

RETENTION MODELS OF CAPACITY FACTOR WITH DIFFERENT COMPOSITIONS OF ORGANIC MODIFIER IN RP-HPLC

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Abstract – A chromatographic retention behavior of five deoxyribonucleosides (dCyd, dUrd, dGuo, dThd, and dAdo) with respect to the mobile phase composition was studied under isocratic conditions of reversed phase High Performance Liquid Chromatography (RP-HPLC). The volume fraction (F) of organic modifier was changed from 0.05 to 0.30, and to 0.12 for methanol and acetonitrile, respectively. The experimental data of nitro and steroid compound were also considered for comparison of five retention models with various class of samples. The Langmuir-type retention model ($k' = A + B/F$) with two parameters shows excellent agreements between the experimental capacity factors and calculated values although the values by the log-scale quadratic model with three parameters ($\log k' = LF^2 + MF + N$) are closer. Unlike the other four retention models, the slope B of the Langmuir-type retention model can characterize the properties of solute and organic modifier simultaneously. For each solute, the intercepts A calculated for acetonitrile and methanol as organic modifiers are coincident closely.

Key words: RP-HPLC, Organic Modifier, Retention Model, Capacity Factor, Langmuir Adsorption

INTRODUCTION

As high performance liquid chromatography (HPLC) is widely used as a standard analytical instrument, a number of stationary phases are commercially available. HPLC columns are improved to increase the selectivity and the efficiency for the mixtures separated. The most commonly used technique is reversed-phase high performance liquid chromatography (RP-HPLC), which is usually done by n-octadecyl modified packings [Krstulovic and Brown, 1982]. As the C_{18} are chemically bonded to the surface of the particle, these packings provide stability and reproducibility as well as selectivity [Horvath and Melander, 1977].

Five nitrogenous bases are found in DNA and RNA nucleotide components. Three of the bases, adenine, guanine, and cytosine, are common to DNA and RNA. Thymine is found only in DNA, while uracil is unique to RNA. Adding the bases to deoxyribose five-carbon sugar becomes deoxyribonucleoside. In recent years, HPLC technique to analyze the DNA fragments has significantly increased.

The important parameter for quantitation in HPLC is capacity factor (k'). Retention volume of a sample compound (V_R) can be expressed in terms of the elution volume of a non-retained material (V_0). k' is given as the ratio of ($V_R - V_0$) to V_0 . The capacity factor is proportional to the free energy change associated with the chromatographic distribution process. It is also related with the partition coefficient. Thus solute retention is affected by the thermodynamics of distribution between the two phases. The compositions of mobile phase determine the retention volume of solutes. For RP-HPLC column, the major con-

stituent is highly polar solvent (e.g. water), and the less polar solvent of organic modifiers (e.g. methanol, acetonitrile, etc.) are added to control the hydrophobic nature between solute and C_{18} -coated stationary phase. Snyder Equation has been typically used to describe the relationship between k' and the fraction of mobile phase [Snyder and Quarry, 1987]. But recently, the more elaborate equation based on the adsorption of Langmuir adsorption shows better prediction of k' with different composition of mobile phase [Lee et al., 1995]. For the nitro and steroid samples from the literature [Snyder and Quarry, 1987] as well as the solutes of deoxyribonucleosides experimentally obtained, five retention models including the Langmuir-type retention model were compared with the experimental data. Therefore, the purpose of the work is to compare the five retention models to predict the capacity factors for nitro compounds and steroid compounds as well as deoxyribonucleoside, and the differences in the retention mechanisms will be discussed for the solutes.

RETENTION MODELS

Normally, the prediction of retention time is based on some expected dependences of capacity factor, k' , on mobile phase composition. Retention volume may be expressed as retention time at constant flow rate of mobile phase. More often it is discussed in literature a problem of the extrapolation of experimental data to estimate the value of capacity factor for water as mobile phase (k'_w) [Snyder and Quarry, 1987]. The value of k'_w serves as a good descriptor and predictor of the solute hydrophobicity in biological systems [Dorsey and Khaledi, 1993].

Snyder described the following linear relationship in RP-HPLC [Snyder and Quarry, 1987]:

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$$\log k' = \log k'_w - SF \quad (1)$$

where k' refers to the solute capacity factor, k'_w is the value of k' for water as mobile phase, F is the volume fraction of organic modifier in the mobile phase, and S is a constant for a given solute and mobile phase composition. The slope and intercept values of Eq. (1) are regarded as a measure of the hydrophobic character of the solutes [Valko, 1984]. The considerable amounts of papers reported the use of Eq. (1) for the estimation of the retention of solutes in RP-HPLC, and some are discussed and reviewed in detail [Johnson et al., 1986; Dorsey and Khaledi, 1993; Melander and Horvath, 1980; Horvath and Melander, 1977; Schoenmakers et al., 1979; Row et al., 1995; Snyder and Quarry, 1987; Valco, 1984].

