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Influence of temperature on retention and selectivity in reversed phase liquid chromatography

David Bolliet and Colin F. Poole*

Department of Chemistry, Wayne State University, Detroit, MI 48202, USA.
E-mail: cfp@chem.wayne.edu

The influence of temperature on retention and selectivity in reversed-phase liquid chromatography using a porous organic polymer stationary phase is quantitatively characterized using the solvation parameter model. The predominant influence of increasing temperature (20–60 °C) is to decrease the relative difference in the ease of cavity formation between the aqueous mobile phase and solvated sorbent and to decrease the hydrogen-bond acidity of the aqueous mobile phase, with other polar interactions affected to a lesser extent. Temperature variation and composition variation produce similar trends in retention, but within the easily accessible range for both variables, the capacity to change retention is much greater for composition variation than temperature variation. Temperature variation is a useful parameter for fine tuning an isocratic separation with an outcome that is easily predicted using the solvation parameter model.

Keywords: *Reversed-phase liquid chromatography; solvation parameter model; temperature; method development; styrene–divinylbenzene macroreticular porous polymer*

Temperature could be considered the overlooked optimization parameter in reversed-phase liquid chromatography.[1,2] Most analysts realize the desirability of column thermostating to improve the reproducibility of retention data, although even this is often neglected, and separations are performed at ambient conditions without further definition of those conditions. The use of elevated temperatures to increase sample solubility, improve column efficiency, reduce the column pressure drop, and to reduce separation times is also widely known, and commonly employed in some areas of chromatography, such as size exclusion and ion exchange. Sub-ambient temperatures have been less commonly used to improve the resolution of difficult to separate compounds by increasing band spacing at the expense of poor column efficiency and long separation times. The possibility of employing temperature variations to optimize selectivity in reversed-phase liquid chromatography has received less attention. In nearly all cases, isocratic retention as a function of temperature has been described by the van't Hoff relationship[1–6]

$$\log k = A + (B/T) \qquad (1)$$

where k is the retention factor, T the column temperature (in K), A a function of the standard state entropy of retention and the phase ratio, and B is proportional to the standard state enthalpy of retention. Exceptions to the linear relationship predicted by eqn. (1) are known for systems involving simultaneous contributions from more than one retention mechanism, changes in solute conformation that influence binding, and for solutes that can exist in several chemical states, in which the different forms have different retention characteristics. These problems are more common for biopolymers than low molecular weight compounds that most often conform to model expectations for the relatively narrow temperature ranges employed in liquid chromatography. The slope of plots of the standard state enthalpy against the standard state entropy (referred to as the compensation temperature) has been used to compare the similarity of the retention mechanism for a solute in different chromatographic systems.[7,8]

From a general interpretive point of view the main problem with the van't Hoff approach is that the A and B coefficients vary for each solute and set of experimental conditions. Any fundamental interpretation of retention differences presupposes that the molecular interactions involved can be deduced from compound structures, which is doubtful in the absence of a quantitative model, given that individual solutes are capable of several simultaneous interactions, of which the overall contribution to retention depends on the availability of complementary properties for the chromatographic system. For example, the ability of a solute to function as a hydrogen-bond base is irrelevant in a chromatographic system in which the stationary and mobile phases have similar hydrogen-bond acidity. To gain a quantitative understanding of the influence of temperature on retention, and therefore its use as a general parameter for optimization of separations, a new approach is required, that relates changes in the contribution of defined intermolecular interactions to retention, to changes in temperature, independent of solute identity.

The solvation parameter model has been applied to solvent selection, sorbent characterization, and retention modeling in reversed-phase liquid chromatography using silica-based, alkanesiloxane-bonded,[9–16] cyanopropylsiloxane-bonded,[17–19] spacer-bonded propanediol,[20] and polymer encapsulated[21,22] (including zirconia) sorbents, porous graphitic carbon[23,24] and macroreticular porous polymers.[25,26] The model is set out below in a form suitable for use in reversed-phase liquid chromatography

$$\log k = c + mV_X + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^0 \qquad (2)$$

