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MOLECULAR CONNECTIVITY INDICES IN THE PREDICTION OF THE RETENTION OF OXYGEN-CONTAINING AMINES IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

With a view to improving the possibility of predicting the retention of oxygen-containing amines, log k' was plotted against the zero-order valence-level indices, $^0\chi^v$, and the difference between the lines for non-oxygen-containing amines and oxygen-containing amines was studied. Equations were derived for the prediction of the retention of oxygen-containing amines, when non-oxygen-containing amines are used in the reference mixture.

INTRODUCTION

The concept of molecular connectivity, χ, was introduced by Randic[1] and extensively developed by Kier and Hall[2,3]. Molecular connectivity indices reflect the shape and atomic interactions of a molecule and they are commonly used to correlate liquid chromatographic retention parameters with molecular structure[4–19].

The usefulness of connectivity indices is greatly dependent on their ability to deal with molecules which contain heteroatoms. In my previous studies[7,9] on the relationship between reversed-phase liquid chromatographic (RPLC) retention data and molecular connectivity indices, the retention of oxygen-containing amines could not be predicted when analysed together with non-oxygen-containing amines. Kier and Hall[20] observed a similar effect when comparing the partition coefficients of alcohols, ethers, ketones, acids, esters, amines and hydrocarbons in a water–octanol system with the respective first-order valence-level indices, $^1\chi^v$. Nearly parallel lines relating to the functional group in the molecule were obtained for all compounds except hydrocarbons.

Some improvement can be achieved by adding new terms to the regression equation. Kier and Hall[21] improved the correlation between the water solubility of oxygen-containing compounds and the corresponding molecular connectivity indices by adding the vertex value, δ, of the oxygen atom to the regression equation for alcohols and ethers. For ketones it was omitted. A similar procedure gave a high correlation for boiling points and partition coefficients in the case of primary, secondary and tertiary amines[22].

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These findings prompted me to examine the relationship between the RPLC retention and the molecular connectivity indices of oxygen-containing amines. I have evaluated the possibilities of deriving equations for predicting the retention of oxygen-containing amines, when non-oxygen-containing amines are used in the reference mixture.

EXPERIMENTAL

Apparatus

An Hewlett-Packard Model 1090 chromatograph equipped with an HP 1040 diode-array detector, an HP 85B personal computer, an HP 3392A integrator and an HP 9121 disc-drive unit for data storage was used.

Reagents and chemicals

Ammonia, morpholine, 4-aminobutanol, 3-aminopropanol, pentylamine, 5-dimethylaminonaphthalene-1-sulphonyl chloride (Dns-Cl), ethyl acetate, sodium nitrate, sodium bicarbonate, 1-propanol, 2-propanol and HPLC-grade acetonitrile were obtained from Merck (Darmstadt, F.R.G.). Indole, 3-amino-2-cyclohexen-1-one, 1-aminomethyl-1-cyclohexanol, 2-(2-aminoethoxy)ethanol and 2-pyrrolidone were from EGA (Steinheim, U.S.A.). 2-Methoxyethylamine, 2-methylbutylamine, 2-amino-1-propanol, 2-(methylamino)ethanol, 2-(ethylamino)ethanol and 2-amino-1-pentanol were from Aldrich (Milwaukee, WI, U.S.A.) and isopropylamine from K & K Labs. (New York, NY, U.S.A.). Ethanol was produced by Alko (Helsinki, Finland). The water used was distilled and deionized. All the other reagents were obtained from Fluka (Buchs, Switzerland). All the reagents were of analytical grade and were used without further purification.

Preparation of derivatives for retention studies

A 100-μmol sample of the amine of interest was mixed in a test-tube with 5 ml of acetone–water (60:40) containing 0.01 M sodium bicarbonate, after which 80 μmol of Dns-Cl in acetone were added. This mixture was stirred at room temperature for 2 h and stored in a refrigerator.

Column

A 20 cm × 4.6 mm I.D. column packed with Nucleosil 5 C_{18} (Macherey-Nagel, Düren, F.R.G.) was used.