Due to the dependence of $\log k'$ on the mobile phase composition, attempts have been made to find an alternative chromatographic parameter that is less dependent on the conditions and can be used as a continuous and universal scale. Kaibara and co-workers [Hsieh and Dorsey, 1993] suggested the following form:

$$\log k' = K \log(1/F) + H \quad (2)$$

where K and H are empirical coefficients.

The simple polynomial of quadratic form is adopted and the two types of k' , normal and log scale is as follows,

$$k' = CF^2 + DF + E \quad (3)$$

$$\log k' = LF^2 + MF + N \quad (4)$$

where C , D , E , L , M , and N are empirical coefficients.

Finally, the Langmuir-type relationship between the capacity factor and organic modifier content in the eluent was first proposed by Row and coworkers [1995]. This equation assumed that organic modifier adsorption is described by Langmuir isotherm. The final equation can be expressed as follows:

$$k' = A + B(1/F) \quad (5)$$

where A and B are experimental coefficients. The intercept, A characterizes the adsorption interaction between the organic modifier molecules and adsorbent surface while the slope, B , relates to the solute molecules and adsorbent surface interaction. Unlike the other four equations, Eq. (5) was theoretically developed with a few of assumptions. All equations were linearized by LOTUS 123™ (Ver. 2.0). The resulting correlation coefficients, r^2 , has the following form,

$$r^2 = \frac{[\sum(x_i - \bar{x})(y_i - \bar{y})]^2}{[\sum(x_i - \bar{x})^2][\sum(y_i - \bar{y})^2]} \quad (6)$$

EXPERIMENTAL

All deoxyribonucleosides were chromatographically pure and were purchased from Sigma (St. Louis, MO, U.S.A.). The solutes were dissolved in HPLC-grade water and each concentration was 50 $\mu\text{g/ml}$. HPLC-grade water, methanol and acetonitrile were obtained from Baker (Phillipsburg NJ, U.S.A.). Waters Model 600 liquid chromatograph (Waters Associates, Milford, MA, U.S.A.) equipped with the Waters 600E Multisolute Delivery System, a UV-visible tunable wavelength ad-

sorbance detector (Waters 486), and U6K injector (2 ml sample loop) was used. The data acquisition system was CHROMATE (Ver. 2.1, Interface Eng.) installed in PC. A Waters column (30 \times 0.39 cm) packed by μ -Bondapak C_{18} reversed phase material of 10 μm particle size was used.

The modifier concentrations of methanol and acetonitrile were ranged from 0 to 30% and from 0 to 12% (v/v), respectively. The injection volumes of 5 μl were injected directly for HPLC analysis. The elutions were performed by using an isocratic mode at a flow rate of 1 ml/min. Absorbance was monitored at 254 nm with a sensitivity of 2 and 0.001 a.u.f.c. All separations were done at the ambient temperature. The dead volume was measured by introducing of 20 μl of methanol to be 2.95 ml.

RESULTS AND DISCUSSION

The experimental data of reversed-phase HPLC retention of five investigated deoxynucleosides in water-methanol and water-acetonitrile mobile phases with respect to the content of organic modifier are presented in the Figs. 1 and 2. The experimental data of nitro and steroid compound [Snyder and Quarry, 1987] were also added in the Figures to compare the retention models with various classes of samples. The data of deoxyribonucleosides are characterized by the lower content of organic modifier, while Snyder's data by the higher content of organic modifier. In both cases, the retention of the samples (deoxyribonucleosides, nitro and steroid compound) decreases with an increase in concentration of modifier in a semi-logarithmic relationship. Compared to methanol, acetonitrile offers approximately twice the elution power for nucleosides, but there is no significant difference in their separation selectivity. The elution order of deoxynucleosides is the same in the different mobile phases (Figs. 1, 2). When the modifier concentration is less than 5%, the retention value increases as following: dCyd-dUrd-dThd-dGuo-dAdo. Here dCyd, dUrd, and dThd contain the pyrimidine bases, and dGuo and dAdo contain the purine bases. This result can be related with the increase of molecule size and, therefore, the increase of the surface area of a solute molecule [Johnson et al., 1986]. The retention order on Bon-

