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The equilibrium constant of β-cyclodextrin–phenolphtalein complex; influence of temperature and tetrahydrofuran addition[1]

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Abstract

The temperature influence on creation of a supramolecular complex in which β-cyclodextrin (β-CD) is the host molecule and phenolphtalein (PP) is the guest has been studied in aqueous solution by UV-visible absorption spectroscopy. The decrease of temperature of β-cyclodextrin-phenolphtalein system resulted in a decrease in absorbance of the UV-vis spectrum. Under favourable conditions (0.1 mM β-CD, 30 μM PP) the termochromic effect is very significant (≅ 0.1 U of absorbance/10°C). The formation constant of inclusion complex was determined at various temperatures (from 10 to 70°C) using Scott's equation. The association constants (K_{11}) for the binding in 0.02 M sodium carbonate (pH 10.5) at 10 and 70°C are 7.44 and 0.26×10^4 M^{-1}, respectively. The stoichiometric ratio of investigated complex was found to be 1:1 on wide range of β-cyclodextrin:phenolphtalein concentration ratio (from 0.8:1 to 427:1). Additionally, strong interaction between cyclodextrin and tetrahydrofuran (THF) was observed and the inhibitory effect of tetrahydrofurane on the association of β-CD–PP complex was studied. From linear Van't Hoff plots thermodynamic parameters such as: the change of enthalpy ($\Delta H°$) and change of entropy ($\Delta S°$) were estimated and interpreted.

Keywords: Cyclodextrin inclusion complexes; Phenolphtalein; Tetrahydrofuran; UV-vis absorption spectroscopy; Scott equation; Complex stoichiometry; Binding constant; Temperature effects; Thermodynamic parameters

1. Introduction

The formation of inclusion complexes between small organic molecules and cyclodextrins has proven to be an excellent model system for studying the nature of noncovalent binding forces in solution. They have provided valuble insights concerning electrostatic (van der Waals) interactions and are good models for understanding the specifity of enzyme–substrate interactions. The interior of CD cavities is relatively hydrophobic. It is formed by a circular configuration of hydrogen atoms and glucoside oxygen atoms, while all the hydroxyl groups are on the outside of the

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molecule. Therefore, CD's and their inclusion complexes, even with nonpolar organic compounds, are quite soluble in water [1,2]. The CD-complexation processes are highly stereoselective and can be considered as the method of choice for resolution of various isomers; structural, geometrical, diastereoisomeric and enantiomeric. Moreover, as CD's are composed of D-glucose units they are themselves chiral and therefore, represent a potential tool for formation of diastereoisomeric complexes with other chiral compounds of different chemical natures, including those that are difficult to transform into diastereoisomers [3]. Cyclodextrins are well known as chiral selectors applied in gas chromatography (GC) [4], high-performance liquid chromatography (HPLC) [3], thin-layer chromatography (TLC) [5], and capillary electrophoresis (CE) [6]. They also have been used in the pharmaceutical, cosmetics and food industries in order to improve solubility, dissolution rate, stability, and the bioavailability of drugs [7]. Finally, cyclodextrins were applied to enhance the fluorescence intensity of certain compounds [8].

A supramolecular complex in which β-cyclodextrin is the host molecule and phenolphtalein is the guest, has proven an unusual stabilities and colour intensity change in alkaline solutions [1]. Recently this supramolecular system has been extensively studied particularly using spectrophotometric and potentiometric method [9,10], ^{13}C and ^{1}H NMR spectroscopy [10,11] as well as temperature-jump and flow injection gradient techniques [12,13]. The phenomenon, that the colour intensity of phenolphtalein in alkaline medium diminishes when phenolphtalein is incorporated into the cavity of β- or γ-cyclodextrin, has been employed for indirect photometric detection of cyclodextrins in liquid chromatography [14–16]. Frijlink and co-workers have successfully applied this indirect detection method for pharmacokinetic studies of β-cyclodextrin in rats, after intravenous injection [14]. Nevertheless, despite the number of papers dealing with inclusion association and other various applications of CD–PP complex, little attention has been focused on temperature[10,12]. However, it never has been clearly described before, that this complexation process is very strong temperature dependent, even though temperature plays a basic role in, e.g. chromatographic non-chiral [17,18] and chiral separations [19].

