Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **30:** 151–161, 1998.

Effects of Some Hydrotropic Agents on the Formation of Indomethacin/β-Cyclodextrin Inclusion Compounds

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(Received: 7 January 1997; in final form: 15 May 1997)

Abstract. The influence of the water structure promoters, mannitol and sucrose, on the indomethacin/β-cyclodextrin inclusion process at different pH values was evaluated using the phase solubility method and circular dichroism spectroscopy. The effect of mannitol and sucrose on the total solubilizing activity of β-cyclodextrin as well as on the stabilization of the complex was moderate, and depended strongly on the pH value of the medium. The most pronounced effect on the association constant of the indomethacin inclusion compound was observed in KCl/HCl buffer of pH 1.6, because mannitol and sucrose, in the absence of β-cyclodextrin, approximately double the indomethacin solubility so that the constant value decreases. On the other hand, a very strong complex formation between indomethacin and β-cyclodextrin was observed in acid medium (KCl/HCl buffer, pH = 1.6), a fact not much discussed in the literature. The apparent association constant of the inclusion compound formed in the absence of mannitol or sucrose was found to be of the same order of magnitude as for the complex formed at pH 6.8. Changes in the optical activity of the indomethacin/β-cyclodextrin inclusion complex were registered in the presence of sucrose, which interacts more strongly than mannitol with water molecules, and could probably adhere to the surface of the inclusion complex, thus changing its configuration and environment.

Key words: Indomethacin/β-cyclodextrin inclusion complex, solubility, mannitol, sucrose, circular dichroism spectra.

1. Introduction

The influence of water structure modifiers (disrupters and promoters) on the inclusion of guests by cyclodextrins has not been well studied, and the data reported are contradictory. Hammada *et al*. [1] studied the effect of urea and sodium chloride on the β-cyclodextrin/mefenemic acid interaction, and outlined the high potential of these compounds to change the structure of water which is formed around the solute molecules. More recently, Hüttenrauch and Fricke [2] reported on the

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favourable effect of sorbitol on the stability of vitamin D2 to light. Other structure modifiers, such as urea and methylacetamide, did not affect the stability of dissolved ergocalciferol in solution after 12 days of irradiation by sunlight. Müller and Albers [3] reported that nicotinamide and urea (water structure disrupters), unlike sorbitol, favour the formation of the methyltestosterone/hydroxypropyl β-cyclodextrin complex.

Pedersen [4] studied the effect of different water structure modifiers on the formation and stability of a clotrimazole/β-cyclodextrin inclusion compound and concluded that the water structure promoters, sorbitol and fructose, could stabilize the cyclodextrin complexes but they could not be used to form concentrated drug solutions because they decrease the total drug solubility in β-cyclodextrin solution.

The present paper reports on the effect of the water structure promoters, mannitol and sucrose, on the inclusion of the anti-inflammatory non-steroidal drug indomethacin in β-cyclodextrin at different pH values of the medium.

2. Materials and Method

2.1. Materials

Indomethacin, mannitol and sucrose were all purchased from Fluka (Switzerland). The β-cyclodextrin was supplied by Roquette Frères (France). The buffer substances were of analytical grade.

2.2. Equilibrium solubility analysis [5]

An excess of indomethacin (16 mmol/L) was always added to aqueous or buffered solutions of β-cyclodextrin in increasing concentrations, from 2 to 16 mmol/L, and in the presence or absence of 0.5 mol/L mannitol or sucrose. The buffers used were KCl (0.2 mol/L)/HCl (0.2 mol/L) of pH 1.6 and phosphate (0.2 mol/L)/citrate (0.1 mol/L) buffer of pH 6.8. The samples were agitated for 48 h at 25 $\pm$ 1 °C. The concentration of the dissolved indomethacin was spectrophotometrically assayed by UV after filtration and suitable dilution of the samples.

The phase solubility analysis of indomethacin in acid medium and in solutions of mannitol and sucrose in increasing concentrations, from 0.25 to 1.5 mol/L (without β-cyclodextrin), was carried out at 37 $\pm$ 1 °C in the same experimental conditions as described above.

