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Effect of temperature and mobile phase on the retention of retinoates in reversed-phase liquid chromatography

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ABSTRACT

The effect of temperature on the retention of seven closely related retinoates in reversed-phase liquid chromatography using three organic mobile phases was studied. Retinoates were chromatographed on an RP-18 column with aqueous organic mobile phases (90% methanol, 87% acetonitrile and 55% tetrahydrofuran). The capacity factors were measured at five temperatures (35–55°C). An increase in temperature decreased the retention. The standard enthalpy changes of the retention process ($-\Delta H^0$) were calculated from the slopes of Van't Hoff plots (ln k' *vs.* $1/T$). The effect of the molecular structure on the standard enthalpy change was studied. This effect increased significantly as the organic modifier was changed in the order methanol < acetonitrile < tetrahydrofuran.

INTRODUCTION

Reversed-phase chromatography with bonded alkyl stationary phases is widely used in quantitative structure–retention relationship (QSRR) studies. The main objective in these studies is to obtain information about the physico-chemical properties of the solutes that affect both the retention and, for example, the chemical reactivity or biological activity of the compound. The solvophobic theory developed by Horváth *et al.* [1] can explain the influence of the solvent on the solvophobic interaction between the solute and the stationary phase, but cannot precisely explain the physico-chemical mechanism of the interaction process.

The mechanism behind the separation process can be explored by studying the free-energy relationships of the separation. The equilibrium constant is linearly dependent on the free-energy change of the process. The system is said to express an enthalpy–entropy compensation effect when the free-energy changes are linearly dependent on the corresponding enthalpy change. The retention mechanism in such a system is the same for all participating species [2,3]. A constant retention mechanism is essential for reliable QSRR studies. This should always be verified before making any postulations from the chromatographic data.

The following equation expresses the enthalpy–entropy compensation [4]:

$$\Delta H^0 = \beta \Delta S^0 + \Delta G^0_\beta \quad (1)$$

where ΔG^0_β denotes the Gibbs free energy of physico-chemical interaction at temperature β, and ΔH^0 and ΔS^0 are the corresponding standard enthalpy and entropy, respectively. At temperatures close to β the changes in ΔH^0 are caused by changes in ΔS^0, and the free-energy changes are virtually independent of temperature. The values of β and G^0_β are constant when enthalpy–entropy compensation occurs. β is called the compensation temperature.

By combining two equations for the Gibbs free-energy change ΔG^0,

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (2)$$

where T temperature, and

$$\Delta G^0 = -RT\ln K = -RT\ln(k'/\varphi) \quad (3)$$

where R is the gas constant, ln K is the thermodynamic equilibrium constant, k' is the capacity factor and φ is the phase ratio of the column, we

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obtain an equation describing the relationship between the capacity factor k' and the temperature T:

$$\ln k' = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \varphi \tag{4}$$

When the retention mechanism is the same over the temperature range investigated and the enthalpy is constant, the resulting plot of ln k' against $1/T$ (the Van 't Hoff plot) yields a straight line [2,3].

The $-\Delta H^0$ of the transfer of a solute molecule from the mobile phase to the stationary phase can be calculated from the slope of the Van 't Hoff plots [5]:

$$\text{slope} = \frac{-\Delta H^0}{R} \tag{5}$$

The enthalpy values are related to the carbon number of the solutes [6]. A more specific method for describing the molecular structure is to use a topological index, the molecular connectivity index [7], which is closely related to many physico-chemical [8–10] and biological [11,12] properties and chromatographic retention [13,14] of the molecules.

The relationship between $-\Delta H^0$, the standard enthalpy of the transfer of a solute molecule from the mobile phase to the stationary phase, and the molecular structure has been examined [6]. The slopes of the regression equations have been considered to be the methylene group increment of $-\Delta H^0$ and found to increase with the retention order of the solute groups. The intercepts were estimated to represent the $-\Delta H^0$ of the transfer of the functional group alone and to indicate the preferences of the mobile phase among the different groups.

The polarity of the mobile phase is the most important factor governing the retention. The polarity of solvents can be expressed quantitatively, *e.g.* by the polarity parameter P' of Snyder [15]. The retention (ln k') decreases as the temperature increases, and this reduction is linearly related to the polarity of the mobile phase [16].