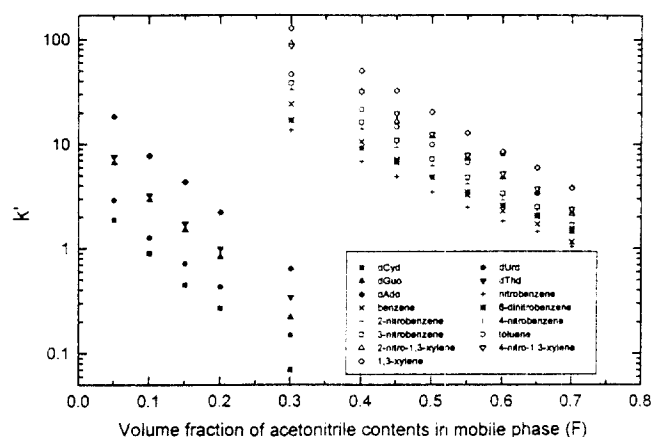


Fig. 1. Effect of volume fraction of methanol content in mobile phase (F) on k' .

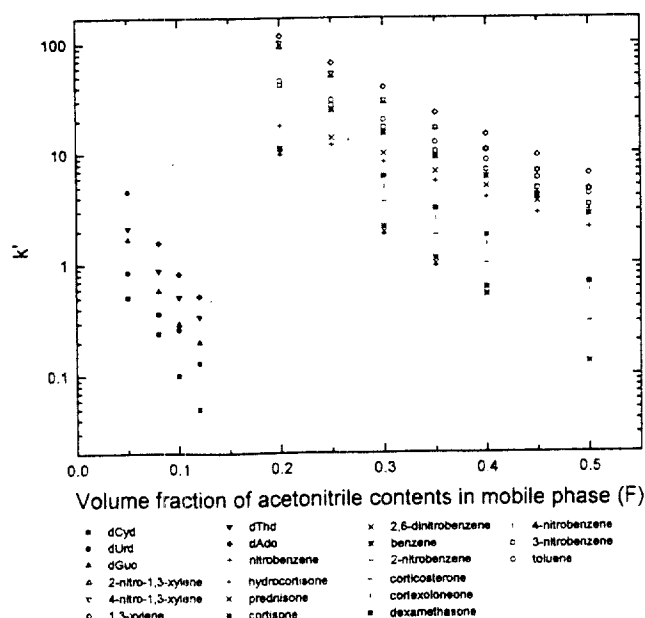


Fig. 2. Effect of volume fraction of acetonitrile content in mobile phase (F) on k' .

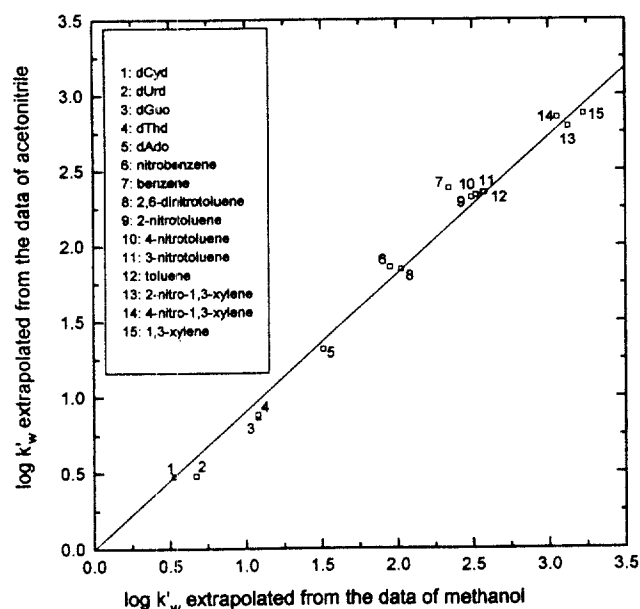


Fig. 3. Comparison of experimental values of k' extrapolated from data with methanol and acetonitrile.

dapak column in pure water probably corresponds to the hydrophobicity of the investigated deoxynucleosides, i.e. their affinity for this surface.

k'_w is the value of capacity factor for water only as mobile phase. In RP-HPLC, without the organic modifier in mobile phase, the retention time of sample is very long because water is passed through but the sample is retained on the hydrophobic C_{18} surface. The k'_w values can be obtained by extrapolation from the experimental dependences of $\log k'$ vs organic modifier content. As seen in Fig. 3, the dependence of the $\log k'_w$ intercepts calculated for acetonitrile vs the $\log k'_w$ intercepts calculated for methanol as organic modifier is fitted by straight

