Binding of Sodium Salicylate by β-Cyclodextrin or 2,6-Di-*O*-methyl-β-cyclodextrin in Aqueous Solution

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Abstract □ Speed of sound and conductivity experiments have been done at 298.15 K to study the encapsulation process of sodium salicylate (NaSA) by β-cyclodextrin (β-CD) and 2,6-di-*O*-methyl-β-cyclodextrin (DIMEB) in aqueous solutions. Since the concentration of the salicyclic form (HSA), coming from the hydrolysis of SA^-, is negligible at biological pH, the binding process studied in this work is that of the SA^- species. The stoichiometries of the complexes DIMEB:SA^- and β-CD:SA^- have been found to be 1:1, as usually determined for most CD:drug complexes. Their association constants and their ionic molar conductivities at infinite dilution have been obtained by fitting the experimental conductivity data with a nonlinear regression method (NLR). For that purpose, a model based on that of Gelb and co-workers has been used. From the values of $K_{\beta\text{-CD:SA}^-} = (105 \pm 15)\ M^{-1}$ and $K_{\text{DIMEB:SA}^-} = (140 \pm 20)\ M^{-1}$ obtained, the bioavailability of the salicylate drug in the complexed form has been discussed.

Introduction

Poor bioavailability is, unfortunately, a frequent problem for drug delivery. Many molecules that are biologically active *in vitro* are inactive *in vivo* due to a variety of possible problems such as limited solubility or stability, adverse side effects, unfavorable tastes and odors, and limited transport across cell membranes.

Many works can be found in the literature regarding the low bioavailability of insoluble and unstable drugs. Among the different methods proposed to overcome this problem, the molecular encapsulation of these drugs by cyclodextrins (CD's) is probably the most widely used.[1−7] Nowdays, CD's, which are well-known nontoxic macrocyclic sugars of natural origin, are considered a whole family of pharmaceutical excipients and drug carriers, and many patents and even commercial products containing CD's are, actually, in course. Their doughnut-shaped structure, with hydrophilic external faces and hydrophobic inner surface, makes them the most important simple organic compounds capable of forming noncovalently bonded inclusion complexes with a wide variety of drug molecules in aqueous solution. The resulting host−guest systems become more soluble and stable, the stability being a result of the concurrence of different contributions, such as van der Waals interactions, hydrophobic effect, solvent reorganization, and hydrogen bonding.[8]

On the other hand, drugs which are highly polar and soluble in aqueous medium can also be poorly bioavailable due to inefficient transport across the hydrophobic lipid bilayer constituting the cellular membranes. This problem, which is particularly significant in the area of antiviral and antisense drugs, has been studied by using model systems which can mimic the cellular membranes, such as liposomes and micelles.[9] Moreover, the encapsulation of these soluble drugs by cyclodextrins can be useful since the host molecules may act as concentration-regulating agents, decreasing the adverse side effects at the organ or cellular level and improving the optimal dose of the drug and its frequency of administration.[2,3,6,7]

The numerous therapeutic effects of salicylates, as well as their possible adverse effects, have been known a long time. They have been used, for example, as diuretics (lithium salicylate), intestinal antiseptics (bismuth salicylate), and anti-inflammatory, analgesic, and antipyretic agents (lysine acetylsalicylate, sodium salicylate). In this work, the encapsulation processes of sodium salicylate (NaSA), a soluble anti-inflammatory drug, by β-CD or by its methylated derivative, DIMEB, are studied by means of very precise speed of sound and conductimetric measurements. Speed of sound has been widely proved to be a physicochemical property capable of detecting any structural change occurring in the solution. Particularly, it is a well-known method[5,10,11] to study the formation of supramolecular aggregates, i.e. micelles and inclusion complexes. On the other hand, conductivity has been widely used to characterize a large amount of different host−guest systems,[5,12−23] many of them with a drug molecule[13−17] as the guest and a cyclodextrin as the host. In this work, the speed of sound measurements allowed us to determine the stoichiometry of the complexes and to analyze qualitatively the affinity of the binding process, while the conductivity data provide us not only with the stoichiometries but also with the association constants of the CD:NaSA inclusion complexes, obtained from the NLR fitting of the conductivity data. These association constants are, probably, the most important thermodynamic parameters regarding the bioavailability of the drug. It is well-known that, if reliable binding constants values are required, accurate experimental data and nonlinear regression (NLR) methods to fit these data are necessary. We have recently demonstrated[24] that the binding constant determination can be problematic in the case of acid−base conjugated systems being the substrates, in the sense that the pH value must guarantee the presence of only the base or the acid species. On the contrary, the result obtained is just an apparent constant, averaged over the encapsulation processes of both species. Since the concentration of the salicyclic acid form, coming from the neutralization of salicylate in aqueous medium, is almost negligible at biological pH, the encapsulation processes of NaSA by β-CD and/or DIMEB at this pH or higher (as is the case of the present work) are reduced to the inclusion of the SA^- species.

