

CHROM. 16,510

## DEVELOPMENT OF A HIGH-PERFORMANCE LIQUID CHROMATOGRAPH WITH ARTIFICIAL INTELLIGENCE

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### SUMMARY

An important aspect of chromatography is the ability to develop a **chromatograph** with artificial intelligence, with which an analytical method can be developed automatically for high-performance liquid chromatography (HPLC), which means that the optimal mobile phase composition can be selected from scouting tests, and optimal operating parameters can be also fed back in order to obtain the final analytical results. Second, qualitative information for the peaks, obtained from the **chromatogram**, especially for an unknown mixture, could be given by the **chromatographic** method. For this purpose, fundamental retention equations were derived for **undissociated** solutes, weak acids, weak organic bases and amphoteric compounds in liquid-solid chromatography with variation of the mobile phase composition.

The program RVPKLC-83 was developed for calculating the parameters of these equations from experimental data, and the validity of the equations was verified experimentally. The program OMPCLC-83 was developed for predicting the optimal mobile phase composition. An HPLC instrument equipped with a rapid-scanning UV detector system was used. The program ChgrA-83 is being developed for determining the purity of peaks and for detecting unresolved peaks. Several examples of the applications of this method are given.

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### INTRODUCTION

High-performance liquid chromatographic (HPLC) columns packed with **microparticulate silica** and chemically bonded silica, have been widely used to solve various analytical problems in different **fields**<sup>1-5</sup>. A long column is required only for analyzing very complex mixtures. A column with a length of less than 25 cm and an inner diameter of less than 2 mm, having a column efficiency of several thousand or even more than 10,000 theoretical plates, is often used for ordinary analysis". Up to now, mobile phase selectivity has been approached experimentally. For this purpose, new models of liquid **chromatographs** for automatic method development have been reported by many workers, and solvent selectivity scouting has been added in recent advances in LC theory and instrumentation<sup>7-18</sup>. A solvent selectivity triangle concept, used together with computerized mapping techniques to formulate a four-solvent, seven-experiment selectivity optimization procedure for isocratic LC, has been **de-**

veloped<sup>7,8</sup>. However, in all these instances, a linear relationship between the logarithm of the capacity factor ( $\ln k'$ ) and the volume fraction ( $C_B$ ) of the stronger solvent has been used. Of course, deviations from linearity are expected from theory and are observed in practice. A simple procedure for the rapid optimization of binary and ternary mobile phase mixtures has been reported by Schoenmaker and co-workers<sup>12-16</sup>. A repetition of the optimization routine can give the final optimal composition for solutes exhibiting a significant deviation from linearity of  $\ln k'$  with solvent composition<sup>16</sup>. Another simple procedure, developed by Berridge<sup>17,18</sup>, is based on the chromatography response function and the second derivative of the chromatogram. However, standard optimal operating conditions must be used for testing any of the simplified procedures. Thus, a remaining problem is the establishment of the relationships between the retention and the composition of the mobile phase over the whole concentration range. In this paper, some new relationships for the whole concentration range of binary and ternary solvent systems and also for ionic solutes are reported. The influence of the concentration of the solutes in the mixture to be analysed on resolution is discussed and a procedure for establishing the optimal mobile phase composition is described. Software for this has been developed and incorporated in the main computer of the chromatography data base at our Institute. In order to test the purity of the separated peaks, a high-performance liquid chromatograph with a photodiode array UV detection system and a curve-fitting method are being developed.

## THEORETICAL

### Optimization criterion

The aim of any chromatographic method is to separate a mixture into individual peaks for qualitative and quantitative determination. A good relationship between the resolution criterion,  $K_1$  and the accuracy of analytical method has been reported<sup>19,20</sup>. For example, we can easily calculate the required value of  $K_1$  from the relative accuracy of the quantitative determination with the peak-height method,  $P_h$ , and the peak-height ratio  $\phi$  according to the following equation:

$$P_h = \phi e^{-2.77K_1^2} \quad (1)$$

The resolution criterion  $K_1$  can be used as an optimization criterion to select the optimal mobile phase composition in considering the effect of the peak-height ratio of the two adjacent peaks on the required value of  $K_1$ .

Hence:

$$\begin{aligned} K_1 &= \frac{t_{r_2} - t_{r_1}}{\Delta t_{1/2(1)} + \Delta t_{1/2(2)}} \\ &= \frac{1}{2.354} (\alpha' - 1)n \cdot \\ &= \frac{1}{2.354} (\alpha' - 1) \frac{1 + k'}{\beta + k'} \cdot \sqrt{n^\infty} \\ &= \frac{1}{2.354} \cdot s \sqrt{n^\infty} \end{aligned} \quad (2)$$

where  $S$  is the selectivity criterion of the column system:

$$S = (\alpha' - 1) \frac{(1 + k')}{(\beta + k')} \quad (2')$$

$n$  is the number of theoretical plates of the column,  $\sqrt{n^\infty}$  is the number of the theoretical plates determined by the solute having a capacity ratio approaching infinity and  $\beta$  is the coefficient of variation of plate number with variation of the capacity ratio. For HPLC with minimization of the extra-column effect,  $n$  may be about constant for all solutes under given operation conditions. Even on changing the mobile phase composition, the difference in  $n$  is not very large. Generally, for an ordinary instrument, it is very easy to have a column with an efficiency about 8000 theoretical plates.  $\alpha'$  is the relative retention time of two adjacent peaks (not the relative adjusted retention time). Therefore, in this instance, we can also use  $\alpha'_{\min}$  directly as an optimization criterion.

If  $n$  for solutes having different  $k'$  is not the same, and the linear relationship between peak width and the retention value can be used, then the required minimal relative retention  $\alpha'_{\min}$  for solutes having different  $k'$  is different and a correction is needed. Therefore, the principle of choosing the optimal composition for a given instrument is to find the optimal mobile phase concentration with which the lowest value of  $\alpha'_{\min}$  of two adjacent peaks is higher than the required value and simultaneously to keep the retention value of the last peak as small as possible. It is clear that the problem is to obtain equations to describe the variation of retention value with mobile phase composition over the whole concentration range.