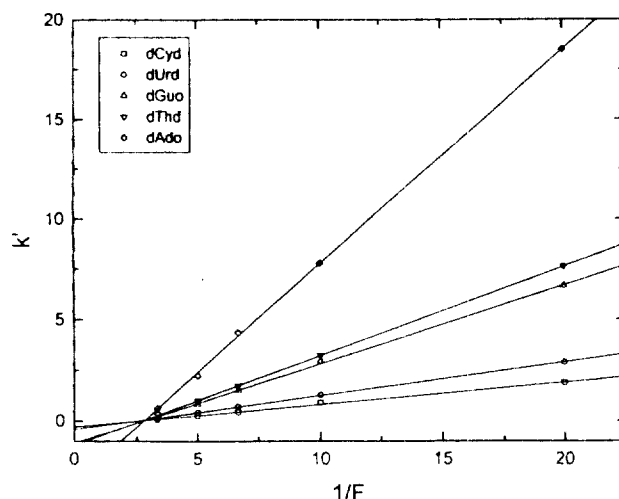


Fig. 4. Comparison of experimental k' and calculated k' from Eq. (5) with inverse of volume fraction of methanol content in mobile phase (F).

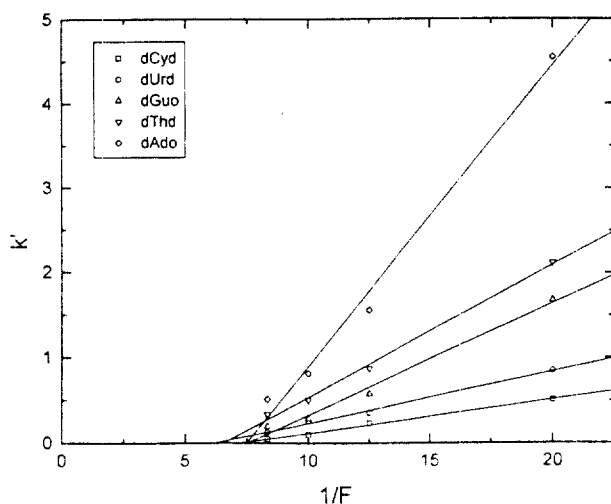


Fig. 5. Comparison of experimental k' and calculated k' from Eq. (5) with inverse of volume fraction of acetonitrile content in mobile phase (F).

line arising from origin. That is, the intercepts of Eq. (1) calculated by using the experimental values of $\log k'_w$ for different organic modifiers are almost coincident. The intercepts calculated are independent on the nature of organic modifier. The extrapolated values of $\log k'_w$ measured in the regions of different content of organic modifier appear to be in the functional dependence on hydrophobic parameters of solutes.

In Figs. 4, 5, the experimental data and linear regression lines are compared, and the lines are calculated by Eq. (5). The dependences of k' vs. $1/F$ plots are characterized by the different magnitudes of slopes for each deoxyribonucleosides. In the following Langmuir-type relationship,

$$k' = A + B(1/F) \quad (5)$$

the intercept, A , and the slope, B , was obtained by the regression analysis for the five deoxyribonucleosides and 10 nitro-compounds. These results are illustrated in Fig. 6 by straight

line arising from origin. As shown in the Figure, the intercepts calculated for acetonitrile and for methanol as organic modifiers are coincident. Specially, for the deoxyribonucleosides with the lower content of the organic modifiers, the values are closely same. At the higher content of the organic modifiers, the data points are slightly deviated from the diagonal line. The slopes (B) are different for deoxyribonucleosides and nitro-compounds, and will be shown later. As shown in Table 1, the ratios of the slopes are greatly divided by the type of sample. The table also shows that their ratios $B_{\text{methanol}}/B_{\text{acetonitrile}}$ of deoxyribonucleosides are varied in comparatively narrow range from 2.750 to 3.000, but the ratios of nitro-compounds are changed in wider range from 0.928 to 1.753. Therefore, in the small content of organic modifier, the ratios of slopes can characterize the properties of organic modifier only. As the content of organic modifier in mobile phase increases, the slopes are af-

ected by both the sample and the organic modifier simultaneously.

