Journal of Chromatography, 360 (1986) 327–341
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 575

PREDICTION OF RETENTION OF HYDROXYL AROMATICS IN NORMAL-PHASE LIQUID CHROMATOGRAPHY WITH SLOPE–INTERCEPT RELATIONSHIPS

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(First received November 20th, 1985; revised manuscript received February 19th, 1986)

SUMMARY

Total solubility parameters for normal-phase *n*-heptane–2-propanol compositions were used to empirically predict the retention of unknown hydroxyl aromatic compounds at different mobile phase compositions on μBondapak NH_2, CN and μPorasil columns. A single experimental k' value was needed to predict k' values at other mobile phase compositions for an unknown compound. The method of predicting retention was based on two linear relationships derived from the experimental data. A linear relationship was obtained from plots of log k' *vs.* log total solubility parameter of the mobile phase for standard hydroxyl aromatic compounds. Next, a linear relationship was obtained by plotting the slope as a function of the intercept from the slope and intercept values derived from the log k' plots. Combined, the two linear relationships permitted the prediction of retention at different mobile phase compositions for compounds structurally similar to the original data set.

INTRODUCTION

Several aspects related to retention mechanisms in normal-phase high-performance liquid chromatography (HPLC) have been discussed[1–14]. Presently, the Snyder–Soczewiński displacement model[2–4] is the most theoretically and experimentally useful model for describing solute retention in normal-phase HPLC. The Snyder–Soczewiński displacement model assumes that the most important interactions are solute–stationary phase and solvent–stationary phase interactions and that solute–mobile phase interactions are generally unimportant and can usually be ignored[15]. However, Snyder[1,3,16] and Snyder and Poppe[4] have pointed out that solute–mobile phase interactions cannot be ignored when hydrogen bonding is possible.

Although the displacement model is very useful and fundamentally important, several of the parameters required to describe solute retention are unknown or can be difficult to determine. This limits the model for practical application in predicting solute retention. Therefore, there is a need for simple methods that can be used to predict solute retention in normal-phase systems.

In this work, we modified an empirical method developed for predicting solute retention in reversed-phase chromatography[17], so it would be applicable to normal-phase HPLC. The prediction of retention for a number of mono- and dihydroxyl aromatic compounds using HPLC is described. Linear relationships were obtained for log k' as a function of log total solubility parameter of the mobile phase. Furthermore, a linear relationship was obtained by plotting the slope as a function of the intercept for the slope and intercept values obtained from the linear log k' *vs.* log total solubility parameter relationships. Combined, the two linear relationships allowed the prediction of retention at most mobile phase compositions for hydroxyl aromatic compounds.

EXPERIMENTAL

High-performance liquid chromatography

The liquid chromatograph used was a Waters Model ALC/GPC 244 equipped with a Model 6000A pump controlled by a Model 680 Automated Gradient Controller operating in the isocratic mode, a U6K injector, a free standing UV detector set at 254 nm and 280 nm, a 10-mV strip chart recorder and a Hewlett-Packard Model 3390A integrator.

Columns

The columns used were 30 cm × 3.9 mm I.D. prepacked columns and were obtained from Waters Assoc. (Milford, MA, U.S.A.). The column packings were μBondapak NH_2 consisting of propylamine groups chemically bonded to Waters 10-μm porous silica, μBondapak CN consisting of propylnitrile groups chemically bonded to Waters 10-μm porous silica and μPorasil consisting of Waters 10-μm porous silica.

Reagents

HPLC grade 2-propanol was obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). HPLC grade *n*-heptane was obtained from MCB Manufacturing Chemists (Cincinnati, OH, U.S.A.). These solvents were filtered through a Millipore type FH 0.5-μm filter prior to use. The hydroxyl aromatic standards were obtained from commercially available sources and were purified when necessary.

Chromatographic systems studied

A μBondapak NH_2 column with *n*-heptane–2-propanol mobile phases at 1.0 ml/min was used for monohydroxyl aromatics and a flow-rate of 2.0 ml/min was used for dihydroxyl aromatics. A μBondapak CN column with *n*-heptane–2-propanol mobile phases at 1.0 ml/min and a μPorasil column with *n*-heptane–2-propanol mobile phases at 1.0 ml/min were employed for the monohydroxyl and dihydroxyl aromatics.

Hydroxyl aromatic standards

Solutions of 1–12 mg/ml of the hydroxyl aromatic standards were prepared in chloroform, 2-propanol or tetrahydrofuran depending on the solubility of the standards. The retention volumes of the hydroxyl standards were determined by injecting

1.0–6.0 μl of the standard solution onto the chromatographic systems described above. The capacity factor k', was calculated by $k' = (V_R - V_M)/V_M$, where V_R is the retention volume (ml) and V_M is the column void volume (ml). The column void volumes were obtained by eluting toluene and were determined to be 3.02 ml for the μBondapak NH_2 column, 3.05 ml for the μBondapak CN column and 3.01 ml for the μPorasil column.

