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## REVERSED-PHASE RETENTION BEHAVIOUR OF Dns-AMIDES AS A FUNCTION OF ELUENT COMPOSITION AND MOLECULAR STRUCTURE IN ETHANOL-WATER AND METHANOL-WATER

PEKKA LEHTONEN

Research Laboratories of the Finnish State Alcohol Company, Alko Ltd., P.O. Box 350, SF-00101 Helsinki 10 (Finland)

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### SUMMARY

The reversed-phase liquid chromatographic retention of eighteen Dns-amides in ethanol-water and methanol-water was investigated. A high correlation was obtained when the volume fractions,  $\varphi$ , that produce the same retention in ethanol-water and methanol-water were plotted against each other. The same retention is obtained using a 0.1–0.2 higher volume fraction of methanol than ethanol in water.

The molecular connectivity indices,  $\chi$ , to sixth order were correlated with the  $\log k'$  values at various eluent compositions. The same parameters were chosen as the best descriptors of retention in the two eluents. Retention can be predicted with great accuracy and the best descriptors of retention are the zero valence level,  ${}^0\chi^v$ , and the first valence level,  ${}^1\chi^v$ , indices.

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### INTRODUCTION

5-Dimethylaminonaphthalene-1-sulphonyl chloride (Dns-Cl) is the most widely used derivatizing agent for amines and especially for amino acids in liquid chromatography. It reacts with both primary and secondary amino groups and forms highly fluorescent products, which are quite stable. Dns derivatives have been formed from catecholamines<sup>1</sup>, diamines<sup>2</sup>, polyamines<sup>2</sup>, alkaloids<sup>3</sup> and from some aliphatic amines<sup>4</sup>. The use of Dns chloride has been mainly restricted to amino acids. Thin-layer chromatography (TLC) has been used<sup>5</sup> to separate Dns derivatives of simple aliphatic amines, but there is no high-performance liquid chromatographic (HPLC) study available on the retention behaviour of these compounds.

Several studies<sup>6–13</sup> have been reported on the variation of sample capacity factor,  $k'$ , as a function of eluent composition. Schoenmakers *et al.*<sup>6</sup> carried out a detailed study of the variation of  $k'$  with the volume fraction of organic solvent in water,  $\varphi$ , in methanol, ethanol and propanol, and observed that  $k'$  is given as a function of  $\varphi$  by

$$\log k' = A\varphi^2 + B\varphi + C \quad (1)$$

where  $A$ ,  $B$  and  $C$  are coefficients.

The solvent strength,  $S$ , of a pure organic solvent is usually calculated from the plot of  $\log k'$  vs.  $\phi$  (ref. 11)

$$\log k' = \log k'_w - S\phi \quad (2)$$

where  $k'_w$  represents the capacity factor of a solute with pure water as mobile phase. Values of  $S$  can vary from 2.0 to 6.0 for different solvents and compounds<sup>11,14,15</sup>, which shows that a given increment in organic modifier concentration causes large differences in retention. In fact, the plots of  $\log k'$  vs.  $\phi$  are slightly curved, and the real  $\log k'_w$  values are more adequately estimated by the complex equation used by Wells *et al.*<sup>16</sup>.

Molecular connectivity indices have frequently been used to correlate chromatographic retention parameters with molecular structure<sup>13,17-23</sup>. These indices reflect the shape and atomic interactions of a molecule. Detailed discussion of this concept and associated calculations have been given by Kier and Hall<sup>17</sup> and Wells *et al.*<sup>18</sup>. When the nature of the atom is not taken into consideration the index is referred to as the connectivity level,  $\chi$ ; otherwise the index is called the valence level,  $\chi^v$ . Connectivity indices have been extended to include indices of different orders, the order being the number of bonds involved in the subgraphs composed of paths, clusters path/clusters and chains.

In this study the reversed-phase chromatographic properties of Dns-amides originating from simple aliphatic amines in ethanol-water and methanol-water are evaluated. We attempt to make comparisons between ethanol and methanol as eluents. The relationships between extended molecular connectivity indices and retention parameters are evaluated, and the observed and calculated  $\log k'$  values are compared at various eluent compositions.

## EXPERIMENTAL

### *Apparatus*

The liquid chromatograph consisted of a Hewlett-Packard Model 1084B equipped with a 79875A variable-wavelength spectrophotometer detector. Absorbance spectra of Dns-amides were determined in the UV region 200-320 nm on a Kontron Uvikon 820 spectrophotometer.