### ***Fundamental retention equations in liquid-solid chromatography***

An equation for liquid-solid chromatography (LSC) relating the retention to some thermodynamic parameters was derived by Snyder in 1968<sup>21</sup> using the displacement adsorption model. Another description of the effects of solvent composition on retention based on a multilayer sorption model in LSC was given by Scott and Kucera<sup>22</sup>. Recently, we proposed the following relationship between the capacity ratio,  $k'$ , and the composition of the binary mobile phase based on the displacement adsorption solution interaction model<sup>23,24</sup>:

$$\ln k' = a + bC_B + c \ln C_B \quad (3)$$

The chromatographic retention behaviour can be described by eqn. 3 over a wide concentration range. The parameters  $a$ ,  $b$  and  $c$  have their own definite physico-chemical meanings. Parameter  $a$  is related to the adsorption energy of the solute, molecular interactions between the solute and solvents, surface area of the adsorbents, phase ratio, molecular weight of the solute, the configuration factor of the mobile phase and temperature. Parameter  $b$  is only involved in the solution interactions existing in the given mobile phase. Parameter  $c$  is an entropy function of an adsorbed solute. For the functional relationships of individual parameters, see refs. 23 and 24.

The validity of eqn. 3 has been established using some PTH-amino acids<sup>24</sup> with normal-phase elution and 16 different compounds with reversed-phase elution<sup>25</sup>.

The accuracy of predicting retention values by eqn. 3 has been reported<sup>26</sup>. A simplified method can be used to calculate the value of  $a$ ,  $b$  and  $c$  in eqn. 3 after four experimental steps, three retention values in the low-concentration range of a stronger solvent and another one with pure or a high concentration of the stronger solvent. Parameters  $a$  and  $c$  can be calculated from the first three retention data as a limiting condition according to the following equation:

$$\ln k' = a + c \ln C_B \quad (4)$$

and  $b$  can be calculated from the fourth datum according to eqn. 5 (with pure stronger solvent) or 6 (in the higher concentration range of stronger solvent).

$$b = \frac{\ln k' - a}{C_B} \quad (5)$$

$$b = \frac{\ln k' - a - c \ln C_B}{C_B} \quad (6)$$

The equations mentioned above are still limited in the case of the retention behaviour of undissociated solutes when a binary mobile phase is used. With advances in HPLC, multi-component elution has been adopted extensively for separating very complex mixtures. However, when  $C_B$  approaches zero, a limiting value of  $\ln k'$  cannot be properly adopted. A series of new equations<sup>27</sup> were derived by the statistical thermodynamic method, using the smoothed-potential model of dilute solutions and the ideal localized monolayer adsorption of solutes and solvents (see Appendix).

$$\ln k' = a' + \sum_{i=1}^n b_i C_{B_i} + c \ln \left( 1 + \sum_{i=1}^n k_i C_{B_i} \right) \quad (7)$$

$$\ln k'_{\text{acid}} = \ln k' - \ln (1 + K_{a(1)}/[H^+] + K_{a(1)}K_{a(2)}/[H^+]^2) \quad (8)$$

$$\ln k'_{\text{base}} = \ln k' - \ln (1 + 10^{14} K_{b_1} [H^+] + 10^{28} K_{b_1} K_{b_2} [H^+]^2) \quad (9)$$

$$\ln k'_{\text{am}} = \ln k' - \ln (1 + K_a/[H^+] + 10^{14} K_b [H^+]) \quad (10)$$

The validity of eqn. 7 was shown in ref. 26. By using a column packed with chemically bonded silica YQG-C<sub>16</sub> and a buffer solution of KH<sub>2</sub>PO<sub>4</sub> with different pH values as the mobile phase, eqns. 8 and 9 were confirmed<sup>28</sup> with weak organic acids such as vanillic acid (VA), vanillmandelic acid (VMA) and 3,4-dihydroxybenzoic acid (DHBA) and organic bases such as epinephrine (E), norepinephrine (NE) and dopamine (DA).

A series of programs which are used to predict optimal mobile phase composition, have been developed for the main computer of the chromatography data base at our Institute. The program RVPKLC-83 was developed for calculating the

parameters  $a$ ,  $a'$ ,  $b_1$ ,  $b_2$ ,  $c$ ,  $k_1$ ,  $k_2$ , the equilibrium constant  $K_a$ , and  $K_b$ , etc., in eqns. 7-9 and giving the accuracy of the method.

Using the program OMPCLC-83 we can calculate the values of  $\alpha'_{\min}$  of two adjacent peaks, compare them and obtain the lowest value of  $\alpha'_{\min}$  and the retention capacity of the last peak,  $k'_{\text{last}}$ , and, after comparison of all these results with variation of the mobile phase composition over the whole concentration range, the optimal mobile phase composition can be suggested initially by computer if the required value of  $K_1$  is calculated according to eqns. 1 and 2.

### *Detecting the unseparated peak*

In practice, it is very important to detect qualitatively the peak shifts of an unknown mixture due to changes in the mobile phase composition. Therefore, we used an on-line fast-scanning photodiode array UV detector to follow the peak shifts and also to detect the components in unseparated peaks. Also, a program (**ChgrA-83**) of the chromatogram analyser is being developed that is able to obtain some information on unseparated peaks. Because any actual peak in a chromatogram is unsymmetrical, the first task of the chromatogram analyser is to describe an unsymmetrical peak with an exponentially modified Gaussian curve<sup>29-32</sup>.

$$h(t) = \frac{1}{\tau\sigma\sqrt{2\pi}} \int_0^{\infty} \exp \left[ - \left( \frac{t - t_g - t'}{\sqrt{2}\sigma} \right)^2 - \frac{t'}{\tau} \right] dt' \quad (11)$$

where  $A$  is the peak area,  $\tau$  is the time constant of the exponential decay,  $\sigma$  is the standard deviation of the Gaussian,  $t_g$  is the centre of gravity of the Gaussian component and  $t'$  is a dummy variable of integration, A Sigma 10 instrument (**Perkin-Elmer**) can be used for on-line calculation of  $t_g$ ,  $\sigma$ ,  $\tau$  and  $A$  directly from the chromatogram.

