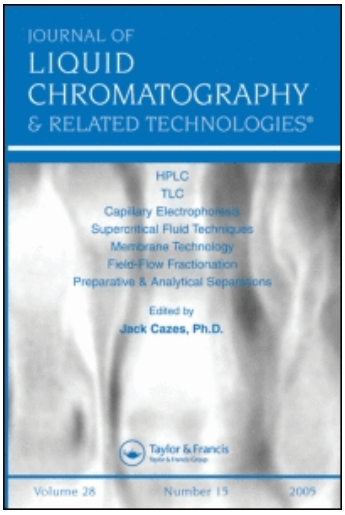

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Chromatographic Determination of the Association Constants Between Psoralen Derivatives and Modified β-Cyclodextrin: Effect of Sucrose as a Co-enhancer Association Agent

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ABSTRACT

The retention of psoralen derivatives, i.e., 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and trimethylpsoralen (TMP) in high performance liquid chromatography (HPLC) was investigated using a

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C_{18} bond silica column, hydroxypropyl-β-cyclodextrin (HP-β-CD) as mobile phase additive (0–19 mM) and a wide range of column temperature (−5°C–55°C). Such a study was carried out to demonstrate the potential drug complexing role of this cyclodextrin for a future application in pharmaceutical formulation. Assuming a 1 : 1 stoichiometry, the association constants (K) were calculated from the chromatographic data. At a column temperature of −5°C, and in a 42% water–58% methanol (v/v) mixture, K was equal to 30, 75, and 40 M^{-1} for the 8-MOP/, 5MOP/, and TMP/, HP-β-CD associations, respectively. The thermodynamic parameters of the complexes were determined from linear van't Hoff plots for the three complexes. From the enthalpy and entropy changes, it appeared that the recognition mechanism of HP-β-CD for 8-MOP and TMP was temperature dependent, and the hydrophobic effect between TMP and the flexible hydroxypropyl groups of HP-β-CD was counterbalanced by its steric hindrance. As well, the role of sucrose on this association was investigated. It was expected, that the sucrose would act on the psoralen derivatives/HP-β-CD association process by modifying the surface tension of the bulk solvent and increase the K_f values.

Key Words: HPLC; Hydroxypropyl-β-cyclodextrin; Psoralen; Association-constant; Thermodynamic; Sucrose.

INTRODUCTION

Several reports have shown the utility of the use of modified β-cyclodextrin in pharmaceutical formulation to improve the bioavailibility of drugs.[1–7] Various methods have been used to determine the association constants between cyclodextrin and drugs. Ultraviolet-visible absorption, NMR, potentiometry fluorescence measurements, capillary electrophoresis, and calorimetry have been described.[8–12] Chromatographic experiments have also been previously carried out for both enantiomeric drug separation and the determination of the apparent association constant of various drugs with both native or derivatized cyclodextrins.[13–31]

The retention behavior of solute in high performance liquid chromatography (HPLC) is based on the partitioning of the solute between the mobile and the stationary phases. When cyclodextrin is added to the mobile phase, solute retention is split into two main physicochemical processes, i.e., solute complexation by cyclodextrin and transfer of free (i.e. uncomplexed) solute from the mobile to the stationary phase. The association constant K between compound and cyclodextrin can be determined using the well known equation:[13–17]

$$\frac{1}{k} = \frac{1}{k_0} + \frac{K[\mathrm{CD}]^x}{k_0}$$

where k is the solute retention factor, k_0 the solute retention factor without cyclodextrin in the mobile phase, [CD] the cyclodextrin concentration, and x the stoichiometry of the complex. For an inclusion complex with a 1 : 1 stoichiometry ($x = 1$), a linear plot of $1/k$ vs. [CD] must be obtained and the K value calculated. Psoralens are regularly used in therapy, in combination with ultraviolet A (UVA) light irradiation to treat skin diseases such as psoriasis, vitiligo, and mycosis fungoides.[32–36] Its poor aqueous solubility poses bioavailability problems in vivo. This could be overcome by the formation of inclusion complexes with cyclodextrins.