Chromatographic procedures

The eluent was pumped at a flow-rate of 1 ml/min, and the oven temperature was 40°C. The elution volume for a 0.5-μl injection of an aqueous solution of sodium nitrate was taken as the column dead volume. The samples were diluted to give the smallest detectable peaks at 250 nm.

Calculation of the molecular connectivity indices

The molecular connectivity indices for dansylamides were calculated using a computer program written in BASIC. The following general equation proposed by Kier and Hall[23] was used for computation of an index of type t and order m

$$ {}^m\chi_t = \sum_{j=1}^{{}^m n_s} {}^m c_j = \sum_{j=1}^{{}^m n_s} \left[\prod_{i=1}^{m} (\delta_i)_j^{-\frac{1}{2}} \right] \qquad (1) $$

where ${}^m c_j$ is the subgraph term for mth order subgraphs and ${}^m n_s$ is the number of mth order subgraphs.

Correlation of retention with the molecular connectivity indices

The IBM Statistical Analysis System (SAS) RSQUARE procedure was used for determining an index best describing the retention. The SYSREG procedure of the same system was used to determine the coefficients of linear regressions[24].

RESULTS AND DISCUSSION

Retention measurements were performed for the Dns derivatives of 22 non-oxygen-containing amines and 17 oxygen-containing amines in volume percentages of acetonitrile, ethanol and methanol in water giving about the same retention times (Table I). When log k' is plotted against the zero-order valence-level index, ${}^0\chi^v$, the correlation in general is not very high (Fig. 1, Table II). For certain structures, high correlations are nevertheless obtained (Table II). The zero-order valence-level index was chosen because it was observed to give the highest correlations for Dns derivatives of amines.

It is obvious from Fig. 1 that different types of compounds are arranged according to their polarities; dihydroxyamines deviate the most from non-oxygen-containing compounds, whereas aminoethers assume a position between non-oxygen-containing compounds and monohydroxamines. However, one of the two aminoketones tested appeared among the non-oxygen-containing compounds.

In the following, monohydroxyamines are used as an example to show how to investigate the difference between non-oxygen-containing and oxygen-containing amines. For this purpose, retention measurements were performed on the Dns derivatives of twelve non-oxygen-containing amines and nine monohydroxyamines (Table III) with various compositions of water–acetonitrile, water–ethanol and water–methanol as eluents. The regression equation is

$$ \log k' = A\,{}^0\chi^v + B \qquad (2) $$

where A and B are regression coefficients. The regression and correlation coefficients are given in Table IV.The slope of the line for non-oxygen-containing amines is slightly higher than that of monohydroxyamines (Table IV). If A_1 and A_2 are the slopes and B_1 and B_2 the intercepts of these lines, respectively, the difference in slopes, ΔS, and intercepts, ΔI, can be expressed according to:

$$ \Delta S = A_1 - A_2 \qquad (3) $$

$$ \Delta I = B_1 - B_2 \qquad (4) $$

The parameters ΔS and ΔI (Table V) are dependent on the volume percentage of

TABLE I

AMINES STUDIED AND RETENTION OF CORRESPONDING Dns-AMIDES ON NUCLEOSIL $5C_{18}$ TOGETHER WITH THE ZERO-ORDER VALENCE-LEVEL INDICES

