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MOBILE PHASE EFFECTS IN REVERSED-PHASE CHROMATOGRAPHY

VII. DEPENDENCE OF RETENTION ON MOBILE PHASE COMPOSITION AND COLUMN TEMPERATURE*

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SUMMARY

The relationship between logarithmic retention factor, κ, in reversed-phase chromatography and operating conditions including mobile phase composition and column temperature is still controversial. Earlier, the following analytical relationship was proposed for use with alkyl benzenes: $\kappa = A_1 \varphi(1-T_c/T) + A_2/T + A_3$ where φ is volume fraction organic co-solvent of the hydro-organic mobile phase, T is absolute temperature and A_1–A_3 are parameters appropriate to the eluite and stationary phase. Insofar as its use had not been tested with polar eluites that are retained by only one mechanism, its description of the retention of 54 polar and apolar eluites was examined and compared to equations with other, usually more complex, relationships between retention and operating conditions. The following four-parameter equation described best most data: $\kappa = A_1 \varphi(1-T_c/T) + A_2/T + A_3 + A_4\varphi$. But neither this nor any other four-parameter equation under examination here emerged solely as the best expression for the dependence of retention on the eluent composition and temperature. The average relative errors were 7.8% and 6% with use of the three- and the four-parameter equations which were found to be best for a given eluite, respectively. In view of the small decrease in error but significant increase in complexity and in data points with the use of the four-parameter equation, the three-parameter equation is recommended for general use.

INTRODUCTION

In order to exploit the full potential of reversed-phase chromatography methods are needed to predict quantitatively the effect of conditions such as mobile phase composition and column temperature on the retention factor. As differences in retention behavior of silica bonded *n*-alkyl stationary phases are believed to arise

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mainly from differences in their phase ratios[1], the parameters in such quantitative expressions for the retention factor would be constants peculiar to the mobile phase composition.

Early in the development of reversed-phase chromatography it was already recognized that the retention factor increases exponentially with the volume fraction of water in the eluent[2] under normal conditions and in the absence of specific effects. Although numerous investigations suggested that the relationships between logarithmic retention factor and composition is non-linear[3,4] the deviation from linearity is usually small under regular conditions in reversed-phase chromatography. Other studies[5] have shown that reversed-phase chromatography systems exhibit enthalpy–entropy compensation behavior. These findings were used to develop a simple three-parameter relationship to express the retention factor as a function of mobile phase composition and temperature[6]. The relationship has been successfully tested with aromatic hydrocarbons as eluites and has been found to be superior to competing thermodynamic models[7] in its treatment of both mobile phase composition and temperature on retention. Other attempts to establish a framework for predicting the dependence of retention on eluent composition[8–13] do not explicitly include the effect of temperature and therefore deal only with the dependence of the retention factor on the eluent composition.

The goal of this study is to expand the scope of our previous treatment to a variety of eluites containing polar functions and to express the effect of mobile phase composition and column temperature on retention in a general way. On the basis of extensive experimental data several relationships between logarithmic retention factor, mobile phase composition and column temperature are critically examined.

THEORY

We have previously shown[6] that the logarithmic retention factor, κ, in reversed-phase chromatography is related to the volume fraction of organic co-solvent in the hydro-organic mobile phase, φ, and the absolute temperature, T, by

$$\kappa = A_1\varphi(1-T_c/T) + A_2/T + A_3 \tag{1}$$

where T_c is the compensation temperature which is usually constant in reversed-phase chromatography. Eqn. 1 was derived by taking advantage of enthalpy–entropy compensation found to occur in reversed-phase chromatography[5] and assuming linear dependence of the logarithmic retention factor on the composition of binary hydro-organic eluents. Whereas the existence of enthalpy–entropy compensation and a common compensation temperature in reversed-phase chromatography has not been shown for both polar and apolar eluites, the validity of eqn. 1 has not been demonstrated for polar compounds.

In a series of papers Jinno and Kawasaki[8–11] have successfully implemented an algorithm for prediction of retention factors of a wide variety of eluites by a cubic equation with respect to the concentration of the strong solvent. Jandera *et al.*[13] have expressed the retention factor in binary mobile phase by the retention factors in the neat solvents[12] as well as the retention factor in ternary mobile phase by the retention factors in the binary mobile phases from which the ternary solvent can be prepared.