The aim of this paper is to investigate the stoichiometry and the association constants for the associations between a series of psoralen derivatives and hydroxypropyl (HP)-β-cyclodextrin (CD), using a chromatographic approach. This was done by using a C_{18} silica as stationary phase and cyclodextrin as mobile phase additive.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a HPLC Waters pump 501 (Saint Quentin, Yvelines, France), an Interchim Rheodyne injection valve model 7125 (Montluçon, France) fitted with a 20 μL sample loop, and a Merck 2500 diode array detector (Nogent-Sur-Marne, France). An Interchim RP18 column 125 mm × 4 mm I.D. were used with a controlled temperature in an Interchim oven, TM N°701 (Montluçon, France) for high temperature, and an Osi Julabo FT200 cryoimmerser (Elancourt, France) for low temperature. The mobile phase was fixed at 1 mL/min and the wavelength at 254 nm.

Reagents

All the psoralen derivatives were obtained from Sigma Aldrich (Saint-Quentin, France) (Fig. 1). The HP-β-CD was provided from Roquette (Lestrem, France) and was a mixture of homologous and isomers. Deuterium oxide (Merck, Nogent-Sur-Marne, France) was used as a dead time marker. Water was obtained from an Elgastat option water purification system (Odil, Talant, France) fitted with a reverse osmosis cartridge. Sucrose was obtained from Prolabo (Paris, France). The mobile phase consisted of a methanol/water mixture 58–42 (v/v) with various HP-β-CD concentrations (0, 5.20, 7.09, 14.01, 14.98, 16.77, and 18.71 mM). Twenty microliters of each psoralen derivative was injected in triplicate and the retention times measured. All the experiments were repeated three times for each temperature and for each cyclodextrin concentration.

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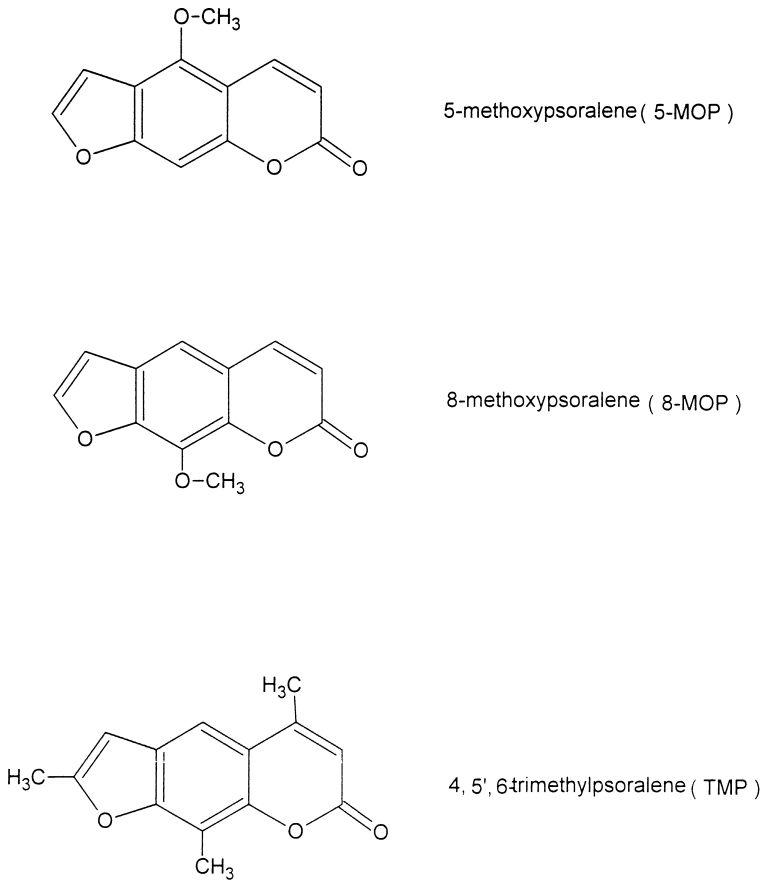

Figure 1. Psoralen derivative structures.

Temperature Study

Compound retention factors were determined over the wide temperature range −5°C–55°C (±0.1°C). The chromatographic system was allowed to equilibrate at each temperature for at least 1 h prior to each experiment. To study this equilibration, the compound retention time of 5-methoxypsoralen (5-MOP) was measured every hour for 7 h and again after 22, 23, and 24 h. The maximum relative difference of the retention time of this compound was 0.6%, making the chromatographic system sufficiently equilibrated for use after 1 h.

Thermodynamic Relationships

ΔH^0 and ΔS^0 are, respectively, the standard enthalpy and entropy of transfer of the psoralen derivatives from the mobile phase to the cyclodextrin. These energies can be calculated using the following thermodynamic relationships as previously described:

$$\ln K = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R}$$

where T is the temperature and R the gas constant. For a linear plot of $\ln K$ vs. $1/T$, the slope and intercept are respectively $-\Delta H^0/R$ and $\Delta S^0/R$.