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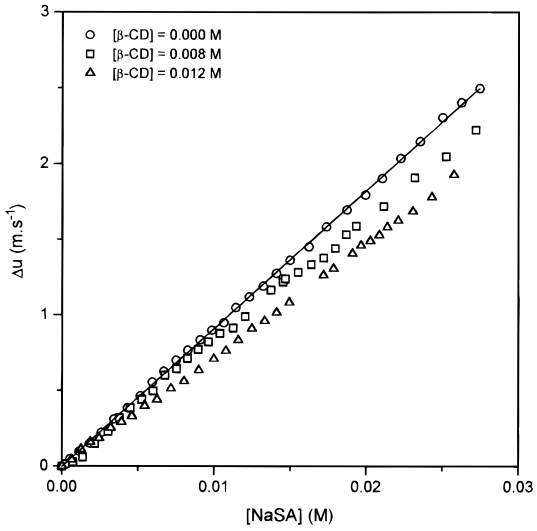


Figure 1—Plot of Δu ($=u - u_0$) versus [NaSA], at several constant β-CD concentrations for the ternary system β-CD/NaSA/H_2O.

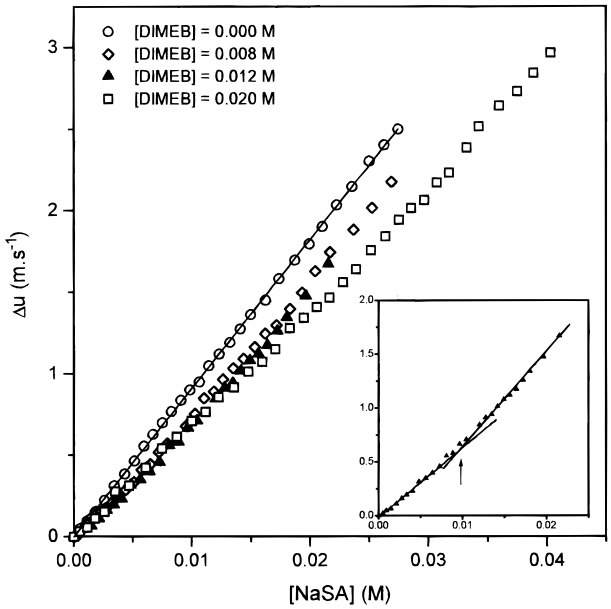


Figure 2—Plot of Δu ($=u - u_0$) versus [NaSA], at several constant DIMEB concentrations for the ternary system DIMEB/NaSA/H_2O. The inset in the bottom indicates, as an example, the method to obtain the stoichiometry, *A*, of the inclusion complex for the concentration 0.012 M of DIMEB.

Experimental Section

Sodium salicylate (NaSA) was purchased from Aldrich Co., with purities of 99% or greater. β-Cyclodextrin (β-CD) and 2,6-di-*O*-methyl-β-cyclodextrin (DIMEB) were obtained from Aldrich Co. and Cyclolab (Budapest, Hungary), respectively. All of them were used without purification. Two thermogravimetric analyses (TG) were done for both cyclodextrins to check the amount of water. The results obtained, which were considered to calculate solute concentrations, were 13.5% of water in β-CD and 1.7% of water in DIMEB. All the solutions were prepared with distilled, deionized (taken from a Millipore Super-Q System, with a conductivity lower than 18 $\mu\Omega^{-1}$ cm^{-1}), and also degassed water.

Speeds of sound, *u*, were measured at 298.15 K by using a pulse−echo−overlap technique of fixed path type at a frequency of 2.25 Mhz. This technique operates in a multiple-echo mode with broadband pulses. The equipment used, as well as the experimental method, have been fully described previously.[25] The transducer−reflector distance of the measuring cell was calibrated from the speed of sound of pure water (1496.74 m s^{-1}) reported by Kroebel and Mahrt.[26] The precision of *u* data is ±0.1 m s^{-1}. The *u* measurements were made (i) as a function of concentration for the aqueous solutions of the pure substances β-CD, DIMEB, and NaSA and (ii) as a function of the drug concentration at different constant values of cyclodextrin concentration, for the systems β-CD + NaSA and DIMEB + NaSA.