This work is a continuation of our earlier contribution concerning the termochromic behaviour of CD–PP supramolecular system, based on host-guest complexation processes and ocurring in solution [20].

2. Experimental

2.1. Reagents

β-Cyclodextrin was purchased from Merck (Darmstadt, Germany) and was recrystallized from a mixture of water and methanol. Phenolphtalein and sodium carbonate were obtained from a commercial supplier and used as received. Tetrahydrofuran (99.9%; HPLC grade) was purchased from Aldrich (Milwaukee, WI). Water was purified by double distillation, filtered through a 1.5 μm membrane and degassed on ultrasonic bath UM-2 (Unitra-Unima, Olsztyn, Poland) prior to use.

2.2. UV measurements

The absorption spectra were recorded using a Philips PU 8750 (UK) UV-vis one beam spectrophotometer. All measurements were carried out using standard 1 cm thick quartz cells, 1 nm sampling wavelenght, 1000 nm min^{-1} scan speed and temperatures from 10 to 70°C, step 10°C. A constant temperature of the samples was maintained using heat exchanger (placed inside cell) which was constructed using stainless stell capillary (ID = 0.5 mm) through which a heat-exchange medium (ethanol–water mixture, 30:70, v/v) was circulated from a constant-temperature bath. Temperature of the sample was measured on-line by thermistor probe placed inside cell and connected to a Metex M-4650CR (Germany) digital multimeter. Using this home-made equipment temperature of the sample was controlled with an accuracy of ± 0.2°C.

Alkaline solution of phenolphtalein at a concentration of 30 μM was prepared in aqueous