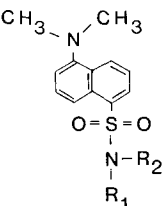



### *Reagents and chemicals*

Dns-Cl, acetone, sodium nitrate, and sodium bicarbonate were from Merck (Darmstadt, F.R.G.). All amines listed in Table I (except pentylamine and morpholine, which were from Merck) were from Fluka (Buchs, Switzerland). Ethanol was from Alko (Helsinki, Finland) and HPLC-grade methanol was from Orion (Espoo, Finland). Water was distilled and deionized. All the reagents were pro analysis grade and were used without further purification.

### *Preparation of derivatives*

A 100- $\mu$ mol amount of the amine was mixed in a test-tube with 5 ml of acetone-water (60:40) containing 0.01  $M$  sodium bicarbonate, and 80  $\mu$ mol of Dns-Cl in acetone were then added. This mixture was stirred for 2 h at room temperature and stored in the refrigerator.

TABLE I  
THE Dns DERIVATIVES INVESTIGATED

Structure	Compound No.	R <sub>1</sub>	R <sub>2</sub>
	1	CH <sub>3</sub>	H
	2	CH <sub>3</sub> CH <sub>2</sub>	H
	3	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>	H
	4	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub> CH <sub>2</sub>	H
	5	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>3</sub> CH <sub>2</sub>	H
	6	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>4</sub> CH <sub>2</sub>	H
	7	Dns—N 	
	8	Dns—N 	
	9	Dns—N 	
	10	CH <sub>3</sub>	CH <sub>3</sub>
	11	CH <sub>3</sub> CH <sub>2</sub>	CH <sub>3</sub> CH <sub>2</sub>
	12	CH <sub>2</sub> CH <sub>2</sub> OH	CH <sub>2</sub> CH <sub>2</sub> OH
	13	CH <sub>3</sub>	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>
	14	CH <sub>3</sub>	C(CH <sub>3</sub> ) <sub>3</sub>
	15	CH <sub>3</sub> CH <sub>2</sub>	CH(CH <sub>3</sub> ) <sub>2</sub>
	16	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>
	17	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>
	18	CH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>

### Column

A 20 cm × 4.0 mm I.D. column packed with Spherisorb S5 ODS2 (5 μm, from Phase Separations, Queensferry, U.K.) was used. The column was packed by a slurry technique using acetone as the suspending medium.

### Chromatographic procedures

The eluent was pumped isocratically at a flow-rate of 1.0 ml/min and the oven temperature was 40°C. The column dead volume was taken as the elution volume for a 2-μl injection of an aqueous solution containing sodium nitrate, with the UV detector operating at 254 nm and 0.01 a.u.f.s. Sufficiently dilute samples were prepared to give least detectable peaks (20% of scale). The UV detector was operated at 266 nm.

The ethanol and methanol content of solvent mixtures was determined by a pycnometric method at 20 ± 0.02°C. Duplicate analyses were performed.

### Calculation of molecular connectivity indices

Valence and connectivity indices, including path, cluster and path/cluster types, were calculated to the sixth order. Calculations were performed with an in-house BASIC program on a Hewlett-Packard 2645A. The number of subgraph terms are listed in Table II.

TABLE II

NUMBER OF SUBGRAPH TERMS FOR PATH (*p*), CLUSTER (*c*) AND PATH/CLUSTER (*pc*) TYPES

See Table I for compound identification.

Compound No.	${}^0\chi$		${}^1\chi$		${}^2\chi$		${}^3\chi$			${}^4\chi$			${}^5\chi$			${}^6\chi$			Total		
	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>	<i>c</i>	<i>p</i>	<i>c</i>	<i>pc</i>	<i>p</i>	<i>c</i>	<i>pc</i>	<i>p</i>	<i>c</i>	<i>pc</i>	<i>p</i>	<i>c</i>	<i>pc</i>	<i>p</i>	<i>c</i>	<i>pc</i>
1	18	19	28	37	9	47	1	32	58	8	78	57	1	188	246	19	298				
2	19	20	29	38	9	50	1	32	60	8	81	60	1	194	257	19	307				
3	20	21	30	39	9	51	1	32	63	8	81	62	1	197	266	19	310				
4	21	22	31	40	9	52	1	32	64	8	81	65	1	197	274	19	310				
5	22	23	32	41	9	53	1	32	65	8	81	66	1	197	280	19	310				
6	23	24	33	42	9	54	1	32	66	8	81	67	1	197	286	19	310				
7	21	23	34	47	10	62	1	40	73	10	101	79	2	251	318	23	392				
8	22	24	35	48	10	63	1	40	79	10	101	79	2	253	328	23	394				
10	19	20	30	40	10	49	1	38	61	10	86	61	2	210	261	23	334				
11	21	22	32	44	10	56	1	40	65	10	99	67	2	237	286	23	376				
13	22	23	33	44	10	55	1	39	68	10	93	69	2	225	292	23	357				
14	22	23	36	46	13	58	2	47	67	13	117	70	3	267	300	31	431				
15	22	23	34	46	11	60	1	43	67	11	110	70	2	253	300	25	406				
16	23	24	34	46	10	60	1	40	73	10	101	72	2	251	309	23	392				
17	25	26	36	48	10	62	1	40	77	10	101	80	2	253	329	23	394				
18	25	27	39	52	11	66	1	42	83	10	101	82	2	247	349	24	390				