TABLE I

VALUES OF COMPENSATION TEMPERATURE, T_c, AND f(φ,T) SUBSTITUTED INTO EQN. 2 TO OBTAIN THE EQUATIONS WHICH ARE DENOTED BELOW AND WHICH WERE EXAMINED FOR RELATIONSHIP BETWEEN LOGARITHMIC RETENTION FACTOR AND MOBILE PHASE COMPOSITION AND TEMPERATURE

Equation number	$f(\varphi,T)$	T_c (°K)
1	0	625
2a	$(1-0.5\varphi)^{-1}$	625
2b	$(1-0.5\varphi)^{-2}$	625
2c	φ	625
2d	φ^2	625
2e	φ/T	625
2f	φ^2/T	625
2g	0	0
2h	0	Variable

these results, together with earlier data[3,4] suggest that the assumption of a linear relationship between mobile phase composition and logarithmic retention factor may not be generally applicable and urge re-examination of eqn. 1. In light of these results, we believe that the retention factor of eluites having a wide range of chemical structures could be represented by an expanded version of eqn. 1 given by

$$\kappa = A_1\,\varphi\,(1 - T_c/T) + A_2/T + A_3 + A_4 f\,(\varphi,T) \tag{2}$$

where A_4 is a constant peculiar to an eluite and f(φ,T) is a function of φ and T and may be appropriate either to members of an eluite class, *e.g.* anions, or to all eluites. As the function f(φ,T) may take various forms a family of equations arises from eqn. 2, each different in the expression of f(φ,T). Those of interest here are presented in a compact form by eqns. 2a–h in Table I.

The relationships given by eqns. 2a–h are semi-empirical in nature. The activities of ions and dipoles in the mobile phase are expected to be linear functions of the reciprocal dielectric constant and its square, respectively. Eqns. 2a and 2b express that dependence through the relationship between dielectric constant and mobile phase composition which has been found for acetonitrile–water mixtures.

Many empirical measures of polarity have been described for neat organic solvents. For many binary solvent mixtures except acetonitrile–water, the $E_T(30)$ scale[14,15] has been introduced to express the polarity as a function of composition[16]. Examination of the dependence of $E_T(30)$ values on the solvent composition has suggested the use of the functions in eqns. 2c–f as given in Table I with different organic co-solvents in hydro-organic mixture.

Eqns. 2g and 2h were used to examine the significance of the value 625°K for compensation temperature by allowing the compensation temperature to be zero and undefined respectively.

EXPERIMENTAL

Equipment

The liquid chromatograph was assembled from a Knauer (West Berlin, F.R.G.) Model FR-30 pump, a Rheodyne (Berkeley, CA, U.S.A.) Model 7010 sample injector with a 20-μl loop and a Kratos (Westwood, NJ, U.S.A.) Model 770 variable-wavelength UV detector. The optical density of the effluent was detected at 254 nm and chromatograms were obtained with a Heath-Zenith (Benton Harbor, MI, U.S.A.) dual pen recorder.

Columns

A 10-μm Partisil ODS-2 column (250 × 4.6 mm I.D.) was obtained from Whatman (Clifton, NJ, U.S.A.).

Materials

Acetonitrile was distilled in glass from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Distilled water was prepared with a Barnstead distilling unit. All the compounds used in this investigation were purchased from Chem Service (West Chester, PA, U.S.A.).

Conditions

Isocratic elution was carried out with various compositions of acetonitrile–water eluents and the column temperature was maintained by circulating water through a water jacket from Messgeraete Werk (Lauda, F.R.G.) Model K2R-D thermostatted bath. Retention factors were evaluated from the chromatograms in the usual way with t_0 evaluated as the first eigenpeak observed with injection of deuterated acetonitrile.

Data analysis

The coefficients of eqns. 1–2h were obtained from multiple linear regression analysis of retention factor–composition–temperature data of each eluite by use of the GLM package in SAS on IBM 370/158 computer at Yale Computer Center.

RESULTS AND DISCUSSION

The expressions examined here for the relationship between logarithmic retention factor mobile phase composition and column temperature are given in Table I. Eqn. 1 is identical to that previously reported to describe the retention of alkylbenzenes. Eqns. 2a and 2b express the dependence of reciprocal dielectric constant and its square on mobile phase composition. These would be expected to reflect the relationship between operating conditions and logarithmic retention factor of ions and dipoles, respectively, if mobile phase interactions dominate the elution. No universal relationship between mobile phase composition and dielectric constant is known to us. However, correlating data[17] published on acetonitrile–water mixtures[18] we have found that eqn. 3 adequately expresses the dependence of the dielectric constant of the mixture, ε, on the composition

$$\varepsilon = \varepsilon_0(1 - 0.5\varphi) \tag{3}$$

where ε_0 is the dielectric constant of neat water. Thus for classical ions and dipoles the functions $f(\varphi,T)$ appropriate to acetonitrile–water systems are given in Table I by eqns. 2a and 2b, respectively.