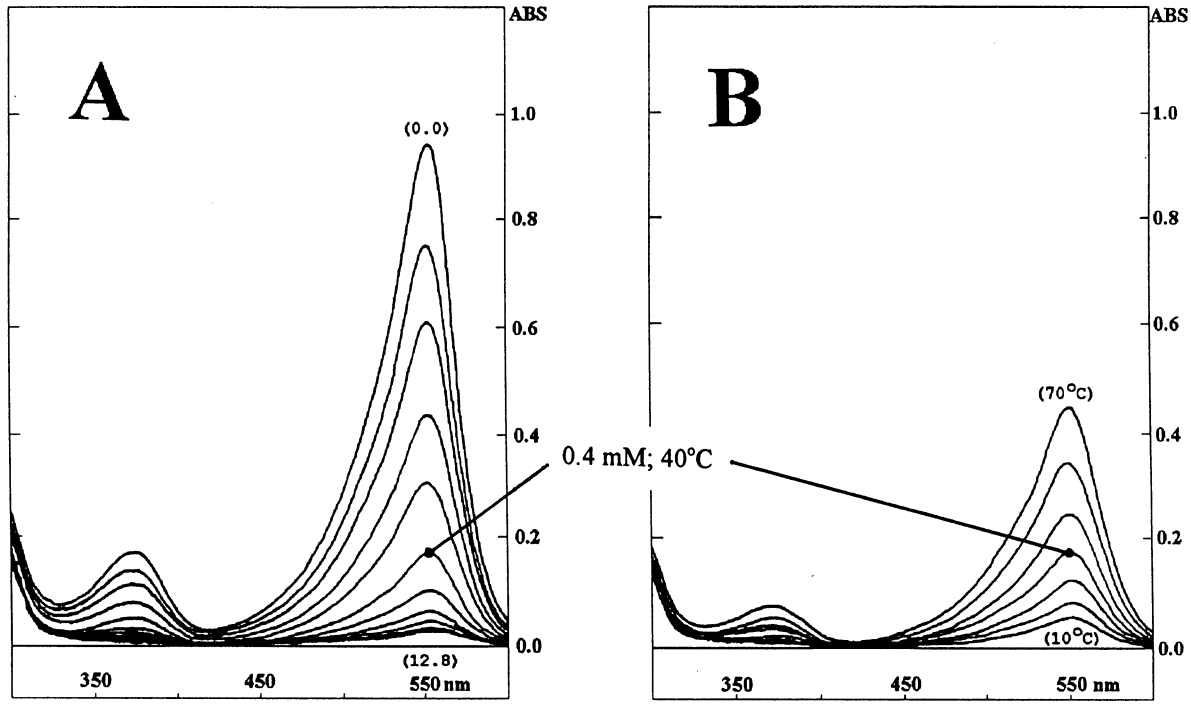

Fig. 1. UV-visible absorption spectra of phenolphtalein (30 μM) measured in alkaline solution (0.02 M Na_2CO_3; pH = 10.5); A, constant temperature (40°C) and different β-cyclodextrin concentrations (0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 mM); B, constant β-cyclodextrin concentration (0.4 mM) and different temperatures (from 10 to 70°C step 10°C).

sodium carbonate 0.02 M, pH = 10.5. The samples were modified by the addition of β-cyclodextrin at a concentration of 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 mM as well by the addition of tetrahydrofuran at a concentration of 37 mM. Appropriate alkaline solution without of the phenolphtalein were used as the references. All of the solutions were prepared freshly in the day of use.

The stoichiometric proportion and binding constants K, from spectrophotometric data, were calculated using Scott's Eq. (1) [21–23]:

$$C_{\beta\text{-CD}}/\Delta A = C_{\beta\text{-CD}}/(C_{\text{PP}} \times \Delta\varepsilon) + 1/(C_{\text{PP}} \times \Delta\varepsilon \times K) \quad (1)$$

where $C_{\beta\text{-CD}}$ and C_{PP} are the total molar concentrations of β-cyclodextrin and phenolphtalein, ΔA is the change in absorbance after addition of β-cyclodextrin ($\Delta A = A_{\text{PP}} - A_{\beta\text{-CD}\cdot\text{PP}}$), K is the binding constant and $\Delta\varepsilon$ is the difference of the molar absorptivities for free and complexed phenolphtalein. If resulting plot of $C_{\beta\text{-CD}}/\Delta A$ against $C_{\beta\text{-CD}}$ yields a straight line, the 1:1 complexing system is expected and the binding constant K_{11} (M^{-1}) can be calculated from Eq. (2):

$$K_{11} = (\text{slope}/\text{intercept}) \times 1000 \quad (2)$$

where slope and intercept correspond to regression coefficients a and b of the regression equation $C_{\beta\text{-CD}}/\Delta A = aC_{\beta\text{-CD}} + b$ (concentration of cyclodextrin in mM).

3. Results and discussion

It is well known, that the addition of β-cyclodextrin to the alkaline solution containing phenolphtalein results in a decrease of its absorbance in UV-visible region [12]. Fig. 1A shows changes in absorbance after addition of various amount of β-CD, from 0.025 to 12.8 mM, at a temperature of 40°C. This phenomenon was widely applied to

indirect postcolumn detection of β-CD in chromatographic methods [14–16]. Fig. 2B shows the influence of temperature on the change in absorbance of investigated complex. The range of temperatures investigated was from 10 to 70°C and the concentration of β-cyclodextrin was set at 0.4 mM. In this case decreasing of temperature induces a decrease in absorbance of both bands observed at $\lambda_{max} \cong 374$ and 554 nm. Due to low absorbance of wavelenght region from 300 to 400 nm for further investigations only the region from 500 to 600 nm was considered. As can be seen from the curves presented on Fig. 2 under favourable conditions (0.1 mM β-CD, 30 μM PP) the termochromic effect is very significant ($\cong 0.1$ U of absorbance/10°C).