Kier and Hall<sup>17</sup> proposed the following general equation for computation of a  $\chi$  index of type *t* and order *m*:

$${}^m\chi_t = \sum_{j=1}^{m_{n_s}} m_{c_j} = \sum_{j=1}^{m_{n_s}} \left[ \prod_{i=1}^m (\delta_i)_j^{-\frac{1}{2}} \right] \quad (3)$$

where  $m_{c_j}$  is the subgraph term for *m*th order subgraphs and  $m_{n_s}$  is the number of *m*th order subgraphs.

## RESULTS AND DISCUSSION

Table I lists the eighteen Dns derivatives investigated, and Tables III and IV detail the solvent composition and the capacity factor, *k'*. Six of the derivatives are of primary amines (compounds 1–6) and the remainder of secondary amines.

### Retention behaviour of Dns-amides

The elution order of the Dns-amides at representative water–organic solvent mixtures is presented in Fig. 1. The order remains the same in ethanol–water and methanol–water throughout the water–organic solvent scale, except for some compounds containing five carbon atoms in the amine part. These are pentyl-Dns (compound 5), piperidine-Dns (8), N-methyl-N-*tert*-butyl-Dns (14) and N-ethyl-N-iso-

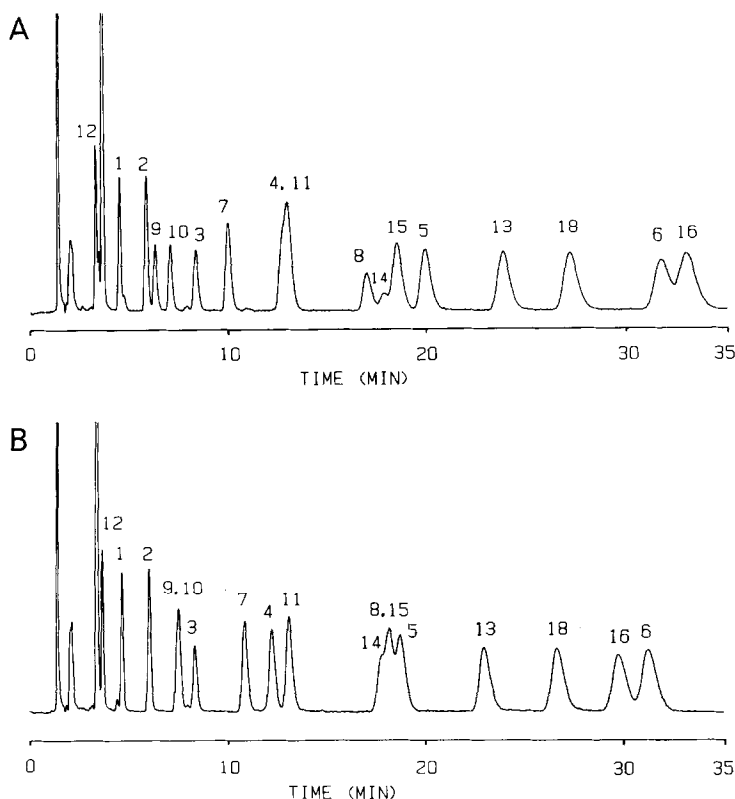


Fig. 1. Separation of Dns-amides: (A) ethanol-water (50:50), (B) methanol-water (65:35). Flow-rate, 1 ml/min; column, 20 cm  $\times$  4.0 mm I.D. packed with Spherisorb S5 ODS2; oven temperature, 40°C, detector wavelength, 266 nm. See Table I for peak identification.

propyl-Dns (15). Pentyl-Dns (No. 5) is more strongly affected by the eluent composition than the others, as is seen from the higher solvent strength value for this compound in Table V. The retention order follows the carbon number in the amine part except for compounds containing oxygen (compounds 9 and 12) and the compound containing the rigid phenyl group (18).