The aim of this work was to study the effect of temperature on the retention of seven closely related retinoates in three mobile phases. The second aim was to study the relationships between the enthalpy change of the retention, the molecular structure of the solutes and the nature of the mobile phase.

EXPERIMENTAL

The apparatus consisted of a Hewlett-Packard 1090 liquid chromatograph equipped with a Pye Unicam UV detector and a Hewlett-Packard 3380A integrator. The straight-chain retinoates, methyl to pentyl (Nos. 1–5, Fig. 1), were synthesized from all-*trans* retinoic acid and a corresponding alkyl halide in the presence of potassium carbonate. The branched-chain retinoates, isopropyl and *tert.*-butylcarbinyl esters (Nos. 6 and 7, Fig. 7), were synthesized from a preformed N-retinylimidazolide *p*-toluenesulphonate and a corresponding alcohol (for more details, see ref. 17). Methanol, acetonitrile and tetrahydrofuran were high-performance liquid chromatography (HPLC) grade (Rathburn, Walkerburn, UK), and water was purified with the Alpha-Q system (Millipore, Bedford, MA, USA). Mobile phases were filtered through a 0.22-μm membrane filter (Millipore).

The compounds were chromatographed on a Spherisorb 5-ODS column (5 μm, 100 × 4 mm I.D., LKB Pharmacia, Uppsala, Sweden) equipped with a 10-mm guard column filled with LiChrosorb RP-18 (10 μm, Merck, Darmstadt, Germany). Aqueous organic solvents (90% methanol, 87% acetonitrile and 55% tetrahydrofuran) were used as the mobile phases. The compositions of the mobile phase were selected to give equal retention at a flow-rate of 1.00 ml/min regardless of the solvent used. The compounds were detected at 350 nm. The re-

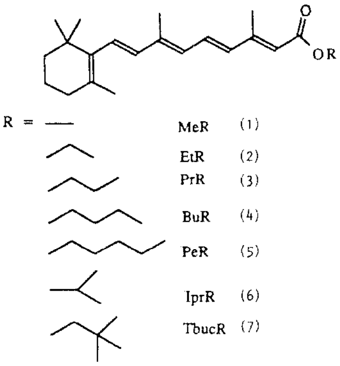

Fig. 1. Structures of the retinoates. MeR, EtR, PrR, BuR, PeR, IprR and TbucR are methyl, ethyl, propyl, butyl, pentyl, isopropyl and *tert.*-butylcarbinyl retinoates, respectively.

tention times were measured at five temperatures (35–55°C), which were controlled by the built-in column oven of the chromatograph. The capacity factors are presented in Table I.

The molecular connectivity indices were calculated using the general equation:

$$^{m}\chi_t = \sum_{j=1}^{^{m}n_s} {}^{m}c_j = \sum_{j=1}^{^{m}n_s}\left[\prod_{i=1}^{m}(\delta_i)_j^{-1/2}\right] \qquad (6)$$

where $^{m}c_j$ is the subgraph term for the mth-order subgraph, and $^{m}m_s$ is the number of subgraphs. The connectivity and valence level path indices of the orders 0–4 were calculated (Table II).

The regression between the capacity factors and temperature ($\ln k'$ *vs.* $1/T$, Van 't Hoff plot) was studied (Table III). The standard enthalpy changes of the retention process ($-\Delta H^0$) were calculated from the slopes of the regression equations using eqn. 5 (Table III). The values of $-\Delta H^0$ were plotted against indices describing the molecular structure.

RESULTS AND DISCUSSION

The retention times of the solutes were fairly equal in all the mobile phases, but the differences between the voild volumes (methanol 1.01 min, acetonitrile 1.05 min, tetrahydrofuran 1.13 min) caused larger variation between the corresponding capacity factors (Table I).

The Van 't Hoff plots of the retinoates were linear and the slopes positive (Table III, Fig. 2). The enthalpy of association between these compounds and the alkyl stationary phase was calculated using eqn. 5, and is constant and negative. In other words, the mechanism of the retention process was similar over the investigated temperature range, and the strength of the hydrophobic interaction decreased with increasing temperature.