In the experiment a wide range of β-cyclodextrin:phenolphtalein concentration ratios (from 0.8:1 to 427:1) was studied (Fig. 2). Hence, the distribution of the adequate data points on the y-axis of Scott's plot are inregular. Therefore, for evaluation of the binding constant, the concentrations of 'host' molecules from 0.2 to 12.8 mM (molar concentration ratio of β-CD:PP from 7:1 to 427:1) were considered. As can be seen, from Table 1 a linear Scott's relationships are observed for all isotherms investigated. A high correlation

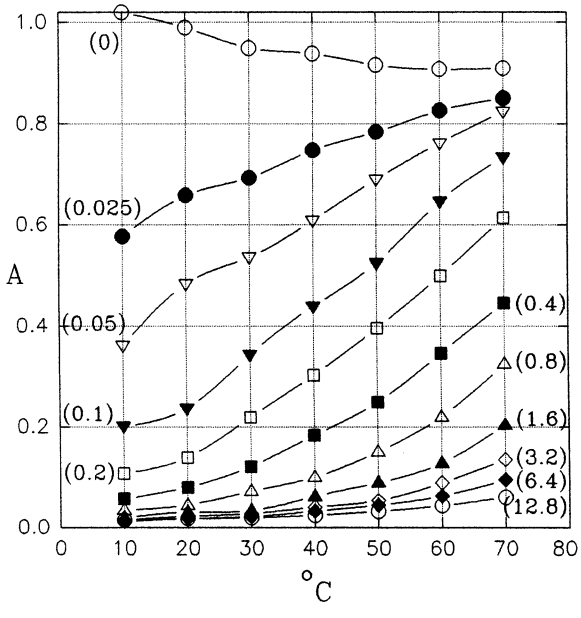

Fig. 2. Influence of temperature on the absorbance ($\lambda_{max} \cong 554$ nm) of phenolphtalein (30 μM) and β-cyclodextrin:phenolphtalein system. The values in parentheses indicate the concentration of β-cyclodextrin. Other condition are given under Fig. 1.

Table 1
Regression coefficients (a, b) and correlation coefficient (r) of the regression equation $C_{\beta\text{-CD}}/\Delta A = aC_{\beta\text{-CD}} + b$

Temperature(°C)	a	b	r
A			
10	0.9933(9E-4)	0.013(5E-3)	0.9999
20	1.0264(5E-4)	0.026(3E-3)	0.9999
30	1.0710(9E-4)	0.049(5E-3)	0.9999
40	1.0874(8E-4)	0.091(4E-3)	0.9999
50	1.121(1E-3)	0.148(8E-3)	0.9999
60	1.138(2E-3)	0.26(1E-2)	0.9999
70	1.146(4E-3)	0.45(2E-2)	0.9999
B			
10	0.9827(7E-4)	0.060(4E-3)	0.9999
20	1.024(1E-3)	0.101(8E-3)	0.9999
30	1.042(1E-3)	0.145(6E-3)	0.9999
40	1.077(3E-3)	0.22(1E-2)	0.9999
50	1.100(4E-3)	0.38(2E-2)	0.9999
60	1.111(3E-3)	0.55(2E-2)	0.9999
70	1.11(1E-2)	0.91(7E-2)	0.9997

The absorbance values of phenolphtalein (30 μM) in alkaline solution (0.02 M Na_2CO_3; pH = 10.5) were measured at $\lambda_{max} \cong 554$ nm using different cyclodextrin concentration (0.2–12.8 mM) without (A) and with addition of 37 mM of tetrahydrofuran (B).
The values in parentheses indicate the standard error at 95% significance level; number of samples: 7.

