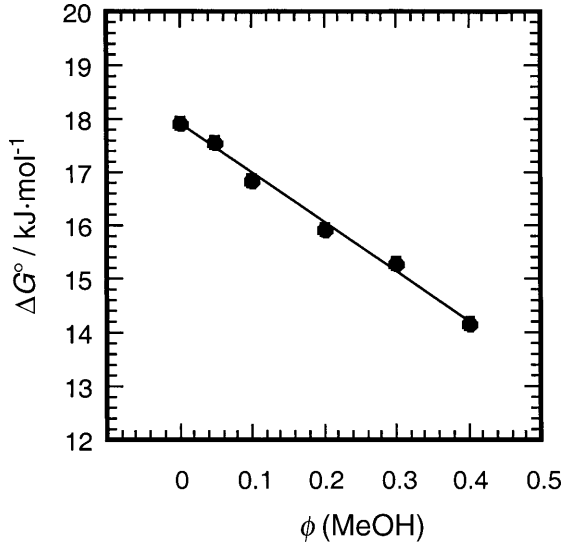

Fig. 2. Variation of the *Gibbs* free energy of binding of mebendazole-*β-CD* with the methanol content in methanol–water mixtures

hydrophobic effect to the driving force of complexation. Therefore, water is the best solvent for inducing strong association between apolar binding partners, and this property is essential for sustaining all functions of life.

Obviously, on the basis of the above discussion one can conclude that the formation of a complex between mebendazole and *CD* is hydrophobically driven. On the other hand, although the hydrophobic interaction is characterized by $\Delta H^{\circ} \approx 0$ and $\Delta S^{\circ} > 0$ [20], the derived enthalpy and entropy changes (Table 2) are not in line with those expected for the classical hydrophobic interaction. Similar results [21, 22] have been obtained for association between guest molecules and an apolar cavity in water. These processes are exothermic and enthalpy driven ($|\Delta H^{\circ}| > T|\Delta S^{\circ}|$). This suggests that forces other than hydrophobic interactions significantly contribute to the stabilization of mebendazole-*CD* complexes. Such processes have been considered as nonclassical hydrophobic interactions. The favourable ΔH° values may be a result of the *van der Waals* interaction of the host and guest molecules. Other contributions to the favourable ΔH° values come

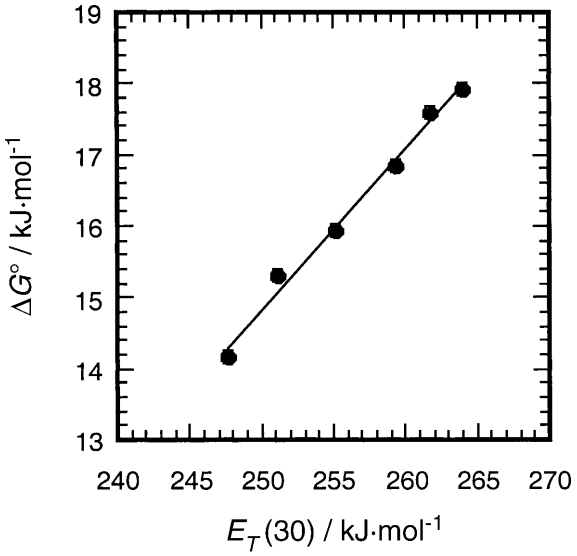

Fig. 3. Dependence of the *Gibbs* free energy of binding of mebendazole-*β-CD* on the solvent polarity as expressed by $E_T(30)$ values of methanol–water mixtures at 298.15 K

from changes in the behaviour of water associated with the host and guest molecules, the replacement of the incorporated water molecules (unstable) from the *CD* cavity by the drug and/or conformational changes of *CD* [23]. *Lewis* and *Hansen* [24] have attributed the large enthalpy change to the formation of hydrogen bonds between water molecules displaced from the cyclodextrin cavity, thus becoming bulk water. The unfavourable entropy change results from the higher ordering of the displaced water molecules from the cyclodextrin cavity.