#### Solvent properties

Although plots of  $\log k'$  vs.  $\phi$  are slightly curved it can be assumed that eqn. 2 gives comparable  $\log k'_w$  values. The retention data of all the compounds include  $\log k'$  values from  $-0.1$  to  $1.4$ . The solvent strengths of ethanol and methanol were calculated over this range from 2. The results are given in Table V.

Regression analysis on  $\log k'_{w(E)}$  and  $\log k'_{w(M)}$ , where the subscripts (E) and (M) refer to ethanol and methanol, respectively, gives

$$\log k'_{w(E)} = 0.651 \log k'_{w(M)} + 0.411 \quad r = 0.996 \quad (4)$$

The values for N,N-diethanol-Dns (compound 12) were omitted because it behaves

TABLE III  
EXPERIMENTAL  $k'$  VALUES IN ETHANOL-WATER

See Table I for compound identification.

Compound No.	Amount of ethanol in water (%)																	
	19	24	29	35	39	47	50	55	60	65	69	74	79	84	87	92	96	
1		34.4	16.0	8.47	4.66	2.41	2.10	1.22	1.15	1.12	0.70	0.59						
2		63.4	27.7	13.9	7.46	3.50	2.73	1.91	1.51	1.08	0.84	0.70	0.56					
3			58.1	26.7	13.2	5.61	4.45	2.77	2.05	1.39	1.06	0.85	0.64					
4					25.5	9.54	7.11	4.20	2.89	1.85	1.35	1.04	0.76	0.60				
5					46.3	16.4	11.4	6.36	4.14	2.50	1.76	1.30	0.91	0.70	0.57			
6						27.7	18.5	9.74	5.99	3.42	2.32	1.63	1.10	0.82	0.65			
7				34.4	16.8	7.28	5.7	3.53	2.55	1.74	1.32	1.04	0.80	0.65				
8					34.4	13.3	10.3	5.95	4.02	2.56	1.87	1.42	1.03	0.82	0.68	0.50		
9			34.2	16.9	8.79	4.00	3.22	2.14	1.65	1.19	0.94	0.79	0.63					
10			42.9	19.6	10.7	4.67	3.93	2.48	1.88	1.34	1.04	0.86	0.67	0.57				
11					24.5	9.91	7.76	4.53	3.16	2.07	1.53	1.18	0.87	0.70	0.58			
12	45.0	19.35	9.13	4.71	2.70	1.41	1.22	0.90	0.79									
13					57.3	19.3	14.8	8.02	5.16	3.10	2.19	1.59	1.13	0.86	0.70	0.50	0.39	
14					38.5	13.8	10.9	6.14	4.11	2.62	1.88	1.41	1.01	0.80	0.66			
15					40.0	14.2	11.0	6.27	4.19	2.63	1.89	1.42	1.01	0.80	0.66	0.48		
16					28.5	20.1	10.8	6.73	3.97	2.72	1.93	1.32	0.98	0.79	0.55			
17					53.6	25.3	14.3	7.49	4.80	3.11	1.99	1.37	1.03	0.67	0.48			
18					67.1	22.8	16.6	8.93	5.62	3.31	2.30	1.67	1.16	0.88	0.71	0.51		

TABLE IV  
EXPERIMENTAL  $k'$  VALUES IN METHANOL-WATER  
See Table I for compound identification.

Compound No.	Amount of methanol in water (%)														
	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
1		50.9	26.4	14.1	8.82	5.40	3.43	2.28	1.55	1.10	0.84	0.64	0.57		
2			46.4	24.3	14.2	8.26	5.12	3.20	2.09	1.45	1.04	0.77	0.61		
3				45.9	25.2	14.1	8.04	4.69	2.93	2.05	1.31	0.93	0.70		
4					47.7	24.0	13.5	7.33	4.34	2.57	1.70	1.13	0.84	0.59	
5					92.2	39.8	22.6	11.5	6.49	3.55	2.28	1.44	0.97	0.65	
6							39.8	22.0	9.93	5.22	3.12	1.86	1.18	0.76	0.55
7				64.9	39.3	20.0	11.0	6.31	4.09	2.57	1.84	1.30	0.95	0.70	
8					76.7	42.2	20.8	11.0	6.69	3.91	2.61	1.68	1.21	0.84	0.66
9			68.6	32.4	20.8	13.2	6.59	3.98	2.74	1.80	1.35	1.04	0.80	0.63	
10			73.1	35.2	21.8	12.9	7.51	4.47	2.85	1.87	1.35	1.03	0.78	0.59	
11					49.4	28.9	15.0	7.94	4.85	3.09	1.98	1.37	0.97	0.69	0.55
12	52.4	33.7	17.7	9.52	5.64	3.43	2.28	1.53	1.10	0.91	0.66				
13					55.7	28.8	15.1	8.26	4.60	2.88	1.84	1.23	0.81	0.63	
14					40.1	20.8	10.7	6.47	3.71	2.45	1.60	1.10	0.77	0.60	
15					40.1	21.7	11.0	6.67	3.71	2.52	1.61	1.11	0.78	0.60	
16					81.3	41.9	20.4	11.0	6.06	3.61	2.12	1.40	0.90	0.66	
17					121	49.0	25.6	11.8	6.59	3.51	2.04	1.17	0.79		
18					69.7	34.8	16.0	9.31	5.13	3.16	1.92	1.28	0.84	0.63	

