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Thermodynamic study of the retention behaviour of selected macrocycles using reversed-phase high-performance thin-layer chromatography plates and methanol–water mobile phases

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Abstract

The influence of temperature and mobile-phase composition on retention of α-, β-, and γ-cyclodextrins and two macrocyclic antibiotics (rifamycin B and rifampicin) has been examined by reversed-phase thin-layer chromatography, using wide-range (0–100%) binary mixtures of methanol–water. Retardation factors (R_F) of the solute molecules were measured at different temperatures from 5 to 60°C. Temperature changes of the chromatographic conditions produce significant differences in migration of the investigated macrocyclic compounds. Generally, the plots of the rate mobility factor (R_M) versus the reciprocal of the absolute temperature are linear. The ranges of mobile-phase composition in which R_F values are equal to unity were wider at higher temperatures. From linear Van't Hoff plots thermodynamic parameters such as the change of enthalpy (ΔH°) and the change of entropy (ΔS°) were estimated. In each case the sign of the calculated parameters is negative. However, the magnitudes of ΔH° and ΔS° indicate significant thermodynamic differences between two groups of solutes, one of which includes α- and γ-cyclodextrins and the other macrocyclic antibiotics and β-cyclodextrin.

Keywords: Thermodynamic parameters; Enantiomer separation; Temperature effects; Mobile-phase composition; Cyclodextrins; Rifamycins; Rifampicin

1. Introduction

Generally, in classical reversed-phase liquid chromatography solute retention is inversely related to temperature. The dependence of the logarithms of the capacity factor ($\ln k'$) on temperature is given by Eq. (1) and is known as the Van't Hoff plot [1–3]

$$\ln k' = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln \phi \quad (1)$$

where k' denotes capacity factor, ΔH° enthalpy change of transfer for the solute from the mobile phase to the stationary phase, ΔS° entropy change of transfer of the solute from the mobile phase to the stationary phase, ϕ phase ratio of the column and R gas constant. This fundamental equation can be easily explained by assuming that ΔH°, ΔS° and ϕ are independent of temperature. When the retention mechanism is the same over the temperature range

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investigated and the above parameters are constant, the resulting plot of $\ln k'$ against $1/T$ yields a straight line [3,4].

Nevertheless, any reversible process that alters the enthalpy or entropy of adsorption in principle gives rise to non-linear Van't Hoff plots. Among others, changes in conformation and changes in the extent to which the mobile phase interacts with either the analyte or the stationary phase are examples of such reversible behaviour [5,6]. Moreover, the presence of multiple types of retention mechanisms or multiple types of binding sites also lead to non-linearity of the Van't Hoff plots. Particularly, multiple types of retention and the importance of conformation can be expected, and therefore the effect of temperature on retention might be very complex [7,8].

Cyclodextrins (CDs) are toroidal-shaped cyclic oligomers of α-1,4-D-glucopyranose units, which contribute to several guest-associated phenomena in solution. In chromatography, CDs are commonly used as chiral selectors and for improving separation of other stereoisomers [9–11]. Despite the enormous number of practical applications, some of the basic properties of cyclodextrins are still unknown. Therefore, the retention properties of CDs have been studied using HPLC [12–14], even though this system has several disadvantages for such purposes. Since CDs have almost no UV absorption, detectors of low sensitivity such as refractive index or polarimetric detectors must be used. This requires overload injections, which, because of an enormous adsorption effect, strongly influences retention measurements. Moreover, the mobile-phase composition which can be investigated is restricted, because under extreme conditions the retention time is too long or the solute does not elute from the column. Considering these disadvantages, TLC seems to be particularly well suited to the study of CD behaviour in chromatographic systems.

As an alternative, mobile-phase additives and stationary-phase modifiers for chromatographic and electrophoretic chiral separations, like macrocyclic antibiotics, have been used [15–18]. Contrary to cyclodextrins, this class of chiral selectors contains groups which enable the separation and enantioseparation via different mechanisms including π–π complexation. Hydrogen bonding, hydrophobic inclusion and steric interaction are also possible. Recently, chromatographic and electrophoretic separations using rifamycin B (RB) as a chiral selector were reported [15,19,20]. Nevertheless, there is no report concerning the influence of temperature on retention behaviour of ansamycins in RP-HPTLC systems.