Linear regression was carried out according to Eqs. (1)-(5) for each solute (deoxyribonucleosides, nitro and steroid compounds) and organic modifier (methanol and acetonitrile). The slopes, intercepts, and correlation coefficients calculated are listed in Tables 2, 3 for the organic modifier of methanol and acetonitrile, respectively. The capacity factors of 6 steroid samples are listed with acetonitrile only in Table 3. For deoxyribonucleosides, one of the best fits are obtained when Langmuir-type relationship, Eq. (5), was used to approximate the experimental data k' as a function of F . The correlation coefficients (r^2) are always higher than 0.990, with the exception of the two cases, where they are higher than 0.987 (see Tables 2, 3). The $\log k'$ vs $\log(1/F)$ plots have the poorest correlations. Eq. (2) approximates in a good manner the data obtained for dGuo, dThd, and dAdo only with acetonitrile in the mobile phase (the correlation coefficients are more than 0.996). The two polynomial models, Eqs. (3) and (4), give relatively good correlation coefficients, but inherently, the parameters $[C, D$

Table 1. Ratio of slopes calculated for methanol and acetonitrile by Eq. (5)

Material	$B_{\text{MeOH}}/B_{\text{ACN}}$
dC	2.750
dU	2.833
dG	3.000
dT	2.750
dA	2.972
Nitrobenzene	1.253
Benzene	0.928
2,6-Dinitrotoluene	1.468
2-Nitrotoluene	1.322
4-Nitrotoluene	1.359
3-Nitrotoluene	1.472
Toluene	1.618
2-Nitro-1,3-xylene	1.580
4-Nitro-1,3-xylene	1.368
1,3-Xylene	1.753

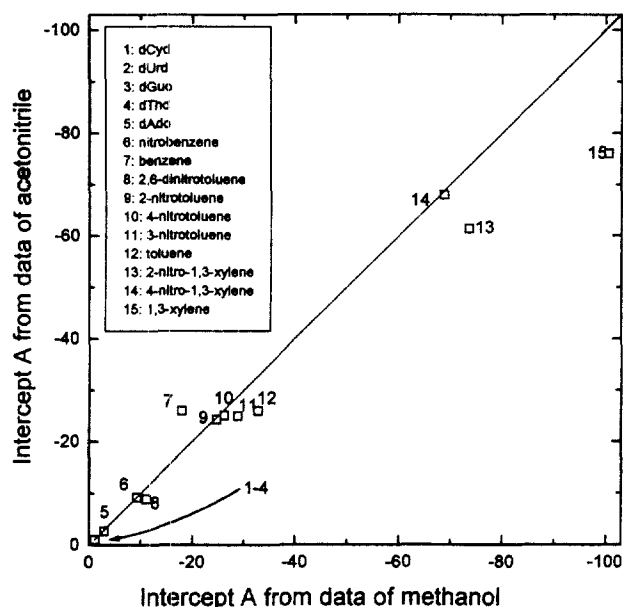


Fig. 6. Comparison of intercept (A) from data of methanol and acetonitrile.

Table 2. Calculated results of the parameters used in Eqs. (1)-(5) in organic modifier of methanol

	(1)			(2)			(3)				(4)				(5)		
	$\log k'$	S	r^2	H	K	r^2	C	D	E	r^2	L	M	N	r^2	A	B	r^2
dC	0.53	-5.64	0.9977	-1.90	1.77	0.9379	42.88	-21.83	2.79	0.9788	-57.82	-4.87	0.10	0.9915	-0.27	0.11	0.9965
dU	0.67	-5.27	0.9933	-1.63	1.67	0.9595	67.67	-33.91	4.27	0.9662	-4.17	-10.44	0.45	0.9898	-0.42	0.17	0.9999
dG	1.08	-5.84	0.9977	-1.45	1.84	0.9453	158.39	-79.37	9.91	0.9732	72.56	-25.69	1.33	0.9979	-1.06	0.39	0.9992
dT	1.08	-5.29	0.9902	-1.24	1.69	0.9683	183.49	-91.03	11.30	0.9658	40.37	-18.30	1.14	0.9996	-1.17	0.44	0.9999
dA	1.51	-5.74	0.9963	-0.98	1.81	0.9497	440.42	-219.94	27.36	0.9669	56.61	-23.21	1.68	0.9995	-2.96	1.07	0.9997
Nitrobenzene	1.95	-2.79	0.9973	-0.41	3.06	0.9913	99.41	-129.18	43.19	0.9925	1.00	-3.79	2.19	0.9993	-9.31	6.68	0.9793
Benzene	2.34	-3.28	0.9979	-0.44	3.60	0.9914	201.76	-254.90	81.71	0.9865	1.11	-4.40	2.61	0.9996	-17.98	12.06	0.9607
2,6-Dinitrotoluene	2.03	-2.66	0.9987	-0.23	2.91	0.9867	112.27	-149.50	51.53	0.9960	0.46	-3.13	2.14	0.9992	-11.15	8.28	0.9889
2-Nitrotoluene	2.49	-3.35	0.9970	-0.35	3.68	0.9926	281.55	-353.58	112.42	0.9852	1.36	-4.72	2.81	0.9995	-24.69	16.40	0.9555
4-Nitrotoluene	2.52	-3.36	0.9972	-0.32	3.68	0.9923	298.90	-375.51	119.43	0.9852	1.31	-4.67	2.83	0.9995	-26.25	17.44	0.9558
3-Nitrotoluene	2.57	-3.38	0.9974	-0.29	3.71	0.9921	328.67	-412.86	131.26	0.9853	1.27	-4.66	2.87	0.9996	-28.89	19.17	0.9557
Toluene	2.58	-3.09	0.9664	-0.03	3.37	0.9509	357.93	-458.66	150.21	0.9770	-0.16	-2.93	2.54	0.9664	-32.79	22.76	0.9621
2-Nitro-1,3-xylene	3.13	-4.04	0.9961	-0.30	4.44	0.9938	903.40	-1102.68	336.45	0.9734	1.89	-5.95	3.58	0.9994	-73.53	46.11	0.9203
4-Nitro-1,3-xylene	3.06	-3.87	0.9967	-0.22	4.25	0.9936	830.78	-1019.93	313.82	0.9760	1.71	-5.95	3.46	0.9997	-68.74	43.61	0.9288
1,3-Xylene	3.23	-3.81	0.9987	-0.01	4.17	0.9893	1167.49	-1449.86	452.57	0.9821	0.94	-4.75	3.45	0.9997	-100.58	64.65	0.9442