where k is the experimentally observed retention property (the retention factor), V_X is the solute's characteristic volume (in cm^3/100 mol), R_2 its excess molar refraction (in cm^3/10), π_2^H the ability of the solute to stabilize a neighboring dipole by virtue of its capacity for orientation and induction interactions, and $\Sigma\alpha_2^H$ and $\Sigma\beta_2^0$ are parameters characterizing the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively. The characteristic volume and excess molar refraction are usually available by simple arithmetic calculations; the other solute descriptors are parameters derived from equilibrium measurements for complexation and distribution processes, with values available for over 2000 compounds. [27] The system constants in eqn. (2) are defined by their complementary interactions with the solute descriptors. The r constant determines the difference in ability of the solvated sorbent and mobile phase to interact with solute n- or π-electrons; the s constant to the difference in ability of the solvated sorbent and mobile phase to take part in dipole–dipole and dipole-induced dipole interactions; the a constant is a measure of the difference in hydrogen-bond basicity of the solvated sorbent and mobile phase; the b constant is a measure of the difference in hydrogen-bond acidity of the solvated sorbent and mobile phase; and the m constant is a measure of the relative ease of forming a cavity for the solute in the solvated sorbent and mobile phase. The system constants are determined by multiple linear regression analysis from experimental values for the observed parameter, log *k*, for a group of varied solutes with known descriptors sufficiently varied to define all interactions in eqn. (2), and of sufficient number to establish the statistical validity of the model.

The solvation parameter model has not been applied previously to studies of the temperature variation of retention in reversed-phase liquid chromatography, but was successful in explaining variation in stationary phase retention with temperature in gas-liquid chromatography. [28–31]

Experimental

All solvents and water were of OmniSolv grade from EM Science (Gibbstown, NJ, USA). Other chemicals were of reagent grade or better and obtained from several sources. The macroreticular porous polymer beads, PLRP-S 100 and PLRP-S 300, average particle diameter 50-70 μm, were obtained from Polymer Laboratories (Church Stretton, Shropshire, UK). Columns for HPLC of 10 or 3.5 cm × 4.6 mm id were prepared from the sorbent using the tap and fill (dry packing) procedure.[1] After filling, the columns were consolidated and freed from air by pumping methanol through them until the pressure stabilized and no further gas bubbles were observed at the detector outlet.

For HPLC a Varian 5000 pump module (Walnut Creek, CA, USA), a Valco 10 μl rotary injection valve (Houston, TX, USA), a Haake Instruments variable temperature circulating water-bath (Paramus, NJ, USA), a Kratos Spectroflow 773 UV–visible absorption detector (Westwood, NJ, USA), and a Hewlett-Packard 3396A integrator (Wilmington, DE, USA) were used. The injection valve was mounted on the top cover of the water-bath in an insulated box and connected to the pump module by several metres of coiled stainless-steel capillary tubing immersed in the water-bath. The column was also completely immersed in the water-bath. A 10 atm back pressure regulator (Valco) was installed on the outlet line from the detector flow cell.

Multiple linear regression analysis and statistical tests were performed on an Epson Apex computer (Torrence, CA, USA) using the program SPSS/PC+ (SPSS, Chicago, IL, USA). The solute descriptors for the solvation parameter model were taken from several sources and are summarized in Table 1.[18,20,26,27] Cross-correlation among the solute descriptors is negligible.