2.3. Circular dichroism measurements

Circular dichroism spectra were recorded on Dichrographe Spectropolarimeter (Jobin Ivon, France) at ambient temperature. The solutions studied contained 8×10^{-5} mol/L indomethacin, 16×10^{-3} mol/L β-cyclodextrin and 0.5 mol/L of the corresponding hydrotropic compound in buffer of pH 6.8.

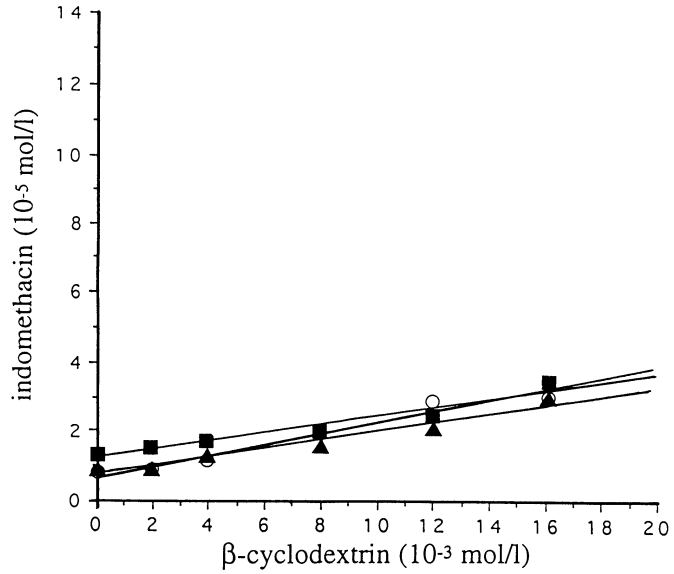

Figure 1. Solubility profiles of indomethacin in the presence of increasing concentrations of β-cyclodextrin and 0.5 mol/L hydrotropic agent in KCl/HCl buffer of pH 1.6: (○) without hydrotropic agent, (■) with sucrose, (▲) with mannitol.

3. Results and Discussion

The study was carried out following the method described by Higuchi and Connors [5] at three pH values of the dissolution medium, since there are data from Lin [6] and Szejtli and Szente [7] that confirm the influence of the pH value on the indomethacin/β-cyclodextrin inclusion compound formation. It was established that the complexation occurs to the greatest extent at pH around 7.0 [6–8]. The apparent stability constant of the complex at pH 7.0 was about six times greater than the constant of the complex obtained in distilled water. This phenomenon was related to the higher solubility of indomethacin. The possible ion-pair formation in a phosphate buffer was not considered as a probable mechanism of the complexation. On the other hand, there are no data about the complex formation in acid medium, probably because of low indomethacin solubility.

These considerations determined the choice of the pH values used in the present investigations. The solubility isotherm diagrams are depicted in Figures 1, 2 and 3. The main parameters derived from the solubility curves are summarized in Tables I and II. It can be seen that all curves are linear with a positive slope supporting interaction of the A_L type, i.e. the inclusion complex formed is water-soluble in all cases so that a 1 : 1 stoichiometry could be assumed [5].

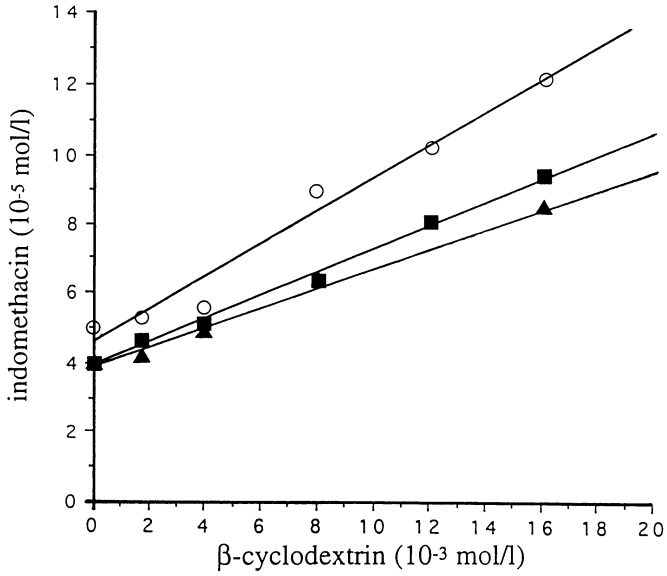

Figure 2. Solubility profiles of indomethacin in the presence of increasing concentrations of β-cyclodextrin and 0.5 mol/L hydrotropic agent in distilled water: (○) without hydrotropic agent, (■) with sucrose, (▲) with mannitol.