TABLE V

PARAMETERS OF THE EQUATION  $\log k' = \log k'_w - S\varphi$  OVER THE  $\log k'$  RANGE FROM  $-0.1$  TO  $1.4$

Subscripts E and M refer to ethanol and methanol, respectively. See Table I for compound identification.

Compound No.	$S_{(E)}$	$S_{(M)}$	$\log k'_{w(E)}$	$\log k'_{w(M)}$
1	3.78	3.64	2.26	2.78
2	3.71	3.78	2.40	3.02
3	3.82	4.03	2.63	3.34
4	3.80	4.24	2.77	3.66
5	3.96	4.53	3.03	4.01
6	4.08	4.85	3.25	4.43
7	3.53	3.83	2.57	3.36
8	3.52	4.05	2.76	3.71
9	3.61	3.63	2.41	3.06
10	3.53	3.70	2.44	3.11
11	3.55	3.99	2.68	3.55
12	4.41	3.78	2.28	2.69
13	3.78	4.33	3.02	3.98
14	3.64	4.12	2.84	3.74
15	3.65	4.13	2.86	3.75
16	3.84	4.54	3.15	4.23
17	4.29	5.15	3.70	4.96
18	3.37	4.47	3.11	4.12

differently from the other derivatives. The high correlation coefficient indicates that  $\log k'_w$  reflects basically the same molecular properties of the solute in both solvents, but these properties contribute differently to retention.

The higher solvent strength of methanol over ethanol is readily seen from Table V. A plot of the solvent strength of ethanol against that of methanol gives

$$S_{(E)} = 0.415 S_{(M)} + 2.028 \quad r = 0.846 \quad (5)$$

The correlation is not statistically as good as between the  $\log k'_w$  values.

To find the volume fractions,  $\varphi$ , of ethanol and methanol that give the same retention for a particular compound, the  $\log k'$  values were plotted against  $\varphi$ . Plots were observed to be slightly curved, and the Schoenmakers equation (eqn. 1) was used to describe the retention behaviour. Regression and correlation coefficients were evaluated with the statistical analysis system (SAS) procedure SYSREG. Correlation coefficients obtained varied from 0.9991 to 0.9999 in ethanol-water and from 0.9985 to 0.9999 in methanol-water for different compounds.

Values of  $\varphi$  that produce the same retention in ethanol-water and methanol-water were calculated over the  $\log k'$  range from  $-0.1$  to  $1.4$  at intervals of  $0.1$  for each compound. The  $\varphi_{(E)}$  values were plotted against  $\varphi_{(M)}$  values to give

$$\varphi_{(E)} = 1.086\varphi_{(M)} - 0.212 \quad n = 288 \quad r = 0.995 \quad (6)$$

The high correlation coefficient indicates that changes in organic modifier concentration have a similar effect whether the solvent is ethanol or methanol. The standard



deviations of intercept and slope are 0.0043 and 0.0060, respectively, which imply that the line does not go through the origin and that the slope differs significantly from unity<sup>24</sup>. From this it follows that the difference in volume fraction of methanol and ethanol to give the same retention is lower at high concentrations of organic modifier. Depending on the amount of organic solvent in water, a 10–20% higher concentration of methanol than ethanol in water has to be used to obtain the same retention.

#### Comparison of $\log k'$ values with molecular connectivity indices

The molecular connectivity indices were calculated for the compounds in Table I. The SAS RSQUARE procedure<sup>25</sup>, which performs all possible regressions for dependent variables on a collection of independent variables and orders them according to correlation coefficient, was run to obtain regressions of  $\log k'$  against all possible one- and two-variable combinations of the indices, their reciprocals, squares, reciprocal squares, square roots and reciprocal square roots, *i.e.* index to the power 1,  $-1$ , 2,  $-2$ , 0.5 and  $-0.5$ , respectively. The regressions were calculated separately for Dns derivatives of primary amines, for Dns derivatives of secondary amines and for all the Dns derivatives combined.

