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INFLUENCE OF TEMPERATURE ON THE RETENTION BEHAVIOUR OF MEMBERS OF HOMOLOGOUS SERIES IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The capacity factors of C_6–C_{16} *n*-alkanols, C_6–C_{12} *n*-alkanal dinitrophenylhydrazones and C_6–C_{11} 2-*n*-alkanone dinitrophenylhydrazones have been determined at various temperatures on MicroPak CH-10 and Nucleosil C-18 octadecyl reversed-phase packings with aqueous methanol eluents. Linear log k' *vs.* C_n, log k' *vs.* $1/T$, $-\Delta H^0$ *vs.* C_n and log k' *vs.* $-\Delta H^0$ relationships have been obtained. Equations are presented that describe the capacity factors as functions of both temperature and carbon number. The compensation temperatures determined according to Melander *et al.* indicate that the retention mechanisms underlying these reversed-phase separations are identical.

INTRODUCTION

The awareness of the role of temperature in reversed-phase (RP) high -performance liquid chromatography (HPLC) has increased in recent years (see, *e.g.*, Horváth and co-workers[1,2], Colin *et al.*[3] and references cited therein). Melander *et al.*[4] applied the concept of enthalpy–entropy compensation to demonstrate the identity of the retention mechanism underlying RP separations in a few phase systems.

As by-products of systematic measurements in this Institute, a set of data relating to the influence of temperature on the retention behaviour of members of homologous series in several RP systems has been accumulated. It seems worthwhile presenting these measurements in an organized form as a contribution to the data base of the temperature dependence of retention in RP-HPLC.

EXPERIMENTAL

Experiments were carried out with a liquid chromatograph assembled from a Model 6000 pump, a U6K injector, an M440 fixed-wavelength photometer, an R401 differential refractometer (Waters Assoc., Milford, MA, U.S.A.), an LC55 variable-wavelength photometer (Perkin-Elmer, Norwalk, CT, U.S.A.) and an A-25 dual-

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channel recorder (Varian Aerograph, Walnut Creek, CA, U.S.A.). A commercial 25 cm × ⅛ in. O.D. column (MicroPak CH-10, Varian Aerograph) and a custom-made 30 cm × 1/4 in. O.D. column slurry-packed with Nucleosil C_{18} (Macherey, Nagel & Co., Bad Dürckheim, G.F.R.) were used. The columns were jacketed over their full lengths[5] and thermostated by a U10 circulating water-bath (MLW, Medingen, G.D.R.) Analytical-reagent grade methanol was obtained from Reanal (Budapest, Hungary).

The water content of the eluents filtered through GF/A and GF/D glass-fibre filters (Whatman, Clifton, NJ, U.S.A.) and vacuum degassed in an ultrasonic bath was determined daily (or after repeated degassing) from samples withdrawn from the inlet manifold of the pump. Water determinations were carried out in triplicate by Karl Fischer titration, with dead-stop end-point indication.

Samples were either analytical-reagent grade chemicals obtained from Merck (Darmstadt, G.F.R.), Fluka (Buchs, Switzerland), Condea (Hamburg, G.F.R.), BDH (Poole, Great Britain), Aldrich Europe (Beerse, Belgium) and Reanal (Budapest, Hungary), or synthesized, purified and analysed as described elsewhere[6].

Retention times were determined from the distance of the peaks from the point of injection on the chromatograms.

RESULTS

The retention of primary C_6–C_{16} *n*-alkanols on a MicroPak CH-10 column was investigated[7]. Aqueous methanol (78.24%) was used as the eluent. t_0 was determined by injecting a sufficient amount of 85% methanol to cause a noticable baseline disturbance. The capacity factors determined in the temperature range 30–60°C are given in Table I. The values are averages from five replicate determinations. The average reproducibility was better than 1% (relative).

TABLE I

CAPACITY FACTORS (k') OF PRIMARY *n*-ALKANOLS AT VARIOUS TEMPERATURES

Column: Micropak CH-10 (Varian Aerograph), 25 cm × 1/8 in. O.D. Eluent: aqueous methanol (78.24%). t_0 was determined by injecting the minimum amount of 85% methanol to cause a noticable disturbance on the baseline.