The effect of the molecular structure on the standard enthalpy change was calculated. The carbon number of the solute can be used in the calculations when the investigated compounds are homologous. The series included two branched-chain analogues. The structures were therefore expressed by the connectivity indices, which have selectivity towards the molecular geometry and were found in earlier retention studies to be good descriptors of the molecular structure of retinoates [18]. Nearly all connectivity indices correlated excellently with the enthalpy change ($r^2_{\text{mean}} = 0.907 \pm 0.091$, $n = 30$), the only exception being $^{1}\chi^{v}$ ($r^2_{\text{mean}} = 0.685 \pm 0.091$, $n = 3$). The first order connectivity index, $^{1}\chi$, had the best

TABLE I

CAPACITY FACTORS (k') OF RETINOATES MEASURED AT FIVE TEMPERATURES AND THREE MOBILE PHASES

Temperature (°C)	MeR	EtR	PrR	BuR	PeR	IprR	TbucR
Methanol (90%)							
35	5.31	6.33	7.79	9.82	12.42	7.12	10.68
40	4.77	5.59	6.96	8.66	10.83	6.32	9.40
45	4.33	5.06	6.20	7.75	9.63	5.72	8.40
50	3.90	4.54	5.56	6.87	8.52	5.11	7.43
55	3.52	4.09	4.95	6.07	7.49	4.57	6.60
Acetonitrile (87%)							
35	4.36	5.37	7.79	8.54	10.93	6.41	10.25
40	3.87	4.69	6.96	7.35	9.34	5.56	8.64
45	3.45	4.15	6.20	6.44	8.07	4.97	7.53
50	3.10	3.72	5.56	5.63	7.02	4.36	6.57
55	2.77	3.25	4.93	4.90	6.02	3.81	5.66
Tetrahydrofuran (55%)							
35	4.29	5.18	6.57	8.19	10.00	6.21	9.42
40	3.63	4.43	5.52	6.79	8.17	5.22	7.76
45	3.23	3.89	4.79	5.87	7.01	4.60	6.67
50	2.81	3.35	4.05	4.95	5.82	3.94	5.61
55	2.44	2.89	3.49	4.17	4.86	3.35	4.75

TABLE II

VALUES OF THE CONNECTIVITY INDICES

	MeR	EtR	PrR	BuR	PeR	IprR	TbucR
$^0\chi$	17.46	18.17	18.87	19.58	20.29	19.04	20.67
$^1\chi$	10.76	11.26	11.76	12.26	12.76	11.61	12.40
$^2\chi$	9.56	9.94	10.30	10.65	11.00	10.21	10.75
$^3\chi$	5.41	5.90	6.17	6.42	6.67	6.00	6.51
$^4\chi$	4.32	4.65	4.71	4.90	5.08	4.73	4.84
$^0\chi^v$	15.40	16.11	16.82	17.52	18.23	16.98	18.60
$^1\chi^v$	7.73	8.42	8.92	9.42	9.92	8.01	8.57
$^2\chi^v$	6.18	6.41	6.82	7.18	7.53	6.56	7.33
$^3\chi^v$	4.11	4.21	4.38	4.67	4.92	4.28	4.60
$^4\chi^v$	2.47	2.59	2.67	2.79	2.99	2.68	2.76

correlation with the following equations for the three mobile phases (Fig. 3):

Methanol 90%:

$$-\Delta H^0 = 1.87\,^1\chi - 3.00 \qquad r^2 = 0.99$$

Acetonitrile 87%:

$$-\Delta H^0 = 3.01\,^1\chi - 13.50 \qquad r^2 = 0.96$$

Tetrahydrofuran 55%:

$$-\Delta H^0 = 3.40\,^1\chi - 13.72 \qquad r^2 = 0.99$$

The ΔH^0 of the ester group is expressed by the intercepts, which increase in the order methanol < acetonitrile < tetrahydrofuran. The slopes increased in the same order.