RESULTS AND DISCUSSION

Chromatographic Determination of the Apparent Association Constant for the Psoralen Derivative–Cyclodextrin Association

Using the solute retention time and the void time, the k values were determined for the three psoralen derivatives, and at all the cyclodextrin concentrations, at temperatures of −5, 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55°C. The coefficients of variation of the k values were <0.4%, indicating a high reproducibility and a good stability for the chromatographic system. The $1/k$ vs. [HP-β-CD] plots were determined and the values of the linear regression coefficients R were calculated. The R values are higher than 0.995 in all cases. For example, Fig. 2 shows the three plots for a column temperature equal to 20°C. The values of apparent association constants are presented in Table 1 for five column temperatures. From the R values, it appears clearly that the behavior of psoralen derivatives is well described by the model, assuming a 1 : 1 stoichiometry for the three complexes. This is consistent with previous results, which have shown by spectroscopic or chromatographic methods, a 1 : 1 inclusion complex between psoralen with β-CD, dimethyl DM-β-CD, and trimethyl TM-β-CD.[37,38] As shown in Table 1, the association constant values varied as follows: For a temperature < to a critical temperature $\beta^* \cong 35°C$, 5-MOP/HP-β-CD > TMP-/HP-β-CD > 8-MOP/HP-β-CD but, for a temperature > β^*, 5-MOP/HP-β-CD > 8-MOP/HP-β-CD > TMP/HP-β-CD. As the HP-β-CD was a mixture of homologous and isomers, the measured binding constants and thermodynamic data are weighted averages for this closely related mixture. The apparent association constant

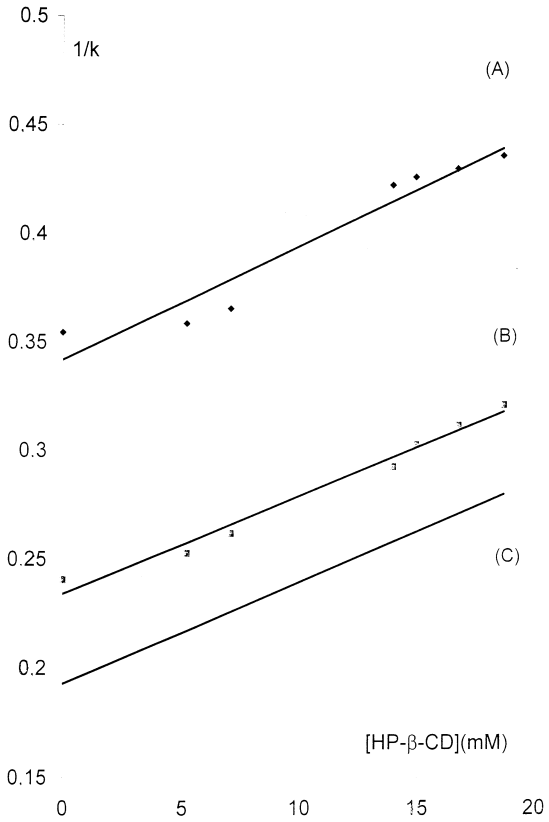

Figure 2. $1/k$ vs. [HP-β-CD](mM) plot (assuming 1 : 1 stoichiometry) for 8-MOP/HP-β-CD (A), 5-MOP/HP-β-CD (B), and TMP/HP-β-CD (C) associations.

values are equal to 30, 75, $40\,M^{-1}$ at $T=-5°C$ and 13, 18, $12\,M^{-1}$ at $T=45°C$ for 8-methoxypsoralen (8-MOP), 5-MOP, and trimethylpsoralen (TMP). These values are in the same order of magnitude as the association constants obtained between psoralen and dimethyl DM-β-CD and trimethyl TM-β-CD, respectively, equal to $603\,M^{-1}$ and $69.6\,M^{-1}$.[37–38]

Thermodynamic Parameters for the Psoralen Derivatives–Cyclodextrin Complexes

In order to gain information about the mechanistic aspect of the difference in the HP-β-CD affinity for 8-MOP, 5-MOP, and TMP, the thermodynamic

Table 1. Apparent association constants K between 8-MOP, 5-MOP or TMP and HP-β-CD determined at various column temperatures.