Size effect

The stability of mebendazole inclusion complexes is in the order *Me-β-CD* ≥ *HP-β-CD* > *β-CD* > *γ-CD* > *α-CD*. This fact emphasizes the importance of the size-fit relation between the host and the guest in the complexation process. Also, the side chain substitutions of *β-CD* have a remarkable effect on the binding of mebendazole.

The *K* values of mebendazole complexes are in good agreement with the results of *Diaz et al*. [25]. The interaction of mebendazole with *α*- and *γ-CD*s is weak as expressed by the lower *K* values, thus indicating poor interactions between the complex components. Higher stability constants are observed for *β-CD* and its substituted derivatives, confirming that mebendazole interacts more strongly with these *CD*s. These observations suggest that the stability of the *CD*-mebendazole complexes varies markedly with the size of the *CD* cavity. Obviously, the stability constants reflect a favourable positioning of the guest molecules inside the cavity of the *CD* host molecules [26]. Therefore, the mebendazole structure is not compatible either with the internal cavity of *α-CD* or *γ-CD*. These cavities are clearly too small to allow penetration or may be too large to allow a close fit, respectively. Interaction of mebendazole with *β-CD* and its derivatives is sterically

more favourable that than with α- or γ-*CD*. *Diaz et al*. [27] have reached similar conclusions for albendazole complexation. The highest stability constant values of the mebendazole cyclodextrin complexes were found for *Me*- and *HP*-β-*CD*s. This may be due to the alkyl chains which may provide additional hydrophobic binding sites and thereby interact with hydrophobic portions of the molecule outside the cyclodextrin cavity [28].

Conclusions and final remarks

The results obtained here obviously show that the studied cyclodextrins enhance the aqueous solubility of mebendazole. The cavity of β-*CD* and its derivatives (*HP*-β-*CD* and *Me*-β-*CD*) are suitable candidates for mebendazole; α-*CD* is too small and γ-*CD* is too large. Therefore, high stability constants are found for β-*CD*, *Me*-β-*CD*, and *HP*-β-*CD*. The hydrophobic effect plays an important role in the formation of *CD*-mebendazole complexes. An increasing apolar character of the medium results in a clear decrease on the affinity of binding. Complex formation is exothermic and enthalpy driven ($|\Delta H^\circ| > T|\Delta S^\circ|$), indicating that a combination of hydrophobic effect ($\Delta H^\circ \approx 0$ and $\Delta S^\circ > 0$), *van der Waals* forces ($\Delta H^\circ < 0$ and $\Delta S^\circ < 0$), and solvent reorganization could account for the main forces governing the interaction between the *CD* and mebendazole. Hence, it is expected that the formulation of mebendazole as a microencapsulate with cyclodextrins may result in a better bioavailability. Such a drug-*CD* inclusion complex may help to avoid undesirable side effects which the administration of the drug alone may provoke, thus improving its therapeutic usefulness. Also, there is a hope that the mebendazole content of medicines may be reduced, and thus the side effects will lessen, *i.e*. the therapeutical risk will be smaller, whereas the pharmacon effect will increase; consequently, the quantity of medicine residues will be smaller. In addition, the use of β-*CD* or any of its derivatives is of utmost economical importance.

Experimental

Materials and reagents

Mebendazole, α-*CD*, β-*CD*, and γ-*CD* were obtained from Sigma Chemical Co. *Me*-β-*CD* and *HP*-β-*CD* were purchased from Aldrich. All chemicals were used as received and without further purification. The cyclodextrins were subjected to a thermogravimetric (TG) analysis to determine the degree of hydration which was considered in calculations of their concentrations. The *Britton-Robinson* buffer solutions were prepared in the usual way by adding appropriate amounts of NaOH (0.2 *M*) to an orthophosphoric acid, acetic acid, and boric acid mixture (0.04 *M* each). All chemicals were reagent grade (Merck, Darmstadt). All solutions were prepared in deionized water (specific conductance $< 2\,\mu S \cdot cm^{-1}$) and used within 24 h. Bidistilled water was purified using a Millipore Super Q system and was degassed prior to the preparation of the solutions. The homogeneity of the initial solutions was assured by sonication for 15 min in an ultrasonic bath.