It can be seen from Table III that the regression coefficients and the intercepts of the regression equations $\ln k' = b \cdot (1000/T) + a$ increased with the size of the solute in all three mobile phases. This dependence was studied in two different ways. First,

TABLE III

REGRESSION BETWEEN THE CAPACITY FACTORS AND TEMPERATURE AS THE SLOPE b, THE INTERCEPT a, THE CORRELATION COEFFICIENT r^2 OF THE REGRESSION EQUATIONS $\ln k' = b \cdot (1000/T) + a$ AND THE $-\Delta H^0$ OF THE SEPARATION PROCESS CALCULATED FROM THE SLOPES OF THE REGRESSION EQUATIONS

	MeR	EtR	PrR	BuR	PeR	IprR	TbucR
Methanol							
b	2.07	2.19	2.28	2.41	2.53	2.22	2.42
a	−5.05	−5.27	−5.36	−5.54	−5.69	−5.25	−5.49
r^2	0.999	0.999	1.000	0.999	0.999	0.999	1.000
$-\Delta H^0$	17.23	18.22	18.98	20.04	21.01	18.48	20.12
Acetonitrile							
b	2.27	2.50	2.56	2.78	2.99	2.59	2.95
a	−5.91	−6.43	−6.41	−6.88	−7.30	−6.56	−7.27
r^2	1.000	0.999	0.999	1.000	1.000	0.998	0.999
$-\Delta H^0$	18.91	20.75	21.28	23.11	24.83	21.57	24.55
Tetrahydrofuran							
b	2.81	2.91	3.18	3.37	3.60	3.07	3.43
a	−7.67	−7.81	−8.44	−8.83	−9.39	−8.13	−8.88
r^2	0.998	0.999	0.999	0.999	0.999	0.998	0.999
$-\Delta H^0$	23.35	24.21	26.42	28.00	29.93	25.50	28.48

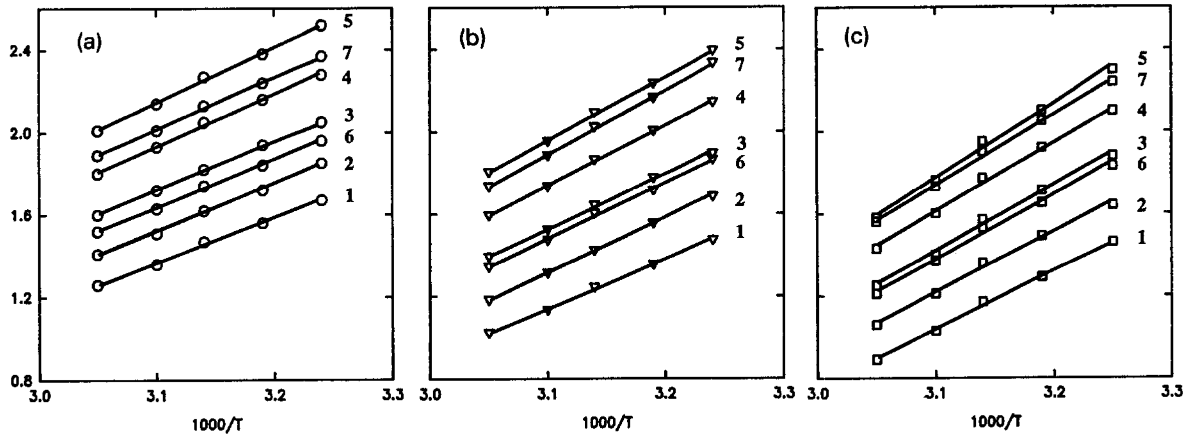

Fig. 2. Capacity factor ln k' *vs.* the reciprocal temperature $1000/T$: mobile phase (a) 90% methanol; (b) 87% acetonitrile; (c) 55% tetrahydrofuran. Retinoates numbered as in Fig. 1.

the separate equations were fitted to the intercepts and the slopes using the ${}^1\chi$ index as a descriptor for molecular structure.

The equations for the intercepts were (Fig. 4):

Methanol:

$$a = -0.30\,{}^1\chi - 1.89 \qquad r^2 = 0.95$$

Acetonitrile:

$$a = -0.70\,{}^1\chi + 1.54 \qquad r^2 = 0.92$$

Tetrahydrofuran:

$$a = -0.88\,{}^1\chi - 1.98 \qquad r^2 = 0.97$$

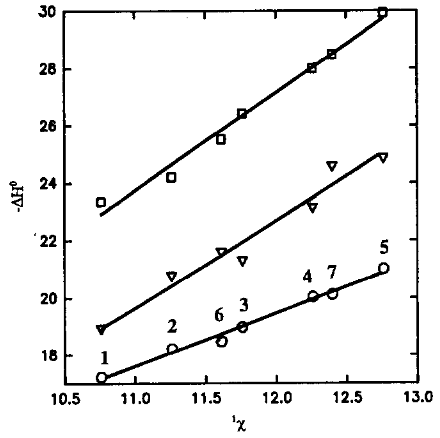

Fig. 3. Dependence of the standard enthalpy change, $-\Delta H^0$, on the molecular structure expressed with the first-order molecular connectivity index${}^1\chi$. Mobile phases: ○ = methanol, ▽ = acetonitrile and □ = tetrahydrofuran. Retinoates numbered as in Fig. 1.