Amine	*Capacity factor, k'*			$^{0}\chi^{v}$
	53% Acetonitrile	*45% Ethanol*	*65% Methanol*	
Non-oxygen-containing amines				
Ammonia	2.32	1.91	1.88	9.713
Methylamine	4.00	2.96	3.04	10.64
Ethylamine	5.49	4.08	4.08	11.34
Isopropylamine	7.34	5.65	5.44	12.21
Dimethylamine	7.77	5.21	5.46	11.58
Propylamine	7.84	5.99	5.75	12.05
Pyrrolidine	10.89	7.60	8.03	12.41
Isobutylamine	11.01	8.72	8.08	12.92
Diethylamine	11.13	7.63	7.45	13.00
Butylamine	11.35	9.14	8.46	12.76
Isopentylamine	15.67	13.22	11.94	13.63
2-Methylbutylamine	15.70	12.96	11.84	13.63
Pentylamine	16.69	14.05	12.83	13.46
Piperidine	17.56	12.70	13.08	13.12
Methyl-*tert.*-butylamine	17.89	13.41	12.80	14.08
Ethylisopropylamine	18.65	13.96	13.10	13.87
Methylbutylamine	23.11	17.58	16.57	13.70
Hexylamine	25.01	21.99	19.98	14.17
Methylbenzylamine	25.08	20.24	20.00	14.68
Histamine	28.18	20.78	19.08	13.65
Dipropylamine	30.57	24.50	22.22	14.41
Heptylamine	37.86	34.50	31.78	14.88
Hydroxyamines				
Ethanolamine	2.03	1.99	2.17	11.50
3-Amino-1-propanol	2.16	2.12	2.27	12.21
4-Amino-1-butanol	2.43	2.35	2.60	12.91
1-Amino-2-propanol	2.46	2.35	2.50	12.37
2-Amino-1-propanol	2.54	2.48	2.68	12.37
2-(Ethylamino)ethanol	3.92	3.45	3.78	13.15
2-Amino-1-pentanol	4.35	4.51	4.36	13.78
1-Aminomethyl-1-cyclohexanol	7.09	7.63	7.68	15.07
2-Butylaminoethanol	7.82	7.38	7.62	14.27
Dihydroxyamines				
3-Amino-1,2-propanediol	1.32	1.45	1.76	12.52
Diethanolamine	1.82	1.91	2.23	13.31
Hydroxy and an ether group in same amine				
2-(2-Aminoethoxy)ethanol	2.00	2.04	2.41	13.32
Aminoethers				
2-Methoxyethylamine	4.36	3.25	3.54	12.46
Morpholine	6.64	4.57	5.42	12.82
3-Ethoxypropylamine	7.11	5.37	5.52	13.87
Aminoketones				
3-Amino-2-cyclohexen-1-one	10.50	7.64	7.45	13.74
2-Pyrrolidone	11.01	7.59	7.45	12.61

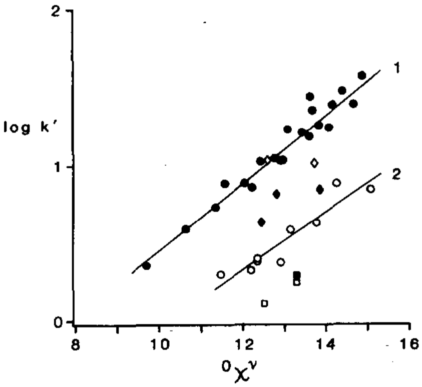

Fig. 1. Logarithm of the capacity factor in acetonitrile–water for different Dns derivatives of amines plotted against the zero-order valence-level index. 1 = Non-oxygen-containing amines (●); 2 = monohydroxyamines (○). □ = Dihydroxyamines; ◆ = aminoethers; ◇ = aminoketones; ■ = ether and hydroxy group in the same amine.

organic solvent in water, Φ

$$\Delta S = C\Phi + D \tag{5}$$

$$\Delta I = E\Phi + F \tag{6}$$

where C, D, E and F are regression coefficients. The regression and correlation coefficients are given in Table VI and the plots are presented in Figs. 2 and 3. The smallest absolute value for the slope, C, is obtained in ethanol–water; in acetonitrile–water it is doubled and in methanol–water the value is about four times higher than in ethanol–water (Table VI). Thus the change in volume percentage of the organic solvent in water causes the strongest effect in methanol–water of the eluents examined.

The retention of monohydroxyamines can be predicted according to:

$$\log k' = (A_1 - \Delta S)^0\chi^v + (B_1 - \Delta I) \tag{7}$$

TABLE II

REGRESSION COEFFICIENTS FOR EQUATIONS OF THE TYPE $\log k' = A^0\chi^v + B$

Compound group	*53% Acetonitrile*			*45% Ethanol*			*65% Methanol*		
	A	*B*	*r*	*A*	*B*	*r*	*A*	*B*	*r*
All (n = 39)	0.202	−1.75	0.576	0.212	−1.96	0.653	0.202	−1.83	0.674
No hetero-atoms (n = 22)	0.222	−1.77	0.971	0.232	−2.01	0.973	0.222	−1.90	0.971
Monohydroxy-amines (n = 9)	0.185	−1.89	0.942	0.194	−2.01	0.956	0.182	−1.83	0.953