In order to analyse the whole chromatogram including the separated and unseparated peaks, we must investigate the variation of  $\sigma$  and  $\tau$  with retention values. A simple linear relationship between  $\sigma$  or  $\tau$  and the retention time has been reported<sup>32</sup>. We can predict the values of  $\sigma$  and  $\tau$  for all peaks, including unresolved ones, with this relationship. In this way, the whole chromatogram can be calculated and compared with others, and give some information on some unresolved peaks. In order to develop a standard optimal method especially for the separation of an unknown mixture, it is evident that not only an optimization criterion and the retention equation over the whole concentration range of the mobile phase are required, but also an on-line fast scanning detector and a chromatogram analyser must be used.

### EXPERIMENTAL

A Model K-2 high-performance liquid chromatograph constructed at our Institute was used. The different kinds of K-2 HPLC columns, with different lengths packed with silica YWG-5 (Qingdao Ocean Chemical Plant, Qingdao, China) or YQG-CH(2)-5 home-made hexadecyldimethylsilica beads, were used. The sampling system was a Model 7413 sample injection valve (Rheodyne) with a 0.5- $\mu$ l loop. A Model BT-3030 UV detector (Biotronik) and a variable-wavelength UV spectrometer

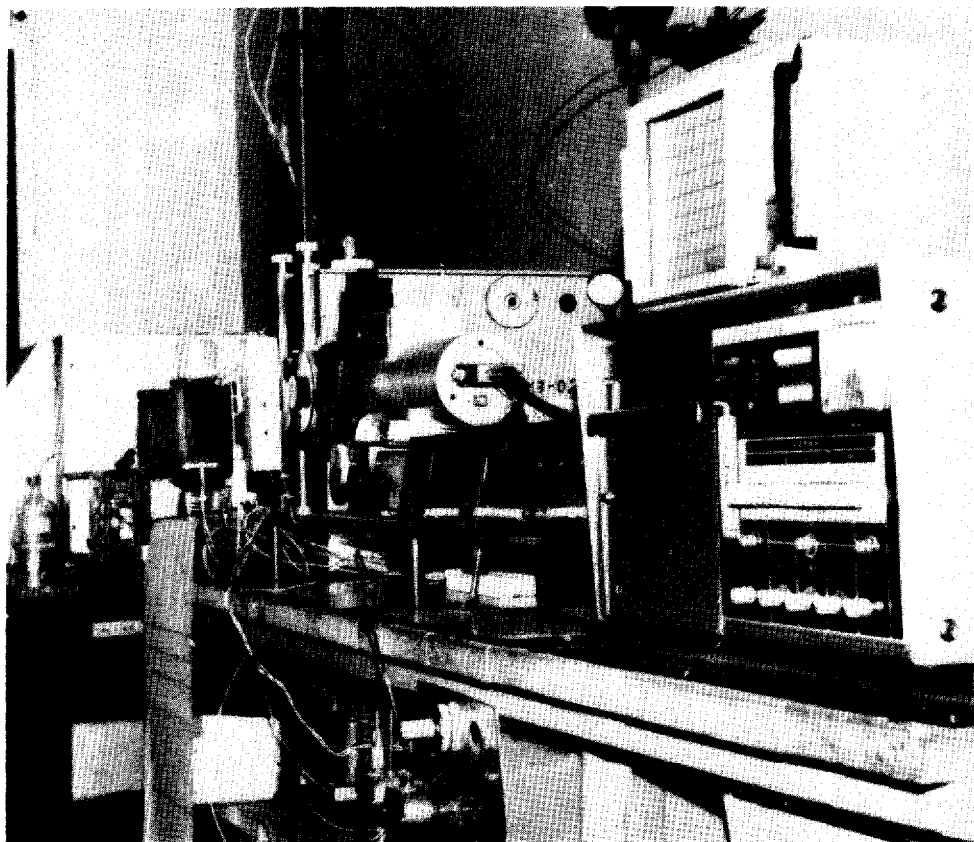


Fig. 1. HPLC instrument with a rapid-scanning UV detector system.

(Knauer) were also used. A Model 1205-A optical multi-channel analyser with a Model 1205-D serial silicon intensified target (Princeton Applied Research), a Model WDS-3 spectrophotometer (Tianjin Optical Factory) and a Model SC-16 oscillograph (Shanghai Electrometer Factory) were connected and used as a rapid scanning UV detector system as shown in Fig. 1.

The solvents used were analytical-reagent grade n-hexane, diethyl ether and methanol and redistilled water. In order to obtain mixed solvents with sufficient accuracy for normal-phase elution, pipettes were used. Tetrachloroethylene was used as an unretained substance to determine the dead time accurately. For reversed-phase elution, the dead time was measured by injecting pure methanol when the eluent had a high water content and was measured by injecting pure water when the eluent had a high methanol content. Ten aromatic hydrocarbons, oxygen and nitrogen compounds were used as solutes.

The Sigma 10 instrument was used for on-line calculation of the parameters of each peak. A Model VS-80 computer (Wang Laboratories) and a Model TRS-80 microcomputer (Radio Shack) were used to program the equations.

## RESULTS AND APPLICATIONS

Applications of the above approach are given to demonstrate normal- and reversed-phase elution. An example is also given of the determination of the purity of separated and unresolved peaks by using the on-line rapid scanning UV detection system and the software of the chromatogram analyser.

**Normal-phase elution**

A **K-2** column (150 × 2 mm I.D.) having an efficiency of about 8000 plates was used. The retention values of six compounds were determined with variation of the mobile phase composition. The program RVPKLC-83 was used for the calculation of  $a'$ ,  $b_i$ ,  $c$  and  $k_i$  in eqn. 7. With the calculated values of these parameters and the program OMPCLC-83, the optimal mobile phase composition was obtained and the chromatogram under these operating conditions was obtained to test the method. The results are shown in Fig. 2 and Table I. The separation is in agreement with prediction.

**Reversed-phase elution**

A **K-2** column packed with **YQG-CH(2)-5** chemically bonded silica beads, with a column efficiency about 7000 plates, was used. The solvents were methanol and redistilled water. The retention values of five compounds were determined with variation of the methanol content of the mobile phase for calculating the parameters  $a'$ ,  $b_i$ ,  $c$  and  $k_i$  in eqn. 7 with the program RVPKLC-83. We predicted the optimal mobile phase composition with the data obtained. The results are shown in Table II. By using the predicted optimal operating conditions, the chromatogram shown in Fig. 3 was obtained, and demonstrates the validity of the method.