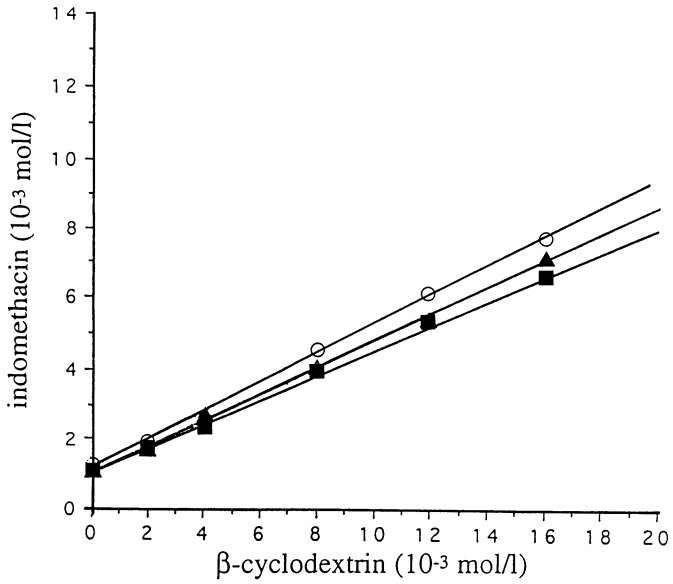

Figure 3. Solubility profiles of indomethacin in the presence of increasing concentrations of β-cyclodextrin and 0.5 mol/L hydrotropic agent in phosphate/citrate buffer of pH 6.8: (○) without hydrotropic agent, (■) with sucrose, (▲) with mannitol.

Table I. Parameters derived from solubility isotherm diagrams.

Sample	pH 1.6	Distilled water	pH 6.8
Indomethacin solubility (S_0) (mol)			
pure indomethacin	3.0×10^{-6}	4.2×10^{-5}	1.0×10^{-3}
indomethacin + mannitol[b]	7.2×10^{-6}	3.9×10^{-5}	0.95×10^{-3}
indomethacin + sucrose[b]	7.8×10^{-6}	4.0×10^{-5}	0.91×10^{-3}
Slope[a]			
pure indomethacin	2.0×10^{-3}	4.1×10^{-3}	0.42
indomethacin + mannitol[b]	1.5×10^{-3}	2.9×10^{-3}	0.36
indomethacin + sucrose[b]	1.6×10^{-3}	3.3×10^{-3}	0.35
Stability constant ($K_{1/1}$) (mol^{-1})[a]			
pure indomethacin	573	98	682
indomethacin + mannitol	213	73	598
indomethacin + sucrose	200	83	556

[a] Samples with β-cyclodextrin concentration range 0 to 16 mmol/L.
[b] The concentration of mannitol and sucrose used was always 0.5 mol/L.

Table II. Maximum indomethacin solubility (mol/L) achieved in 16 mmol/L β-cyclodextrin solutions of different pH values.

Sample	pH 1.6	Distilled water	pH 6.8
Pure indomethacin[a]	3.0×10^{-6}	4.2×10^{-5}	1.0×10^{-3}
Indomethacin	3.5×10^{-5}	12.1×10^{-5}	7.8×10^{-3}
Indomethacin + mannitol[b]	3.2×10^{-5}	8.7×10^{-5}	7.0×10^{-3}
Indomethacin + sucrose[b]	3.8×10^{-5}	9.5×10^{-5}	6.5×10^{-3}

[a] Indomethacin apparent solubility in the absence of β-cyclodextrin.
[b] The concentration of mannitol and sucrose is 0.5 mol/L.

3.1. INTERACTIONS BETWEEN INDOMETHACIN AND β-CYCLODEXTRIN IN THE ABSENCE OF HYDROTROPIC AGENTS

The solubility of indomethacin in β-cyclodextrin solutions without hydrotropic agents was evaluated under the same experimental conditions used to study the hydrotropic effect of mannitol and sucrose and served as a basis for comparison. The results revealed some very important facts which have not been well described or thoroughly discussed in the literature.