Results and discussion

For the solutes identified in Table 1 the influence of temperature (range 20–60 °C) on retention and selectivity was studied for three binary solvents of approximately equal solvent strength but different selectivity. For 60% v/v methanol in water the experimental retention factors changed by a factor of 2–3 over the temperature range studied; for 37% v/v propan-2-ol in water the change in retention factors was by a factor of 2–4; and for 49% v/v acetonitrile in water the change in retention factors was much smaller at less than a factor of 2. Plots of log *k* as a function of temperature (°C) were linear with a correlation coefficient greater than 0.99. The variation in slope for individual solutes is not great: 0.007–0.021 for 60% v/v methanol in water; 0.006–0.018 for 37% v/v propan-2-ol in water; and 0.002–0.006 for 49% v/v acetonitrile in water, with the majority of compounds clustered around the mean values. However, within the temperature range studied peak crossovers are occasionally observed, and in these cases, the ease of prediction from the linear plots provides a simple means of temperature optimization for some separations. It is not as simple to relate the slope of the plot to solute structure so that chemical intuition could be used to predict those compounds with the greatest susceptibility to change in retention with variation of temperature. For example, the chemically diverse compounds cyclohexanone, benzyl alcohol, acetanilide, pentan-2-one, 2-phenylethanol, and 4-methylpentan-2-one have virtually identical slopes in the solvent system 49% v/v acetonitrile in water. To understand better the physical processes involved in controlling retention as a function of temperature a detailed solvation model for the retention process, in our case represented by the solvation parameter model, is required. For perspective, changes in solvent composition over the range 5–50% (v/v) for the three binary solvents at a constant temperature of 20 °C yield changes in the retention factor of about 30–100-fold, indicating that changes in composition are more powerful for optimizing retention than usual changes in temperature.[26]

To explain the changes in retention observed in the above studies the solvation parameter model was fit to the various data sets and the results are summarized in Table 2. The statistics for the model fits are reasonable and the system constants make chemical sense. The general trends of ease of cavity formation (*m* constant) and lone pair electron attraction (*r* constant) promoting retention by the stationary phase and polar interactions of a dipole-type (*s* constant), and hydrogen bonding (*a* and *b* constant) favoring the mobile phase are consistent with expectations for reversed-phase liquid chromatography. The general behavior of the three binary mobile phases with respect to variation of temperature is similar, Table 2, hence, for the purpose of illustration we will consider only the data for 37% v/v propan-2-ol in water, Fig. 1. Increasing temperature has the most significant effect on the *m* and *b* system constants with the change in the *r*, *s*, and *a* system constants being small but statistically significant. Increasing temperature decreases the ability of the stationary phase to compete for lone pair electron and dipole-type interactions and decreases the ability of the

Table 1 Solute descriptors used in the solvation parameter model

	Solute descriptor				
Compound	V_X	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^0$
Benzene	0.716	0.610	0.52	0	0.14
Benzaldehyde	0.873	0.820	1.00	0	0.39
Acetophenone	1.014	0.818	1.01	0	0.48
Benzonitrile	0.871	0.742	1.11	0	0.33
Nitrobenzene	0.891	0.871	1.11	0	0.28
Phenol	0.775	0.805	0.89	0.60	0.31
3-Methylphenol	0.916	0.822	0.88	0.57	0.34
4-Methylphenol	0.916	0.820	0.87	0.57	0.32
2,6-Dimethylphenol	1.057	0.860	0.79	0.39	0.39
2-Chlorophenol	0.898	0.853	0.88	0.32	0.31
4-Chlorophenol	0.897	0.915	1.08	0.67	0.20
3-Bromophenol	0.950	1.080	1.17	0.67	0.20
2-Nitrophenol	0.949	1.015	1.05	0.05	0.37
4-Nitrophenol	0.949	1.070	1.72	0.82	0.26
4-Phenylphenol	1.383	1.560	1.41	0.59	0.45
Benzyl alcohol	0.916	0.803	0.87	0.33	0.56
4-Nitrobenzyl alcohol	1.090	1.064	1.39	0.44	0.62
2-Phenylethanol	1.057	0.811	0.91	0.30	0.65
Acetanilide	1.113	0.870	1.40	0.50	0.67
Benzamide	0.972	0.990	1.50	0.49	0.67
Pyridine	0.675	0.631	0.84	0	0.52
Cyclohexanone	0.861	0.403	0.86	0	0.56
Pentan-2-one	0.829	0.143	0.68	0	0.51
Hexan-2-one	0.968	0.136	0.68	0	0.51
4-Methyl-pentan-2-one	0.968	0.111	0.65	0	0.51
Nitromethane	0.424	0.313	0.95	0.06	0.31
1-Nitropropane	0.706	0.242	0.95	0	0.31
1-Nitrobutane	0.846	0.227	0.95	0	0.29
Dibromomethane	0.600	0.714	0.67	0.10	0.10

