EFFECTS OF TEMPERATURE AND PERCENTAGE OF ORGANIC MODIFIER ON RETENTION AND SELECTIVITY IN RP-HPLC USING SOLVATION PARAMETER MODEL

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Abstract

Effects of temperature and percentage of organic modifier were studied on retention and selectivity in RP-HPLC using solvation parameter model. The system constants were determined by multiple linear regression analysis from experimental values in the retention factor for a group of different solutes with known descriptors by computer using the program SPSS/PC. The experimental results showed that the variation of percentages of organic modifier (5-20% V/V CH_3CN) changes cavity formation (m = +3.55 to +2.53), dipole-dipole interactions (s = +0.62 to −0.64) and hydrogen-bond acidity (b = −2.41 to −3.27) terms. The variation of temperature (5-45°C) also changes cavity formation (m = +3.21 to +2.56) and dipole-dipole interactions (s = +0.34 to −0.22) terms.

Keywords: Reversed-phase liquid chromatography; Solvation parameter model

Introduction

Temperature could be considered the overlooked optimization parameter in RP-HPLC. 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 the conditions [1,2]. To gain a quantitative understanding of the influence of temperature on retention, and therefore its use as a general parameter for optimization of separation, 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, stationary phase characterization, and retention modeling in RP-HPLC using silica-based, alkanesiloxane-bonded [3-10], cyanopropylsiloxane-bonded [11-13], spacer-bonded propanediol [14], polymer encapsulated stationary phase [15,16], porous graphitic carbon [17,18] and macroreticular porous polymers [19-21].

The model set out below is a form suitable for use in RP-HPLC:

$$\log k' = c + mV_x + rR_2 + s\pi_2^H + a\Sigma a_2^H + b\Sigma \beta_2^H \quad (1)$$

where K′ is the solute retention factor and the solute descriptors are Mc Gowan's characteristic volume V_x (in $cm^3/100$ mol) and excess molar refraction R_2 (in $cm^3/10$), π_2^H is the ability of the solute to stabilize a

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neighboring dipole by virtue of its capacity for orientation and induction interactions, $\sum\alpha_2^H$ and $\sum\beta_2^H$ are the soulte's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively. The system constants in Equation (1) are defined by their complementary interactions with the solute descriptors. The constant r determines the difference in capacity of the stationary phase and mobile phase to interact with solute n- or π-electrons; the constant s is related to the difference in the capacity of the stationary phase and mobile phase to take part in dipole-dipole and dipole-induced dipole interactions; the constants a and b are measure of the hydrogen-bond basicity and hydrogen-bond acidity of the stationary phase and mobile phase, respectively; and the constant m is a measure of the relative ease of cavity formation and general dispersion interactions for the solute on the stationary phase and mobile phase. The system constants were determined by multiple linear regression analysis from experimental values of the retention factor for a group of different solutes with known descriptors by computer using the program SPSS/PC.

Experimental

Apparatus

The chromatographic analysis of the samples was performed using an isocratic system. the HPLC system consisted of the series 10 liquid chromatography pump model (Perkin Elmer, Norwalk, CT, USA) with model 7125 Manual Injector (Rheodyne Inc., Cotati, CA, USA) fitted with a 15 µl loop and model 440 absorbance detector (waters ASSO., Milford, MA, USA), AR 55 singel Pen Linear Recorder (Pye Unicam, Holland), LCT-100 laboratory computing integrator (Perkin Elmer, Norwalk, CT, USA), SRD Nuclesil 100-5C$_{18}$ (150 × 4.6 mm i.d., 5 µm particle size) and Jenway pH meter 3030 (Jenway, Itd, UK). The UV detector was set at 254 nm to monitor the compounds.

Reagents

Acetonitrile and Methanol were of HPLC grade and all other inorganic chemicals of analytical grade such as NaH$_2$PO$_4$.2H$_2$O and H$_3$PO$_4$ were obtained from Fluka Company (Fluka AG, Chemische Fabrik CH-9470 Buchs, Switzerland). The samples used were a mixture of different compounds summarized in Table 1.

Preparation of Standard Solution and Mobile Phases

Stock solutions of 1 mg/ml of samples were prepared

separately in methanol. Freshly prepared mobile phases were mixture of different percentages (5-20% V/V) of acetonitrile and doubly distilled de-ionized water. Before use, all mobile phases were filtered through a 0.45 µm millipore solvent filter and degassed. The column was thermostated using a glass water-Jacket.

Results and Discussion

The influence of temperature (5-45°C) and percentage of organic modifier (5-20% V/V CH$_3$CN) on retention and selectivity were studied for the solutes given in Table 1. The experimental retention factor, K′, changed by a factor of 2-3 for temperature changes (5-45°C) and 3-9 for variation of CH$_3$CN (5-20%) at 25°C.

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 Tables 2 and 3. 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.

Figure 1 shows variation of system constants with temperature at 15% V/V CH$_3$CN. Increasing temperature has the most significant effect on the m and s system constants with the change in the r, a, and b system constants being small but statistically significant. 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 temperature, making cavity formation easier than at lower temperatures. For the s constant, increasing temperature decreases the ability of the stationary phase to compete dipole-type interactions. Variation of temperatures is not as powerful as an optimization strategy variation in mobile phase composition.