The variables that gave the highest correlation coefficients at each of the eluent

TABLE VI

MOST POWERFUL MOLECULAR CONNECTIVITY INDICES FOR PREDICTING  $\log k'$  IN ETHANOL-WATER

	Ethanol (%)	One-variable model	Correlation coefficient	Two-variables model		Correlation coefficient
		$\chi_1$		$\chi_1$	$\chi_2$	
All amides (1-18)	50	${}^0\chi^v$	0.9792	${}^0\chi^v$	$({}^4\chi_{pc}^v)^2$	0.9900
	55	${}^0\chi^v$	0.9806	${}^0\chi^v$	$({}^1\chi^v)^2$	0.9903
	60	${}^0\chi^v$	0.9798	${}^0\chi^v$	$({}^1\chi^v)^2$	0.9893
	65	$({}^0\chi^v)^2$	0.9801	$({}^0\chi^v)^2$	$({}^1\chi^v)^2$	0.9882
	69	$({}^0\chi^v)^2$	0.9784	${}^0\chi^v$	$({}^6\chi_p^v)^2$	0.9862
	74	$({}^0\chi^v)^2$	0.9765	$({}^1\chi^v)^2$	$({}^4\chi_{pc}^v)^{-1}$	0.9866
Secondary amides (1-6)	45	$({}^2\chi^v)^2$	0.9998			
	50	$({}^0\chi^v)^2$	0.9999			
	55	$({}^2\chi^v)^2$	0.9999			
	60	$({}^2\chi^v)^2$	0.9999			
	65	$({}^0\chi^v)^2$	0.9999			
	69	$({}^2\chi^v)^2$	0.9999			
74	$({}^2\chi^v)^2$	0.9999				
Tertiary amides (7-18)	50	$({}^0\chi^v)^2$	0.9641	${}^1\chi^v$	$({}^4\chi_p^v)^{-0.5}$	0.9947
	55	$({}^0\chi^v)^2$	0.9648	$({}^1\chi^v)^{0.5}$	$({}^4\chi_p^v)^{-2}$	0.9963
	60	$({}^0\chi^v)^2$	0.9628	$({}^1\chi^v)^{0.5}$	$({}^4\chi_p^v)^{-2}$	0.9960
	65	$({}^1\chi^v)^2$	0.9620	$({}^1\chi^v)^{0.5}$	$({}^4\chi_p^v)^{-2}$	0.9956
	69	$({}^1\chi^v)^2$	0.9626	$({}^1\chi^v)^{0.5}$	$({}^4\chi_p^v)^{-2}$	0.9950
	74	$({}^1\chi^v)^2$	0.9631	${}^1\chi^v$	$({}^4\chi_p^v)^{-2}$	0.9943
	80	$({}^1\chi^v)^2$	0.9666	${}^1\chi^v$	$({}^4\chi_p^v)^{-2}$	0.9940
	85	$({}^1\chi^v)^2$	0.9646	${}^1\chi^v$	$({}^4\chi_p^v)^{-2}$	0.9921