Computer

Calculations and plots of data were obtained with a Hewlett-Packard Model 87 computer programmed in BASIC and equipped with a Model 82908A 65K Expansion Memory Module, a Model 82901M Flexible Disc Drive, and a Model 82095B Printer.

RESULTS AND DISCUSSION

General considerations

Considering only solute–adsorbent and solvent–adsorbent interactions, Snyder[3] has derived the following equation for normal-phase chromatography with binary mobile phases

$$\log k' = \log k'_0 - \left(\frac{A_s}{n_b}\right) \log X_s \tag{1}$$

where k' is the capacity factor for a solute eluted with the binary mobile phase, k'_0 is the capacity factor for a solute eluted with just strong solvent, A_s is the molecular area of the solute, and n_b is the molecular area of the strong solvent and X_s is the mole fraction of the strong solvent in the binary mobile phase. Eqn. 1 predicts a linear dependence of $\log k'$ as a function of $\log X_s$. In this work, eqn. 1 was considered for predicting the retention of unknown hydroxyl aromatic compounds using a slope–intercept relationship. Specifically, the prediction of retention of several hydroxyl aromatic unknowns at different mobile phase compositions was investigated using amino, cyano and silica gel high-performance columns.

Empirical considerations

As shown below, it was experimentally observed that a linear relationship exists for $\log k'$ as a function of log total solubility parameter ($\log \delta_{m,T}$) of the normal-phase binary mobile phase

$$\log k' = I - S \log \delta_{m,T} \tag{2}$$

where I is the intercept and S is the slope. The total solubility parameter ($\delta_{m,T}$) in eqn. 2 is assumed to be dependent on the mole fraction of the two constituent solvents. Thus

$$\delta_{m.T} = X_s\, \delta_{s,T} + (1 - X_s)\, \delta_{w,T} \tag{3}$$

where $\delta_{s,T}$ is the total solubility parameter of the strong solvent, $\delta_{w,T}$ is the total solubility parameter for the weak solvent and $(1 - X_s)$ is the mole fraction of the weak solvent in terms of the mole fraction of the strong solvent X_s. Solubility parameter theory and total solubility parameter values have been discussed in detail elsewhere[18-22].

Plotting the slope (S) *vs.* intercept (I) values obtained from eqn. 2 for structurally similar compounds results in a linear slope–intercept relationship. The linear slope–intercept relationship has the general form

$$S = -pI - q \tag{4}$$

where p and q are the linear regression coefficients for the slope and intercept, respectively. On the other hand, no linear correlation was observed for eqn. 4 using the slope and intercept values obtained from eqn. 1. Currently, we have no theoretical justification for eqns. 2 and 4. Linear slope–intercept relationships similar to eqn. 4 have been reported for reversed-phase systems[17,23-25], but apparently not for normal-phase systems prior to this work. The significance of eqn. 4 is that substituted into eqn. 2 a means of predicting retention at different compositions for unknown compounds is available. The resulting equation for predicting retention in normal-phase systems is

$$\log k' = (1 + p \log \delta_{m,T})I + q \log \delta_{m,T} \tag{5}$$

We have reported a similar equation for predicting retention with reversed-phase systems in earlier work[17].

To predict the retention of an unknown at different mobile phase compositions requires a single determination of log k' at a given mobile phase composition (total solubility parameter value, $\delta_{m,T}$) for the unknown. Substitution of the experimental values for log k' and $\delta_{m,T}$ into eqn. 5 allows the calculation of I which apparently is unique for a given compound. Eqn. 5 can then be used to predict log k' values at different $\delta_{m,T}$ values for the unknown. In addition, the intercept value, I, can be used to obtain the slope for the unknown via eqn. 4 and thus the calculated linear log k' relationship for the unknown as a function of log $\delta_{m,T}$.

Prediction of retention for hydroxyl aromatic compounds

Twenty-one monohydroxyl and thirteen dihydroxyl aromatic compounds were investigated with μBondapak NH_2, CN and μPorasil columns with several *n*-heptane–2-propanol mobile phase compositions. Table I gives the compounds investigated and the chromatographic data obtained on the μBondapak NH_2 column. Table II gives the least-squares slope and intercept values from log k' *vs.* log X_s plots (eqn. 1) and the corresponding linear correlation coefficients for all the compounds in Table I. Table III gives the least-squares slope and intercept values from log k' *vs.* log $\delta_{m,T}$ plots (eqn. 2) and the corresponding linear correlation coefficients for all the compounds in Table I.