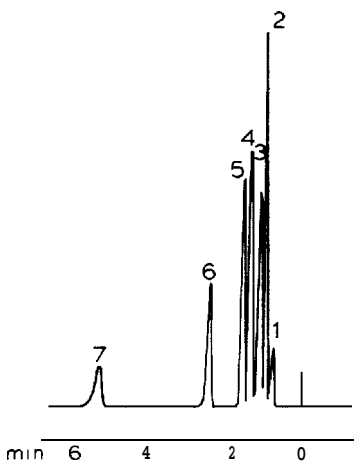


Fig. 2. Chromatogram of normal-phase separation with the optimal mobile phase composition predicted by the program OMPCLC-83. Column, **YWG-5u**, 150 × 2 mm I.D.; mobile phase, *n*-hexane-diethyl ether (84.7: 15.3); flow-rate, 0.40 ml/min; detector, UV (254 nm). Compounds: 1, tetrachlorethylene; 2, ethyl phenyl ether; 3, butyl benzoate; 4, o-nitrotoluene; 5, nitrobenzene; 6, 2,6-dimethylphenol; 7, *m*-dinitrobenzene.

TABLE I

EXAMPLE FOR PREDICTING THE OPTIMAL MOBILE PHASE COMPOSITION WITH THE PROGRAM OMPCLC-83

N = 8000;  $t_0 = 1.2$  min; packing, YWG, 5  $\mu\text{m}$ ; mobile phase, *n*-hexane-diethyl ether (84.7: 15.3); minimal  $a'$  value, 1.11.

No.	Compound	$k'$	$t_R$	$a'$
1	Ethyl phenyl ether	0.14	1.36	1.11
2	Butyl benzate	0.26	1.51	1.11
3	<i>o</i> -Nitrotoluene	0.4	1.68	1.13
4	Nitrobenzene	0.58	1.9	1.18
5	2,6-Dimethylphenol	0.87	2.24	2.34
6	m-Dinitrobenzene	3.37	5.24	

**Detecting the purity of the peaks and unresolved peaks by a rapid scanning UV detector system and a chromatogram analyser**

The column (500  $\times$  2 mm I.D.) was packed with YWG-5 irregular microparticulate silica. The instrument used is shown in Fig. 1. Because of the connection of two detectors, the extra-column effect was larger. Even in this instance there is the linear relationship between  $a$ ,  $\tau$  and the retention values of separated peaks, as shown in Fig. 4. For this relationship we can predict the values of  $\sigma$  and  $\tau$  for unseparated peaks, and then the whole chromatogram can be calculated as shown in Fig. 5. The symbols \* are the actual values and the points are the calculated values.

Fig. 6 shows the fast-scanning UV spectra of separated peaks. These peaks may be pure compounds, as shown by comparison with the spectra of the corresponding pure compounds. This shows the possibility of using this method to test the purity of a peak. The method is being developed further.

CONCLUSIONS

From the examples mentioned above, it can be concluded that to elaborate a rapid method development strategy, it is necessary to develop a standard method first. Therefore, fundamental equations to describe the variation of retention values

TABLE II

PREDICTING THE OPTIMAL MOBILE PHASE COMPOSITION FOR REVERSED-PHASE ELUTION WITH THE PROGRAM OMPCLC-83

N = 7000;  $t_0 = 1.5$  min; packing, YQG-CH (2), 5  $\mu\text{m}$ ; mobile phase, methanol-water (86.1: 13.9); minimal  $a'$  value, 1.11.

No.	Compound	$k'$	$t_R$	$a'$
1	Aniline	0.19	1.79	1.28
2	Nitrobenzene	0.53	2.29	1.11
3	Anisole	0.7	2.56	1.17
4	Benzophenone	1	3	1.17
5	Naphthalene	1.35	3.53	



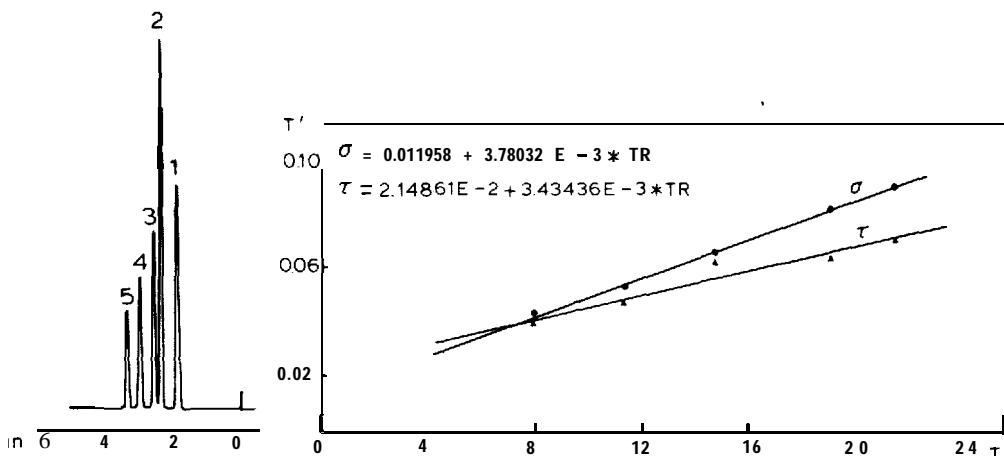


Fig. 3. Chromatogram of reversed-phase elution under the optimal operating conditions predicted by the program OMPCLC-83. Column, YQG-CH (2), 5  $\mu\text{m}$ , 150  $\times$  5 mm I.D.; mobile phase, methanol-water (86:14); flow-rate, 1.2 ml/min; detector, UV (254 nm). Compounds: 1, aniline; 2, nitrobenzene; 3, anisole; 4, benzophenone; 5, naphthalene.