mobile phase to compete as a hydrogen-bond base. The main contributing factor for retention by the stationary phase is the relative ease of cavity formation, which becomes less favorable with increasing temperature. The *m* constant is determined by properties of both the solvated stationary phase and the mobile phase, but it seems likely that the main contribution to the system constant in this case is the reduction in cohesion of the mobile phase at higher temperatures, making cavity formation easier than at lower temperatures. For the *b* constant, increasing temperature either makes the stationary phase more effective as a hydrogen-bond acid or the mobile phase a weaker hydrogen-bond acid. The latter option would seem to be the more logical. In deciding which solutes will show the greatest range of retention difference with variation in temperature the most important solute characteristics will be size differences and solute hydrogen-bond basicity. Contrarily, the two trends oppose each other.

The general trends in the system constants as a function of temperature are independent of the solvent strength. Table 3 summarizes the variation in the system constants with temperature for 30% v/v acetonitrile in water for PLRP-S 100 and 1% v/v acetonitrile in water for PLRP-S 300. The results for 1% v/v acetonitrile in water (Fig. 2) are interesting, since in this case, the mobile phase contribution to retention and selectivity must be dominated by the properties of water, and the observed trends are entirely consistent with the results obtained for the binary mobile phases containing a significant volume fraction of organic solvent. This is a clear indication of the primacy of the characteristic properties of water, its high cohesive energy and hydrogen-bond acidity, on the general retention mechanism in reversed-phase liquid chromatography.

Variation of temperature is not as powerful an optimization strategy as variation in mobile phase composition. Fig. 3 shows the changes in the system constants for acetonitrile–water mixtures containing from 1 to 50% v/v acetonitrile at 20 °C. The general trends observed for variation of composition are similar to those for variation of temperature, but the magnitude of the changes in the system constants is much larger for composition.

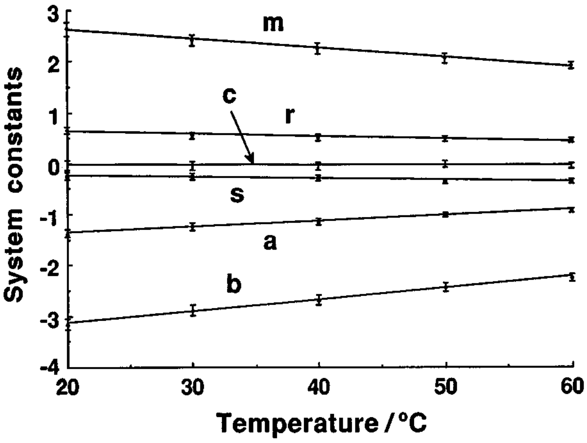

Fig. 1 Variation of the system constants with temperature for 37% v/v propan-2-ol in water on PLRP-S 100.

Table 2 System constants for binary mobile phases as a function of temperature on the porous organic polymer PLRP-S 100