The utilization of cyclodextrin and antibiotic inclusion processes in chromatography has been carried out by two different approaches. The first relies on the use of inclusion agents chemically [21] or physically [22,18] bonded to the stationary phase. In this case the selector should be strongly adsorbed on the support material, which means that in TLC its R_F value should be equal to zero. In the second approach inclusion agents are applied as mobile-phase components. Therefore, the inclusion agent should be present in the mobile phase during the whole chromatographic process. In TLC, this means that the R_F value of the inclusion component should be very close, or even equal, to unity [23].

The aim of this paper is to demonstrate that temperature can be a very useful parameter for designing an enantioselective chromatographic system in which R_F values of the chiral phase modifier should be equal to zero or unity.

2. Experimental

2.1. Reagents

Cyclodextrins (α, β and γ) were purchased from Reanal-Chinoin (Budapest, Hungary). Rifamycin B (RB) was a product of Sigma (St. Louis, MO, USA). Rifampicin (R) was obtained from Polfa (Tarchomin, Poland). Methanol used as component of mobile phases was purchased from P.O.CH. (Gliwice, Poland). It was purified by double distillation. Acetonitrile (99.9%, HPLC grade) was obtained from Aldrich (Milwaukee, WI, USA) and was used as received. Double-distilled water was used for preparation of the stock solutions and the binary mobile phases. Stock solutions of the investigated compounds (1 mg ml^{-1}) were prepared in pure methanol and in a mixture of acetonitrile–water (30:70, v/v) for ansamycins and cyclodextrins, respectively.

2.2. Chromatography

Chromatography was performed on 4×10 cm RP-18W HPTLC plates (wettable with water, without fluorescent indicator) obtained from Merck (Darmstadt, Germany). The chromatographic chambers (110×60 mm wide and 15 mm deep) were saturated with the vapour of the mobile phase under 1 atm pressure. Chromatographic experiments were performed at temperatures of 5, 10, 20, 30, 40, 50 and 60°C, controlled by circulating water from the thermostat with an accuracy of ±0.5°C. The plates were thermostated 25 min before development in order to obtain proper temperature equilibrium. When a new temperature was started, the chromatographic device was thermostated for at least 30 min.

The cyclodextrins were visualized by spraying the plates with concentrated sulfuric acid–methanol (1:4, v/v) and heating at 140°C for 2–5 min. After this time the cyclodextrins were visualized as grey and black spots on the white background.

3. Results and discussion

The retardation factor (R_F) values of the studied macrocyclic compounds were measured using a wide range (from 0 to 100% v/v) of methanol in water binary mobile phases and a wide range of temperatures from 5 to 60°C. Thus, the total number of chromatographic data obtained during these experiments for all solutes, temperatures and solvent systems was 385. Fig. 1A–E illustrates the retention behaviour of α-, β-, and γ-cyclodextrin, and rifamycin B and rifampicin, respectively, on RP-18W HPTLC plates using different mobile-phase compositions and temperatures. It is noteworthy that under favourable chromatographic conditions the solutes were well separated, as is shown in Fig. 2. The retention behaviour of cyclodextrins using different mobile phases and temperature set at 30°C has been recently reported and interpreted in our previous papers [24,25]. The results of the present study confirm our earlier observation, that in RP-TLC systems cyclodextrins do not elute when the concentration of methanol is less than 15% (Fig. 1A–C). Similar to the retention behaviour of the CDs, the macrocyclic antibiotics do not elute when the concentration of organic modifier is close to zero (Fig. 1D,E). For rifampicin the range of retention maximum (i.e. $R_F=0$) is wider than those observed for remaining macrocycles. On the mobile-phase compositions studied, rifampicin is strongly adsorbed on the stationary phase in comparison to rifamycin B, particularly when mobile phases containing more than 50% (v/v) of water have been applied. With the exception of favourable chromatographic conditions (90% of methanol in water, 60°C) observed R_F values of rifampicin are less than 0.9. For that reason, using RP-18W plates, this compound should not be used as a modifier of mobile phases. However, rifampicin should be the best modifier for physical immobilization on the support, because its R_F value is equal to zero in the widest range of binary mobile-phase compositions (see Fig. 1E).