Table 3. Calculated results of the parameters used in Eqs. (1)-(5) in organic modifier of acetonitrile

	(1)			(2)			(3)				(4)				(5)		
	log k'	S	r ²	H	K	r ²	C	D	E	r ²	L	M	N	r ²	A	B	r ²
dC	0.48	-14.62	0.9844	-3.62	2.61	0.9453	66.69	-18.00	1.25	0.9968	1.39	-6.13	0.57	0.9981	-0.28	0.04	0.9906
dU	0.48	-11.14	0.9898	-2.67	2.01	0.9742	126.41	-31.37	2.09	0.9906	4.39	-6.83	0.77	0.9976	-0.37	0.06	0.9956
dG	0.86	-13.45	0.9847	-2.97	2.46	0.9964	368.36	-83.45	4.92	0.9987	2.54	-6.73	1.14	0.9989	-0.98	0.13	0.9876
dT	0.88	-11.48	0.9940	-2.38	2.09	0.9963	377.08	-89.26	5.63	0.9990	6.13	-7.46	1.22	0.9984	-1.02	0.16	0.9961
dA	1.32	-13.66	0.9917	-2.57	2.49	0.9973	979.15	-223.30	13.25	0.9980	3.25	-6.89	1.59	0.9983	-2.67	0.36	0.9892
Nitrobenzene	1.86	-2.96	0.9973	-0.36	2.36	0.9927	197.90	-188.50	47.24	0.9947	1.80	-4.38	2.06	0.9998	-9.20	5.33	0.9943
Benzene	2.38	-3.69	0.9966	-0.43	2.99	0.9941	575.33	-524.20	121.61	0.9982	2.63	-5.79	2.67	0.9999	-25.93	13.00	0.9782
2,6-Dinitrotoluene	1.84	-2.64	0.9978	-0.26	2.36	0.9957	159.40	-163.09	44.56	0.9886	1.68	-3.93	1.86	0.9997	-8.91	5.64	0.9956
2-Nitrotoluene	2.32	-3.46	0.9949	-0.35	2.85	0.9957	547.78	-497.41	116.08	0.9878	3.04	-5.88	2.66	0.9998	-24.47	12.41	0.9787
4-Nitrotoluene	2.33	-3.47	0.9953	-0.33	2.84	0.9955	564.05	-512.75	119.83	0.9876	2.92	-5.79	2.66	0.9999	-25.06	12.83	0.9791
3-Nitrotoluene	2.35	-3.51	0.9971	-0.29	2.81	0.9913	531.11	-492.74	117.92	0.9948	2.04	-5.14	2.58	0.9994	-24.93	13.02	0.9881
Toluene	2.35	-3.36	0.9980	-0.13	2.64	0.9896	538.17	-508.29	124.72	0.9962	1.50	-4.55	2.52	0.9994	-25.91	14.07	0.9926
2-Nitro-1,3-xylene	2.79	-3.96	0.9938	-0.30	3.30	0.9969	1445.20	-1275.47	284.93	0.9786	3.93	-7.10	3.23	0.9999	-61.35	29.18	0.9588
4-Nitro-1,3-xylene	2.85	-4.05	0.9926	-0.34	3.41	0.9975	1622.98	-1423.24	314.95	0.9763	4.44	-7.58	3.35	0.9999	-67.92	31.89	0.9530
1,3-Xylene	2.87	-3.91	0.9957	-0.11	3.19	0.9951	1736.00	-1551.07	352.34	0.9837	3.15	-6.41	3.23	0.9999	-75.94	36.87	0.9691
Hydrocortisone	2.16	-6.15	0.9955	-2.17	4.59	0.9882	181.86	-157.21	33.55	0.9286	3.10	-8.32	2.51	0.9981	-7.84	3.33	0.9580
Prednisone	2.21	-6.24	0.9970	-2.19	4.66	0.9904	191.14	-165.36	35.31	0.9307	2.72	-8.15	2.51	0.9989	-8.27	3.51	0.9550
Cortisone	2.29	-6.35	0.9976	-2.18	4.72	0.9832	208.38	-180.57	38.63	0.9308	1.22	-7.20	2.43	0.9979	-9.06	3.86	0.9606
Corticosterone	2.15	-5.37	0.9991	-1.97	4.87	0.9978	105.09	-100.31	24.20	0.9872	2.33	-7.25	2.52	0.9998	-4.92	2.47	0.9801
Cortexolone	2.03	-4.57	0.9947	-1.49	4.16	0.9997	135.36	-129.37	31.45	0.9872	5.46	-8.98	2.89	0.9998	-6.18	3.21	0.9814
Dexamethasone	2.17	-4.73	0.9943	-1.47	4.31	0.9998	175.46	-166.85	40.26	0.9863	5.89	-9.49	3.10	0.9999	-7.86	4.03	0.9783