Temperature/ °C	System constants						Statistics*			
	m	*r*	*s*	*a*	*b*	*c*	*R*	SE	*F*	*n*
60% *v/v methanol in water*:										
20	2.57	0.59	−0.09	−1.28	−2.61	−0.30	0.983	0.085	106	24
	(0.16)	(0.09)	(0.09)	(0.09)	(0.15)	(0.12)				
30	2.50	0.53	−0.09	−1.22	−2.43	−0.38	0.982	0.083	98	24
	(0.16)	(0.08)	(0.09)	(0.09)	(0.15)	(0.12)				
40	2.45	0.50	−0.21	−1.19	−2.42	−0.32	0.986	0.076	129	25
	(0.14)	(0.08)	(0.08)	(0.08)	0.12)	(0.11)				
50	2.26	0.46	−0.24	−1.08	−2.22	−0.30	0.986	0.069	134	25
	(0.13)	(0.07)	(0.07)	(0.08)	(0.11)	(0.10)				
60	2.12	0.45	−0.26	−1.04	−2.09	−0.34	0.990	0.058	169	24
	(0.11)	(0.06)	(0.06)	(0.06)	(0.09)	(0.08)				
37% *v/v propan-2-ol in water*:										
20	2.63	0.65	−0.25	−1.37	−3.16	−0.03	0.992	0.080	281	30
	(0.13)	(0.07)	(0.08)	(0.08)	(0.11)	(0.09)				
30	2.41	0.55	−0.26	−1.24	−2.88	−0.04	0.992	0.070	300	30
	(0.11)	(0.07)	(0.07)	(0.07)	(0.10)	(0.08)				
40	2.24	0.51	−0.29	−1.14	−2.68	−0.06	0.993	0.061	347	30
	(0.10)	(0.06)	(0.06)	(0.06)	(0.09)	(0.07)				
50	2.04	0.48	−0.36	−1.02	−2.44	−0.03	0.994	0.054	366	29
	(0.09)	(0.05)	(0.05)	(0.05)	(0.08)	(0.06)				
60	1.90	0.44	−0.36	−0.94	−2.25	−0.06	0.995	0.047	425	29
	(0.07)	(0.04)	(0.05)	(0.04)	(0.07)	(0.05)				
49% *v/v acetonitrile in water*:										
20	1.60	0.20	−0.13	−0.87	−2.07	−0.08	0.989	0.053	196	28
	(0.07)	(0.05)	(0.05)	(0.05)	(0.08)	(0.06)				
30	1.58	0.20	−0.16	−0.85	−2.00	−0.12	0.989	0.052	193	28
	(0.07)	(0.05)	(0.05)	(0.05)	(0.08)	(0.06)				
40	1.53	0.20	−0.16	−0.82	−1.80	−0.20	0.986	0.059	160	29
	(0.08)	(0.05)	(0.06)	(0.06)	(0.08)	(0.07)				
50	1.49	0.19	−0.18	−0.80	−1.74	−0.22	0.988	0.053	183	29
	(0.07)	(0.05)	(0.05)	(0.05)	(0.07)	(0.06)				
60	1.47	0.17	−0.20	−0.77	−1.65	−0.24	0.988	0.050	196	29
	(0.07)	(0.04)	(0.05)	(0.05)	(0.07)	(0.06)				

* *R* = overall correlation coefficient, SE = standard error in the estimate, *F* = *F*-statistic, *n* = number of solutes, and the numbers in parentheses represent the standard error in the coefficient.

Table 3 System constants for acetonitrile–water mobile phases as a function of temperature on the porous organic polymers PLRP-S 100 and PLRP-S 300

Temperature/	System constants						Statistics*			
°C	*m*	*r*	*s*	*a*	*b*	*c*	*R*	SE	*F*	*n*
30% *v/v acetonitrile in water on PLRP-S* 100:										
20	2.43	0.31	−0.18	−1.00	−2.90	0	0.991	0.063	223	27
	(0.09)	(0.06)	(0.07)	(0.06)	(0.11)	(0.08)				
30	2.37	0.29	−0.20	−0.98	−2.73	−0.02	0.992	0.057	251	27
	(0.08)	(0.05)	(0.06)	(0.06)	(0.10)	(0.07)				
40	2.35	0.27	−0.22	−0.96	−2.61	−0.08	0.992	0.056	245	27
	(0.08)	(0.05)	(0.06)	(0.05)	0.10)	(0.07)				
50	2.31	0.25	−0.27	−0.94	−2.51	−0.08	0.992	0.056	278	28
	(0.08)	(0.05)	(0.06)	(0.05)	(0.09)	(0.07)				
60	2.27	0.21	−0.28	−0.90	−2.39	−0.13	0.992	0.055	295	29
	(0.08)	(0.05)	(0.06)	(0.05)	(0.08)	(0.06)				
1% *v/v acetonitrile in water on PLRP-S* 300:										
20	5.17	0.72	−0.52	−1.33	−3.96	−0.15	0.989	0.107	130	21
	(0.23)	(0.15)	(0.12)	(0.22)	(0.21)	(0.17)				
30	5.10	0.63	−0.46	−1.27	−3.75	−0.31	0.991	0.094	156	21
	(0.20)	(0.13)	(0.10)	(0.19)	(0.19)	(0.15)				
40	4.98	0.66	−0.63	−1.31	−3.56	−0.24	0.990	0.093	155	21
	(0.20)	(0.13)	(0.10)	(0.19)	(0.19)	(0.14)				
50	4.73	0.60	−0.60	−1.26	−3.33	−0.25	0.989	0.096	129	21
	(0.21)	(0.14)	(0.11)	(0.20)	(0.19)	(0.15)				
60	4.53	0.68	−0.56	−1.34	−3.27	−0.32	0.989	0.092	128	20
	(0.20)	(0.14)	(0.10)	(0.19)	(0.19)	(0.15)				