Fig. 4. Relationship between  $\sigma$ ,  $\tau$  and the retention values of separated peaks.

with mobile phase composition for undissociated and weakly dissociated organic compounds in liquid-solid chromatography were derived. The program RVPKLC-83 was developed for calculating the parameters of these equations with a few experimental data. On the basis of these data, the optimal mobile phase composition can be predicted by using the program OMPCLC-83.

APPENDIX

*Derivatization of the fundamental retention equations*

Considering an isolated system which contains the solute A, solvents  $\mathbf{B}_1, \mathbf{B}_2, \dots, \mathbf{B}_n$ , and adsorbent S, there is no exchange of material between the system and the outside.

In dilute solution, the total partition function  $Q$  is equal to the product of the particular partition functions of all molecular species involved:

$$Q = (T_A J_A)^{N_A} \prod_{i=1}^n (T_{B_i} J_{B_i})^{N_{B_i}} \Omega(T) \tag{A1}$$

where  $T_A, T_{B_i}$  are translational partition functions,  $J_A, J_{B_i}$  the internal partition functions of the solute A and solvents  $B_i$ , respectively, and  $N_A \ll N_{B_1}, N_{B_2}, \dots, N_{B_n}$  and therefore the interactions between molecules of solute A can be neglected. The configuration partition function,  $\Omega(T)$ , can be written as

$$\Omega(T) = \frac{1}{N_A! \prod_{i=1}^n N_{B_i}!} \int \dots \int e^{-\frac{1}{kT}(X_A N_A) + \sum_{i=1}^n (X_{B_i} N_{B_i})} (dw_A)^{N_A} \prod_{i=1}^n (dw_{B_i})^{N_{B_i}} \tag{A2}$$

PERK NO 21

NI TO N22409.443

8 8

SGM= 3.91634E-2

NO= 25.1116 TR= 4.88624E-2  
TR= 8.18198 TP= 8.21



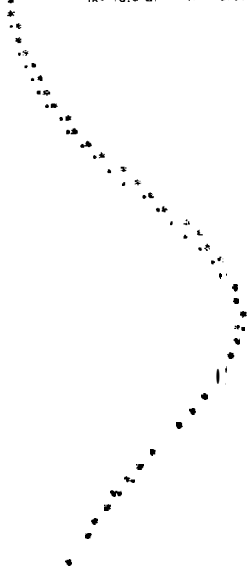
PERK NO 24

NI TO N221460.1514

8 8

SGM= 8.214E-2

NO= 24.1831 TR= 5.01331E-2  
TR= 18.0181 TP= 18.86



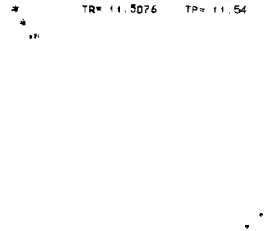
PERK NO 22

NI TO N22738.777

8 8

SGM= 5.26039E-2

NO= 19.2215 TR= 4.96992E-2  
TR= 11.5076 TP= 11.54



PERK NO 23

NI TO N221689.1747

8 8

SGM= 9.15129E-2

NO= 20.8971 TR= 6.00061E-2  
TR= 21.136 TP= 21.10



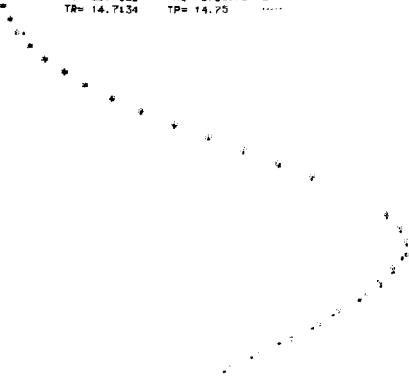
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NI TO N221085.1103

8 8

SGM= 6.45601E-2

NO= 35.9822 TR= 5.33976E-2  
TR= 14.7134 TP= 14.75



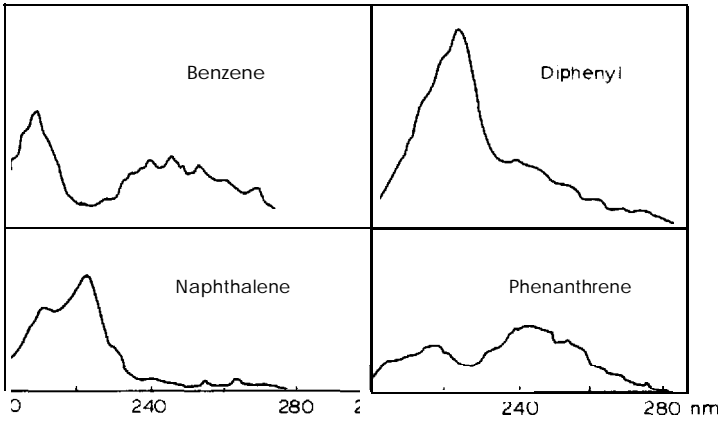
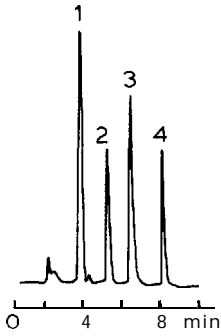


Fig. 6. On-line fast-scanning UV spectra of separated peaks. Column, YQG-CH, 5  $\mu\text{m}$ , 250  $\times$  2 mm I.D.; mobile phase, methanol-water (85:15); flow-rate, 0.2 ml/min; detector, UV (254 nm). Peaks: 1, benzene; 2, naphthalene; 3, diphenyl; 4, phenanthrene.

where the term  $X$  refers to the potential in the solution. According to the assumptions of the smoothed-potential model, we obtained

$$\Omega(T) = \frac{1}{N_A! \prod_{i=1}^n N_{B_i}!} e^{-\frac{1}{kT} (X_A N_A + \sum_{i=1}^n X_{B_i} N_{B_i})} \cdot V_0^{(N_A + \sum_{i=1}^n N_{B_i})} \tag{A3}$$

where  $V_0 = N_A V_A + \sum_{i=1}^n N_{B_i} V_{B_i}$  is the total volume of solution in this system.  $V_A$  and  $V_{B_i}$  refer to the volumes of individual molecule.

Fig. 5. Comparison of an actual (points \*) with a calculated chromatogram (points •).