Chain carbon number, C_n	*Temperature (°C)*					
	30	*35*	*40*	*45*	*50*	*60*
	$1/T \cdot 10^3$ (°K^{-1})					
	3.30	*3.25*	*3.19*	*3.14*	*3.10*	*3.00*
6	0.91	0.87	0.78	0.74	0.72	0.61
7	1.47	1.34	1.26	1.15	1.07	0.95
8	2.29	2.14	1.89	1.75	1.64	1.36
9	3.54	3.18	2.90	2.60	2.39	2.02
10	5.53	5.05	4.28	3.91	3.58	2.88
11	8.43	7.59	6.41	5.75	5.31	4.13
12	12.91	10.94	9.62	8.38	7.46	5.99
13	19.9	17.5	14.2	12.59	11.53	8.28
14	31.2	26.2	22.4	18.9	16.3	12.50
15	48.8	41.9	33.4	28.8	26	17.6
16	74.3	61.2	51.9	42.5	36	26.6

In connection with a study[6] of the retention behaviour of various saturated and unsaturated aldehyde and 2-*n*-alkanone dinitrophenylhydrazones (DNPHs), the temperature dependence of their capacity factors was also investigated. These separations were effected on a Nucleosil C_{18} column. Again, aqueous methanol (80.0%) was used as the eluent. t_0 was determined by injecting a sufficient amount of 68% methanol to cause a noticable baseline disturbance. The capacity factors of the C_6–C_{12} *n*-alkanal and 2-*n*-alkanone DNPHs are given in Table II. The values are averages from nine replicate determinations. The reproducibility was generally better than 0.8% (relative).

TABLE II

CAPACITY FACTORS (k') OF *n*-ALKANAL AND 2-*n*-ALKANONE DNPHs AT VARIOUS TEMPERATURES

Column: Nucleosil C_{18} (Macherey, Nagel & Co.), 30 cm × 1/4 in. O.D. Eluent: aqueous methanol (80%). t_0 was determined by injecting the minimum amount of 68% methanol to cause a noticable disturbance on the baseline.

Compound type	*Chain carbon number, C_n*	*Temperature (°C)*					
		20	*30*	*40*	*43.6*	*50*	*60*
		$1/T \cdot 10^3$ (°K^{-1})					
		3.41	*3.30*	*3.19*	*3.16*	*3.10*	*3.00*
n-Alkanal DNPHs	6	6.05	4.80	3.78	3.65	3.10	2.57
	7	8.91	6.95	5.38	5.14	4.30	3.51
	8	13.31	10.19	7.72	7.31	6.04	4.85
	9	20.0	16.9	11.32	10.60	8.55	6.67
	10	30.2	22.2	16.0	15.9	12.04	9.27
	12	70	48.8	33.7	30.7	24.2	17.8
2-*n*-Alkanone DNPHs	6	6.41	5.12	3.98	3.88	3.26	2.73
	7	9.39	7.36	5.62	5.43	4.53	3.72
	8	13.94	10.70	8.04	7.70	6.36	5.12
	11	48.2	34.7	24.3	22.7	1.80	13.59

DISCUSSION

A number of conclusions can be drawn from the data in Tables I and II. It is known that an approximately linear relationship exists between log k' and the carbon number of members of a homologous series:

$$\log k' = \log k'_n + aC_n \tag{1}$$

The intercepts, slopes and correlation coefficients (r^2) for *n*-alkanols and *n*-alkanal and 2-*n*-alkanone DNPHs at different temperatures are given in Table III. It can be seen that the fit is very good.