The effect of electrostatic interactions can also be taken into account by use of empirical relationships for the mobile phase polarity. A variety of polarity scales have been described, of which but a few are applicable to hydro-organic mixtures. According to a recent review[19] on probes for polarity of non-aqueous or hydro-organic solvents, the values of the different probes are highly correlated. The $E_T(30)$ scale, which is based on the shift in π–π^* adsorption of 2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridino)phenolate at 25°C (see refs. 14 and 15) with solvent change was regarded as the most comprehensive. Values of E_T are available for selected hydro-organic mixtures, *e.g.* those with methanol, ethanol, 2-propanol, 1,4-dioxane, pyridine, 2-methylpyridine, 2,6-dimethylpyridine and piperidine as organic co-solvent[16]. Inspection of the data suggests that the E_T-values decrease linearly with increase in organic co-solvent volume fraction in some systems but in others the relationship is more nearly quadratic. The literature does not explicitly state the temperature dependence of E_T. Therefore, the functions given by eqns. 2c–f were used to examine the temperature–eluent composition relationship.

Eqn. 1 and eqns. 2a–h were tested for all eluites by linear regression of logarithmic retention factor *vs.* appropriate functions of mobile phase composition and temperature. The mobile phase compositions were 0.3–0.9 volume fractions, acetonitrile in water at 298°K, 308°K, 323°K, and 338°K. In each analysis, the coefficients A_1–A_4, as well as the correlation coefficient, coefficient of variation and standard error of estimate were obtained. Insofar as the regression analysis was done on the logarithmic retention factor which is usually nearly zero in value, the coefficient of variation was frequently observed to be exceedingly large. Its value as an index of merit of the model was small in comparisons of the fits of different models among several eluites. Therefore, the correlation coefficients and standard error were used to compare the equations.

The mean value of absolute relative error, MARE, was also calculated for each model. this quantity is defined by eqn. 4

$$\mathrm{MARE} = 100/N \sum_{i=1}^{N} |k_{\mathrm{pred},i}/k_{\mathrm{obs},i} - 1| \qquad (4)$$

where $k_{\mathrm{pred},i}$ is the value of retention factor obtained by use of the appropriate equation and eluite parameters A_1–A_4) for eluite i and $k_{\mathrm{obs},i}$ is the experimentally observed value.

The equations which give optimal and penoptimal (second-best) fit for each eluite are indicated in Table II. The mean absolute relative error obtained for each equation as well as that for eqn. 1 are also given. In the last column is found T_c as calculated by use of eqn. 2h.

Modification of eqn. 1 by use of eqn. 2h in which the value of T_c is allowed to be unique for each eluite failed to produce a new value of T_c that is consistent for all eluites. Indeed no relationship between the magnitude of T_c determined in this way and the equation of optimal fit or chemical class of the eluite are evident from Table II. However, the use of eqn. 2g in which T_c is set equal to zero consistently produces lower quality of fit than does eqn. 1. Therefore, the use of values other than 625°K for T_c is not sustained by the results. Inspection of the results suggests that the mean relative error obtained with use of eqn. 1 is 7.8%.

TABLE II

THE EQUATIONS WHICH PROVIDED OPTIMAL AND PENOPTIMAL DESCRIPTION OF MOBILE PHASE AND TEMPERATURE EFFECTS FOR EACH ELUITE AND THE MEAN ABSOLUTE RELATIVE ERROR, MARE, OBTAINED WITH THEIR USE

In addition the MARE obtained with use of eqn. 1 and the value of T_c obtained by use of eqn. 2h is given.