The maximum indomethacin solubilities achieved in 16 mmol/L β-cyclodextrin solutions at different pH, which are presented in Table II, clearly show that the solubilizing effect of β-cyclodextrin does not increase regularly when the pH of the medium increases. For example, this effect is greatest in an acid medium (pH

1.6) where the maximum indomethacin solubility was about ten times higher than the drug solubility in solution without β-cyclodextrin. This increase is about eight times in buffer of pH 6.8 and about three times in distilled water.

It was also established that the inclusion of indomethacin in the β-cyclodextrin cavity in acid medium pH 1.6 can take place to approximately the same extent as in phosphate/citrate buffer at pH 6.8. The association constant of the complex was found to be of the same order of magnitude (570–680 mol^{-1}) as for the complex formed at pH 6.8.

It is probable that the strong inclusion complex formation in acid medium might not be due only to the predominant existence of the [HA] form of indomethacin (the difference between pK_a (4.5) and pH (1.6) is 2.9 and corresponds theoretically to 99.999% [HA] form). It can be assumed that the ion composition (KCl/HCl) of the buffer exerts a very marked, positive influence on the inclusion process. There are data in the literature [9, 10] showing that the type and concentration of some inorganic salts could influence β-cyclodextrin hydration and solubility so that the apparent association constant of the drug/β-cyclodextrin inclusion compound could be significantly changed.

The results of our study also outline another very important point. Regardless of the fact that in water about 98% of the indomethacin is theoretically ionized, the increase of indomethacin solubility in an aqueous solution of β-cyclodextrin is about 2.6 times lower than that achieved in buffer of pH 6.8. The apparent association constant of the complex formed in water is about six times lower if it is compared with that of the inclusion compound formed at pH 6.8. The constant value obtained in water coincides with the value of 99 mol^{-1} reported by Myles *et al*. [8].

This fact is not surprising if interactions of indomethacin with sodium ions of the phosphate/citrate buffer of pH 6.8 are assumed. Inagi *et al.* [11] reported on the ability of indomethacin to form ion-pairs with some monovalent ions, especially in the region of pH much higher than the pK_a of the indomethacin. So it can be expected that, in sodium phosphate/citrate buffer of pH 6.8, indomethacin will be in the form of lipophilic ion-pairs with Na cations, and will show greater affinity for the cyclodextrin cavity. On the other hand, according to the data in Ref. [12], the phosphate and sodium ions of the buffer could decrease the apparent dissociation constant of the complex or exert a stabilizing effect because of the decrease in the activity of the water caused by their hydration.

It is evident that the interactions between indomethacin and β-cyclodextrin in KCl/HCl buffer, as well as in water and in phosphate/citrate buffer pH 6.8, need further more detailed investigations which are beyond the scope of the present paper.

3.2. EFFECTS OF HYDROTROPIC AGENTS ON THE INTERACTIONS BETWEEN INDOMETHACIN AND β-CYCLODEXTRIN

3.2.1. *Influence of Mannitol and Sucrose on Indomethacin Solubility in the Absence of β-Cyclodextrin*

The study showed that increasing the concentrations of mannitol and sucrose alone does not enhance the solubility of indomethacin in distilled water and in a buffer of pH 6.8. But in acid medium (pH 1.6, achieved with KCl/HCl buffer), the increase in the solubility of indomethacin is about double. Nevertheless, all further investigations were carried out with a constant concentration (0.5 mol/L) of the corresponding hydrotropic compound.

In order to cast more light on the phenomenon observed, the solubility of indomethacin in acid medium was studied in the presence of increasing concentrations of mannitol and sucrose. The temperature chosen was 37 °C, which favours the potential interactions. A linear relationship of A_{L} type was established when the indomethacin solubility was plotted against the concentration of the hydrotropic compound. The calculation of the stability constant of the assumed 1 : 1 drug/hydrotropic agent complex was done according to Equation (1):

$$K_{\mathrm{compl}} = \mathrm{slope}/S_0(1 - \mathrm{slope}) \tag{1}$$

where S_0 is the solubility of the pure indomethacin: 0.84×10^{-5} mol/L at 37 ± 1 °C.