Another linear relationship exists at a particular carbon number between log k', and the reciprocal of the absolute temperature of the separation (van 't Hoff plots):

$$\log k' = \log k'_T + \frac{b}{T} \tag{2}$$

TABLE III

PARAMETERS OF LOG $k' = \text{LOG}\ k_n + a\text{C}_n$ RELATIONSHIPS FOR THE THREE COMPOUND CLASSES AT VARIOUS TEMPERATURES

Temperature (°C)	*1/T·10³ (°K⁻¹)*	*n-Alkanols*			*n-Alkanal DNPHs*			*2-n-Alkanone DNPHs*		
		Intercept	*Slope*	*r^2*	*Intercept*	*Slope*	*r^2*	*Intercept*	*Slope*	*r^2*
20	3.41	—	—	—	−0.2894	0.1773	0.9997	−0.2556	0.1759	0,9997
30	3.30	−1.1662	0.1901	0,9999	−0.3312	0.1689	0,9970	−0.2981	0.1668	0,9997
35	3.25	−1.1583	0.1846	0,9997	—	—	—	—	—	—
40	3.19	−1.1677	0.1797	0,9997	−0.3772	0.1586	0,9998	−0.3512	0.1577	0,9998
43.6	3.16	—	—	—	−0.3684	0.1544	0,9999	−0.3502	0.1540	0,9998
45	3.14	−1.1617	0.1744	0,9998	—	—	—	—	—	—
50	3.10	−1.1543	0.1700	0.9996	−0.40901	0.1491	0,9998	−0.3824	0.1487	0,9998
60	3.00	−1.1650	0.1614	0.9995	−0.4353	0.1403	0,9999	−0.4049	0.1397	0,9999

The intercepts, slopes and correlation coefficients (r^2) for *n*-alkanols and *n*-alkanal and 2-*n*-alkanone DNPHs are given in Table IV. It can be seen that the fit is excellent.

The standard enthalpy of the transfer of a sample component from the mobile to the stationary phase can be calculated from the slope of the log k' *vs.* $1/T$ relationship as

$$\text{slope} = \frac{-\Delta H^0}{2.3R} \tag{3}$$

The $-\Delta H^0$ values thus calculated are given in Table IV. It can be seen that the $-\Delta H^0$ values increase regularly with increasing carbon number. Therefore, another series of linear relationships can be derived:

$$-\Delta H^0 = -\Delta H^0_n - c\mathrm{C}_n \tag{4}$$

The slopes and intercepts are given in Table V. The slope can be considered as the methylene group increment of $-\Delta H^0$. This increment increases in the order *n*-alkanol < *n*-alkanal DNPH < 2-*n*-alkanone DNPH, which agrees with the retention order. The intercepts represent the magnitude of $-\Delta H^0$ associated with the transfer of the functional group alone. The intercepts increase in the order 2-*n*-alkanone DNPH < *n*-alkanal DNPH < *n*-alkanol, indicating that the mobile phase prefers a hydroxyl group to the aldehyde DNPH group, and the latter to the ketone DNPH functional group.

It can be also seen in Table II that both the intercept and the slope change regularly with the carbon number for all the three compound classes, *i.e.*, two linear equations can be fitted to the data: for the intercepts

$$\log k'_T = d + e\mathrm{C}_n \tag{5}$$

and for the slopes

$$b = f + g\mathrm{C}_n \tag{6}$$

The parameters of eqns. 5 and 6 are given in Table V. Eqns. 5 and 6 combined with eqn. 2 result in a relationship that describes retention as a function of both temperature and carbon number:

$$\log k' = d + e\mathrm{C}_n + \frac{f}{T} + g \cdot \frac{\mathrm{C}_n}{T} \tag{7}$$

After substitution, these equations describing the capacity factors are as follows:

(a) for the *n*-alkanols:

$$\log k' = -1.09 - 0.128\ \mathrm{C}_n - \frac{22}{T} + \frac{96.5}{T} \cdot \mathrm{C}_n \tag{8}$$

(b) for the *n*-aldehyde DNPHs:

$$\log k' = -1.52 - 0.134\ \mathrm{C}_n + \frac{359}{T} + \frac{92}{T} \cdot \mathrm{C}_n \tag{9}$$

(c) for the 2-*n*-alkanone DNPHs:

$$\log k' = -1.52 - 0.124\ \mathrm{C}_n + \frac{370}{T} + \frac{88}{T} \cdot \mathrm{C}_n \tag{10}$$

TABLE IV

PARAMETERS OF LOG $k' = \mathrm{LOG}\, k'_T + b/T$ RELATIONSHIPS FOR THE THREE COMPOUND CLASSES WITH VARIOUS CARBON NUMBERS