TABLE III

AMINES USED FOR TESTING THE PREDICTION OF CHROMATOGRAPHIC RETENTION BY THE MOLECULAR CONNECTIVITY INDICES WITH VARIOUS ELUENT COMPOSITIONS

Non-oxygen-containing amines	*Monohydroxyamines*
Ammonia	Ethanolamine
Methylamine	3-Amino-1-propanol
Ethylamine	4-Amino-1-butanol
Isopropylamine	1-Amino-2-propanol
Dimethylamine	2-Amino-1-propanol
Propylamine	2-(Ethylamino)ethanol
Pyrrolidine	2-Amino-1-pentanol
Diethylamine	1-Aminomethyl-1-cyclohexanol
Butylamine	2-Butylaminoethanol
Isopentylamine	
Pentylamine	
Piperidine	

The parameters A_1 and B_1 are also dependent on the volume percentage of the organic solvent in water according to

$$A_1 = G\Phi + H \quad (8)$$

$$B_1 = I\Phi + J \quad (9)$$

where G, H, I and J are regression coefficients. The regression and correlation coefficients are given in Table VII.

When non-oxygen-containing amines are used for calibration, eqns. 10–12 (obtained by combining eqns. 5–9) can be used for predicting the retention of mo-

TABLE IV

REGRESSION DATA FOR CORRELATIONS OF THE TYPE $\log k' = A^0\chi^v + B$ FOR Dns DERIVATIVES OF COMPOUNDS IN TABLE III

Eluent	*Non-oxygen-containing amines*			*Monohydroxyamines*		
	A	*B*	*r*	*A*	*B*	*r*
49.0% Acetonitrile	0.237	−1.82	0.978	0.203	−2.01	0.944
53.0% Acetonitrile	0.217	−1.72	0.976	0.185	−1.89	0.943
57.0% Acetonitrile	0.198	−1.62	0.973	0.168	−1.77	0.940
61.0% Acetonitrile	0.182	−1.53	0.971	0.153	−1.66	0.934
41.3% Ethanol	0.246	−2.03	0.981	0.216	−2.17	0.957
45.2% Ethanol	0.219	−1.86	0.981	0.190	−1.97	0.956
49.0% Ethanol	0.195	−1.72	0.980	0.167	−1.79	0.954
52.9% Ethanol	0.169	−1.52	0.977	0.142	−1.57	0.952
61.0% Methanol	0.238	−1.94	0.975	0.207	−2.03	0.956
65.0% Methanol	0.213	−1.79	0.973	0.186	−1.76	0.948
69.0% Methanol	0.186	−1.61	0.970	0.161	−1.63	0.950
73.0% Methanol	0.161	−1.44	0.965	0.141	−1.52	0.943

TABLE V

DIFFERENCES IN SLOPES, ΔS, AND IN INTERCEPTS, ΔI, OF log k' *vs.* ${}^0\chi^v$ FOR NON-OXYGEN-CONTAINING AMINES AND MONOHYDROXYAMINES (EQNS. 3 AND 4)

Eluent	ΔS	ΔI	*Eluent*	ΔS	ΔI
49.0% Acetonitrile	0.0340	0.190	61.0% Methanol	0.0312	0.0803
53.0% Acetonitrile	0.0322	0.167	65.0% Methanol	0.0270	0.0310
57.0% Acetonitrile	0.0303	0.147	69.0% Methanol	0.0246	−0.0242
61.0% Acetonitrile	0.0284	0.130	73.0% Methanol	0.0207	−0.0876
41.3% Ethanol	0.0294	0.146			
45.2% Ethanol	0.0284	0.111			
49.0% Ethanol	0.0278	0.0763			
52.9% Ethanol	0.0265	0.0502			

nohydroxyamines in acetonitrile–water, ethanol–water and methanol–water, respectively:

$$\log k' \text{ (acetonitrile)} = -0.413\ {}^0\chi^v\Phi + 0.405\ {}^0\chi^v + 2.926\Phi - 3.438 \quad (10)$$

$$\log k' \text{ (ethanol)} = -0.632\ {}^0\chi^v\Phi + 0.477\ {}^0\chi^v + 5.160\Phi - 4.306 \quad (11)$$

$$\log k' \text{ (methanol)} = -0.558\ {}^0\chi^v\Phi + 0.547\ {}^0\chi^v + 5.670\Phi - 5.492 \quad (12)$$

When using eqns. 10–12, however, we have to keep in mind that the regression coefficients are derived from the retention data obtained on Nucleosil 5 C_{18} and they may be different on another C_{18} material or on other types of bonded phase.