The K_{compl} values calculated were 1.2 and 2.3 mol^{-1} respectively for the systems with mannitol and sucrose. Obviously, the stability constants are too low to confirm the formation of a stable complex. Furthermore, the amounts of solubilized indomethacin/hydrotropic agent complex, of free, non-complexed indomethacin, as well as the ratio (free indomethacin)/S_0 were calculated according to Refs. [5,15] by means of Equations (2) and (3):

$$(\mathrm{indomethacin\ complex}) = C_{\mathrm{hydr}} S_{\mathrm{t}} K_{\mathrm{compl}}/1 + C_{\mathrm{hydr}} K_{\mathrm{compl}}, \tag{2}$$

where: C_{hydr} is the molar concentration of the hydrotrope; S_{t} is the total solubility of indomethacin at the corresponding hydrotropic agent concentration; and K_{compl} is the stability constant of the assumed indomethacin/hydrotropic agent 1 : 1 complex.

$$(\mathrm{free\ indomethacin}) = S_{\mathrm{t}} - (\mathrm{indomethacin\ complex}). \tag{3}$$

The data are presented in Table III. It can be seen that in all cases the ratio (free indomethacin)/S_0 is close to or higher than unity. This fact, and the very low K_{compl} values, clearly show that the increased indomethacin solubility in the presence of mannitol and sucrose in acid medium cannot be due to the formation of a stable complex. It is more probable that in this case mannitol and sucrose are involved in interactions with the ionic components of the KCl/HCl buffer. As a result, their

Table III. Indomethacin solubility parameters in acid medium and in the presence of increasing hydrotrope concentrations.

Hydrotrope concentration (mol/L)	Indomethacin total solubility (S_t) (10^{-5} mol/L)	Concentration of indomethacin complex (10^{-5} mol/L)	Concentration of free indomethacin (10^{-5} mol/L)	Free indomethacin/S_0
Mannitol				
0.25	–	–	–	–
0.50	1.25	0.47	0.78	0.93
1.00	1.82	0.99	0.83	0.99
1.50	2.26	1.45	0.81	0.96
Sucrose				
0.25	1.33	0.48	0.85	1.01
0.50	2.04	1.07	0.97	1.15
1.00	2.85	1.99	0.85	1.02
1.50	3.90	3.03	0.87	1.03

ability to organize the water molecules decreases strongly, so that the indomethacin solubility increases.

3.2.2. *Influence of Mannitol and Sucrose on the Total Solubilizing Effect of β-Cyclodextrin and on the Apparent Association Constant*

The curves depicted in Figures 1–3, as well as the data summarized in Tables I and II, clearly show that the effect of mannitol and sucrose on the total solubilizing effect of β-cyclodextrin is very similar, but moderate, and depends strongly on the pH of the dissolution medium. The decrease in indomethacin solubility is more pronounced in distilled water, in which the total amount of indomethacin dissolved was about 25% lower than that in a solution of β-cyclodextrin without hydrotropic agents (Table II, Figures 1–3).

It can be assumed that, in water, mannitol and sucrose are not engaged in interactions with ions, so that they display their strong capacity to provoke the formation of an organized water structure, thus inhibiting total drug solubility.

The stability constant values of the inclusion compound formed in the presence of hydrotropic agents were calculated according to Equation (1) and the results are presented in Table I. In general, mannitol and sucrose do not influence the stability of the indomethacin inclusion complex. The lower values of the stability constant in acid medium (about 2.2 times lower than the value obtained in the absence of hydrotropic agent) are related to the observed increase in apparent solubility.