Figure 2 shows the changes in the system constants for acetonitrile-water mixtures containing from 5 to 20% V/V acetonitrile at 25°C. The general trends observed for variation of composition are similar to those for variation of temperature, but magnitude of the changes in the system constants is much larger for composition. System constants change over a wide range (m from 3.55 to 2.54, s from 0.62 to −0.62 and b from −2.41 to −3.27) for the composition range shown

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Table 1. Solute descriptors used in the solvation parameter model [22]

Compounds	Solute Descriptors				
	V_x	R_2	π_2^H	$\sum\alpha_2^H$	$\sum\beta_2^H$
Benzoic acid	0.932	0.730	0.90	0.59	0.40
4-Hydroxy-benzoic acid	0.990	0.930	0.92	0.87	0.53
O-Toluic acid	1.073	0.730	0.90	0.60	0.43
P-Toluic acid	1.073	0.730	0.90	0.60	0.40
m-Toluic acid	1.073	0.73	0.90	0.59	0.38
3-Choloro-benzoic acid	1.054	0.840	0.95	0.65	0.30
4-Choloro-benzoic acid	1.054	0.840	0.99	0.63	0.26
3-Bromo-benzoic acid	1.107	1.000	1.04	0.65	0.27
4-Bromo-benzoic acid	1.107	1.000	1.07	0.63	0.26
Benzene	0.716	0.610	0.52	0	0.14
Benzaldehyde	0.873	0.820	0	0	0.39
Benzonitrile	0.871	0.742	1.11	0	0.33
Benzyl alcohol	0.916	0.803	0.87	0.33	0.56
Phenol	0.775	0.805	0.89	0.60	0.31
3-Methyl-Phenol	0.916	0.822	0.88	0.57	0.34
4-Methyl-Phenol	0.916	0.820	0.87	0.57	0.32
2-Choloro-Phenol	0.898	0.853	0.88	0.32	0.31
4-Choloro-Phenol	0.897	0.915	1.08	0.67	0.20
2-6-Dimethyl-Phenol	1.057	0.860	0.79	0.39	0.39
4-Nitro-Phenol	0.949	1.070	1.72	0.82	0.26

Table 2. System constants as a function of temperature for appropriate conditions (15% V/V CH_3CN and pH = 3)

Temperature (°C)	System Constants						Statistics[*]			
	c	m	r	s	a	b	R	SE	F	n
5	−0.47	3.21	−0.45	0.34	−0.87	−2.82	0.990	0.075	44	20
15	−0.45	2.94	−0.55	−0.15	−0.79	−3.24	0.992	0.069	54	20
20	−0.42	2.85	−0.60	−0.80	−0.93	−3.05	0.990	0.075	44	20
25	−0.44	2.88	−0.43	0.22	−0.97	−2.74	0.993	0.065	56	20
30	−0.40	2.69	−0.40	−0.82	−0.57	−3.47	0.981	0.110	19	20
35	−0.39	2.82	−0.51	−0.33	−0.89	−3.20	0.985	0.095	26	20
40	−0.41	2.71	−0.63	−0.19	−0.87	−2.94	0.988	0.077	39	20
45	−0.38	2.56	−0.42	−0.22	−0.89	−2.75	0.985	0.095	26	20

[*] R: Overall correlation coefficient, SE: Standard Error, F: F-Statistic, n: number of solutes.
Conditions: column 150×4.6 mm, 5 μm C_{18} column, Flow rate 1.2 ml/min, UV detector at λ=254 nm.
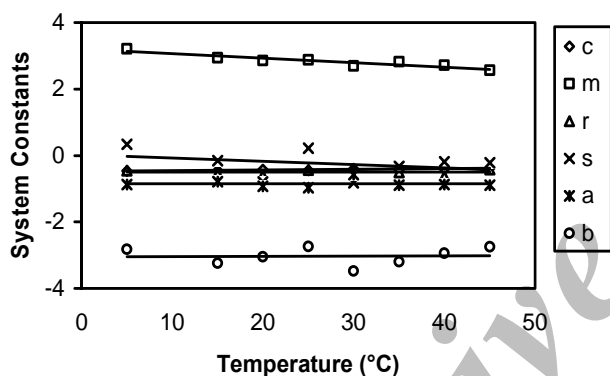
Table 3. System constants as a function of percentage of acetonitrile for appropriate conditions (pH=3 at 25°C)

| %CH₃CN	System Constants						Statistics			
	c	m	r	s	a	b	R	SE	F	n
5	−0.51	3.55	−0.20	0.62	−1.01	−2.41	0.985	0.095	26	20
10	−0.48	3.25	−0.05	0.30	−0.86	−3.00	0.981	0.101	17	20
15	−0.44	2.88	−0.43	0.22	−0.97	−2.74	0.993	0.065	56	20
20	−0.42	2.54	−0.31	−0.64	−0.89	−3.27	0.984	0.098	25	20

Conditions similar to recorded in Table 2.