As can be seen in Fig. 1, temperature changes produce significant differences in the migration of the investigated macrocyclic compounds. For practical purposes, when the macrocycles are used as additives to the mobile phase in order to improve selectivity, they should constantly migrate with the mobile phase, i.e. their R_F values should equal unity. From this point of view, in the methanol–water system, the best candidate as mobile-phase modifier is γ-CD, then α-CD, β-CD, and RB. As mentioned above, rifampicin should be excluded because its R_F never reaches unity.

Comparing the results obtained at different temperatures it is noteworthy that the range of mobile-phase compositions in which R_F values are equal to unity is wider with higher temperatures. It can be easily explained according to the Van't Hoff plot behaviour of the investigated compounds. The phenomenon that retention decreases with temperature increase was observed many times in LC and other chromatographic methods. However, in TLC little attention has been focused on the influence of temperature on retention or separation, and this problem is still poorly recognized [23]. Fig. 3 shows the influence of temperature on chromatographic behaviour of solutes when a methanol–water (50:50) binary mobile phase was used. In this case, rates of mobility factors (R_M) have been used as retention parameters. Hence, the slope of the linear plots $y=bx+a$ (where x denotes $1000/T$ and y denotes R_M values) corresponds to the enthalpy change

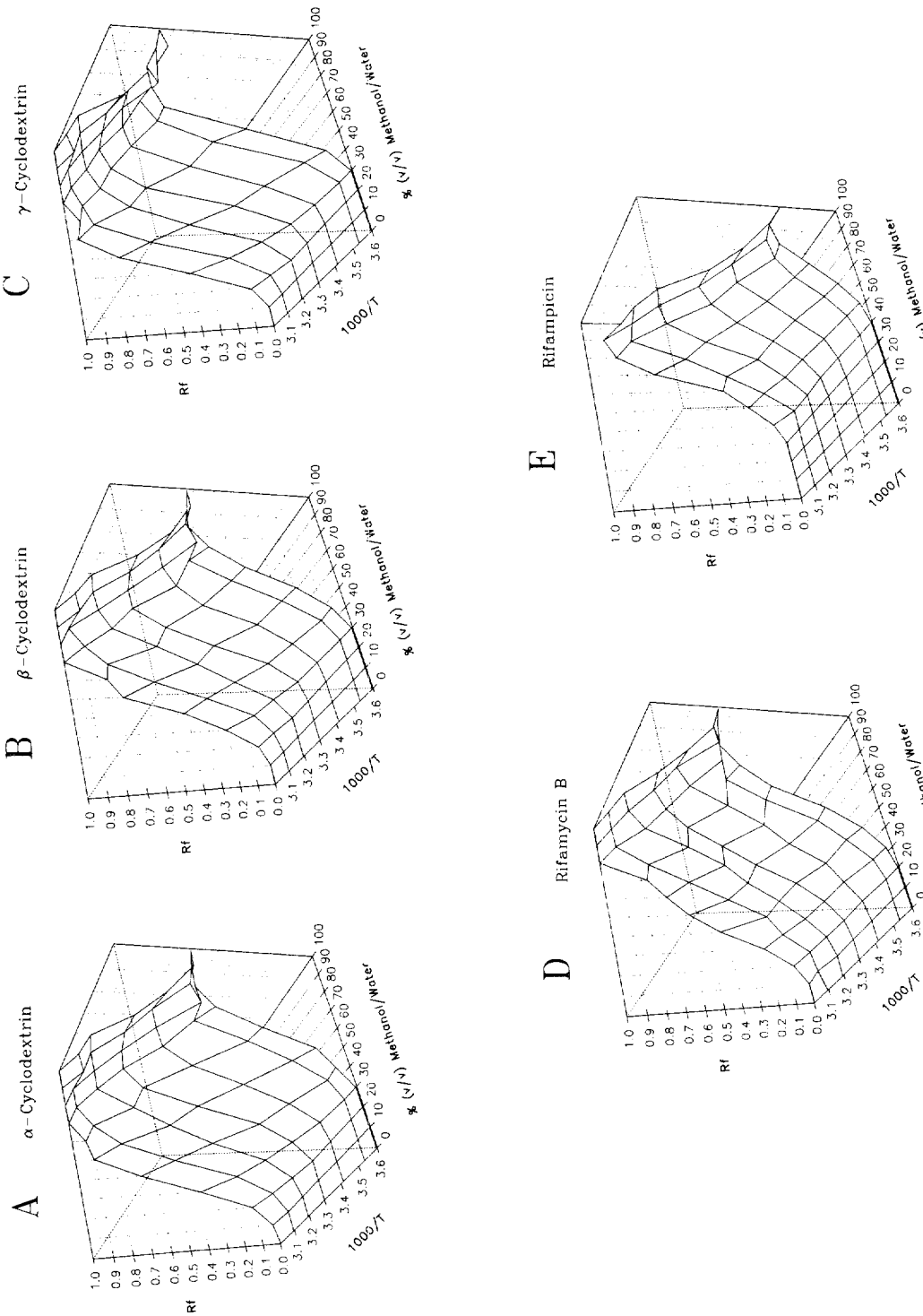

Fig. 1. Relationships between R_f values of α- (A), β- (B), and γ-cyclodextrin (C), rifamycin B (D) and rifampicin (E) versus different mobile-phase compositions and reciprocal of absolute temperature.

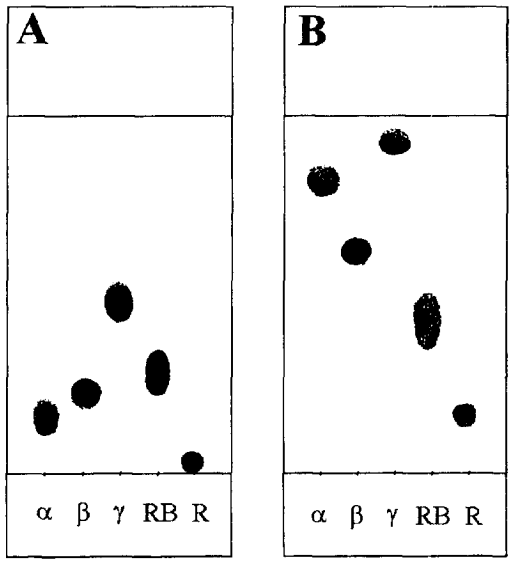

Fig. 2. Separation of studied macrocycles using methanol–water (50%, v/v) as mobile phase. The temperature of chromatographic process was 5 (A) and 50°C (B), respectively.

($b = -\Delta H^\circ/2.303R$) and intercept to the entropy change ($a = \Delta S^\circ/2.303R$) of transfer of the solute from the mobile phase to the stationary phase according to Eq. (2), which is an equivalent formulation of Eq. (1):

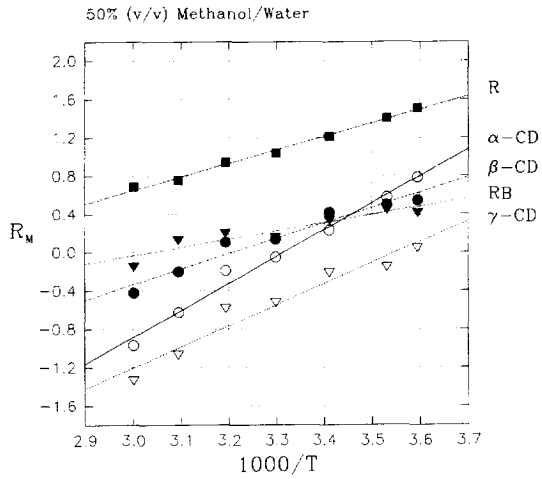

Fig. 3. Plots of R_M versus $1000/T$ for α- (○), β- (●), and γ-cyclodextrin (▽), rifamycin B (▼) and rifampicin (■). Mobile phase: methanol–water (50%, v/v).

