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Thermodynamic Study of the β-Cyclodextrin Benzyl-2-pyridyl Ketone 2-pyridyl Hydrazone Inclusion Process

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Abstract. Benzyl-2-pyridylketone-2-pyridylhydrazone and β-cyclodextrin form a 1:1 adduct in water with a standard free energy change, $\Delta G°$, in the region of 14.2 kJ mol^{-1} at 25°C, and an association constant of 275 M^{-1} in the pH range 5–7. These values have been obtained by using the fluorimetric spectral changes associated with the inclusion process.

Key words. Cyclodextrins, BPKPH, association constants.

1. Introduction

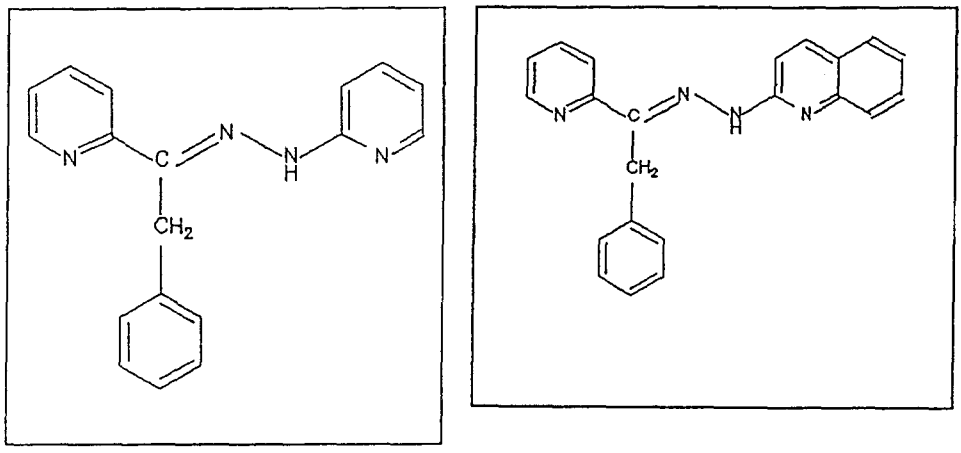
Cyclodextrins are cyclic doughnut-shaped molecules composed of several (6 to 9) D-glucopyranose units in the C-1 chain conformation. The interior of the torus of these molecules is relatively apolar compared to water. Because of their truncated cone structure they tend to include guest molecules in their cavities, and show a selectivity depending on their relative size compared to that of the guest molecules [1–4]

We have found that valuable information concerning the interactions of chromophoric (lumiphoric) sites in the excited state is revealed by a study of the emission properties of these complexes.

Because of the organizing ability of cyclodextrin media, luminescent phenomena are enhanced, the molecules in the internal cavity are isolated from the surrounding environment and their excited states shielded from extinction processes. On the other hand, for a multichromophoric guest such as BPKPH (benzyl-2-pyridylketone-2-pyridylhydrazone) whose size impedes its inclusion into the cavity because of steric considerations, the more apolar part of the molecule is preferred by cyclodextrin. Frequently, this apolar moiety is responsible for the hydrophobic behaviour of many organic ligands which means that mixed solvent media and multiple extraction steps have to form part of the analytical procedure. Thus, the use of cyclodextrin included ligands avoids this experimental complication.

The applications of this behaviour in inorganic analytical chemistry were shown by the use of an organic ligand (BPKPH) coordinated with metal ions, the entire complex being included in β-cyclodextrin [5]. Figure 1 shows the molecular structures of BPKPH and BPKQH.

In the present study, we report on the thermodynamics of the binding of BPKPH to β-cyclodextrin as a function of pH. Qualitative evidence for host-guest binding comes from a study of the spectroscopic behaviour of BPKPH included into



BPKPH BPKQH

Fig. 1. Molecular structures of BPKPH and BPKQH.

β-cyclodextrin. Quantitative information in the form of association constants has been obtained from the spectroscopic data. From the temperature dependence of these association constants, we have calculated values of the thermodynamic parameters ΔH, ΔS and ΔG°.

2. Experimental

2.1. APPARATUS

All fluorimetric measurements were performed on a Perkin-Elmer LS-5 Luminescence Spectrometer, equipped with a xenon discharge lamp pulsed at the line frequency (50 Hz) and an $f/3$ Monk-Gillieron (9.9 W) type monochromator. The spectrometer was connected to a Perkin-Elmer, Model 3600 Data Station. The LS-5 fluorimeter was checked daily for reproducibility using a polymer fluorescence sample of p-terphenyl (10^{-7} M) which gives a relative fluorescence intensity of 90% at $\lambda_{em} = 340$ nm with $\lambda_{ex} = 295$ nm, slits 2.5/2.5 and sensitivity factor 0.593.