Therefore, the Heimholtz function of solution is

$$\begin{aligned}
 F &= -RT \ln Q \\
 &= -RT \left\{ -\ln N_A! - \sum_{i=1}^n \ln N_{B_i}! \right. \\
 &\quad - \frac{1}{kT} \left( X_A + \sum_{i=1}^n X_{B_i} \frac{\partial N_{B_i}}{\partial N_A} \right) + \left( 1 + \sum_{i=1}^n \frac{\partial N_{B_i}}{\partial N_A} \right) \ln V_0 \\
 &\quad \left. + \ln (T_A J_A) + \sum_{i=1}^n \left[ \frac{\partial N_{B_i}}{\partial N_A} \ln (T_{B_i} J_{B_i}) \right] \right\} \tag{A4}
 \end{aligned}$$

The chemical potential of solute A in the solution can be obtained:

$$\begin{aligned}
 \mu_A &= \left. \frac{\partial F}{\partial N_A} \right|_{T,v} \\
 &= -RT \left\{ -\ln N_A - \sum_{i=1}^n \frac{\partial N_{B_i}}{\partial N_A} \ln N_{B_i} \right. \\
 &\quad - \frac{1}{kT} \left( X_A + \sum_{i=1}^n X_{B_i} \frac{\partial N_{B_i}}{\partial N_A} \right) + \left( 1 + \sum_{i=1}^n \frac{\partial N_{B_i}}{\partial N_A} \right) \ln V_0 \\
 &\quad \left. + \ln (T_A J_A) + \sum_{i=1}^n \left[ \frac{\partial N_{B_i}}{\partial N_A} \ln (T_{B_i} J_{B_i}) \right] \right\} \tag{A5}
 \end{aligned}$$

The number of the absorbed molecule A,  $B_1, \dots, B_i$  which have potentials  $X_A^a, X_B^a, \dots, X_{B_i}^a$ , is denoted by  $N_A^a, N_B^a, \dots, N_{B_i}^a$ . According to the ideal localized monolayers, the total partition function describing the surface situation of the absorbent is

$$Q^a = \frac{N_S!}{N_A^a! \prod_{i=1}^n N_{B_i}^a!} e^{-\frac{1}{kT} \left( X_A^a N_A^a + \sum_{i=1}^n X_{B_i}^a N_{B_i}^a \right)} \cdot (J_A^a)^{N_A^a} \prod_{i=1}^n (J_{B_i}^a)^{N_{B_i}^a} \tag{A6}$$

where  $J$  is the internal partition functions.  $N_s, N_A^a$  and  $\sum_{i=1}^n N_{B_i}^a$  refer to the number of the sites or the absorbed molecule A or B on the absorbent surface. The Helmholtz function for the molecules absorbed on the surface is

$$\begin{aligned}
 F^a &= -RT \ln Q \\
 &= -RT \left\{ \ln N_S! - \ln N_A^a! - \sum_{i=1}^n \ln N_{B_i}^a! \right.
 \end{aligned}$$

$$- \frac{1}{kT} \left[ X_A^a N_A^a + \sum_{i=1}^n (X_{B_i}^a N_{B_i}^a) \right] + N_A^0 \ln J_A^a + \sum_{i=1}^n (N_{B_i}^a \ln J_{B_i}^a) \} \quad (A7)$$

Similarly, the chemical potential of a solute A on the surface can be obtained as

$$\begin{aligned} \mu_A^a = & -RT \left\{ \left( 1 + \sum_{i=1}^n \frac{\partial N_{B_i}^a}{\partial N_A^a} \right) \ln N_S \right. \\ & - \ln N_A^a - \sum_{i=1}^n \left( \frac{\partial N_{B_i}^a}{\partial N_A^a} \ln N_{B_i}^a \right) \\ & - \frac{1}{kT} \left[ X_A^a + \sum_{i=1}^n \left( \frac{\partial N_{B_i}^a}{\partial N_A^a} X_{B_i}^a \right) \right] \\ & \left. + \ln J_A^a + \sum_{i=1}^n \left( \frac{\partial N_{B_i}^a}{\partial N_A^a} \ln J_{B_i}^a \right) \right\} \quad (A8) \end{aligned}$$

Because the system discussed is the isolated one and the substance cannot be interchangeable, then

$$\partial N_A^a = -\partial N_A \quad (A9)$$

$$\partial N_{B_i}^a = -\partial N_{B_i} \quad (A10)$$

Therefore,

$$\frac{\partial N_{B_i}^a}{\partial N_A^a} = \frac{\partial N_{B_i}}{\partial N_A} \quad (A11)$$

Substituting eqn. A11 into eqn. A8, we obtain

$$\begin{aligned} \mu_A^a = & -RT \left\{ \left( 1 + \sum_{i=1}^n \frac{\partial N_{B_i}}{\partial N_A} \right) \ln N_S \right. \\ & - \ln N_A^a - \sum_{i=1}^n \left( \frac{\partial N_{B_i}}{\partial N_A} \ln N_{B_i}^a \right) \\ & - \frac{1}{kT} \left[ X_A^a + \sum_{i=1}^n \left( \frac{\partial N_{B_i}}{\partial N_A} X_{B_i}^a \right) \right] \\ & \left. + \ln J_A^a + \sum_{i=1}^n \left( \frac{\partial N_{B_i}}{\partial N_A} \ln J_{B_i}^a \right) \right\} \quad (A12) \end{aligned}$$

The condition for equilibrium is that chemical potentials both in the solution and on the absorbent surface must be equal. We therefore derive the expression for the capacity ratio,  $k'$ :

$$\begin{aligned} \ln k' = & \sum_{i=1}^n \left( \frac{\partial N_{B_i}}{\partial N_A} \ln \frac{N_{B_i}}{N_{B_i}^a} \right) + \left( 1 + \sum_{i=1}^n \frac{\partial N_{B_i}}{\partial N_A} \right) \ln \frac{N_S}{V_0} \\ & + \ln \left( \frac{J_A^0}{T_A J_A} \right) + \sum_{i=1}^n \left[ \frac{\partial N_{B_i}}{\partial N_A} \ln \left( \frac{J_{B_i}^a}{T_{B_i} J_{B_i}} \right) \right] \\ & + \frac{1}{kT} \left\{ X_A - X_A^a + \sum_{i=1}^n \left[ \frac{\partial N_{B_i}}{\partial N_A} (X_{B_i} - X_{B_i}^a) \right] \right\} \end{aligned} \quad (\text{A13})$$