Comparison of Tables II and III shows that overall the correlation coefficients in Table II are somewhat better than those in Table III. This was also the case for silica gel column; however, for the cyano column the converse was true. Table IV gives the least-squares slope (p) and intercept (q) values from the slope–intercept plots of the data in Tables II and III using eqn. 4 for various sets of compounds. Table IV shows that little correlation exists for the slope–intercept plots of the data in Table II. Therefore, the slope and intercept data in Table II obtained from eqn. 1 cannot be used to accurately predict retention using a slope–intercept relationship. In contrast, Table IV shows that a good correlation exists for the slope–intercept plots of the data in Table III obtained from log k' *vs.* log $\delta_{\mathrm{m,T}}$ plots. Similar results were obtained with the cyano and silica gel columns.

Fig. 1 shows the graph of the slope and intercept values obtained from eqn. 2 for the hydroxyl aromatics chromatographed on μBondapak NH_2. The straight line in Fig. 1 is defined by eqn. 4. Note that there are three points which lie off of the linear regression line more than the other points in Fig. 1. Two of these outlying points correspond to compounds which showed anomalous retention behavior on μBondapak NH_2, namely 1,2-dihydroxybenzene and 2,3-dihydroxynaphthalene, which are discussed below. The third point corresponds to 1,4-dihydroxybenzene which showed somewhat unusual values for the slope and intercept in Table III compared to the other dihydroxyl compounds. The reason for this result is not clear. Deletion of these compounds from Fig. 1 resulted in a correlation coefficient of 0.980 which is improved over the value shown in Table IV.

Eqn. 5 was used to predict the retention of eight "unknown" monohydroxyl and five "unknown" dihydroxyl compounds. The "unknown" monohydroxyl compounds were comprised of hydroaromatic and aliphatic alcohol types and aromatic hydroxyl types listed in Table I (compounds 1, 2, 3, 7, 10, 12, 16 and 18). These compounds were chosen because of their varied acidities and thus should challenge the predictive method more extensively. The "unknown" dihydroxyl compounds (compounds 22, 25, 26, 29 and 30) were chosen based on a variety of structural features with the hydroxyl substituents *ortho*, *meta* or *para* to one another or substituted on separate aromatic rings. The mono- and dihydroxyl "unknowns" were omitted from the least-squares slope–intercept plots and the values for the slope (p), intercept (q) and correlation coefficients were obtained as shown in Table IV. Inspection of Table IV shows that after deleting the "unknowns" the best correlation coefficient (0.970) was obtained when mono- and dihydroxyl compounds were grouped together. This result was also observed for the cyano and silica gel columns. Therefore, the corresponding values for the slope (p), -0.661, and intercept (q), -3.00, were used to predict the retention of the mono- and dihydroxyl "unknowns" on μBondapak NH_2 at various mobile phase compositions. The values for the slope (p) and intercept (q) in Table IV discussed above were used to predict k' at a $\delta_{\mathrm{m,T}}$ of 10.09 ($X_s = 0.657$) from k' at a $\delta_{\mathrm{m,T}}$ of 8.12 ($X_s = 0.176$) and *vice versa* for the "unknown" monohydroxyl compounds. The appropriate values of $\delta_{\mathrm{m,T}}$ for the dihydroxyl compounds are given in Table V. The situation in Table V represents predicting k' values at both compositional extremes investigated. The experimental k' values at a $\delta_{\mathrm{m,T}}$ of 8.12 and 10.09 were obtained from Table I for each "unknown". The results in Table V show a good correlation between the predicted k' values (k'_{pred}) and the experimental k' values (k'_{exp}), calculated from the linear regression data in Table III, for values of k' at a $\delta_{\mathrm{m,T}}$ of 10.09 predicted from k' at a $\delta_{\mathrm{m,T}}$ of 8.12. The average relative error in k' values was 16.4%.

However, the values for the relative error shown in Table V can be misleading

TABLE I

LOG k' VALUES FOR HYDROXYL AROMATICS ON μBONDAPAK NH_2 WITH *n*-HEPTANE–2-PROPANOL MOBILE PHASES

Conditions: A: total solubility parameter $(\delta_{m,T})^*$ = 8.12; mole fraction 2-propanol (X_s) = 0.176; *n*-heptane–2-propanol (90:10, v/v). B: $\delta_{m,T}$ = 8.73; X_s = 0.324; *n*-heptane–2-propanol (80:20). C: $\delta_{m,T}$ = 9.25; X_s = 0.451; *n*-heptane–2-propanol (70:30). D: $\delta_{m,T}$ = 9.70; X_s = 0.561; *n*-heptane–2-propanol (60:40). E: $\delta_{m,T}$ = 10.09; X_s = 0.657; *n*-heptane–2-propanol (50:50). F: $\delta_{m,T}$ = 9.70; X_s = 0.561; *n*-heptane–2-propanol (60:40). G: $\delta_{m,T}$ = 10.09; X_s = 0.657; *n*-heptanol–2-propanol (50:50). H: $\delta_{m,T}$ = 10.44; X_s = 0.742; *n*-heptane–2-propanol (40:60). I: $\delta_{m,T}$ = 10.75; X_s = 0.817; *n*-heptane–2-propanol (30:70). J: $\delta_{m,T}$ = 11.03; X_s = 0.885; *n*-heptane–2-propanol (20:80).