Carbon number, C_n	*n-Alkanols*				*n-Alkanal DNPHs*				*2-n-Alkanone DNPHs*			
	Intercept	*Slope*	r^2	$-\Delta H^\circ$ *(kcal/mole)*	*Intercept*	*Slope*	r^2	$-\Delta H^0$ *(kcal/mole)*	*Intercept*	*Slope*	r^2	$-\Delta H^0$ *(kcal/mole)*
6	−1.9707	586	0.9855	2.68	−2.3288	912	0.9983	4.17	−2.2886	908	0.9983	4.15
7	−1.9215	632	0.9930	2.89	−2.4398	994	0.9984	4.54	−2.3735	981	0.9989	4.49
8	−2.1563	763	0.9978	3.49	−2.5542	1079	0.9986	4.93	−2.4763	1062	0.9992	4.85
9	−2.1373	813	0.9973	3.72	−2.8473	1224	0.9858	5.59	—	—	—	—
10	−2.3951	952	0.9981	4.35	−2.7910	1254	0.9923	5.73	—	—	—	—
11	−2.4834	1033	0.9985	4.72	—	—	—	—	−2.8939	1343	0.9996	6.14
12	−2.5477	1106	0.9963	5.06	−3.1342	1461	0.9993	6.68	—	—	—	—
13	−2.8491	1258	0.9965	5.75	—	—	—	—	—	—	—	—
14	−2.9042	1332	0.9975	6.09	—	—	—	—	—	—	—	—
15	−3.1261	1460	0.9970	6.67	—	—	—	—	—	—	—	—
16	−3.7030	1497	0.9972	6.84	—	—	—	—	—	—	—	—

TABLE V

PARAMETERS OF THE LOG k' *vs.* C_n, $1/T$ AND THE $-\Delta H^0$ *vs.* C_n FUNCTIONS FOR THE THREE COMPOUND CLASSES

Equation No.	*Dependent variable*	*n-Alkanols*			*n-Alkanal DNPHs*			*2-n-Alkanone DNPHs*		
		Intercept	*Slope*	r^2	*Intercept*	*Slope*	r^2	*Intercept*	*Slope*	r^2
4	$-\Delta H^0$	0.10	−0.441	0.9941	−1.64	0.419	0.9886	−1.69	0.402	0.9974
5	Log k'_T	−1.0920	−0.1285	0.9662	−1.5168	0.1345	0.9520	−1.5189	−0.1236	0.9902
6	b	−22	96.5	0.9941	359	91.7	0.9886	370	88	0.9973

Almost identical coefficients are obtained when the intercepts and slopes in Table III are fitted as functions of $1/T$.

Eqns. 8–10 can be used to determine the selectivity of the separation of two successive members of a homologous series, the so-called methylene group selectivity, $\alpha_{n+1/n}$. The expressions are as follows:

(a) for the *n*-alkanols:

$$\log \alpha_{n+1/n} = -0.128 + \frac{96}{T} \tag{11}$$

(b) for the *n*-alkanal DNPHs:

$$\log \alpha_{n+1/n} = -0.134 + \frac{92}{T} \tag{12}$$

(c) for the 2-*n*-alkanone DNPHs:

$$\log \alpha_{n+1/n} = -0.124 + \frac{88}{T} \tag{13}$$

The methylene group selectivities are generally similar. However, there is a subtle trend: the *n*-alkanols show the highest group selectivity, followed by the *n*-alkanal DNPHs and the 2-*n*-alkanone DNPHs. This order is opposite to the retention order. It seems that the lower the retention at a given carbon number (the more hydrophilic is the molecule), the higher is the effect of the addition of a methylene group on the retention.

Melander *et al.*[4] applied the principle of enthalpy–entropy compensation to the retention data observed in reversed-phase systems to test the identity of the retention mechanisms underlying the separations effected on different columns with different eluents. They derived the equation

$$\log k'_T = -\frac{\Delta H^0}{2.3R}\left(\frac{1}{T} - \frac{1}{\beta}\right) - \frac{\Delta G_B}{2.3R\beta} + \log \varphi \tag{14}$$

where

k'_T is the capacity factor of the sample component at temperature T;

β is the compensation temperature;

ΔH^0 is the standard enthalpy of transfer of the sample component from the mobile to the stationary phase;

ΔS^0 is the associated change in standard entropy;

ΔG_B is the change of Gibbs' free energy; and

φ is the phase ratio of the column.