The potential function  $X_A$  is equal to the sum of the various interactions, including Van der Waals interactions and the hydrogen bond energy involved the molecule A, and can be expressed as

$$\begin{aligned} X_A = & \sum_{i=1}^n Z_{AB_i} E_{AB_i} + E_{AH} \\ = & \sum_{i=1}^n K_{AB_i} E_{AB_i} C_{B_i} + E_{AH} \end{aligned} \quad (\text{A14})$$

where the  $E_{AB_i}$  represent the Van der Waals energy of the each pair of adjacent molecules A and B,  $Z_{AB_i}$  the relative number of molecule B surrounding molecule A,  $C_B$  the volume concentration of solvent B,  $K_{AB_i}$  is the proportional constant and  $E_{AH}$  is the hydrogen bond energy of molecule A.

The potential energy of the absorbed molecule A can be written in a similar form:

$$X_A^a = \sum_{i=1}^n K_{AB_i}^a E_{AB_i} C_{B_i} + E_A^a \quad (\text{A15})$$

where the  $E_A^a$  refer to the absorption energy of molecule A (if a hydrogen bond is formed between the absorbed molecule A and solvent molecules, the term  $E_A^a$  also includes the possible hydrogen bond energy).

$$X_A - X_A^a = \sum_{i=1}^n (K_{AB_i} - K_{AB_i}^a) E_{AB_i} C_{B_i} + E_{AH} - E_A^a \quad (\text{A16})$$

and

$$x_{B_i} - x_{B_i}^a = \sum_{i=1}^n (K_{B_i B_j} - K_{B_i B_j}^a) E_{B_i B_j} C_{B_j} + E_{B_i H} - E_{B_i}^a \quad (\text{A17})$$

For the Langmuir adsorption isotherm of multi-component adsorption:

$$N_{B_i}^a = \frac{N_s k_i C_{B_i}}{1 + \sum_{i=1}^n k_i C_{B_i}} \tag{A18}$$

where  $k_i$  is a constant, and

$$C_{B_i} = \frac{V_{B_i} N_{B_i}}{V_o} \tag{A19}$$

We can obtain the following expression:

$$\frac{N_{B_i}^a}{N_{B_i}} = \frac{N_s k_i V_{B_i}}{V_o \left( 1 + \sum_{i=1}^n k_i C_{B_i} \right)} \tag{A20}$$

Substituting eqns. A1 6, A1 7 and A20 into eqn. A1 3, the fundamental retention equation of an undissociated solute in a multi-component mobile phase system in LSC can be derived as

$$\begin{aligned} \ln k' &= \ln \frac{N_s}{V_o} + \sum_{i=1}^n \left[ \frac{\partial N_{B_i}}{\partial N_a} \ln \left( \frac{J_{B_i}^a}{T_{B_i} J_{B_i} V_{B_i} k_i} \right) \right] + \ln \left( \frac{J_A^a}{T_A J_A} \right) \\ &+ \frac{1}{kT} \left[ E_{AH} - E_A^a + \sum_{i=1}^n \frac{\partial N_{B_i}}{\partial N_A} (E_{B_i,H} - E_{B_i}^a) \right] \\ &+ \left( \sum_{i=1}^n \frac{\partial N_{B_i}}{\partial N_A} \right) \ln \left( 1 + \sum_{i=1}^n k_i C_{B_i} \right) + \frac{1}{kT} \sum_{i=1}^n \left\{ (K_{AB_i} - K_{AB_i}^a) E_{AB_i} \right. \\ &+ \left. \sum_{i=1}^n \left[ \frac{\partial N_{B_i}}{\partial N_A} (K_{B_i,B_j} - K_{B_i,B_j}^a) E_{B_i,B_j} \right] \right\} C_{B_i} \\ &= a' + \sum_{i=1}^n b_i C_{B_i} + c \ln \left( 1 + \sum_{i=1}^n k_i C_{B_i} \right) \end{aligned}$$

where

$$\begin{aligned} a' &= \ln \frac{N_s}{V_s} + \ln \frac{V_s}{V_o} + \sum_{i=1}^n \left[ \frac{\partial N_{B_i}}{\partial N_A} \ln \left( \frac{J_{B_i}^a}{T_{B_i} J_{B_i} k_i V_{B_i}} \right) \right] \\ &+ \ln \frac{J_A^a}{T_A J_A} + \frac{1}{kT} \left[ E_{AH} - E_A^a + \sum_{i=1}^n \frac{\partial N_{B_i}}{\partial N_A} (E_{B_i,H} - E_{B_i}^a) \right] \end{aligned}$$

$$b_i = \frac{1}{kT} \sum_{i=1}^n \left\{ \left( K_{AB_i} - K_{AB_i}^a \right) E_{AB_i} + \sum_{i=1}^n \left[ \left( K_{B_i B_j} - K_{B_i B_j}^a \right) \frac{\partial N_{B_i}}{\partial N_A} - E_{B_i B_j} \right] \right\}$$

$$c = \sum_{i=1}^n \frac{\partial N_{B_i}}{\partial N_A}$$

For a weak organic acid, base or amphoteric compound, the dissociation will take place when a strong solvent is used as the mobile phase. Let the solute be an organic acid  $H_2A$ , then the dissociation equilibria occurring in the mobile phase will be



On the other hand, the processes might occur on the absorbent surface as shown in following equations:



In the above equilibria, the superscript a represents adsorption and m the mobile phase. The equilibrium constants are represented by  $K$ . When the equilibria between the mobile phase and absorbent surface are established, according to the equilibrium principle of a weak electrolyte, the following expressions can be written for equilibria A22–A25:

$$K_{a(1)} = \frac{[HA^{-m}][H^+]}{[H_2A^m]} \quad (A27)$$

$$K_{a(2)} = \frac{[A^{2-m}][H^+]}{[HA^{-m}]} \quad (A28)$$

$$K_{a(3)} = \frac{[H_2A^a]}{[H_2A^m]} \quad (A29)$$

$$K_{a(4)} = \frac{[HA^{-a}][H^+]}{[H_2A^a]} \quad (A30)$$



The mass distribution ratio  $\alpha$  of a solute  $H_2A$  between the stationary phase and mobile phase is

$$\alpha = \frac{[H_2A^a] + [HA^{-c}]}{[H_2A^m] + [HA^{-c}] + [A^{*-m}]} \tag{A31}$$