Spectrophotometric measurements were carried out using a Shimadzu UV-240 Graphicord spectrophotometer.

2.2. REAGENTS

Benzyl-2-pyridylketone-2-pyridylhydrazone (BPKPH) and its quinolyl homologue (BPKQH) (Figure 1) were synthesized as described previously [6, 7]. 1×10^{-3} M solutions were prepared in absolute ethanol.

β-Cyclodextrin (Sigma Chemical Co.) was recrystallized once from boiling water; a 1×10^{-2} M aqueous solution was used. The inclusion complex between BPKPH and β-cyclodextrin was prepared by evaporating 10 mL of a 1×10^{-2} M BPKPH ethanolic solution (28.8 mg) and diluting to 250 mL with aqueous 1×10^{-2} M β-cyclodextrin. The mixture so prepared was sonicated for 10 min, this solution was stable for at least 1 month. Solutions were buffered at pH 5.0 and pH 10.5.

3. Estimation of Thermodynamic Parameters

Values of ΔH and ΔS for the complexation reaction

$$\text{BPKPH} + \beta\text{-CD} \rightleftharpoons \text{BPKPH} - \beta\text{-CD} \quad (1)$$

were obtained from the temperature-dependence of the equilibrium constant. Van 't Hoff plots of $RT \ln K$ vs. T and $R \ln K$ vs. $1/T$ were linear (Figure 2), and we conclude that ΔH and ΔS are both essentially constant over the temperature range studied (15–45°C).

BPKPH was found to form a (1 : 1) inclusion complex with β-cyclodextrin, as demonstrated by application of the Bent and French method [8] to our spectrophotometric data.

The information provided by the fluorescence data of 1×10^{-5} M BPKPH solutions with increasing concentrations of β-cyclodextrin is significant and suggests that the inclusion process has a photophysical effect on the excited state which is more pronounced than on the ground state. This is evident from Figure 3, in which an hypsochromic shift from 370 to 335 nm occurs as the β-cyclodextrin concentration increases.

Quantitative data may be obtained from the enhanced fluorescence emission which occurs as the β-cyclodextrin concentration increases. We attribute this effect

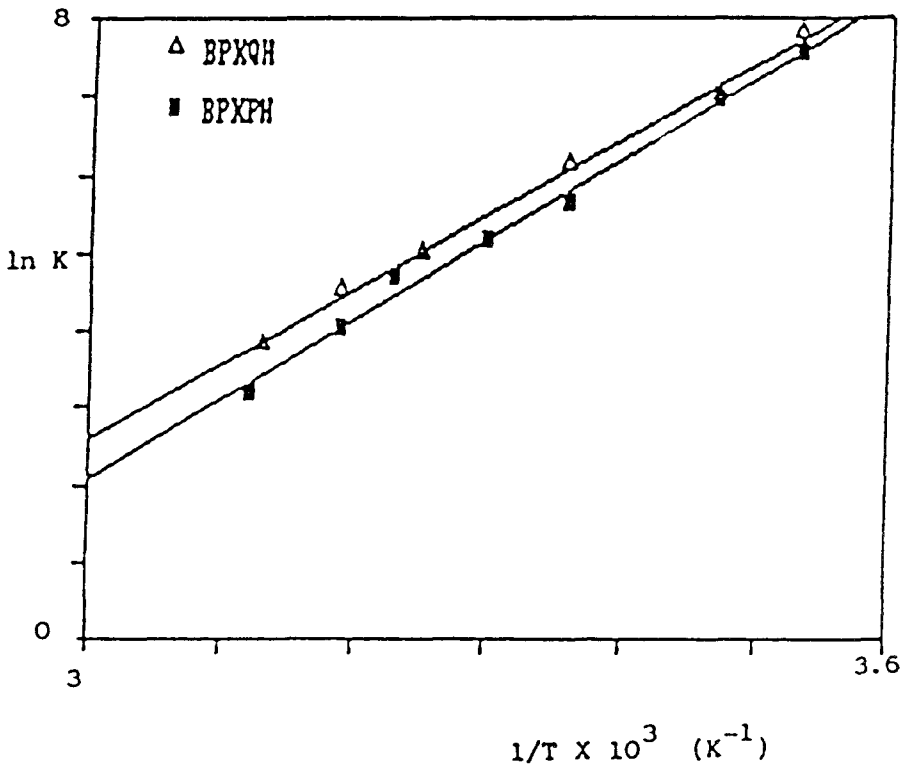


Fig. 2. Van 't Hoff plots of BPKPH and BPKQH association constants with β-cyclodextrin. Temperature range studied 15–45°C.