Conductivity data were collected at 298.15 K with a Hewlett-Packard 4263A LCR meter, using a Metrohm electrode with a cell constant of 0.8129 cm^{-1}. The experimental procedure, fully computerized, was widely described elsewhere.[27] The accuracy on the specific conductivity, κ, obtained as an average of 2400 measurements for each concentration, is believed to be better than 0.03%. The conductivity measurements were made for the pure NaSA and for the systems β-CD + NaSA and DIMEB + NaSA (i) as a function of NaSA concentration at different constant values of [CD], (ii) as a function of cyclodextrin concentration at several constant [NaSA], and (iii) as a function of [CD] and [NaSA] at a constant stoichiometric ratio [CD]/[NaSA] equal to 1.

The temperature control in both speed of sound and conductimetric experiments, as previously reported,[25,27] is better than ±1 mK.

Results and Discussion

Speed of Sound Study—In Figure 1, the experimental values of Δu ($=u - u_o$, where u_o is the speed of sound for the CD initial solution) are plotted as a function of concentration for the system β-CD + NaSA at two different cyclodextrin concentrations, together with the Δu values for the pure NaSA. As might be expected, no change in the slope of Δu vs concentration has been found for the pure NaSA water solution, since the concentration at which NaSA form aggregates in water (cmc), behaving as a classical surfactant,[28] is around 0.67 M, completely out of the concentration range studied in this work. When the β-CD is added, a change of the slope of Δu vs [NaSA], attributed to the inclusion complex formation, might be expected. This change, which has been observed in most of the systems previously studied,[11,19−22] does not seem to appear in Figure 1 for the system β-CD + NaSA. A first interpretation of this fact could be that in this case the inclusion complex β-CD:SA^- is not formed. Nevertheless, it can be observed as well as in Figure 1 that at a given [NaSA] the Δu value for the system is always lower than that for the pure NaSA, and the higher the β-CD concentration is, the wider this difference becomes. This feature must be attributed only to the formation of the complex β-CD:SA^-, although the stoichiometry cannot be determined from the speed of sound experiments. The most plausible explanation of this fact is that, although the inclusion complex CD:SA^- is formed, its association is not strong enough to provoke a detectable change in the slope of Δu vs [NaSA] in this system.

In Figure 2, the experimental values of Δu are plotted for the system DIMEB + NaSA at three constant concentrations of DIMEB. The same behavior found in Figure 1 can be observed in Figure 2: as long as the constant DIMEB concentration increases, the curve is further away than that of pure NaSA, indicating that the DIMEB:SA^- complex is formed as well. Nevertheless, in this case, as can be seen in the zoomed window for one of the DIMEB concentrations, a slight change in the slope due to the complex formation can be noticed, in contrast with the previous system, β-CD + NaSA. This fact, which is a qualitative indication of a higher binding constant, makes possible the determination of the complex stoichiometry from the [NaSA] value at which a change in the slope of Δu is observed. The stoichiometry is thus obtained as the ratio [DIMEB]/[NaSA], where [DIMEB] is the constant cyclodextrin concentration and [NaSA] is the intersection point of the two straight lines at which the experimental Δu values are fitted below and above the change in the slope. The average value, $\bar{A}$, for the three experi-

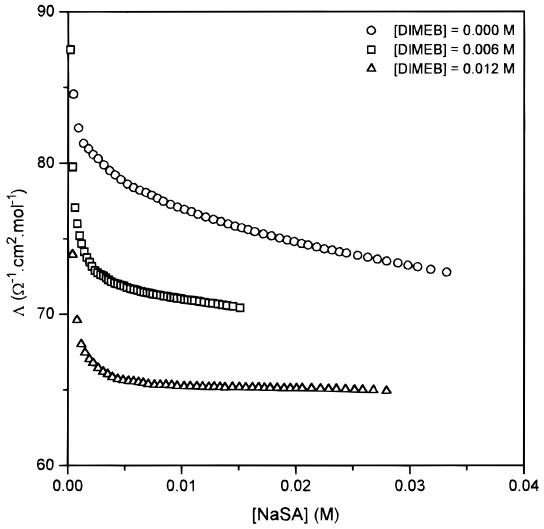


Figure 3—Plot of the molar conductance, Λ, versus [NaSA], at several constant DIMEB concentrations for the ternary system DIMEB/NaSA/H_2O.