T(°C)	8-MOP	5-MOP	TMP
−5	30.1	75.9	40.3
0	27.4	64.5	35.2
15	20.9	40.9	24.1
30	16.4	27.1	17.1
45	13.1	18.7	12.6
55	11.5	14.9	10.4

parameters were obtained from van't Hoff plots. The $\ln K$ vs. $1/T$ plots were obtained for the three psoralen derivatives. Linear van't Hoff plots were obtained with correlation coefficients higher than 0.991. Figure 3 shows the van't Hoff plots corresponding to the three inclusion complexes. ΔH^0 and ΔS^0 for the three complexes are presented in Table 2 with the corresponding Gibbs free energy ΔG^0 at −5°C. For all the complexes, the enthalpic and entropic terms are both negative, demonstrating that the association is enthalpically driven.

The formation of an inclusion complex with cyclodextrin is classically attributed to interactions such as hydrogen bonding with the OH groups at the periphery of the cavity, van der Waals interactions, and hydrophobic effect.[38] In most cases, the solute inclusion in the cyclodextrin cavity is associated to large negative values of ΔH^0 and ΔS^0.[15,39–41] The enthalpic term value for psoralen derivative complexation by HP-β-CD is interpreted as indicating that the binding forces included strong van der Waals–London dispersion interactions. This is associated to a negative value of ΔS^0 related to the apparent low degrees of freedom of the solute included in the rigid cyclodextrin cavity. 5-Methoxypsoralen exhibited the lowest enthalpy and entropic data because it had the strongest van der waals and polar interactions with the HP-β-CD cavity. This was linked with the lowest entropy state due to the lowest degree of freedom when it was transferred inside the HP-β-CD. Water molecules in contact with non polar species adopt an ordered organisation. Thus, the release of these water molecules, when the solute is transferred from a polar to a non polar bulk phase, results in an entropy increase associated to a weak negative value of ΔH^0.

This hydrophobic effect is much more important than hydrophobicity of the solute. Nevertheless, for TMP, the highest hydrophobic compound, its association with HP-β-CD did not exhibit he highest negative value for ΔS^0 (Table 2). This suggests, that the TMP/HP-β-CD association is not only dependent on the hydrophobic effect. Thus, the association mechanism for TMP with HP-β-CD is, as well, governed by the steric hindrance between the

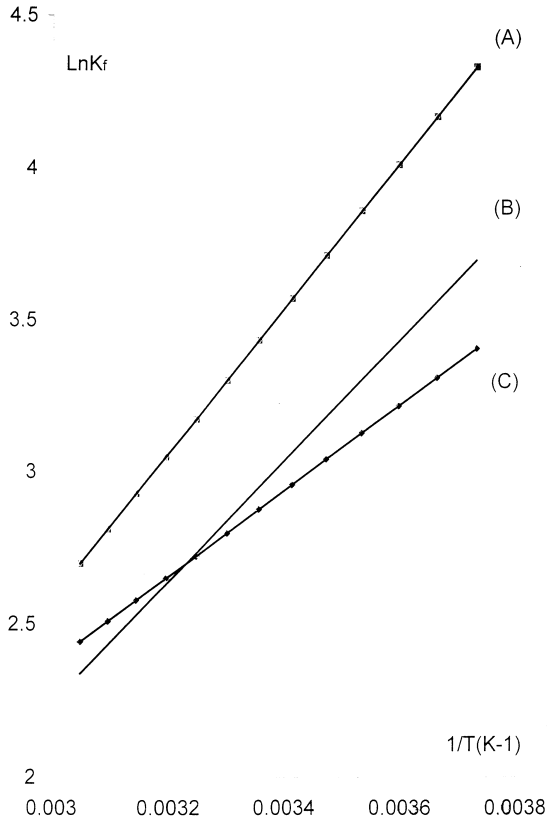

Figure 3. van't Hoff plots ($\ln K$ vs. $1/T$) for 5-MOP/HP-β-CD (A), TMP/HP-β-CD (B), and 8-MOP/HP-β-CD (C) associations.

three non polar groups, i.e., $-CH_3$, of TMP and the hydroxypropyl groups of the modified cyclodextrin. For the temperature $\beta^* = 35^\circ C$, there is an inversion of the strength of the 8-MOP/HP-β-CD and TMP/HP-β-CD inclusion complexes (Table 1). This can be explained by the fact, that temperature β^* was the compensation temperature for which there was a perfect equilibrium between the entropic and enthalpic effects governing the recognition mechanism of the HP-β-CD for its guest molecule TMP or 8-MOP. As well, β^* can be given by the following equation:

$$\beta^* = \frac{\Delta\Delta H^0}{\Delta\Delta S^0}$$

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Table 2. Thermodynamic parameters ΔH^0, ΔS^0, and ΔG^0 (at -5°C) for the associations between 8-MOP, 5-MOP or TMP and HP-β-CD.