in Figure 2. For the b constant, increasing percentage of organic modifier makes the mobile phase less effective as a hydrogen-bond acid. Both the m and s 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 s system constants. However, variation in temperature can be expected to have a greater influence on retention and selectivity with mobile phase that contain low amounts of organic solvent.
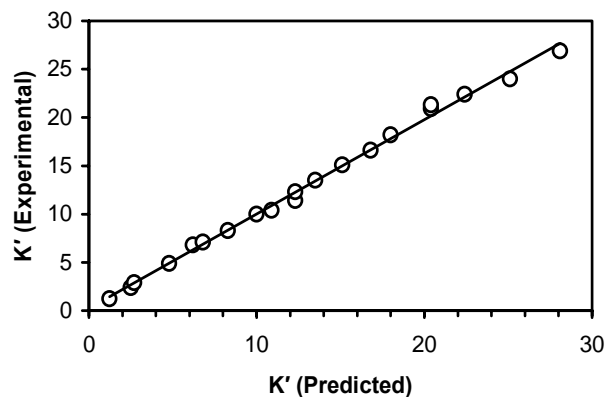
Figure 3 shows the predicted *vs.* experimental capacity factors of compounds using the solvation parameter model. The high correlation coefficient ($r = 0.995$) is an indication that solvation parameter model is a powerful strategy for prediction of experimental capacity factors of solutes with large K'.

Figure 3. Predicted *vs.* experimental retention factors of compounds using the solvation parameter model.

Figure 1. Plots of system constants as a function of temperature at appropriate conditions (15% V/V CH$_3$CN and pH=3).
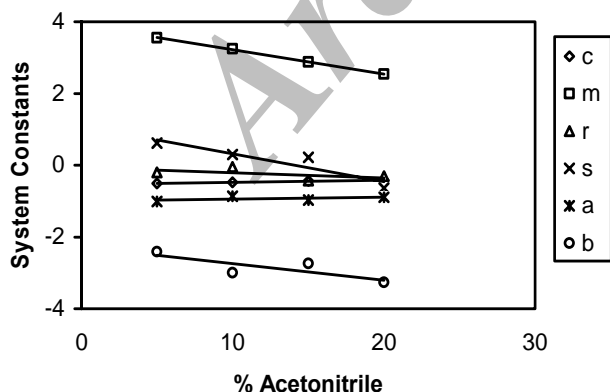
Figure 2. Plots of system constants as a function of percentage of acetonitrile at appropriate conditions (15% V/V CH$_3$CN at 25°C).

References

1. Poole C.F. and Poole S.K. *Chromatography Today.* Elsivier, Amsterdam (1991).
2. Snyder L.R., Kirkland J.J., and Glajch J.L. *Practical HPLC Method Development.* Wiley, New York (1997).
3. Miller K.G. and Poole C.F. *J. High Resolut. Chromatogr.*, **17**: 125 (1994).
4. Poole C.F., Poole S.K., Seibert D.S., and Chapman C.M. *J. Chromatogr. B*, **689**: 245 (1997).
5. Abraham M.H., Roses M., Poole C.F., and Pool S.K. *J. Phys. Org. Chem.*, **10**: 358 (1997).
6. Abraham M.H., Poole C.F., and Pool S.K. *J. Chromatogr. A*, **746**: 201 (1996).
7. Abraham M.H. and Roses M. *J. Phys. Org. Chem.*, **7**: 672 (1994).
8. Abraham M.H., Chadha H.S., and Leo A.J. *J. Chromatogr. A*, **685**: 203 (1994).
9. Tan L.C., Carr P.W., and Abraham M.H. *Ibid.*, **752**: 1 (1996).
10. Carr P.W., Tan L.C., and Park J.H. *Ibid.*, **724**: 1 (1996).
11. Seibert D.S. and Poole C.F. *J. High Resolut. Chromatogr.*, **18**: 226 (1995).
12. Seibert D.S. and Poole C.F. *Chromatographia*, **41**: 51 (1995).
13. Kiridena W. and Poole C.F. *Anal. Commun.*, **34**: 195 (1997).
14. Seibert D.S., Poole C.F., and Abraham M.H. *Analyst.*, **121**: 511 (1996).
15. Nasal A., Haber P., Kaliszan R., Forgacs E., Cserhati T., and Abraham M.H. *Chromatographia*, **43**: 484 (1996).
16. Li J. and Carr P.W. *Anal. Chim. Acta*, **334**: 239 (1996).
17. Jackson P.T., Schure M.R., Weber T.P., and Carr P.W. *Anal. Chem.*, **69**: 416 (1997).
18. Poole S.K. and Poole C.F. *Anal. Commun.*, **34**: 247 (1997).
19. Abraham M.H., Chadha H.S., Leitao R.A.E., Mitchell R.C., Lambert W.J., Kaliszan R., Nasal A., and Haber P. *J. Chromatogra. A*, **766**: 35 (1997).
20. Bolliet D. and Poole C.F. *Chromatographia*, **46**: 381 (1997).
21. Kiridena W. and Poole C.F. *Analyst.*, **123**: 1265 (1998).
22. Abraham M.H. *Chemical Society Reviews*, 73 (1993).

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