TABLE VII

MOST POWERFUL CONNECTIVITY INDICES FOR PREDICTING  $\log k'$  IN METHANOL-WATER

		Methanol (%)	One-variable model	Correlation coefficient	Two-variables model		Correlation coefficient
			$\chi_1$		$\chi_1$	$\chi_2$	
All amides (1-18)	60		$(^1\chi^v)^2$	0.9771	$^0\chi^v$	$(^5\chi_{pc})^2$	0.9892
	65		$(^1\chi^v)^2$	0.9747	$^0\chi^v$	$(^5\chi_{pc})^2$	0.9851
	70		$(^0\chi^v)^2$	0.9779	$^0\chi^v$	$(^1\chi^v)^2$	0.9890
	75		$(^0\chi^v)^2$	0.9757	$^1\chi^v$	$(^4\chi_{pc})^{-1}$	0.9884
	80		$(^0\chi^v)^2$	0.9737	$(^1\chi^v)^2$	$(^4\chi_{pc})^{-2}$	0.9913
	85		$^0\chi^v$	0.9951	$(^1\chi^v)^2$	$(^4\chi_{pc})^{-2}$	0.9889
	90		$(^0\chi^v)^2$	0.9515	$(^1\chi^v)^2$	$(^4\chi_{pc})^{-2}$	0.9893
Secondary amides (1-6)	60		$(^2\chi^v)^2$	0.9991			
	65		$(^2\chi^v)^2$	0.9978			
	70		$(^2\chi^v)^2$	0.9998			
	75		$(^1\chi^v)^2$	0.9995			
	80		$(^2\chi^v)^2$	0.9996			
	85		$(^1\chi^v)^2$	0.9997			
	90		$^3\chi_p^v$	0.9990			
Tertiary amides (7-18)	50		$(^0\chi^v)^2$	0.9637	$^1\chi^v$	$(^4\chi_p^v)^{-1}$	0.9967
	55		$(^0\chi^v)^2$	0.9602	$(^1\chi^v)^{0.5}$	$(^4\chi_p^v)^{-2}$	0.9944
	60		$(^0\chi^v)^2$	0.9693	$^1\chi^v$	$(^4\chi_p^v)^{-2}$	0.9964
	65		$(^1\chi^v)^2$	0.9714	$^1\chi^v$	$(^4\chi_p^v)^{-1}$	0.9967
	70		$(^1\chi^v)^2$	0.9768	$^1\chi^v$	$(^4\chi_p^v)^{-2}$	0.9961
	75		$(^1\chi^v)^2$	0.9745	$^1\chi^v$	$(^4\chi_p^v)^{-0.5}$	0.9965
	80		$(^1\chi^v)^2$	0.9784	$^1\chi^v$	$(^4\chi_p^v)^{-1}$	0.9960
	85		$(^1\chi^v)^2$	0.9777	$^1\chi^v$	$(^4\chi_p^v)^{-0.5}$	0.9890

compositions are given in Tables VI and VII. The zero valence level and the first valence level indices ( $^0\chi^v$  and  $^1\chi^v$ ) were selected most often. The path term of the fourth-order valence level index,  $^4\chi_p^v$ , was often selected to describe retention of derivatives originating from secondary amines in two-variable combinations.

Valence level indices are chosen when the nature of the atom (carbon, nitrogen or oxygen) is important to the correlation. This was usually done (Tables VI and VII), showing that the nature of the atom is an important factor in these correlations. The same parameters were chosen as the best descriptors of retention in either eluent. This suggests that the same structural features are important to the chromatographic retention process at various eluent compositions in ethanol-water and methanol-water. The values of the indices chosen are given in Table VIII.

Regression coefficients were evaluated with the SAS procedure SYSREG and are given in Table IX. For example, the regression equation at 50% of ethanol in water is (from Tables VI and IX)

$$\log k' = 0.277 \ ^0\chi^v - 0.0422 \ (^4\chi_{pc}^v)^2 - 2.602 \quad r = 0.9900 \quad (7)$$

Some of the  $\log k'$  values predicted by equations of this type, using regression coef-

TABLE VIII

## MOLECULAR CONNECTIVITY INDICES SELECTED FOR Dns-AMIDES

See Table I for compound identification.

Compound No.	$^0\chi^v$	$^1\chi^v$	$^2\chi$	$^2\chi^v$	$^3\chi_p^v$	$^4\chi_p$	$^4\chi_p^v$	$^4\chi_{pc}$	$^4\chi_{pc}^v$	$^5\chi_{pc}$	$^6\chi_p^v$
1	10.6361	5.5977	8.0460	4.2304	2.8991	5.1104	1.9988	4.0631	1.2796	6.0713	0.7030
2	11.3432	6.1583	8.4425	4.5242	3.9647	5.6778	2.1566	3.8400	1.2452	6.3722	0.7471
3	12.0503	6.6583	8.7960	4.9206	3.1725	5.6660	2.2030	3.8400	1.2452	6.2144	0.7837
4	12.7574	7.1583	9.1496	5.2742	3.4528	5.8642	2.3499	3.8400	1.2452	6.2144	0.8626
5	13.4645	7.6583	9.5031	5.6277	3.7028	6.0410	2.5482	3.8400	1.2452	6.2144	0.8858
6	14.1716	8.1583	9.8567	5.9813	3.9528	6.2177	2.7249	3.8400	1.2452	6.2144	0.9592
7	12.4117	7.2086	9.5066	5.6340	4.0237	6.7743	2.9470	4.6456	1.5474	7.4881	1.0258
8	13.1188	7.7086	9.8602	5.9874	4.2737	6.9510	3.1238	4.6456	1.5474	7.4881	1.0495
10	11.5833	5.9705	8.6799	4.8103	3.0995	5.2298	2.0762	5.1779	1.6000	6.6188	0.7492
11	12.9975	7.1228	9.0387	5.1122	3.6641	6.4453	2.5821	4.7302	1.6008	7.8915	0.8282
13	13.7046	7.5467	9.5685	5.7030	3.8557	6.0494	2.5483	4.9221	1.5901	7.1471	0.8920
14	14.0833	7.2469	10.6130	6.6662	3.9240	6.2388	2.3875	6.2269	2.6458	10.2116	0.8399
15	13.8677	7.5123	9.7840	5.8250	3.8151	6.9333	2.8184	5.0484	1.8942	8.9881	0.8564
16	14.4117	8.1228	9.7995	5.9269	3.9047	6.6897	2.8695	4.6456	1.5474	7.5479	1.0054
17	15.8259	9.1228	10.5066	6.6340	4.4808	6.8938	3.0397	4.6456	1.5474	7.4881	1.2487
18	14.6771	8.1038	11.1660	6.2693	4.2772	7.3937	2.9729	5.3021	1.7521	7.7360	1.0234