No.	*Compound*	*log k'*									
		A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*
	Monohydroxyl compounds										
1	1-Acenaphthenol	0.10	−0.21	−0.42	−0.51	−0.74					
2	5H-Dibenzo[*a,d*]cycloheptene-5-ol	0.23	−0.08	−0.27	−0.35	−0.53					
3	7,12-Dimethyl-9-hydroxybenz[*a*]-anthracene	0.85	0.44	0.19	0.11	0.06					
4	2-Hydroxybenzo[*c*]phenanthrene	0.87	0.47	0.24	0.15	0.02					
5	3-Hydroxybenzo[*c*]phenanthrene	0.99	0.56	0.32	0.24	0.09					
6	1-(1-Hydroxymethyl)pyrene	0.17	−0.14	−0.34	−0.43	−0.52					
7	1-(Hydroxymethyl)benzo[*a*]pyrene	0.47	0.13	−0.08	−0.18	−0.28					
8	4-Hydroxymethylpyrene	0.36	0.04	−0.17	−0.26	−0.37					
9	9-Hydroxyphenanthrene	0.87	0.47	0.25	0.16	0.04					
10	13-Hydroxypicene	1.07	0.63	0.40	0.26	0.20					
11	1-Hydroxypyrene	1.16	0.75	0.50	0.41	0.28					
12	4-Hydroxypyrene	0.98	0.57	0.35	0.24	0.14					
13	1-Indanol	−0.13	−0.42	−0.64	−0.73	−0.95					

14	5-Indanol	0.36	0.02	−0.20	−0.31	−0.46					
15	1-Naphthol	0.71	0.33	0.11	0.02	−0.12					
16	2-Naphthol	0.71	0.34	0.12	−0.03	−0.14					
17	3-Phenylphenol	0.61	0.23	0.01	−0.08	−0.23					
18	1,2,3,4-Tetrahydro-4-hydroxy-4-methylphenanthrene	−0.23	−0.51	−0.69	−0.79	−0.91					
19	1,2,3,4-Tetrahydro-1-naphthol	−0.20	−0.50	−0.70	−0.78	−0.99					
20	5,6,7,8-Tetrahydro-1-naphthol	0.29	−0.05	−0.26	−0.35	−0.48					
21	5,6,7,8-Tetrahydro-2-phenanthrol	0.59	0.21	−0.01	−0.03	−0.24					
	Dihydroxyl compounds										
22	*p,p'*-Biphenol						0.74	0.50	0.35	0.24	0.12
23	1,2,-Dihydroxybenzene						0.90	0.74	0.63	0.53	0.42
24	1,3-Dihydroxybenzene						0.72	0.51	0.37	0.25	0.13
25	1,4-Dihydroxybenzene						0.55	0.31	0.14	0.00	−0.12
26	1,3-Dihydroxynaphthalene						1.14	0.96	0.82	0.69	0.60
27	1,6-Dihydroxynaphthalene						0.83	0.64	0.49	0.37	0.26
28	1,7-Dihydroxynaphthalene						0.91	0.72	0.58	0.46	0.38
29	2,3-Dihydroxynaphthalene						1.30	1.15	1.04	0.94	0.87
30	2,6-Dihydroxynaphthalene						0.73	0.50	0.35	0.22	0.12
31	2,7-Dihydroxynaphthalene						0.84	0.62	0.46	0.34	0.22
32	2,5-Dihydroxynaphthalene						1.02	0.86	0.70	0.59	0.47
33	2,6-Dihydroxytoluene						0.39	0.23	0.07	−0.04	−0.14
34	3,5-Dihydroxytoluene						0.54	0.34	0.17	0.05	−0.05

* Individual values for *n*-heptane and 2-propanol taken from ref. 22.