and E in Eq. (3), L, M and N in Eq. (4)] do not correlate with any properties of solutes or organic modifiers. The two equations are empirical equations only, and each has three parameters, one more parameter compared to the other three equations. Eq. (4) is specially useful when the content of organic modifier is higher (normally between 0.3 and 0.7 of F). The equation fits better because of logarithmic scale and more parameters to be fixed. Disappointed results are observed when the correlation coefficients in Eq. (5) are relatively low in the samples of nitro and steroid compounds. As mentioned before, the data of the samples are obtained at higher content of organic modifier. In such case, the competitive adsorption of sample and organic modifier is done on the C₁₈ surface. This means the Langmuir-type relationship of Eq. (5) is not adequate, and more complex equation considering the interactions between sample and organic modifier is required. Finally, the slopes S of different solutes calculated by Eq. (1) for each organic modifier are approximately coincided. Their ratio $S_{\text{acetonitrile}}/S_{\text{methanol}}$ of deoxyribonucleosides varies in comparatively narrow range from 2.11 to 2.60 with an average value of 2.30, while the ratios of nitro-compound are close to 1.00. In fact, these slopes are practically same for different deoxyribonucleosides. This conclusion for deoxyuridine and its derivatives was reported [Valko et al., 1989], where the slope values had not correlated to the hydrophobic properties of solutes. So the slopes of Eq. (1) characterize only the properties of organic modifier in the case of solute considered.

Fig. 7 (1-5) shows the comparison of the experimental data (deoxyribonucleosides, nitro and steroid compounds) and the calculated values from the five retention models investigated. Apparently the agreement is the best with Eq. (4), log-scale polynomial-type relationship. In Eq. (5), the deviated points from the diagonal line are mainly caused by the higher content of or-

ganic modifier, while the data of deoxyribonucleosides are almost on the line.

CONCLUSION

The capacity factors of the deoxyribonucleosides with respect to the composition and type of mobile phase were measured under isocratic RP-HPLC. From results of comparison of the five retention models, Eqs. (1)-(5), the Langmuir-type retention model with the two parameters of A and B shows excellent agreements between the experimental capacity factors and calculated values especially in the small content of organic modifier. This new model is established based on the Langmuir adsorption, so the parameters have the physical meaning. But it does not fit well in the large content of organic modifier. For a wider use, Eq. (2) needs modification by adding a term of the interference effect between organic modifier and solute. This will be a subject for further improvement. Over the whole range of organic modifier, it is cautiously recommended that Eq. (4) will be a better predictable approach than the simpler form of Snyder relation, Eq. (1).