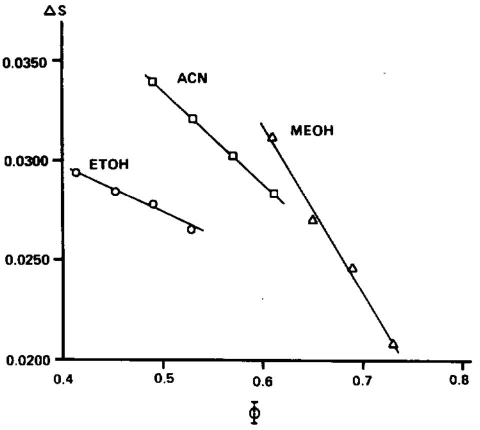

Fig. 2. Plot of the difference in slopes, ΔS, of log k' *vs.* ${}^0\chi^v$ between non-oxygen-containing amines and monohydroxyamines against the volume percentage, Φ, of acetonitrile (ACN), ethanol (ETOH) and methanol (MEOH) in water.

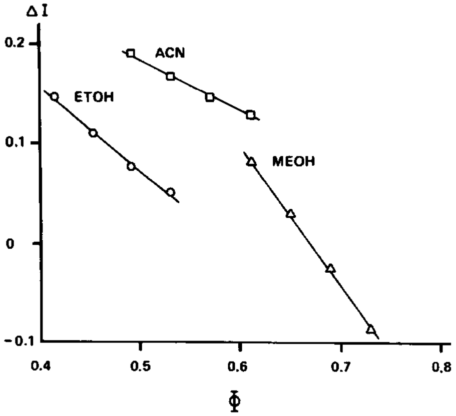

Fig. 3. Plot of the difference in intercepts, ΔI, of log k' *vs.* ${}^0\chi^v$ between non-oxygen-containing amines and monohydroxyamines against the volume percentage, Φ, of acetonitrile (ACN), ethanol (ETOH) and methanol (MEOH) in water.

TABLE VI

REGRESSION DATA FOR CORRELATIONS OF THE TYPE $\Delta S = C\Phi + D$ (EQN. 5) AND $\Delta I = E\Phi + F$ (EQN. 6)

Eluent	*C*	*D*	*r*	*E*	*F*	*r*
Acetonitrile	−0.0468	0.0569	0.999	−0.500	0.434	0.996
Ethanol	−0.0241	0.0394	0.991	−0.834	0.489	0.998
Methanol	−0.0848	0.0827	0.995	−1.397	0.936	0.998

TABLE VII

REGRESSION DATA FOR CORRELATION OF THE TYPE $A_1 = G\Phi + H$ (EQN. 8) AND $B_1 = I\Phi + J$ (EQN. 9)

Eluent	*G*	*H*	*r*	*I*	*J*	*r*
Acetonitrile	−0.460	0.462	0.999	2.426	−3.004	0.999
Ethanol	−0.656	0.516	0.999	4.326	−3.817	0.998
Methanol	−0.643	0.630	0.999	4.273	−4.556	0.999

The valence-level indices should reflect the role of heteroatoms[25]. For example, the zero-order valence-level value of an hydroxyl group is 0.447 which is smaller than that of a methyl group, 1.000. In RPLC, however, an hydroxyl group lowers the retention because of its polarity, requiring the addition of negative correction terms. The higher the valence vertex value, δ^v, the smaller is the contribution of this term to the final zero-order valence-level connectivity index. However, as the vertex value can never be negative, it is not possible to use the normal calculation rules in the case of hydroxyl groups and the procedure presented here is needed.

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