TABLE II

SLOPE, INTERCEPT AND CORRELATION COEFFICIENT VALUES ON μBONDAPAK NH_2 FROM LOG k' *vs.* LOG X_s GRAPHS

Compound No.	*Slope*	*Intercept*	*Correlation coefficient*
1	−1.38	−0.91	0.985
2	−1.26	−0.71	0.993
3	−1.55	−0.32	0.997
4	−1.47	−0.25	0.998
5	−1.54	−0.18	0.997
6	−1.20	−0.74	0.999
7	−1.31	−0.52	0.999
8	−1.27	−0.60	0.999
9	−1.44	−0.22	0.999
10	−1.55	−0.12	0.998
11	−1.52	−0.01	0.998
12	−1.45	−0.13	0.999
13	−1.36	−1.12	0.987
14	−1.40	−0.68	0.998
15	−1.41	−0.36	0.999
16	−1.48	−0.40	0.999
17	−1.44	−0.48	0.998
18	−1.16	−1.10	0.997
19	−1.30	−1.16	0.988
20	−1.33	−0.71	0.999
21	−1.37	−0.46	0.989
22	−3.10	−0.05	0.999
23	−2.40	0.30	0.998
24	−2.92	−0.01	0.999
25	−3.37	−0.30	0.999
26	−2.75	0.45	0.999
27	−2.87	0.11	0.999
28	−2.66	0.24	0.999
29	−2.16	0.75	0.999
30	−3.07	−0.05	0.999
31	−3.11	0.06	0.999
32	−2.75	0.34	0.998
33	−2.68	−0.28	0.999
34	−2.99	−0.21	0.999

due to the small k' values used in calculating the relative error in some cases. In fact, in terms of retention volumes, the relative error between the predicted and experimental k' values for all the "unknowns" is less than 11% except for compound 29 (2,3-dihydroxynaphthalene). Ignoring compound 29, the average relative error in terms of retention volume for this case was 6.25% for all the "unknown" compounds. The compound, 2,3-dihydroxynaphthalene, showed anomalous retention behavior on the amino and silica gel columns. Significant tailing was observed on the amino column for this compound. In addition, 1,2-dihydroxybenzene showed considerable tailing on the amino and silica gel columns. Moreover, 2,3-dihydroxynaphthalene could not be eluted from the silica gel column with the mobile phases investigated. Recent work has shown that this compound can complex metal ions on the silica gel surface in reversed-phase systems causing anomalous retention behavior[26]. This phe-

TABLE III

SLOPE, INTERCEPT AND CORRELATION COEFFICIENT VALUES ON μBONDAPAK NH_2 FROM LOG k' *vs.* LOG $\delta_{m,T}$ GRAPHS

Compound No.	*Slope*	*Intercept*	*Correlation coefficient*
1	−8.46	7.78	0.993
2	−7.65	7.16	0.992
3	−9.31	9.25	0.985
4	−8.84	8.85	0.983
5	−9.25	9.34	0.982
6	−7.22	6.69	0.987
7	−7.86	7.57	0.987
8	−7.65	7.28	0.989
9	−8.59	8.62	0.981
10	−9.25	9.41	0.979
11	−9.13	9.40	0.983
12	−8.69	8.81	0.981
13	−8.33	7.44	0.994
14	−8.48	8.04	0.993
15	−8.50	8.38	0.986
16	−8.94	8.79	0.992
17	−8.70	8.47	0.988
18	−7.08	6.18	0.995
19	−7.93	6.99	0.993
20	−8.03	7.55	0.990
21	−8.25	8.03	0.977
22	−10.96	11.54	0.996
23	−8.51	9.29	0.998
24	−10.33	10.90	0.999
25	−11.93	12.31	0.998
26	−9.74	10.74	0.999
27	−10.17	10.86	0.999
28	−9.43	10.20	0.998
29	−7.67	8.85	0.999
30	−10.84	11.41	0.996
31	−11.02	11.70	0.998
32	−9.77	10.66	0.999
33	−9.50	9.76	0.999
34	−10.58	10.97	0.999

nomenon may also explain our results with this compound and presumably 1,2-dihydroxybenzene on amino and silica gel columns in normal-phase work.

Predicting k' values for k' at a $\delta_{m,T}$ of 8.12 from k' values at a $\delta_{m,T}$ of 10.09, the converse of the discussion above, results in higher overall relative errors as shown in Table V. The average relative error for this case was 18.5%. Nevertheless, a number of "unknowns" show very good agreement between the k'_{exp} and k'_{pred} values in Table V. The poorer results apparently stem in part from the fact that some k' values at a $\delta_{m,T}$ of 10.09 are close to the column void volume. The use of these values to predict k' values at other compositions can lead to a substantial error in the k'_{pred} values for some compounds. In addition, it appears that predicting higher k' values from lower k' values can lead to a significant error in the case where a given compound has a wide k' range over the compositions investigated and a somewhat poorer

TABLE IV

SLOPE–INTERCEPT REGRESSION DATA AND CORRELATION COEFFICIENT VALUES FOR DATA IN TABLES II AND III

	p	*q*	*r*
Table II			
All compounds	−1.05	−2.25	0.639
Monohydroxyl	−0.251	−1.52	0.761
Dihydroxyl	0.801	−2.92	0.764
Table III			
All compounds	−0.706	−2.60	0.960
Monohydroxyl	−0.644	−3.01	0.965
Dihydroxyl	−1.13	2.03	0.980
All compounds*	−0.661	−3.00	0.970
Monohydroxyl**	−0.631	−3.28	0.951
Dihydroxyl***	−0.991	0.532	0.963

* Mono- and dihydroxyl "unknowns" deleted.