Solubility studies

Solubility measurements were performed as described by *Higuchi* and *Connors* [13]. An excess of mebendazole was added to 10 cm^3 of *BR* buffer solution ($pH = 7.2$) containing various concentrations

of the studied cyclodextrins. The suspensions formed were sonicated for 15 min and shaken for 3 days at 40°C. After equilibrium was attained, an aliquot was filtered through a Millipore membrane (0.45 μm). The filtered solutions were appropriately diluted, and the amount of dissolved mebendazole was determined spectrophotometerically at 285 nm. The mebendazole concentration was then determined *via* absorbance measurements in a matched quartz cells using a Shimadzu UV-2101PC UV/Vis scanning spectrophotometer. Previous determinations showed that the *CD*s did not interfere with the spectrophotometeric measurements.

References

[1] Cook GC (1990) Parasitol Today **6**: 133
[2] Sharma S (1989) Drugs of Today **25**: 249
[3] Davis A (1986) Bull WHO **64**: 384
[4] Bassani VL, Krieger D, Duchene D, Wouessidjewe D (1996) J Incl Phen Macrocycl Chem **25**: 149
[5] Saenger W (1980) Angew Chem Int Ed Eng **19**: 344
[6] Szejtle T (1982) Cyclodextrin and Their Inclusion Complexes. Academiai Kiado, Budapest
[7] Jones SP, Grant DJW, Hadgraft J, Parr GP (1984) Acta Pharm Technol **30**: 213
[8] Bastos M, Briggner L-E, Shehatta I, Wadsö I (1990) J Chem Thermodyn **22**: 1181
[9] Hallen D, Schon A, Shehatta I, Wadsö I (1992) J Chem Soc Faraday Trans **88**: 2859
[10] Shehatta I (1996) React Funct Polymers **28**: 183
[11] Shehatta I, Ibrahim M (2001) Can J Chem **79**: 1431
[12] Ibrahim M, Shehatta I, Al-Naiyle A (2001) J Pharm Biomed Anal
[13] Higuchi T, Connors KA (1965) Adv Anal Chem Instrum **4**: 117
[14] Blanco TL, Vila-Jato J, Otero F, Anguianco S (1991) Drug Dev Ind Pharm **17**: 943
[15] Fromming KH, Szejtli J (1994) Cyclodextrins in Pharmacy. Kluwer, Dordrecht
[16] Loftsson T, Brewster ME (1996) Pharm J Pharm Sci **85**: 1017
[17] Loftsson T, Brewster ME (1997) Pharm Techn Eur **9**: 26
[18] Mounoz de la Pena A, Ndou TT, Zung JB, Greene KL, Live DH, Warner IM (1991) J Am Chem Soc **113**: 1572
[19] Reichardt C (1988) Solvents and Solvent Effects in Organic Chemistry, 2nd edn. VCH, Weinheim
[20] Tanford C (1980) The Hydrophobic Effect: Formation of Micelles and Biological Membranes, 2nd edn. Wiley, New York
[21] Junquera E, Aicart E (1997) J Phys Chem B **101**: 7163
[22] Valero M, Costa SB, Ascenso JR, Velazquez MM, Rodriguez LJ (1999) J Incl Phenom Macro Chem **35**: 663
[23] Bender MI, Komiyama M (1977) In: van Tamelen (ed) Bioorganic Chemistry, vol 1. Academic Press, New York
[24] Lewis EA, Hansen LD (1973) J Chem Soc Perkin Trans II, 2081
[25] Diaz D, Bernard MJB, Mora JG, Llanos CME (1999) Drug Dev Ind Pharm **25**: 111
[26] Duchene D, Glomot F, Vaution C (1986) Pharmaceutical Applications of Cyclodextrins. In: Duchene D (ed) Cyclodextrins and Their Industrial Uses. Les Editions de Santé, Paris
[27] Diaz D, Bernard MJB, Mora JG, Llanos CME (1998) Pharm Dev Techn **3**: 395
[28] Zia V, Rajewski RA, Stella VJ (2000) Pharm Res **17**: 936

Received November 30, 2001. Accepted (revised) December 27, 2001