From eqns. A27-A30, we obtain

$$\alpha = K_{a(3)} \frac{1 + K_{a(4)}/[H^+]}{1 + K_{a(1)}/[H^+] + K_{a(1)}K_{a(2)}/[H^+]^2} \tag{A32}$$

where

$$K_{a(4)}/[H^+] = [HA^{-a}]/[H_2A^a] \tag{A33}$$

On non-polar adsorbents, the adsorption of ion HA would be much smaller than that of molecule HA, then  $K_{a(4)}/[H^+] \ll 1$ , and eqn. A32 can be reduced to

$$\alpha = K_{a(3)}/(1 + K_{a(1)}/[H^+] + K_{a(1)}K_{a(2)}/[H^+]^2) \tag{A34}$$

Therefore, the capacity ratio of an organic acid can be expressed as

$$\begin{aligned} \ln k'_{acid} &= \ln \left( t \cdot \frac{V_S}{V_0} \right) \\ &= \ln \left( K_{a(3)} \cdot \frac{V_S}{V_0} \right) - \ln (1 + K_{a(1)}/[H^+] + K_{a(1)}K_{a(2)}/[H^+]^2) \\ &= \ln k' - \ln (1 + K_{a(1)}/[H^+] + K_{a(1)}K_{a(2)}/[H^+]^2) \end{aligned} \tag{A35}$$

where  $V_S/V_0$  is the phase ratio. Combining eqns. A2 1 and A35, the retention equation of organic acid can be obtained:

$$\begin{aligned} \ln k'_{acid} &= a' + \sum_{i=1}^n b_i C_{B_i} + c \ln \left( 1 + \sum_{i=1}^n k_i C_{B_i} \right) \\ &\quad - \ln (1 + K_{a(1)}/[H^+] + K_{a(1)}K_{a(2)}/[H^+]^2) \end{aligned} \tag{A36}$$

For a monoacid,  $K_{a(2)} = 0$ , and then

$$\ln k'_{acid} = a' + \sum_{i=1}^n b_i C_{B_i} + c \ln \left( 1 + \sum_{i=1}^n k_i C_{B_i} \right) - \ln (1 + K_{a(1)}/[H^+]) \tag{A37}$$

The derivations for a weak organic base and an amphoteric compound can be carried

out similarly and the results are expressed by the following equations:

$$\ln k'_{\text{base}} = a' + \sum_{i=1}^n b_i C_{B_i} + c \ln \left( 1 + \sum_{i=1}^n k'_i C_{B_i} \right) - \ln \left( 1 + 10^{14} K_{b(1)} [H^+] + 10^{28} K_{b(1)} K_{b(2)} [H^+]^2 \right) \quad (\text{A38})$$

$$\ln k'_{\text{am}} = a' + \sum_{i=1}^n b_i C_{B_i} + c \ln \left( 1 + \sum_{i=1}^n k'_i C_{B_i} \right) - \ln \left( 1 + K_a/[H^+] + 10^{14} K_b [H^+] \right) \quad (\text{A39})$$

### LIST OF SYMBOLS

$a, b, c$	parameters related to the given chromatographic system
$A$	peak area, solute
$B_i$	solvents $i$
$C_B$	concentration of stronger solvent B in a binary mobile phase
$E_{AH}$	hydrogen bond energy of solute A
$E_A^a$	absorption energy of solute A
$E_{AB_i}$	Van der Waals energy of each pair of adjacent molecules $A-B_i$
$F$	Helmholz function
$h(t)$	exponentially modified Gaussian function
$[H^+]$	concentration of $H^+$ in mobile phase
$J_A, J_{B_i}$	internal partition functions of solute A and solvents $B_i$
$k$	Boltzmann constant
$k_i$	constant in <b>Langmuir</b> adsorption isotherm of solute $i$
$k'$	capacity ratio
$K_1$	resolution criterion
$k'_{\text{acid}}$	capacity ratio of organic acid in reversed phase
$k'_{\text{base}}$	capacity ratio of organic base in reversed phase
$k'_{\text{am}}$	capacity ratio of amphoteric component in reversed phase
$K_i$	equilibrium constants
$k'_{\text{last}}$	capacity ratio of the last peak
$n$	number of theoretical plates of the column
$n^\infty$	number of theoretical plates of the column determined by the solute having an infinite capacity ratio
$N_A, N_{B_i}$	number of molecules of solute A, solvents $B_i$
$N_A^a, N_{B_i}^a$	number of molecules of adsorbed solute A, solvents $B_i$
$N_S$	number of surface sites on absorbent
$P_h$	relative accuracy of the quantitative determination by the peak-height method
$Q$	total partition function
$R$	gas constant
$T$	absolute temperature

$T_A, T_B$	translational partition functions of solute A and solvents $B_i$
$t_R$	retention time
$\Delta t_{1/2}$	peak width at half-height in time units
$i'$	dummy variable of Gaussian integration
$i_g$	centre of gravity of Gaussian peak
$V_0$	total volume of solution
$V_A, V_{B_i}$	volumes of solute A, solvents $B_i$
$V_s/V_0$	phase ratio
$X$	potential function
$X_A^a, X_{B_i}^a$	potential functions of absorbed solute A and solvents $B_i$
$Z_{AB_i}$	relative number of molecules of $B_i$ surrounding solute A
$\alpha$	mass distribution ratio
$\alpha'$	relative retention time of two adjacent peaks
$\alpha'_{\min}$	minimal relative retention
$\beta$	coefficient of variation of plate number with variation of the capacity ratio
$\tau$	time constant of exponential decay
$\sigma$	standard deviation of Gaussian function
$\varphi$	peak-height ratio
$\Omega(T)$	configuration partition function
$\mu_A^a$	chemical potential of absorbed solute A

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