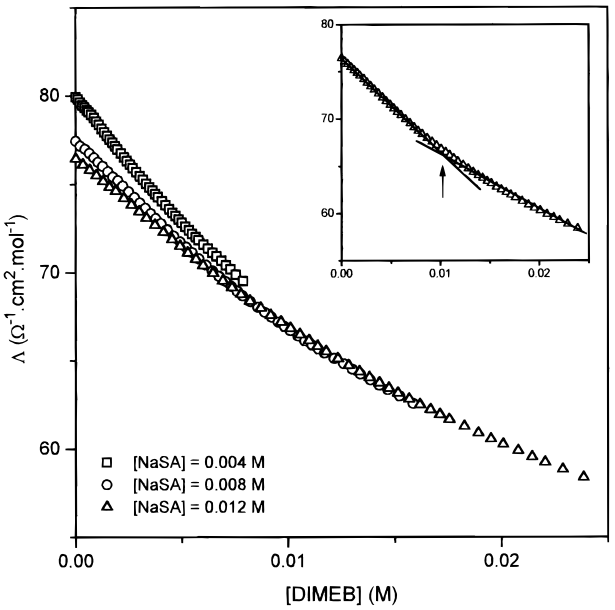


Figure 4—Plot of the molar conductance, Λ, versus [DIMEB], at several constant NaSA concentrations for the ternary system DIMEB/NaSA/H_2O. The inset in the top indicates, as an example, the method to obtain the stoichiometry, *A*, of the inclusion complex for the 0.012 M of NaSA.

ments is 1.0 ± 0.05, as usually found for most CD:guest systems.[20–22,29–34]

Conductivity Study—In Figures 3 and 4, the values of the molar conductance (Λ = 1000κ/[NaSA]) of the drug solutions are plotted as a function of sodium salicylate concentration (at various constant [DIMEB]) and as a function of DIMEB concentration (at various constant [NaSA]), respectively. Similar plots have been obtained for the system β-CD + NaSA.

As can be seen in Figure 3, the molar conductivity Λ of pure NaSA decreases appreciably in the presence of DIMEB, the magnitude of this decrease being higher as long as the constant DIMEB concentration increases. This fact can be attributed to the formation of the DIMEB:SA^- complex. The SA^- species loose mobility as long as it is included in the CD cavity, and the mobility of the CD:SA^- complex is lower than that of the SA^- alone. This loss of mobility justifies the decrease in Λ observed in the figure. The same trend has been found in the system β-CD + NaSA.

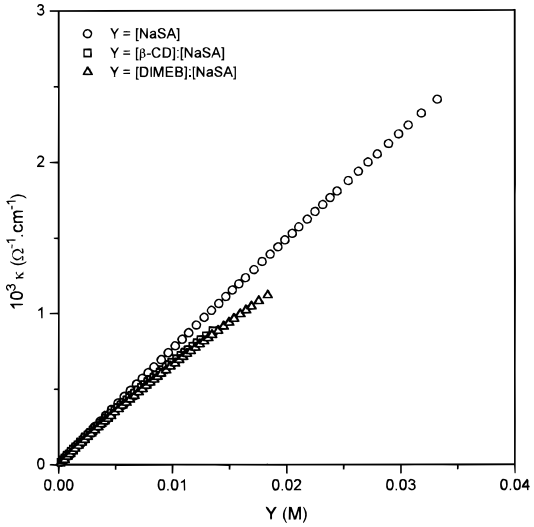
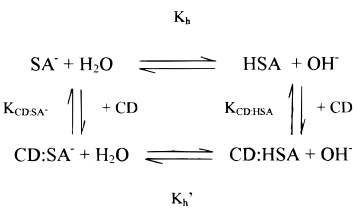


Figure 5—Plot of the specific conductivity, κ, versus [CD] and [NaSA], the concentration ratio being *A* = [CD]/[NaSA] equal to 1.

In Figure 4, the stoichiometry, *A*, of the complex can be estimated following the same procedure previously explained for the speed of sound experiment (Figure 2). It means that A is determined as the ratio [DIMEB]/[NaSA], where [NaSA] is the constant drug concentration and [DIMEB] is the cyclodextrin concentration at which a change of the slope of Λ vs [DIMEB] is observed (see zoomed window). It is noteworthy that this change is not as sharp as that one found for the CD:guest complexes with higher association constants.[20–22] The resulting average value, $\bar{A}$, is in agreement with that obtained from the speed of sound data, confirming the 1:1 stoichiometry for the DIMEB:SA^- complex. In the case of the β-CD:SA^- complex, whose stoichiometry could not be determined from the speed of sound experiments, a value of 1.0 ± 0.05 has been found as well.