NOMENCLATURE

- A, B : empirical constants used in Eq. (5)
- C, D, E : empirical constants used in Eq. (3)
- F : volume fraction of organic modifier in mobile phase
- k' : capacity factor
- k_w' : capacity factor for pure water as mobile phase
- K, H : empirical constants used in Eq. (2)
- L, M, N : empirical constants used in Eq. (4)
- r² : regression coefficient defined by Eq. (6)
- S : empirical constant used in Eq. (1)

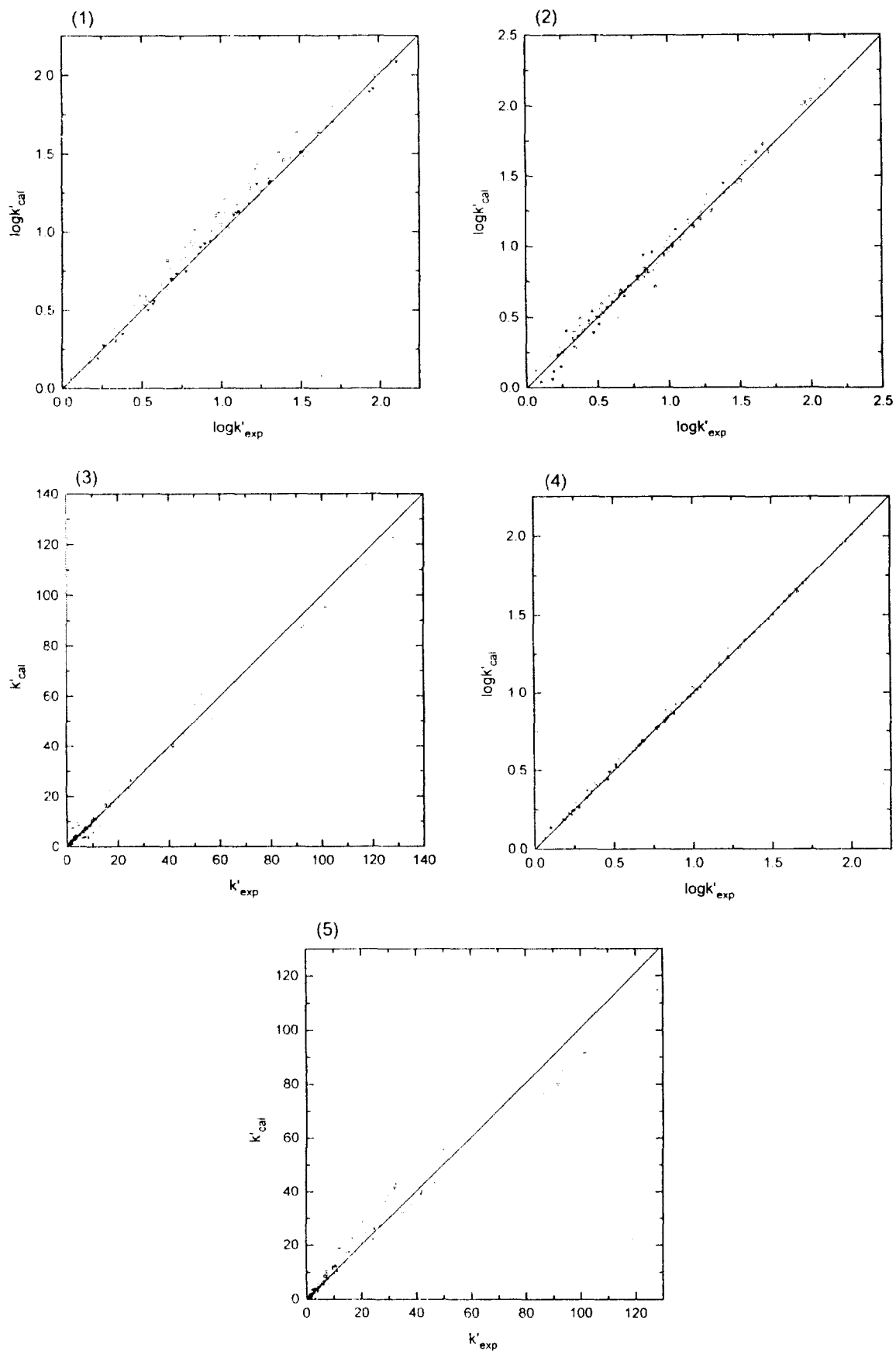


Fig. 7. Comparison of experimental k' and calculated k' in organic modifier of methanol and acetonitrile. (1) from Eq. (1), (2) from Eq. (2), (3) from Eq. (3), (4) from Eq. (4), (5) from Eq. (5).

V_0 : retention volume of unretained component [cm^3]

V_R : retention volume of component [cm^3]

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