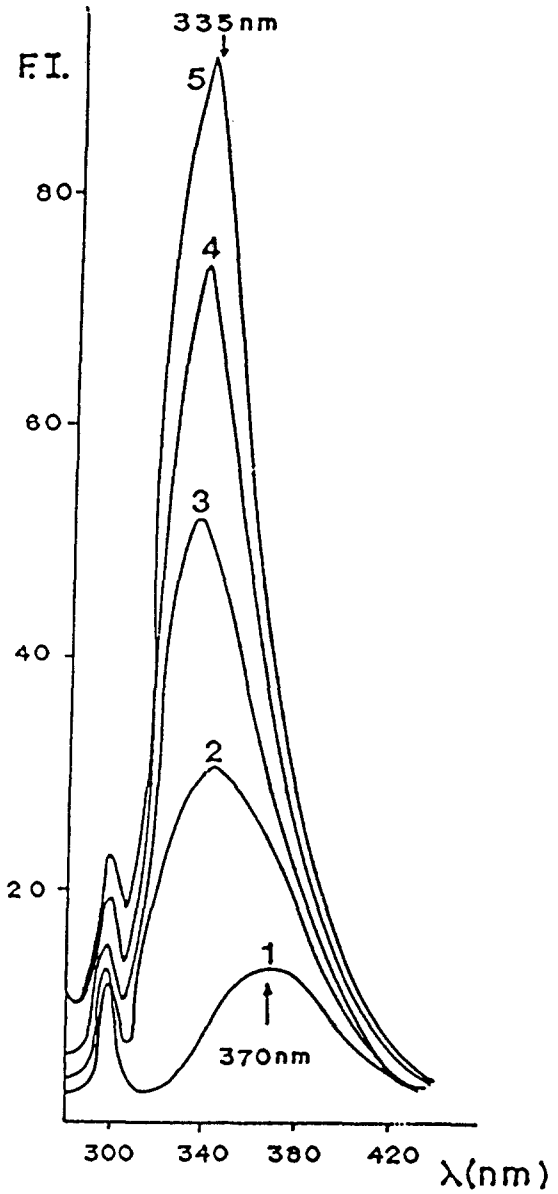


Fig. 3. Fluorescence intensity of aqueous BPKPH solutions (1×10^{-5} M) at various concentrations of β-cyclodextrin: (1) 0 M, (2) 1×10^{-3} M, (3) 2.5×10^{-3} M, (4) 5×10^{-3} M. pH = 6.5, $\lambda_{excitation} = 265$ nm.

to a diminution in rotational freedom in the medium of the hydrophobic cavity, where the chromophores are protected from collisions and, the lack of water molecules surrounding the fluorescent molecules in aqueous solutions, as a result of insertion of the guest into the host.

The binding constants of BPKPH with β-cyclodextrin were obtained by the

Benesi-Hildebrand method [8–10], using the expression:

$$\Delta F^{-1} = (\alpha[\text{guest}]_0 K[\beta\text{-cyclodextrin}])^{-1} + \alpha[\text{guest}]^{-1} \quad (2)$$

where ΔF = the change of fluorescence intensity upon addition of β-cyclodextrin; $[\text{guest}]_0$ = initial concentration of guest molecules; and α = proportionality constant.

The linear relation between ΔF^{-1} and $[\beta\text{-cyclodextrin}]^{-1}$ gives K. The thermodynamic parameters obtained for the inclusion reaction are presented in Table I. In this table we also include data obtained for BPKQH, the quinolyl homologue of BPKPH. Comparison of the association constants of both dyes suggest that the inclusion does not involve the pyridyl or quinolyl groups, but it is the more hydrophobic phenolic group, which incorporates into the β-CD cavity.

Table I also shows K values at several pH values. As expected the association constant is affected by the pH of the medium which in turn modifies the distribution of charge in the dye molecule because this parameter governs the distribution equilibria of the protonic species. In this respect BPKPH and BPKQH have pK_1 values of 2.96 and 1.26, pK_2 values of 5.85 and 5.14, and a pK_3 value of 9.61, respectively, corresponding to the dication, monocation and neutral form of the dyes [12, 13].

The K values of Table I suggest that in acidic or neutral media the binding constant is affected by the decreasing hydrophobic character of the charged molecules of dye. At pH values in which uncharged molecules exist, better inclusion binding is obtained.

The thermodynamic parameters ΔH, ΔS and ΔG° for the inclusion reaction of the guest molecules were determined from the temperature dependence of the association constants. The results obtained are shown in Table I, from which it may be seen that the values for both dyes are almost in the same order of magnitude and do not depend on the pyridyl and quinolyl moieties of the guest molecules.