$$R_M = \log k' = -\Delta H^\circ/2.303RT + \Delta S^\circ/2.303R + \ln \phi \quad (2)$$

It is important to note that ΔH° values are independent of the phase ratio. In addition, any uncertainty in the phase ratio affects ΔS° values equally, and thus trends in ΔS° as a function of eluent composition are unaffected [26].

Enthalpy and entropy changes calculated accordingly to Eq. (2) were listed in Table 1. It is obvious that to calculate R_M values from R_F parameters, the extreme R_F values, i.e. 0 and 1, must be excluded. Therefore, the number of data points shown in Fig. 1 do not correspond to those listed in Table 1. Thus, it should be emphasized, that thermodynamic calculations could help when the separation mechanisms of the solutes itself are investigated, but not necessarily in selection of the best chromatographic system for improving selectivity or enantioseparation, because in these cases the extreme values play the most important role. Generally, nearly linear Van't Hoff behaviour was observed over the whole temperature ranges and concentrations of methanol in water for all solute molecules. However, in the case of γ-CD and RB, when medium to high concentrations of methanol were used, the correlation coefficients (r) are low (less than 0.9). Therefore, for these data points ΔH° and ΔS° parameters were not calculated. Also, this suggests non-linearity in Van't Hoff plots. Non-linear Van't Hoff behaviour may be indicative of a change in the retention mechanism. Moreover, it should be concluded, that the retention mechanism of the solutes considered is very complex, and therefore calculated thermodynamic parameters are valid only at a given temperature and mobile-phase composition ranges.

In all the cases listed in Table 1 the values of enthalpy and entropy changes are negative. Negative values of enthalpy changes indicate that the transfer of solute from mobile to stationary phase is favoured. Moreover, values of enthalpy changes are different for various solute molecules, using the same composition of mobile phase. Therefore, the elution sequences should be different at various temperatures. This assumption is well documented experimentally in Fig. 2, where at 5°C the elution order is γ-CD, RB, β-CD, α-CD and R, while at 50°C the elution sequence is γ-CD, α-CD, β-CD, RB

Table 1
Thermodynamic parameters ΔH° and ΔS° for α-, β-, and γ-cyclodextrins, rifamycin B and rifampicin measured by RP-TLC and different mobile-phase compositions; (r) correlation coefficient, (n) number of samples

Compound	CH_3OH (%, v/v)	Temp. range (°C)	n	r	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})
α-CD	30	20–60	5	0.9901	−62.17	−182.98
	40	5–60	7	0.9895	−50.10	−156.40
	50	5–60	7	0.9930	−53.67	−177.99
	60	5–60	7	0.9764	−45.94	−160.46
	70	5–40	5	0.9799	−49.12	−178.27
	80	5–40	5	0.9504	−45.03	−164.21
	90	5–50	6	0.9524	−38.27	−140.58
	100	5–50	6	0.9244	−40.59	−146.66
β-CD	30	20–60	5	0.9922	−58.64	−164.79
	40	5–60	7	0.9920	−33.90	−99.14
	50	5–60	7	0.9746	−30.68	−98.45
	60	5–60	7	0.9388	−24.68	−86.20
	70	5–50	6	0.9431	−35.04	−127.72
	80	5–40	5	0.9064	−31.49	−116.24
	90	5–50	6	0.9428	−34.50	−126.60
	100	5–50	6	0.9000	−36.81	−132.87
γ-CD	30	20–60	5	0.9990	−67.27	−198.77
	40	5–60	7	0.9969	−45.59	−146.03
	50	5–60	7	0.9707	−41.93	−148.86
	60	5–60	7	0.8945	-	-
	70	5–40	5	0.9622	−33.33	−134.71
	80	5–40	5	0.8816	-	-
	90	5–50	6	0.9154	−34.32	−132.54
	100	5–50	6	0.8678	-	-
Rifamycin B	20	20–60	5	0.9696	−26.89	−57.21
	30	5–60	7	0.9768	−29.97	−79.54
	40	5–60	7	0.9240	−19.85	−54.35
	50	5–60	7	0.9302	−16.40	−49.86
	60	5–60	7	0.7066	-	-
	70	5–60	7	0.7335	-	-
	80	5–50	6	0.8993	-	-
	90	5–50	6	0.9602	−40.95	−151.76
	100	5–50	6	0.9468	−44.28	−161.64
Rifampicin	50	5–60	7	0.9961	−26.95	−68.44
	60	5–60	7	0.9627	−18.31	−49.22
	70	5–60	7	0.9928	−25.44	−80.76
	80	5–60	7	0.9730	−35.18	−118.99
	90	5–60	7	0.9695	−37.10	−126.28
	100	5–60	7	0.9740	−33.95	−113.09