ficients from Table IX and the selected indices from Tables VI and VII, are given in Table X. When mixtures of ethanol and water were used as solvent the mean error of calculated  $\log k'$  was 0.0285, with a standard error of 0.0027, and for methanol

TABLE IX

REGRESSION COEFFICIENTS FOR EQUATIONS OF THE TYPE  $\log k' = A\chi_1 + B\chi_2 + C$ , WHERE  $\chi_1$  AND  $\chi_2$  ARE THE CHOSEN INDICES GIVEN IN TABLES VI AND VII

	A	S.E.*	B	S.E.	C	S.E.
<i>Ethanol (%)</i>						
50	0.277	0.0112	-0.0422	0.0113	-2.602	0.141
55	0.129	0.030	0.0111	0.0003	-1.581	0.239
60	0.113	0.027	0.00958	0.0028	-1.460	0.219
65	0.0038	0.0009	0.00755	0.0025	-0.721	0.053
69	0.125	0.011	0.161	0.055	-1.561	0.114
74	0.0126	0.0006	-1.150	0.260	-0.332	0.073
<i>Methanol (%)</i>						
60	0.301	0.013	-0.00376	0.0010	-2.520	0.156
65	0.262	0.013	-0.00345	0.0010	-2.292	0.159
70	0.113	0.030	0.0114	0.0031	-1.358	0.240
75	0.0185	0.0008	-1.375	0.349	-1.179	0.098
80	0.0158	0.0006	-3.175	0.593	-0.363	0.050
85	0.0126	0.0006	-3.253	0.548	-0.366	0.047
90	0.00965	0.0005	-2.958	0.420	-0.372	0.036

\* Standard error of the regression coefficient.

TABLE X

EXPERIMENTAL AND PREDICTED  $\log k'$  VALUES OF SIXTEEN Dns-AMIDES AT VARIOUS ELUENT COMPOSITIONS

See Table I for compound identification.

Compound No.	Ethanol-water (50:50)		Ethanol-water (69:31)		Methanol-water (65:35)		Methanol-water (85:15)	
	$\log k'$ (obs.)	$\log k'$ (calc.)	$\log k'$ (obs.)	$\log k'$ (calc.)	$\log k'$ (obs.)	$\log k'$ (calc.)	$\log k'$ (obs.)	$\log k'$ (calc.)
1	0.263	0.270	-0.155	-0.156	0.357	0.366	-0.196	-0.168
2	0.436	0.469	-0.076	-0.057	0.505	0.539	-0.112	-0.108
3	0.639	0.665	0.024	0.040	0.670	0.731	-0.031	-0.028
4	0.822	0.861	0.132	0.149	0.865	0.916	0.053	0.059
5	1.049	1.056	0.245	0.244	1.060	1.101	0.159	0.153
6	1.268	1.252	0.364	0.354	1.342	1.286	0.268	0.252
7	0.729	0.729	0.119	0.156	0.800	0.765	0.115	0.138
8	0.985	0.925	0.271	0.252	1.040	0.950	0.226	0.232
10	0.564	0.493	0.018	-0.027	0.650	0.591	0.012	-0.038
11	0.890	0.884	0.185	0.170	0.900	0.897	0.137	0.128
13	1.139	1.081	0.341	0.276	1.180	1.121	0.264	0.218
14	1.006	0.997	0.273	0.308	1.030	1.037	0.204	0.212
15	1.042	1.082	0.277	0.286	1.040	1.061	0.208	0.218
16	1.303	1.282	0.434	0.398	1.310	1.286	0.327	0.315
17	1.729	1.673	0.681	0.663	1.690	1.659	0.545	0.532
18	1.189	1.327	0.362	0.437	1.203	1.346	0.283	0.346

and water the values were 0.0295 and 0.0026. The retention of Dns-amides can be predicted with great accuracy with these indices, and the small errors obtained are not dependent on eluent composition.

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