* *R* = Overall correlation coefficient, SE = standard error in the estimate, *F* = *F*-statistic, *n* = number of solutes, and the numbers in parentheses represent the standard error in the coefficient.

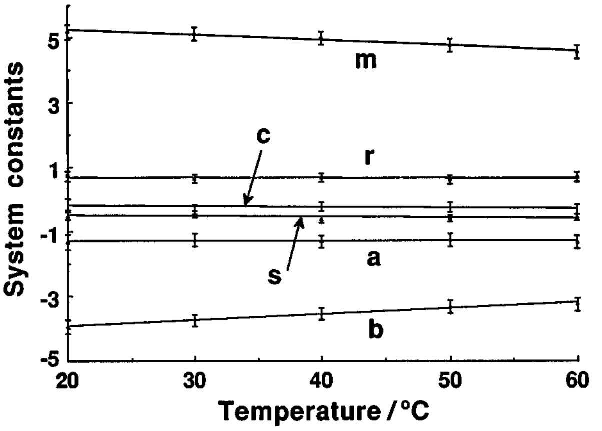

Fig. 2 Variation of the system constants with temperature for 1% v/v acetonitrile in water on PLRP-S 300.

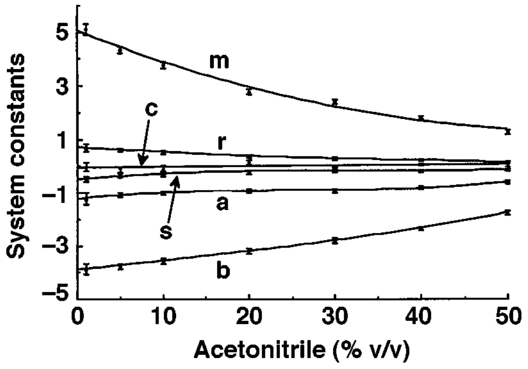

Fig. 3 Plot of the system constants as a function of mobile phase composition for PLRP-S 100 and acetonitrile–water mixtures at 20 °C.

Changes in the *r*, *s*, and *a* coefficients are small but statistically significant for variation in composition, while the *m* and *b* system constants change over a wide range (*m* from 5.11 to 1.26 and *b* from −3.85 to −1.75 for the composition range shown in Fig. 3). The change in system constants for a variation in temperature from 20 to 60 °C is generally about an order of magnitude smaller. Both the *m* and *b* system constants are numerically larger relative to the other system constants at low levels of organic solvent, and given that temperature variation has a greater influence on the values of the *m* and *b* system contants than the other system constants, variation in temperature can be expected to have a greater influence on retention and selectivity with mobile phases that contain low amounts of organic solvent. It has been shown elsewhere[32] that composition variation and temperature variation are uncorrelated, suggesting that simultaneous optimization of composition and temperature is the most effective strategy for difficult separations, a conclusion that we entirely agree with.

Conclusions

The solvation parameter model provides a quantitative interpretation of the influence of temperature on the retention properties of a porous polymer sorbent under reversed-phase chromatographic conditions in terms of changes in the capacity of the chromatographic system for fundamental intermolecular interactions which would not be possible by other means. The predominant influence of higher temperatures is to reduce retention by a reduction in the difference in cohesive energy between the mobile and stationary phases and to decrease the hydrogen-bond acidity of the mobile phase relative to the stationary phase. Changes in other polar interactions are less significant. Temperature variation in reversed-phase liquid chromatography, therefore, will have the largest effect on the band spacing of compounds that differ by size and hydrogen-bond basicity. The range of variation in retention caused by changes in temperature is small compared with those possible by composition variation, but still of value for fine tuning band spacing, and readily predictable using the solvation parameter model.

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