Eluite	*Optimal eqn.*	*Penoptimal eqn.*	*MARE*			T_c (°K)
			Eqn. 1	*Optimal eqn.*	*Penoptimal eqn.*	
o-Nitrophenol	1	2d	6.1	6.1	6.1	−3398
p-Nitroaniline	1	2f	4.4	4.4	4.4	759
Iodobenzene	1	2f	10.3	10.3	11.0	1484
Phenol	2c	1	2.9	2.1	2.9	−4120
1-Chloronaphthalene	2c	1	6.9	5.7	6.9	− 625
tert.-Butylbenzene	2c	1	9.6	7.7	9.6	408
Ethylbenzene	2c	1	14.1	10.5	14.1	− 114
1-Methylbenzyl alcohol	2c	1	4.7	4.0	4.7	− 190
p-Cymene	2c	1	10.2	8.7	10.2	− 482
Durene	2c	1	4.1	2.4	4.1	− 685
Mesitylene	2c	1	10.6	8.0	10.6	− 58.7
Acenaphthene	2c	1	11.0	9.1	11.0	− 465
Fluoranthene	2c	1	10.1	6.7	10.1	− 225
p-Iodotoluene	2c	1	5.1	5.1	5.1	−1739
m-Chlorotoluene	2c	1	9.6	4.5	9.6	75.1
Phenanthrene	2c	1	13.0	11.4	13.0	− 396
Cumene	2c	1	9.6	7.2	9.6	− 263
Diphenylmethane	2c	1	6.1	5.2	6.1	13660
Toluene	2c	1	6.3	3.9	6.3	− 232
4-Chloro-2-nitrophenol	2c	2f	4.4	3.3	4.2	− 4.0
p-Dichlorobenzene	2c	2f	5.2	4.1	4.2	− 318
3-Phenyl-1-propanol	2c	2f	7.0	7.0	7.0	− 91.2
1′,1′-Dimethylbenzyl alcohol	2c	2f	6.3	3.6	6.2	16.5
1-Methylnaphthalene	2c	2f	11.6	9.8	10.9	− 134
Naphthalene	2c	2f	10.0	8.7	9.2	− 108
Biphenyl	2c	2f	12.8	10.7	10.9	− 223
1,3-Dimethylnaphthalene	2c	2f	10.8	8.8	9.5	− 626
1,2,4,-Trimethylbenzene	2c	2f	10.4	7.8	10.0	− 34.5
Mesitylene	2c	2f	10.7	8.0	10.4	58.7
Benzene	2c	2b	7.1	3.8	5.4	56.3
p-Terphenyl	2c	2b	8.0	5.0	7.2	− 24
Chlorobenzene	2c	2b	7.6	4.3	5.9	− 248
o-Xylene	2c	2b	9.2	6.7	9.1	− 13.1
m-Xylene	2c	2b	9.6	6.9	9.8	− 32.2
Benzyl alcohol	2c	2a,b	4.1	2.0	2.9	− 71
2-Phenethyl alcohol	2c	2a,b	6.6	4.3	6.4	− 130
Pyrene	2c	2a,b	8.1	6.5	8.1	− 410
Diethylbenzene	2d	2f	7.8	6.4	6.6	1131
o-Chlorophenol	2d	2a	7.6	5.6	6.2	529
1,3,5-Triethylbenzene	2f	1	9.8	8.3	9.8	− 804
m-Dichlorobenzene	2f	2c	6.1	4.0	5.1	− 116
o-Dichlorobenzene	2f	2c,d	9.7	6.9	8.0	− 267
Bromobenzene	2f	2d	9.5	10.2	8.6	1560
Fluorobenzene	2f	2d	6.2	6.4	6.2	683

TABLE II *(continued)*

Eluite	*Optimal eqn.*	*Penoptimal eqn.*	*MARE*			T_c (°K)
			Eqn. 1	*Optimal eqn.*	*Penoptimal eqn.*	
Nitrobenzene	2f	2d	5.1	3.6	4.4	−2355
p-Dibromobenzene	2f	2d	6.2	4.7	5.2	−1004
1-Bromo-4-chlorobenzene	2f	2b	6.6	3.5	4.8	102
p-Chlorophenol	2f	2b	10.9	4.4	5.4	36.8
p-Nitrophenol	2f	2b	5.8	2.9	3.8	1219
Benzylamine	2f	2a,b	5.8	4.7	5.5	−2742
Triphenylmethane	2f	2d	9.6	7.5	8.3	1086
p-Bromotoluene	2b	2d	1.2	0.5	0.5	36.7
Benzaldehyde	2a	2b	5.4	3.7	3.7	447