** Monohydroxyl "unknowns" (1, 2, 3, 7, 10, 12, 16 and 18) deleted.

*** Dihydroxyl "unknowns" (22, 25, 26, 29 and 30) deleted.

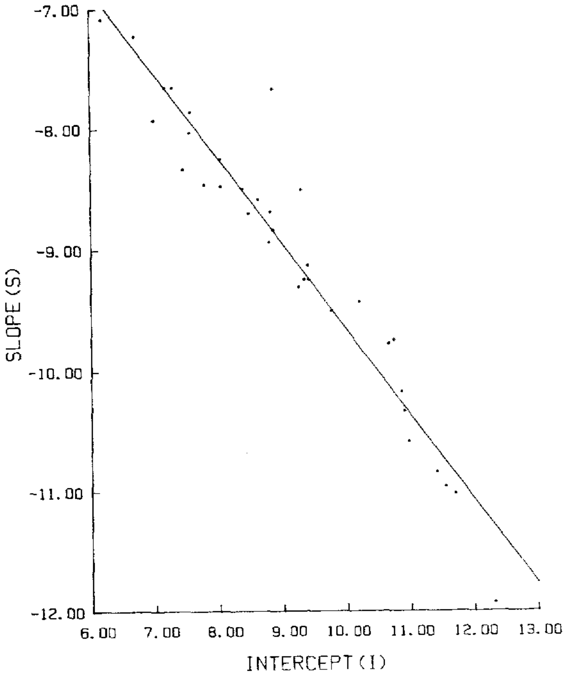

Fig. 1. Graph of slope and intercept values obtained from eqn. 4 for mono- and dihydroxyl aromatics.

log k' *vs.* log $\delta_{m,T}$ correlation coefficient compared to other compounds. In general, a lower relative error resulted when predicting k' values at high $\delta_{m,T}$ (low X_s) values from k' values at low $\delta_{m,T}$ (high X_s) values. Furthermore, the best accuracy was obtained by predicting k' values at adjacent compositions in a series of mobile phase compositions studied. A similar observation was reported earlier for reversed-phase systems[17].

Table V shows the results of predicting k' values at the low and high values of $\delta_{m,T}$ investigated, 10.09 and 8.12 respectively, from the k' values obtained at the $\delta_{m,T}$ values adjacent to 10.09 and 8.12 in Table I (9.70 and 8.73 respectively). Predicting k' at a $\delta_{m,T}$ of 10.09 from k' at a $\delta_{m,T}$ of 9.70 shows an excellent agreement between the k'_{exp} and k'_{pred} values for the "unknowns" with an average relative error of 5.64% as shown in Table V. In terms of retention volumes, the average relative error for this case was 2.43% with individual "unknowns" having relative errors of less than 9%. Predicting k' at a $\delta_{m,T}$ of 8.12 from k' at a $\delta_{m,T}$ of 8.73 shows a good agreement between the k'_{exp} and k'_{pred} values as shown in Table V. The average relative error for this case was 10.3%. In terms of retention volumes the average relative error was 8.21% with individual "unknowns" having relative errors of less than 14.5%. This is very good considering the large retention volumes of some of the "unknowns".

However, the relative error for an individual compound depends on the composition ($\delta_{m,T}$) at which log k' was experimentally determined. For instance, predicting k' at a $\delta_{m,T}$ of 8.12 from k' at a $\delta_{m,T}$ of 9.70 results in a 17.7% relative error for compound 2, whereas, predicting k' at a $\delta_{m,T}$ of 8.12 from k' at a $\delta_{m,T}$ of 8.73 results in a 6.96% relative error. These results are shown in Table VI which gives the relative errors that result from predicting k' values at all $\delta_{m,T}$ values investigated from k' values at each $\delta_{m,T}$ value for two typical "unknowns". Table VI clearly shows that the relative error for each determination depends on the $\delta_{m,T}$ value at which k' was experimentally determined. Notice that the overall average relative error was 6.56% for compound 2 and 8.10% for compound 16 for predicting k' values at each composition investigated.

Analogous to the previous discussion, Table VII shows the results of predicting k' values at the high value of $\delta_{m,T}$ investigated from k' values at the next highest $\delta_{m,T}$ (adjacent) value investigated for the "unknowns" on μBondapak CN and μPorasil. The appropriate values of $\delta_{m,T}$ are given in Table VII. The data in Table VII shows good agreement between the k'_{exp} and k'_{pred} values for the "unknowns" as evidenced by the relatively low average relative error for each column. These results are comparable to those obtained on NH_2.