In a third study, the conductivity has been measured as a function of [CD] and [NaSA] at stoichiometric ratio. It means that both [CD] and [NaSA] increase simultaneously, the 1:1 ratio being kept constant. Figure 5 shows the specific conductivity κ vs [CD] and [NaSA], together with the values obtained for the pure NaSA. Again, it can be observed that the curves for the CD + NaSA systems run below that of NaSA in the absence of cyclodextrin, corroborating the features observed in the two previous studies. Moreover, the curve for the system DIMEB + NaSA runs slightly below that for the β-CD + NaSA system. This behavior can be considered again a qualitative indication of a tighter affinity of DIMEB upon binding NaSA, with respect to that of β-CD.

Association Constant Determination—The encapsulation of the drug molecule into the CD cavity is an equilibrium process governed by the binding constant. In the present case, since the salicylate species is the conjugated base of an acid/base system, the equilibria involved in this encapsulation process are shown in the following scheme:



where

$$K_h = (a_{OH^-} a_{HSA})/(a_{SA^-}) \quad (1)$$

$$K_{CD:SA^-} = (a_{CD:SA^-})/(a_{CD} a_{SA^-}) \quad (2)$$

$$K_{CD:HSA} = (a_{CD:HSA})/(a_{CD} a_{HSA}) \quad (3)$$

$$K_h' = (a_{CD:HSA} a_{OH^-})/(a_{CD:SA^-}) \quad (4)$$

The constants K_h and K_h' are the hydrolysis constants of the salicylate species SA^- in the free and complexed form respectively, while $K_{CD:SA^-}$ and $K_{CD:HSA}$ are the binding constants of the inclusion complexes formed by the cyclodextrin and the nonprotonated and protonated forms of HSA, respectively.

These association constants, together with the stoichiometry, are thermodynamic parameters necessary to characterize these CD:drug inclusion complexes. Factors such as the bioavailability and dosage of the drug, of great importance in the pharmacological industry, can be controlled with the knowledge of the binding constant.

It is necessary to find a model to determine the binding constants by relating them with the experimental values of the studied physicochemical property as a function of concentration. Several features regarding CD/drug/water systems need to be remarked upon in order to choose the most suitable model: (i) Most drug molecules are known to form 1:1 inclusion complexes with CD's.[5,6,30–34] This fact, together with the well-known cavity size of the β-CD and derivatives[35–37] and the small size of the SA^- molecule, leads us to assume that complexes such as CD_2:SA^- or CD:$(SA^-)_2$ or those with even higher stoichiometries are not expected. Furthermore, this fact has been confirmed in this work through speed of sound and conductivity measurements. (ii) Several authors[18,38] have found that the counterion of small molecules are not associated to the inclusion complex, opposite to the behavior found for CD:surfactant inclusion complexes.[22,38,39] Thus, Na^+ ion can be considered in this work as a mere "spectator" of the CD:drug inclusion process. (iii) Since the pK_a of the salicyclic acid is 2.95[24] at 25 °C, the hydrolysis of NaSA in concentrations around 10^{-4}–10^{-2} M is moderately basic. As a consequence, almost all of NaSA in solution is in the salicylate form, with a negligible contribution of the presence of HSA species. Thus, the previously presented equilibria are reduced in this work to that for the encapsulation of the salicylate species by the cyclodextrin:CD + $SA^- \leftrightarrow$ CD:SA^-.

Since all the species in the solution (i.e. H_2O, CD, SA^-, Na^+, and CD:SA^-) will contribute to the speed of sound value, while in the case of conductivity only the charged species (SA^-, Na^+ and CD:SA^-) have to be considered, a model based on conductivity data, with a lower number of variables, seems to be less complicated. Thus, keeping in mind all the above mentioned premises, the model proposed by Gelb et al.[18] has been chosen to obtain the binding constant of the inclusion complexes studied in this work, from conductivity results. Gelb and co-workers have studied a number of CD:substrate complexes by using their model with satisfactory results.[18] On the basis of this model, the specific conductivity, κ, of a solution of sodium salicylate and cyclodextrin can be expressed as a function of the ionic molar conductivities, λ_i, of all the ionic species at their corresponding concentration as follows,