Retention factors predicted by eqn. 1 were calculated. Fig. 1 shows the divergence between predicted and observed retention factors, plotted as log (k_{pred}/k_{obs}) as a function of mobile phase composition. Fig. 2 shows the corresponding plot as a function of observed retention factor. Inspection of the distribution in divergence of the predicted retention factor from the experimental retention factor as a function of mobile phase composition or observed retention factor indicates that the errors are random functions of mobile phase composition and retention factor. Corresponding plots with temperature as independent variable showed errors to be randomly distributed with respect to temperature. Thus eqn. 1 is seen to reproduce experimental retention factors over the entire range of mobile phase composition and temperatures studied here and its use is justified if relative errors in retention factor of the order 7.8% are acceptable in the intended use. The number of times each equation was

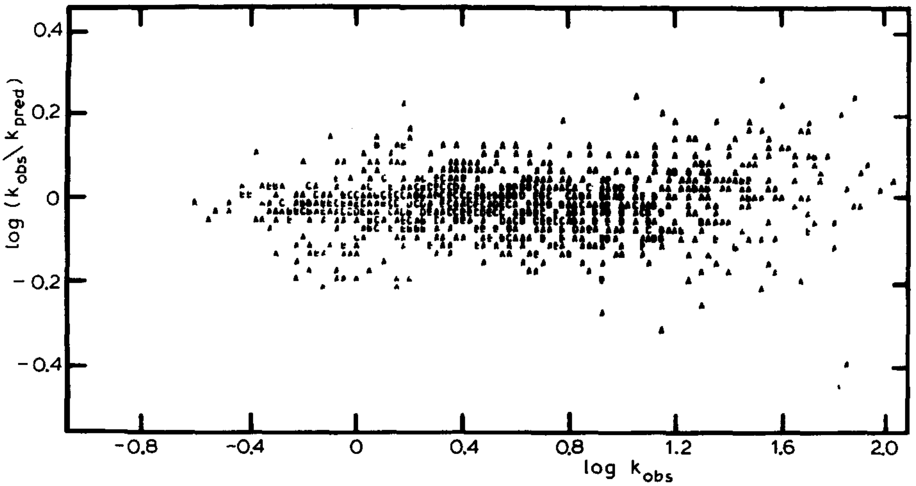

Fig. 1. Errors in calculated retention factor according to eqn. 1 are randomly associated with value of observed retention factor. The difference between predicted and observed logarithmic retention factor is plotted as log (k_{pred}/k_{obs}) against observed logarithmic retention factor.

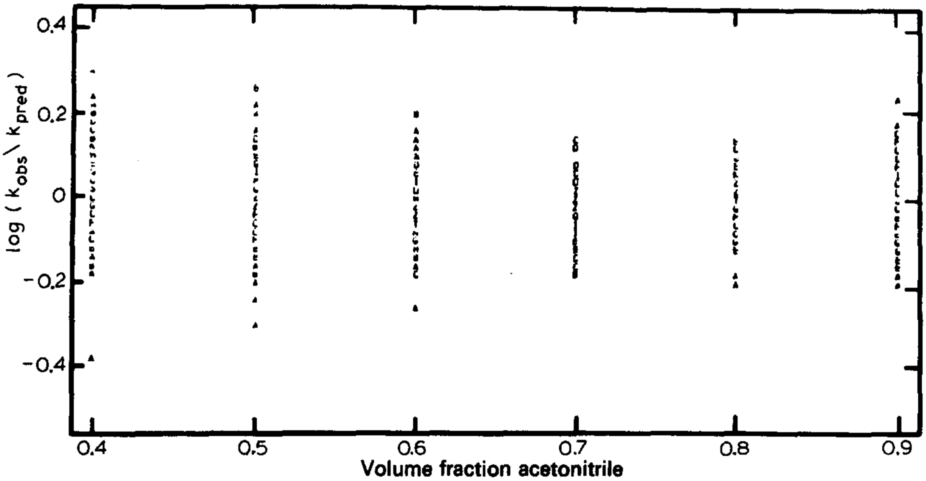

Fig. 2. Errors in retention factor calculated according to eqn. 1 are randomly associated with composition of acetonitrile–water mobile phase. The difference between predicted and observed logarithmic retention factor is plotted as log (k_{pred}/k_{obs}) against volume fraction acetonitrile.

optimal or penoptimal in its description of retention can be determined from Table II. The results of that census are presented in Table III.