The total solubility parameter of the mobile phase is useful in predicting retention in the normal-phase systems discussed here using the empirical method presented in this work. The method is best suited for predicting lower k' values from higher k' values and for predicting k' values at adjacent mobile phase compositions. The method is applicable to mono- and dihydroxyl compounds and gives good estimates of retention for these compounds chromatographed on amino, cyano and silica gel columns with *n*-heptane–2-propanol mobile phases. More work is needed to establish the generality of the method. However, additional work in this laboratory has shown that the method described is applicable to nitrogen containing compounds and furthermore, to other polar mobile phases.

TABLE V

COMPARISON OF k'_{exp} AND k'_{pred} VALUES FOR "UNKNOWN" MONO- AND DIHYDROXYL COMPOUNDS ON μBONDAPAK NH_2

S_e = Standard error estimate[28].

Compound	*k' ($\delta_{m,T}$ = 10.09)* from k' ($\delta_{m,T}$ = 8.12)***			*k' ($\delta_{m,T}$ = 8.12)** from k' ($\delta_{m,T}$ = 10.09)**			*k' ($\delta_{m,T}$ = 10.09)* from k' ($\delta_{m,T}$ = 9.70)****			*k' ($\delta_{m,T}$ = 8.12)** from k' ($\delta_{m,T}$ = 8.73)§*		
	k'_{exp}	k'_{pred}	*Relative error*	k'_{exp}	k'_{pred}	*Relative error*	k'_{exp}	k'_{pred}	*Relative error*	k'_{exp}	k'_{pred}	*Relative error*
1	0.19	0.24	26.3	1.22	0.92	13.5	0.19	0.23	21.1	1.22	1.08	11.5
2	0.30	0.30	0.00	1.58	1.65	2.71	0.30	0.33	10.0	1.58	1.48	6.33
3	0.81	1.01	24.7	6.13	5.92	2.95	0.81	0.90	11.1	6.13	5.12	16.5
7	0.48	0.48	0.00	2.63	3.22	16.3	0.48	0.48	0.00	2.63	2.43	7.60
10	1.31	1.55	18.3	9.80	11.8	18.6	1.31	1.27	3.05	9.80	8.24	15.9
12	1.22	1.30	6.56	8.07	10.3	24.2	1.22	1.23	0.82	8.07	7.08	12.3
16	0.67	0.78	16.4	4.64	4.79	2.66	0.67	0.67	0.00	4.64	4.09	11.9
18	0.12	0.12	0.00	0.55	0.58	1.94	0.12	0.12	0.00	0.55	0.52	5.45
22	1.28	1.52	18.8	5.22	4.68	8.68	1.28	1.33	3.91	5.22	4.72	9.58
25	0.74	1.03	39.2	3.42	2.54	19.9	0.74	0.79	6.76	3.42	2.96	13.5
26	3.88	3.44	11.3	13.6	16.1	17.5	3.88	3.69	4.90	13.6	13.8	2.14
29	7.23	4.74	34.4	19.4	32.3	63.6	7.23	6.50	10.1	19.4	21.9	13.0
30	1.27	1.48	16.5	5.10	4.72	6.23	1.27	1.29	1.57	5.10	4.67	8.43
		Average	16.4		Average	18.5		Average	5.64		Average	10.3
	$k'_{pred} = (0.66 \pm 0.04)\,k'_{exp} + 0.40 \pm 0.11$ S_e = 0.31 and r = 0.975			$k'_{pred} = (1.58 \pm 0.10)\,k'_{exp} - 2.23 \pm 0.85$ S_e = 1.95 and r = 0.977			$k'_{pred} = (0.89 \pm 0.01)\,k'_{exp} + 0.11 \pm 0.02$ S_e = 0.07 and r = 0.999			$k'_{pred} = (1.10 \pm 0.05)\,k'_{exp} - 0.84 \pm 0.37$ S_e = 0.85 and r = 0.991		

* For dihydroxy "unknowns" (22, 25, 26, 29 and 30) $\delta_{m,T}$ = 11.03.

** For dihydroxy "unknowns" (22, 25, 26, 29 and 30) $\delta_{m,T}$ = 9.70.

*** For dihydroxy "unknowns" $\delta_{m,T}$ = 10.75.

§ For dihydroxy "unknowns" $\delta_{m,T}$ = 10.09.

TABLE VI

COMPARISON OF k'_{exp} AND k'_{pred} VALUES FOR TWO "UNKNOWN" MONOHYDROXYL COMPOUNDS AT VALUES OF $\delta_{m,T}$ INVESTIGATED ON μBONDAPAK NH_2