$$\kappa = \lambda_{Na^+}[Na^+] + \lambda_{SA^-}[SA^-] + \lambda_{CD:SA^-}[CD:SA^-] \quad (5)$$

where the concentrations are related to each other through the binding constant (eq 2), the mass balances of the salicylate (free and complexed, eq 6) and the cyclodextrin (eq 7),

$$[drug]_{total} = [SA^-] + [CD:SA^-] \quad (6)$$

$$[CD]_{total} = [CD] + [CD:SA^-] \quad (7)$$

and the charge balance (eq 8)

$$[H^+] + [Na^+] = [SA^-] + [CD:SA^-] + [OH^-] \quad (8)$$

Besides, we have considered the following particularities in the model: (a) In spite of the low concentrations used in this work for SA^- (10^{-4}–10^{-2} M), the activity coefficients of the charged species, obtained through the extended Debye–Hückel theory, have been taken into account. (b) The ionic molar conductivities of the charged species, λ_i, have been related with the corresponding values at infinite dilution, λ_i^o, through the well-known Debye–Hückel–Onsager theory.[40] (c) The ionic molar conductivity at infinite dilution of SA^- ion, $\lambda_{SA^-}^o$, has been directly determined by a NLR method, from the conductivity measurements of the system NaSA + H_2O reported in this work. The obtained value and that reported in the literature for the Na^+ ion[41] are shown in Table 1. (d) The ion size hydrodynamic parameters, a_n, necessary for the calculation of the activity coefficients, have been either taken from the literature for the Na^+ ion[42] or estimated from the ionic molar conductivity at infinite dilution, λ_i^o, using the Brüll equation[43] for the SA^- ion (Table 1).

Table 1—Molar Ionic Conductivities at Infinite Dilution, λ_i^o, Ion Size Hydrodynamic Parameters, a_n, and Association Constants, K, of the Inclusion Complexes

ion	λ_i^o/Ω^{-1} cm^2 mol^{-1}	a_n/Å	K/M^{-1}
Na^+	50.1[41]	4[42]	
SA^-	34 ± 2	7 ± 1	
β-CD:SA^-	22 ± 3	10 ± 2	105 ± 15
DIMEB:SA^-	17 ± 3	13 ± 2	140 ± 20

A nonlinear regression method, based on a nonlinear Newton–Raphson and a Marquardt algorithm, has been used to fit the experimental conductivity data, according with the above explained model. The values obtained for the binding constant $K_{CD:SA^-}$ and the ionic molar conductivity at infinite dilution $\lambda_{CD:SA^-}^o$, are reported in Table 1 for both β-CD:SA^- and DIMEB:SA^- complexes. The values in Table 1 indicate that the mobility of the associated salicylate ion decreases between 40 and 50% with respect to the mobility of the free salicylate ion, causing a decrease in the conductivity of the solution as long as the complex CD:SA^- is formed, justifying the behavior found in Figures 3 and 5. It can also be noticed that the β-CD derivative (DIMEB) binds NaSA with a higher affinity (around 35% higher) than the unsubstituted β-CD does, probably due to the higher flexibility of the DIMEB molecule,[35–37] which makes it easier for the CD–drug interaction to occur. This feature, which has been noticed in the speed of sound and conductimetric experiments, can justify why only the stoichiometry of the complex DIMEB:SA^- could be obtained. Anyway, it should be remarked that both binding constants are moderate, in contrast with those found for the complexes formed by CD's and the salicylic acid, HSA, which are in the range of 1000 M^{-1} [24,44] (at pH ≈ 1), around 8 times higher. A value of $K = 220 \pm 40$ M^{-1} has been reported[24] for the complex HPBCD:SA^- (at pH = 7) using a fluorescence technique, and only a value of $K_{\beta\text{-CD:SA}^-} = (51 \pm 7)$ M^{-1} has been reported at pH = 7.4 by Sideris et al.,[45] using a dynamic dialysis technique. The value

reported by Sideris et al., although of the same order of magnitude, differs from that obtained by us in this work within the experimental uncertainties. We believe that these differences can be attributed to the method used by Sideris et al. to obtain the binding constant, a Scatchard plot method which, in contrast with NLR methods, is well-known to give unreliable values for the association constant in most cases. In contrast, the *K* values obtained for the complexes β-CD:SA^- or DIMEB:SA^- (this work) and for HPBCD:SA^-[24] are all determined under analogous saturation degree[46] range and by using NLR methods, although with different experimental techniques and mathematical models. They reveal that the affinity of NaSA upon binding a cyclodextrin is greater in the cases of the β-CD derivatives than for the partner cyclodextrin (β-CD), behavior usually found in most CD + guest systems.