Inspection of Tables II or III indicates that the three-parameter eqn. 1 is the best or second-best equation for 20 of the 54 eluites under examination. However, retention of eluites with polar groups should be better described, at least theoretically, by a four-parameter expression with φ and T in various combinations as independent variables. The use of eqns. 2a and 2b for ions and dipoles respectively is justified in part on grounds of classical electrostatic theory. The use of eqns. 2c–f to express the variation in electrostatic free energy with composition and temperature are suggested from empirical measures of solvent polarity in a number of systems. Inspection of Tables II and III suggests that, of four-parameter equations, eqn. 2c is most universal

TABLE III

THE NUMBER OF ELUITES FOR WHICH THE INDICATED EQUATION PROVIDED THE OPTIMAL AND PENOPTIMAL FIT TO RETENTION DATA AND THE CORRESPONDING AVERAGE MARE

The average MARE for all eluites was 7.8 + 3.0 with the use of eqn. 1.

Equation number	*Number of eluites*		*Average MARE for optimal and penoptimal eqns.*
	Optimal eqn.	*Penoptimal eqn.*	
1	3	17	8.1 ± 3.2
2c	34	2	6.6 ± 2.7
2f	12	10	6.5 ± 2.6
2b	1	13	5.6 ± 2.5
2a	1	5	6.2 ± 2.9
2d	2	10	7.4 ± 3.1

insofar as it is of first choice in its description of 34 eluites and second for 2. The next most universal equation was eqn. 2f which was best for 12 eluites and second best for 12.

In light of these results, none of the four-parameter equations examined is found to describe the retention behavior of all eluites with high accuracy. Furthermore, no relationship between the molecular structure of eluite and the equations which best described the behavior is evident from the data. Inspection of Table II reveals that the difference in the quality of fit between the two best equations is small; it is very nearly equal to the difference between the best and worst four-parameter equations. Thus, casual inspection of the results in Tables II and III suggests that the difference in the predictions of these fitting functions for individual eluites is small and the choice of any one is not clearly mandated. However, if use of a standard four-parameter equation is desired, the use of eqn. 2c for solvent polarity effects is preferred insofar as it gives best fit to retention data of 34 of 52 eluites while eqn. 2f, its nearest competitor, was superior in describing the retention of only 12 eluites.

The use of a four-parameter equation can be justified on the basis of its usually greater accuracy in describing retention. The chief disadvantages of a four-parameter equation are that (i) the particular equation used may be poorer in describing retention than is eqn. 1, (ii) the complexity of calculation is greater than that required for eqn. 1 and (iii) greater number of data points are required to establish the parameters. Insofar as numerical methods can be used to establish the best of a set of equations and even the most complex calculation for the analyses used here can be easily handled on readily available microcomputers, the first two problems usually can be overcome readily. However, with use of four-parameter equations it remains necessary to obtain 33% more data points in order to obtain parameters of precision comparable to those used in eqn. 1. Thus, according to the rule of thumb that at least four points are required for each parameter, retention data at 12 and 16 comnations of mobile phase composition and temperature are required for use of eqn. 1 and any four-parameter equation, respectively.

One advantage in use of an appropriate four parameter equation is the greater precision. A second is the increased utility of non-linear retention–composition relationships in the optimization of separation in algorithms based on analytical models of retention. Thus their use may be mandated for this purpose.

Examination of Tables II and III suggests that increase in the number of parameter from three to four results in reduction of the relative error. However, uncertainty in the parameters frequently increases. Of greater practical importance is that the decrease in relative error may not justify the increase in complexity associated with four-parameter equations. In short, it appears that any four-parameter expression with φ and T as independent variables describes the retention well but the result is scarcely better than that found with use of eqn. 1. Therefore two comprehensive functions which can be recommended for use in analyzing retention data over a wide range of temperatures and composition of acetonitrile–water mixtures as mobile phase are eqn. 1 unless a four-parameter equation is required, in which case eqn. 2c is the most generally useful.

CONCLUSIONS

In this study we examined various mathematical relationships between mobile phase composition, temperature and logarithmic retention factor in order to account for polar or electrostatic effects in reversed-phase chromatography. This aim was pursued through extension of eqn. 1 that has previously been found to be adequate for use with apolar eluites. With use of acetonitrile–water mixtures as mobile phases, the retention behavior of individual eluites is best described in general by eqn. 2c. However, the improvement in agreement between predicted and observed logarithmic retention factor is not especially great so that the use of eqn. 1 may be desirable in practice on the basis of its greater simplicity. In practice the use of the extended equation may be mandated only when optimization schemes based on a retention model are used in which case the sensitivity of selectivity to mobile phase composition may be more adequately modelled.

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