Compound 2				*Compound 16*		
$\delta_{m,T}$	k'_{exp}	k'_{pred}	*Relative error*	k'_{exp}	k'_{pred}	*Relative error*
k' ($\delta_{m,T}$) from k' ($\delta_{m,T}$ = 8.12)						
8.12	1.58	1.69	6.96	4.64	5.14	10.8
8.73	0.91	0.95	4.40	2.43	2.74	12.8
9.25	0.58	0.60	3.45	1.45	1.65	13.8
9.70	0.41	0.42	2.44	0.95	1.09	14.7
10.09	0.30	0.30	0.00	0.67	0.78	16.4
k' ($\delta_{m,T}$) from k' ($\delta_{m,T}$ = 8.73)						
8.12	1.58	1.48	6.33	4.64	4.09	11.9
8.73	0.91	0.84	7.69	2.43	2.20	9.47
9.25	0.58	0.54	6.90	1.45	1.34	7.59
9.70	0.41	0.37	9.76	0.95	0.90	5.26
10.09	0.30	0.27	10.0	0.67	0.64	4.48
k' ($\delta_{m,T}$) from k' ($\delta_{m,T}$ = 9.25)						
8.12	1.58	1.49	5.70	4.64	4.02	13.4
8.73	0.91	0.85	6.59	2.43	2.17	10.7
9.25	0.58	0.54	6.90	1.45	1.32	8.97
9.70	0.41	0.37	9.76	0.95	0.88	7.37
10.09	0.30	0.27	10.0	0.67	0.63	5.97
k' ($\delta_{m,T}$) from k' ($\delta_{m,T}$ = 9.70)						
8.12	1.58	1.86	17.7	4.64	4.30	7.33
8.73	0.91	1.04	14.3	2.43	2.31	4.94
9.25	0.58	0.66	13.8	1.45	1.41	2.76
9.70	0.41	0.45	9.76	0.95	0.94	1.05
10.09	0.30	0.33	10.0	0.67	0.67	0.00
k' ($\delta_{m,T}$) from k' ($\delta_{m,T}$ = 10.09)						
8.12	1.58	1.65	4.43	4.64	4.79	3.23
8.73	0.91	0.93	2.20	2.43	2.56	5.35
9.25	0.58	0.59	1.72	1.45	1.55	6.90
9.70	0.41	0.41	0.00	0.95	1.03	8.42
10.09	0.30	0.30	0.00	0.67	0.73	8.96
			Average 6.56			Average 8.10

Practically, the empirical method could be used to predict the retention of unknowns present in complex, structurally similar mixtures. This could facilitate the development of separation methods for complex mixtures as well as providing a method of characterization for such mixtures.

In addition, it may be feasible to use the relationship described here, in a manner similar to those proposed by Schoenmakers *et al.*[24,27] for reversed-phase systems, to determine optimal solvent gradients and to estimate isocratic retention from gradient data.

The method presented in this work is best suited for individual compound classes with k' values between 1 and 10. Some of the k' values reported in this work

TABLE VII

COMPARISON OF k'_{exp} AND k'_{pred} VALUES FOR "UNKNOWN" MONO- AND DIHYDROXYL COMPOUNDS ON μBONDAPAK CN AND μPORASIL

Compound No.	*k' ($\delta_{m,T}$ = 7.78) from k' ($\delta_{m,T}$ = 8.12) μBondapak CN*			*k' ($\delta_{m,T}$ = 7.78) from k' ($\delta_{m,T}$ = 7.85) μPorasil*		
	k'_{exp}	k'_{pred}	*Relative error*	k'_{exp}	k'_{pred}	*Relative error*
1	0.89	0.81	8.99	0.85	0.87	2.35
2	1.14	1.12	1.75	0.26	0.24	7.69
3	2.19	2.05	6.39	0.40	0.39	2.50
7	1.99	1.98	0.50	1.32	1.40	6.06
10	3.41	3.36	1.47	0.29	0.26	10.3
12	2.14	2.04	4.67	0.33	0.32	3.03
16	–	–	–	0.42	0.40	4.76
18	0.51	0.49	3.92	0.32	0.31	3.13
22	10.0	8.66	13.5	1.41	1.45	2.84
25	6.89	5.69	17.4	2.52	2.62	3.97
26	5.99	5.20	13.2	1.51	1.52	0.66
29	4.26	4.32	1.41	–	–	–
30	10.5	8.84	16.1	1.61	1.69	4.97
			Average 7.44			Average 4.36
	k'_{pred} = (0.83 ± 0.02) k'_{exp} − 0.26 ± 0.11 S_e = 0.24 and r = 0.997			k'_{pred} = (1.06 ± 0.01) k'_{exp} − 0.03 ± 0.01 S_e = 0.02 and r = 0.999		

are not in this range since they were obtained as a result of group separating mono- from dihydroxyl compounds rather than optimizing the separation of the individual compounds. Thus, the results of this work may be improved by obtaining all k' values between 1 and 10. In addition, the slope and intercept values derived from eqn. 2 are dependent on the chromatographic system and solutes under study. Finally, the method is useful over a wide composition range and is not limited to a specific mobile phase composition like some predictive methods.

ACKNOWLEDGEMENT

Financial support for this project was provided by the U.S. Department of Energy under contract No. DE-AC22-83PC60015.

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