Table 2
The values of the binding constant (K_{11}) of β-cyclodextrin-phenolphtalein complex without (**I**) and with the addition of tetrahydrofuran (**II**) calculated at different temperatures

Temperature (°C)	K_{11} (**I**) ($\times 10^4$ M^{-1})	K_{11} (**II**) ($\times 10^4$ M^{-1})
10	7.44	1.64
20	3.94	1.02
30	2.20	0.72
40	1.20	0.48
50	0.76	0.29
60	0.44	0.20
70	0.26	0.12

Table 3
Regression coefficients (a, b) and correlation coefficient (r) of the regression equation $\ln K_{11} = a(1000/T)+b$ and the thermodynamic parameters ($\Delta H°$ and $\Delta S°$) for investigated complex without (**I**) and with the addition of tetrahydrofuran (**II**); temperature range: 10–70°C

	a	b	r	ΔH° (kJmol^{-1})	ΔS° (Jmol^{-1} °K^{-1})
I	5.39 (0.07)	−7.8 (0.2)	0.9996	−44.8	−64.9
II	4.1 (0.2)	−4.8 (0.5)	0.9964	−34.1	−40.0

The values in parentheses indicate the standard error at 95% significance level; number of samples: 7.

($r = 0.9999$; Table 1A) suggests a β-CD + PP = β-CD·PP equilibrium model. Similarly, an excellent linear plots were observed for concentration ratios of β-CD:PP from 0.8:1 to 27:1, which confirms the 1:1 stoichiometric ratio of investigated complex. Moreover, the data from Table 1B suggest, that for systems in which tetrahydrofuran in a concentration of 37 mM was added, 1:1 stoichiometric proportion can be expected for β-CD–PP complex. The binding constant values (K_{11}) of β-CD–PP complex without an with addition of tetrahydrofuran, calculated from Scott's equation are presented in Table 2. The K_{11} values obtained for temperatures rangeing from 20 to 30°C (2.2 and 3.9×10^4 M^{-1}) are in agreement with literature values: $1.9–3.7 \times 10^4$ M^{-1} [9–13]. It is noteworthy, that the values of binding constants are remarkably lowered after the addition of tetrahydrofuran to β–CD·PP system. Matsui and Mochida [24] have studied the association of α- and β-cyclodextrin with alkohols, using cyclodextrin-azo dye system. Similarly the addition of tetrahydrofuran to β-CD–PP system results in an increase in absorbance which indicate, that a part of amount of THF is included by cyclodextrin and the strong interaction between cyclodextrin and tetrahydrofuran exist.

Table 2 shows the influence of temperature on K_{11} values of investigated complex without and with the addition of tetrahydrofuran. In both cases the linear relationships between $\ln K_{11}$ and $1000/T$ values are observed (Table 3). From linear Van't Hoff plots thermodynamic parameters such as; the change of enthalpy ($\Delta H°$) and change of entropy ($\Delta S°$) were estimated. The calculated values of changes of enthalpy are similar to values reported by Okubo and Kuroda, where inclusional association of phenolphtalein was studied using the temperature-jump technique [12]. In all cases listed in Table 3, the values of enthalpy and entropy changes are negative, what probably indicate, that stiff complex between cyclodextrin and phenolphtalein is created and the contribution of van der Waals interactions to the binding is very large [24].

4. Conclusions

The experiment described confirms that a creation of a supramolecular complex in which β-cyclodextrin is the host molecule and phenolphtalein is the guest is temperature controlled. Decreasing of temperature induce a decrease in UV-visible absorbance of phenolphtalein. Under favourable conditions (0.1 mM β-CD, 30 μM PP) the termochromic effect is very significant ($\cong 0.1$ U of absorbance/10°C).

The addition of tetrahydrofuran to CD–PP system resulted in an increase in absorbance, indicating strong competition in binding site between PP and THF. On the basis of this observation it can be now easily explained, that relatively high concentration of CD is needed, when THF-water binary system is used in chromatography.

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