and for the slopes (Fig. 5):

Methanol:

$$b = 0.23\,{}^1\chi - 0.35 \qquad r^2 = 0.99$$

Acetonitrile:

$$b = 0.36\,{}^1\chi + 1.62 \qquad r^2 = 0.96$$

Tetrahydrofuran:

$$b = 0.41\,{}^1\chi - 1.65 \qquad r^2 = 0.99$$

The slopes increased and the intercepts decreased in the order methanol < acetonitrile < tetrahydrofuran.

The equations were also calculated using two parameters, the temperature and a connectivity index, using all the indices calculated. Nearly all the correlations were highly significant ($r^2_{\text{mean}} = 0.931 \pm 0.065$, $n = 30$), the only exception being again ${}^1\chi^v$ ($r^2_{\text{mean}} = 0.759 \pm 0.030$, $n = 3$). The best correlation was achieved with the ${}^1\chi$ index:

Methanol:

$$\ln k' = 2.31\frac{1000}{T} + 0.41{}^1\chi - 10.29 \qquad r^2 = 0.992$$

Acetonitrile:

$$\ln k' = 2.63\frac{1000}{T} + 0.45{}^1\chi - 11.86 \qquad r^2 = 0.979$$

Tetrahydrofuran:

$$\ln k' = 3.16\frac{1000}{T} + 0.41{}^1\chi - 13.15 \qquad r^2 = 0.979$$

The regression coefficient for the temperature remained the same irrespective of the index used in

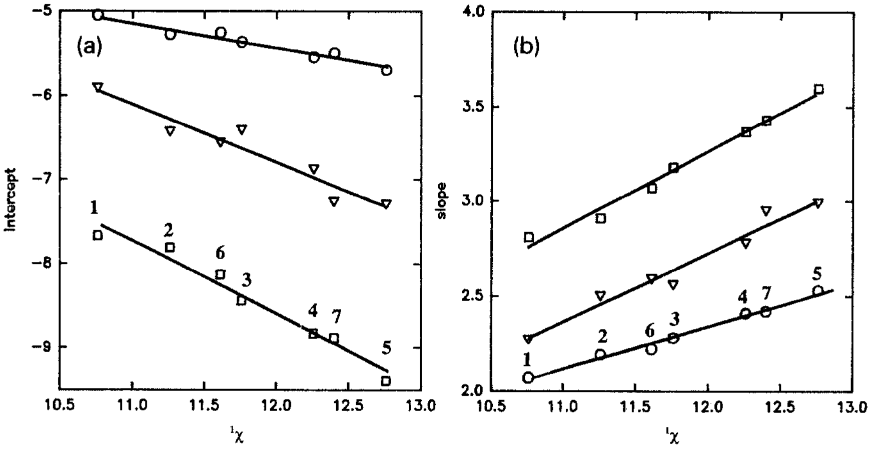

Fig. 4. Relationship between the molecular structure as the first-order molecular connectivity index $^1\chi$ and (a) the slopes and (b) the intercepts of the equations in Table III. Mobile phases: ○ = methanol, ▽ = acetonitrile and □ = tetrahydrofuran. Retinoates numbered as in Fig. 1.

the calculation for a specific mobile phase, but it varied depending on the mobile phase.

The total polarities of the mobile phases were not known. The polarity parameter values, P' [14], of the neat organic modifiers are 3.0 (methanol), 3.1 (acetonitrile) and 4.4 (tetrahydrofuran). The effect of temperature on the retention exhibits a clear trend of being linearly related to the polarity of the organic modifier.

In conclusion, the increase in temperature significantly decreased the capacity factors of the retinoates, resulting in linear Van 't Hoff plots. The decrease in retention in reversed-phase HPLC was linearly correlated with the molecular structure expressed by the molecular connectivity indices, but its magnitude was dependent on the nature of the organic modifier.

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