Several chemical phenomena have been proposed [1, 14] as *driving forces* for complexation: a hydrophobic effect, release of strain in the cyclodextrin ring, release of cavity bound *high energy* water, and hydration of the complex. Hydrophobic interaction essentially involves a favourable positive entropy ($\Delta S > 0$), together with a slightly positive enthalpy change ($\Delta H > 0$). However thermodynamic parameters determined for the formation of cyclodextrin complexes showed that the

Table I. Association constants and thermodynamic parameters for the inclusion reactions with β-cyclodextrin.

Guest		BPKPH	BPKQH
K (M^{-1})	pH = 5.0	275 ± 10	432 ± 25
	pH = 7.0	278 ± 20	432 ± 25
	pH = 10.5	550 ± 50	700 ± 50
ΔH (kJ mol^{-1})		−86.2	−78.2
ΔS (J mol^{-1} K^{-1})		−240.9	−221.7
ΔG (kJ mol^{-1})		−14.2	−15.0

inclusion process is more governed by a negative enthalpy change ($\Delta H < 0$) than by a positive entropy change [15].

The enthalpy stabilization is explained by the expenditure of less energy in the guest desolvation step. Matsui *et al.* [16] explained thermodynamic data (similar to those Table I) in terms of the release of high energy water from the cyclodextrin cavity. The expulsion of these enthalpy-rich molecules into bulk water upon substrate inclusion results in a negative enthalpy change, together with a negative entropy change. This hypothesis is consistent with the results obtained because a large negative enthalpy change (86.2 kJ mol^{-1}) was measured. However, the importance of this binding force is still controversial [17].

BPKPH and BPKPH-β-cyclodextrin have been used as chelating reagents for the fluorimetric determination of gallium [5, 6]. The pH was similar (5.0) in both methods, but in the absence of β-cyclodextrin, it is necessary to work in ethanolic media: (a) to solubilize the reagent; and (b) a high content of ethanol (90%) is found to be essential to obtain a high sensitivity. In ethanolic media the fluorescence intensity is temperature-dependent in the range 10–30°C; however, in β-cyclodextrin, molecular vibrations are most restricted because of the greater rigidity produced by inclusion. The emission maxima of the chelates are identical (545 nm) but there is an hypsochromic shift (7 nm) of the excitation spectra of the chelate β-cyclodextrin BPKPH gallium, probably due to the complex inclusion formation between β-cyclodextrin and BPKPH, which stabilizes the ground state of the hydrazone so that more energy is necessary to electronically excite the molecule.

Acknowledgements

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References

1. M. L. Bender and M. Komiyama: *Cyclodextrin Chemistry*, Springer-Verlag, New York (1977).
2. W. Saenger: *Angew. Chem. Int. Ed. Engl.* **19**, 344 (1980).
3. L. J. Cline Love, M. L. Grayesky, and J. Novoski: *Anal. Chim. Acta* **170**, 3 (1985).
4. F. García Sanchez, M. Hernandez, and A. Heredia: *Anal. Chim. Acta* **187**, 147 (1986).
5. F. García Sanchez, M. Hernandez, and J. C. Márquez: *Fresenius Z. Anal. Chem.* **328**, 499 (1987).
6. J. Laserna, A. Navas, and F. Garcia Sanchez: *Anal. Chim. Acta* **121**, 295 (1980).
7. M. Santiago, A. Navas, J. Laserna, and F. Garcia Sanchez: *Mikrochim. Acta* **II**, 197 (1983).
8. H. Bent and C. French: *J. Am. Chem. Soc.* **63**, 1568 (1941).
9. H. Benesi and J. Hildebrand: *J. Am. Chem. Soc.* **71**, 2703 (1949).
10. H. Kondo, H. Nakatani, and K. Hiromi: *J. Biochem.* **79**, 393 (1976).
11. K. Kano, I. Takenoshita, and T. Ogawa: *J. Phys. Chem.* **86**, 1833 (1982).
12. F. García Sanchez, A. Navas, and J. Laserna: *Anal. Chem.* **55**, 253 (1983).
13. F. García Sanchez, C. Cruces, and J. Medinilla: *Talanta* **33**, 847 (1986).
14. Y. Hul, J. Winkle, and D. Witten: *J. Phys. Chem.* **87**, 23 (1983).
15. I. Tabushi: *J. Am. Chem. Soc.* **100**, 3 (1978).
16. Y. Matsui and T. Nishioka: *Top. Curr. Chem.* **128**, 61 (1985).
17. K. Connors and D. Pendergast: *J. Am. Chem. Soc.* **106**, 7607 (1984).