Psoralene derivatives	ΔH^0 (kJ/mol)	ΔS^0 (J/mol/K)	ΔG^0 (kJ/mol)
8-MOP	−11.7	−15.5	−7.6
5-MOP	−19.9	−38.2	−9.7
TMP	−16.5	−30.9	−8.2

where $\Delta\Delta H^0$ (respectively $\Delta\Delta S^0$) was the difference of the thermodynamic parameters ΔH^0 (respectively ΔS^0) for the associations between 8-MOP and TMP for HP-β-CD (Table 2). For a temperature $T > \beta^*$, the magnitude of $T\Delta\Delta S^0$ was always greater than that of $\Delta\Delta H^0$. Thus, the recognition mechanism of HP-β-CD for 8-MOP and TMP was governed by the difference in their steric constraints in the HP-β-CD cavity. In this temperature domain, the recognition mechanism was led entropically. For a temperature $T < \beta^*$, the reverse was observed. The magnitude of $\Delta\Delta H^0$ was always greater than that of $T\Delta\Delta S^0$. This was attributed to a favorable contribution of selective interactions (van de walls) between 8-MOP and 5-MOP with HP-β-CD. Therefore, in this temperature domain, the recognition mechanism was led enthalpically.

Effect of Sucrose

In an effort to demonstrate the role of the hydrophobic effect on the psoralen derivatives/HP-β-CD association, the influence of a surface tension modifier such as sucrose was studied. The variation of the constants K_f of the psoralen derivative/HP-β-CD associations vs. sucrose concentration in the bulk solvent at $T = -5^\circ$C, was the same for all psoralene derivatives. An example of the plot (for 8-MOP) was given in Fig. 4. The variation observed for K_f with sucrose concentration was confirmed by the fact that the sucrose increased the hydrophobic effect in the bulk solvent[42–43] and, thus, the psoralen derivative/HP-β-CD association. These results showed the role of the sucrose agent as a co-enhancer of the psoralen derivative/HP-β-CD association process.

CONCLUSION

This paper described the psoralen derivative/HP-β-CD association mechanism using a chromatographic method. The apparent association constants were determined experimentally for the first time at very low temperatures ($<0^\circ$C). It was shown, that HP-β-CD has an affinity $\sim$2.5 fold more important for 5-MOP

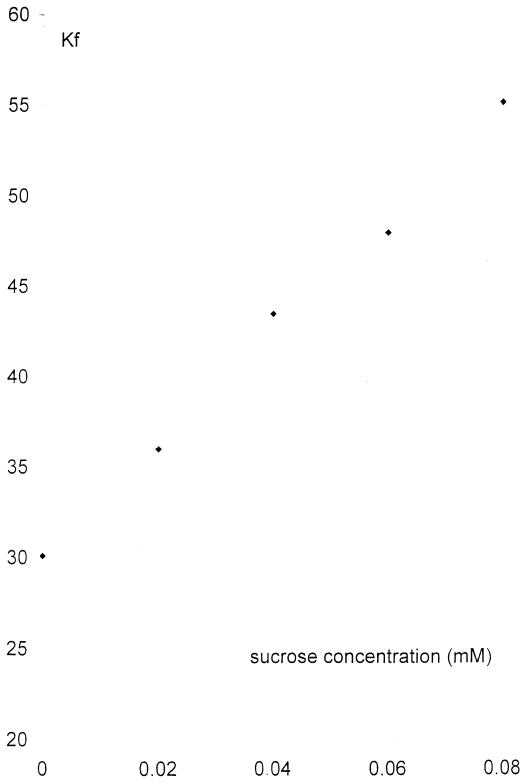

Figure 4. *K* for 8-MOP vs. sucrose concentration in the mobile phase at −5°C.

than for TMP and 8-MOP. From the thermodynamic results, it was shown that the steric hindrance created by the three methyl group of TMP with the apolar moiety of the flexible hydroxypropyl groups of HP-β-CD played a great role. As well, it was demonstrated that the recognition mechanism of HP-β-CD for 8-MOP and TMP was temperature dependent, and the role of the hydrophobic steps on the psoralen derivative inclusion mechanism in the HP-β-CD cavity was clearly visualized by addition of sucrose in the bulk solvent.

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