If a linear relationship can be observed between log k' determined at the harmonic average temperature, T_{harm}, or close to the harmonic average temperature, T_{eval} and the $-\Delta H^0$ values determined as described previously, then the entropy–enthalpy compensation is indeed operative and the compensation temperature, β, can be evaluated from the slope of the log k' *vs.* $-\Delta H^0$ relationship:

$$\log k' = \log k'_0 + \frac{1}{2.3R}\left(\frac{1}{T_{\text{eval}}} - \frac{1}{\beta}\right)(-\Delta H^0) \tag{15}$$

The harmonic average temperature, T_{harm} and T_{eval}, the actual evaluation temperature at which log *k'* values were taken, the parameters of eqn. 15, the intercept expressed as ln k'_0 (to allow for direct comparison with the data in ref. 4) and the compensation temperature, β, are given in Table VI for the three compound classes. The extreme limits of ln k'_0 and β determined by Melander *et al.*[4] are also given.

TABLE VI

COMPENSATION TEMPERATURE AND RELATED DATA FOR THE THREE COMPOUND CLASSES

Compound type	T_{harm} *(°K)*	T_{eval} *(°K)*	*Log k' vs. −ΔH° relationships*			*ln* k'_0	*Compensation temperature,* β *(°K)*
			Intercept	*Slope*	r^2		
n-Alkanols	316	318	−1.11	0.3932372	0.9936	−2.56	743
n-Alkanal DNPHs	317	317	−0.963	0.3674046	0.9844	−2.22	676
2-*n*–Alkanone DNPHs	317	317	−0.984	0.3822803	0.9986	−2.27	709
Melander *et al.*[4]	—	313	—	—	0.965	−2.97	647
						−2.17	596
						−3.13	639

The high r^2 values indicate that the compensation mechanism is indeed operative in each instance. Both the intercepts and the compensation temperatures are within the ranges determined by Melander *et al.*[4]. This means that although the sample components, the eluents and the stationary phases were different (octadecylsilicas with both bulk and brush-type bonded phases) in the two studies the mechanism underlying the separations was identical.

CONCLUSIONS

The data set obtained from studies on the reversed-phase retention behaviour of *n*-alkanols and *n*-alkanal and 2-*n*-alkanone DNPHs at different temperatures was analysed. The separations were carried out on octadecylsilica columns with both bulk and brush-type bonded phases, using aqueous methanol solutions as eluents. In all instances linear relationships were found between log *k'* and C_n, log *k'* and $1/T$, $-\Delta H^0$ and C_n and log *k'* and $-\Delta H^0$. Equations describing log *k'* as function of both temperature and carbon number were obtained. Entropy–enthalpy compensation behaviour could be observed with all three compound classes. The compensation temperatures are similar to those obtained by Melander *et al.* for other octadecylsilica packings[4] with buffered aqueous acetonitrile eluents and very polar sample components, indicating that the underlying separation mechanisms are identical.

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NOTE

Since the completion of this manuscript, another paper dealing with enthalphy–entropy compensation was published by Melander *et al.*[8]. It was concluded that there is a linear relationship between the log k' and both the eluent composition and the reciprocal of the absolute temperature (eqn. 12a and Table I in ref. 8). For *n*-alkylbenzenes in an acetonitrile–water/Partisil ODS-2 system linear relationships were observed between the parameters of eqn. 12a in ref. 8 and the alkyl carbon number. By selecting a constant eluent composition, φ, as in this paper, and substituting for A_1, A_2 and A_3, the parameters of eqn. 12a in ref. 8, the observed linear relationship, one arrives at an equation equivalent to eqn. 7 in this paper. This also means that eqn. 12a (or 12b) in ref. 8 can be extended to include the carbon number as a third variable, resulting in an equation containing all of the primary parameters that influence the magnitude of retention in RP chromatographic systems. Although it would be instructive, one cannot check our data against eqn. 7 in ref. 8 because the dependence of k' on the solvent composition is not reflected by these data.

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