From simple mathematical operations with the values of $K_{\beta\text{-CD:SA}^-} = 105\ M^{-1}$ and $K_{\text{DIMEB:SA}^-} = 140\ M^{-1}$ obtained in this work, it can be concluded that a normal dose of sodium salicylate, if administered encapsulated by β-CD or DIMEB at biological pH, leaves in the medium an initial quantity of free and available SA^- which ranges from 40 to 60% of the total amount of the administered drug. And, what is more important, as long as the organism "makes use" of the drug, the equilibrium (eq 2) is shifted by mass action toward the release of the active principle, keeping and regulating its presence in the medium. These effects would be even more emphasized when the HSA species is absolutely predominant, which occurs at a strongly acidic pH, characteristic for example of the stomach. Thus, repeating the same simple calculations with the binding constants of the complexes formed by cyclodextrins and salicylic acid, it can be concluded that with similar doses, just 25% of the drug would be free in the medium.

These estimations rebound the advantages of the use of cyclodextrins as drug carriers, widely commented on in the literature:[1−6] (i) the adverse side effects can be substantially reduced since the quantity of drug which is really free and available in the medium may be much lower than the administered dose; (ii) the time of action of the drug can be prolonged, which in the case of the NSAID therapy (salicyclic and related drugs) is particularly important, given its well-known fast onset of action and subsequent short elimination half-life; and (iii) as a consequence, the number of doses and their frequency can be reduced with the subsequent decrease of the side effects as well. The confirmation of all these estimations *in vivo*, although out of the scope of this work, would be welcomed.