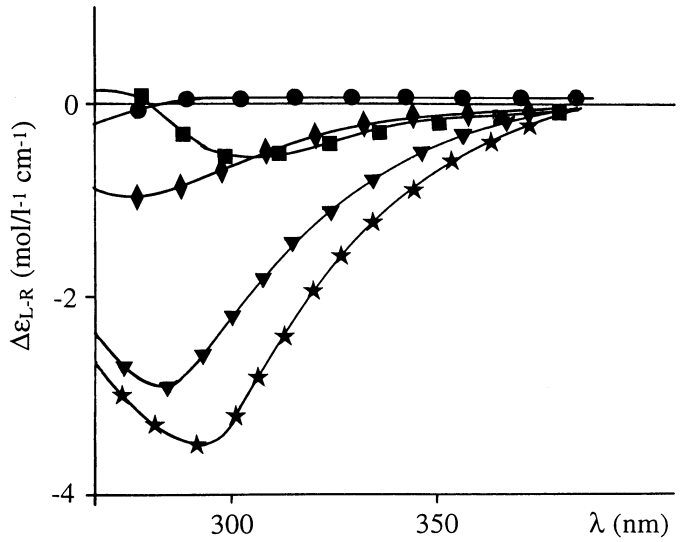

Figure 4. Circular dichroism spectra of indomethacin (8×10^{-5} mol/L) in the presence of β-cyclodextrin (16×10^{-3} mol/L) and sucrose (0.5 mol/L). –●– indomethacin; –◆– β-cyclodextrin; –■– indomethacin/β-cyclodextrin; –▼– indomethacin/sucrose; –★– indomethacin/β-cyclodextrin/sucrose.

3.3. INFLUENCE OF HYDROTROPIC AGENTS ON THE INDUCED CIRCULAR DICHROISM OF INDOMETHACIN IN SOLUTIONS OF β-CYCLODEXTRIN

The study was carried out at constant concentrations of indomethacin (8×10^{-5} mol/L), β-cyclodextrin (16 mmol/L) and hydrotropes (0.5 mol/L) in binary and ternary systems to confirm the results from the phase solubility analysis. The high molar excess of β-cyclodextrin corresponded to the maximum indomethacin solubility, i.e. to the highest complex concentration. The circular dichroism spectra of the samples with sucrose are depicted in Figure 4. The results show, in general, that:

- indomethacin in solution is not optically active in the wavelength range studied but has absorption in this range;
- mannitol shows optical activity but no absorption under these conditions;
- β-cyclodextrin, because of its chiral glucose units, shows a weak negative intrinsic Cotton effect around 280 nm;
- the inclusion of the achiral indomethacin molecule in the β-cyclodextrin cavity generates a negative extrinsic Cotton effect, which is induced around the indomethacin wavelength absorption: the negative peak is at 308 nm, i.e. hypsochromically shifted compared with the indomethacin maximum in the UV region at 320 nm;
- the optical activity of the inclusion complex in the presence of sucrose appears as a negative maximum at 298 nm which is also hypsochromically shifted: it is

interesting to note that a negative extrinsic Cotton effect at 288 nm also arises in the binary indomethacin/sucrose system;

- unlike sucrose, mannitol does not influence the optical activity of the inclusion complex: the induced circular dichroism spectrum of indomethacin in the ternary system indomethacin/β-cyclodextrin/mannitol coincides with that of the binary system indomethacin/β-cyclodextrin: it is well known that the spatial relationship between the centre of asymmetry and the perturbed chromophore, as well as the rigidity of the inclusion complexes, are of great importance for the character of the induced optical activity [13].

On the basis of these statements, it may be assumed that sucrose can change the configuration of the cyclodextrin complex because of its high potency as a water structure promoter. In the lyotropic series of non-electrolytes arranged in order of increasing disruptive effect on water structure [3], sucrose has the least effect and mannitol the greatest effect, i.e. the two hydrotropic agents possess different activity in the organization of water molecules. Moreover, according to Ref. [14], sucrose, when used as a protector by spray-drying at sufficiently high concentrations, may arrange itself in the form of a rigid network of molecules that adhere to each other and to the surface of many biological macromolecules by hydrogen bonds and van der Waals forces. It can therefore be assumed that sucrose creates a more hydrophilic environment around indomethacin chromophores so that hypsochromic shifts in the circular dichroism spectra of the systems with sucrose can take place.

The results of circular dichroism spectroscopy need further, more detailed study, to confirm similar assumptions.

4. Conclusion

In general, the influence of sucrose and mannitol (0.5 mol/L) on the total solubilizing effect of β-cyclodextrin as well as on the complex stability constants could be considered moderate and without practical significance, even in acid medium where these hydrotropic agents increase the apparent indomethacin solubility. Another very important fact has been established: the indomethacin/β-cyclodextrin interaction is found in acid medium to the same extent as in buffer of pH 6.8 and the complex constants are of the same order. Interactions with buffer ions and ion-pair formation can be assumed.

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