and R. Negative values for entropy changes are ascribed to increasing order due to sorption. The more compact solute molecules are expected to involve greater ordering during the interactions with mobile phase than the less compact ones. It is evident that cyclodextrins, which are cyclic glucose oligomers, are more compact than macrocyclic antibiotics with the ansa-bridge and substituents. Thus, the entropy changes for ansamycins are generally of smaller magnitude than those of cyclodextrins.

Enthalpy–entropy compensation is a term used to describe a compensation temperature which is system independent for a class of similar experimental systems. It has been suggested by Cole and Dorsey

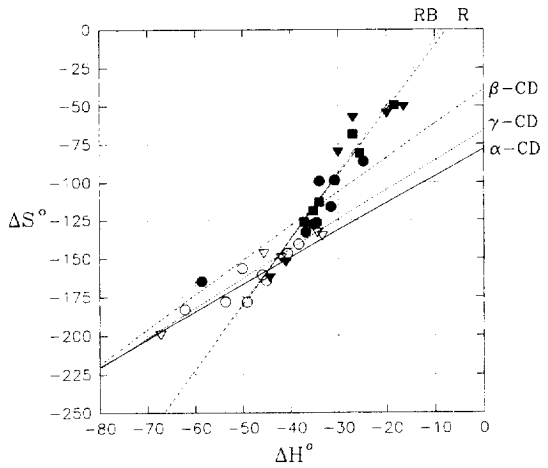

Fig. 4. Plot of enthalpy–entropy compensation for α-CD (○), β-CD (●), γ-CD (▽), rifamycin B (▼) and rifampicin (■).

that the slope of the enthalpy–entropy plot is constant for processes exhibiting similar interaction mechanisms [27]. The compensation plots are shown in Fig. 4 and the corresponding regression equations are listed in Table 2. The data in Table 2 suggest that the compounds studied fall into three groups. Group 1 consists of ansamycins, group 2 includes α-CD and γ-CD, and the behaviour of β-CD can be classified as intermediate between these groups. This conclusion is in agreement with earlier observations by Nowakowski et al. that γ-CD and α-CD are less retained than β-CD [28].

Table 2
Regression coefficients (*a*,*b*) and correlation coefficient (*r*) of the regression equation $\Delta S^\circ = b \times \Delta H^\circ + a$ for macrocycles using the different mobile-phase compositions listed in Table 1: the values in parentheses indicate the standard error at 95% significance level

Macrocycle	*a*	*b*	*r*
α-Cyclodextrin	−78 (20)	1.8 (0.4)	0.8680
γ-Cyclodextrin	−66 (9)	1.9 (0.2)	0.9836
β-Cyclodextrin	−39 (16)	2.2 (0.4)	0.9004
Rifamycin B	37 (21)	4.7 (0.7)	0.9557
Rifampicin	32 (14)	4.2 (0.5)	0.9757

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