References and Notes

1. Luzzi, L.; Palmieri, A. *Biomedical Applications of Microencapsulation*; F. Lim Ed.: CRC Press, Boca Ratón, FL, 1984.
2. Duchêne, D.; Wouessidjewe, D. *Drug Dev. Ind. Pharm.* **1990**, *16*, 2487.
3. (a) Szejtli, J. *Med. Res. Rev.* **1994**, *14*, 353. (b) Szejtli, J. *J. Inclusion Phenom.* **1992**, *14*, 25.
4. Loftsson, T.; Brewster, M. E. *J. Pharm. Sci.* **1996**, *85*, 1017.
5. Szejtli, J.; Osa, T. *Comprehensive Supramolecular Chemistry. Vol. 3. Cyclodextrins*; J. M., Lehn, Ed.; Pergamon Press: Oxford, UK, 1996, and references therein.
6. Thompson, D. O. *Crit. Rev. Ther. Drug Carrier Syst.* **1997**, *14*, 1.
7. Rajewski, R. A.; Stella, V. J. *J. Pharm. Sci.* **1996**, *85*, 1142.
8. Schneider, H.-J. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1417.
9. (a) Nowick, J. S.; Chen, J. S.; Noronha, G. *J. Am. Chem. Soc.* **1993**, *115*, 7636. (b) Nowick, J. S.; Cao, T.; Noronha, G. *J. Am. Chem. Soc.* **1994**, *116*, 3285.
10. Junquera, E.; Aicart, E.; Tardajos, G. *J. Phys. Chem.* **1992**, *96*, 4533.
11. Junquera, E.; Tardajos, G.; Aicart, E. *Langmuir* **1993**, *9*, 1213.
12. Connors, K. A. *Binding Constants: The Measurement of Molecular Complex Stability*; John Wiley & Sons: New York, 1987.
13. Cramer, F.; Saenger, W.; Spatz, H.-Ch. *J. Am. Chem. Soc.* **1967**, *89*, 14.
14. Connors, K. A.; Lipari, J. M. *J. Pharm. Sci.* **1976**, *65*, 379.
15. Uekama, K.; Otagiri, M.; Kanie, Y.; Tanaka, S.; Ikeda, K. *Chem. Pharm. Bull.* **1975**, *23*, 1421.
16. Otagiri, M.; Miyaji, T.; Uekama, K.; Ikeda, K. *Chem. Pharm. Bull.* **1976**, *24*, 1146.
17. Miyaji, T.; Kurono, Y.; Uekama, K.; Ikeda, K. *Chem. Pharm. Bull.* **1976**, *24*, 1155.
18. (a) Gelb, R. I.; Schwartz, L. M.; Murray, C. T.; Laufer, D. A. *J. Am. Chem. Soc.* **1978**, *100*, 3552. (b) Gelb, R. I.; Schwartz, L. M.; Laufer, D. A. *J. Am. Chem. Soc.* **1978**, *100*, 5875.
19. (a) Satake, I.; Ikenoue, T.; Takeshita, T.; Ayakawa, K.; Maeda, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 2746. (b) Satake, I.; Yoshida, S.; Ayakawa, K.; Maeda, T.; Kusumoto, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3991.
20. (a) Palepu, R.; Reinsborough, V. C. *Aust. J. Chem.* **1990**, *43*, 2119. (b) Palepu, R.; Reinsborough, V. C. *Can. J. Chem.* **1988**, *66*, 325.
21. (a) Tawarah, K. M.; Wazwaz, A. A. *J. Chem. Soc. Faraday Trans.* **1993**, *89*, 1729. (b) Tawarah, K. M.; Wazwaz, A. A. *Ber. Bunsenges. Phys. Chem.* **1993**, *97*, 727.
22. (a) Junquera, E.; Peña, L.; Aicart, E. *Langmuir*, **1995**, *11*, 4685. (b) Junquera, E.; Peña, L.; Aicart, E. *J. Inclusion Phenom.* **1996**, *24*, 233.
23. Pospísil, L.; Colombini, M. P. *J. Inclusion Phenom.* **1993**, *16*, 255.
24. Junquera, E.; Aicart, E. *J. Inclusion Phenom.* **1997**, *29*, 119.
25. Tardajos, G.; Diaz Peña, M.; Aicart, E. *J. Chem. Thermodyn.* **1986**, *18*, 683.
26. Kroebel, W.; Marht, K. M. *Acustica* **1976**, *35*, 134.
27. Junquera, E.; Aicart, E. *Rev. Sci. Instrum.* **1994**, *65*, 2672.
28. Treiner, C.; Khodja, A. A.; Fromon, M.; Chevalet, J. *J. Sol. Chem.* **1989**, *18*, 217.
29. Martin Davies, D.; Savage, J. *J. Chem. Soc. Perkin Trans. 2* **1994**, 1525.
30. Fronza, G.; Mele, A.; Redenti, E.; Ventura, P. *J. Pharm. Sci.* **1992**, *81*, 1162.
31. Senel, S.; Çakoglu, Ö.; Sumnu, M.; Duchêne, D.; Hincal, A. A. *J. Inclusion Phenom.* **1992**, *14*, 171.
32. Puglisi, G.; Ventura, C. A.; Fresta, M.; Vandelli, M. A.; Cavallaro, G.; Zappala, M. *J. Inclusion Phenom.* **1996**, *24*, 193.
33. Esclusa-Díaz, M. T.; Guimaraens-Méndez, M.; Pérez-Marcos, M. B.; Vila-Jato, J. L.; Torres-Labandeira, J. J. *Int. J. Pharm.* **1996**, *143*, 203.
34. Loukas, Y. L.; Vraka, V.; Gregoriadis, G. *Int. J. Pharm.* **1996**, *144*, 225.
35. Bender, M.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: Berlín, 1978.
36. Saenger, W. *Inclusion Compounds*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Eds.; Academic Press: London, 1984.
37. Clarke, R. J.; Coates, J. H.; Lincoln, S. F. *Adv. Carbohydr. Chem. Biochem.* **1988**, *46*, 205.
38. MacPherson, Y. E.; Palepu, R.; Reinsborough, V. C. *J. Inclusion Phenom.* **1990**, *9*, 137.
39. Palepu, R.; Reinsborough, V. C. *Can. J. Chem.* **1989**, *67*, 1550.
40. Robinson, R. A.; Stokes, R. M. *Electrolytes Solutions*; Butterworths: London, 1965.
41. Tinner, U. *Electrodes in Potentiometry*; Metrohm, AG: Herisau, 1989.
42. Kielland, J. *J. Am. Chem. Soc.* **1937**, *59*, 1675.
43. Brüll, L. *Gazz. Chim. Ital.* **1934**, *64*, 624.
44. Vromans, H.; Eissens, A. C.; Lerk, C. F. *Acta Pharm. Technol.* **1989**, *35*, 250.
45. Sideris, E. E.; Georgiou, C. A.; Koupparis, M. A.; Macheras, P. E. *Anal. Chim. Acta* **1994**, *289*, 87.
46. Derenlau, D. A. *J. Am. Chem. Soc.* **1969**, *91*, 4044.

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