Journal of Chromatography, 550 (1991) 313–324
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2302

Review

Structure–retention correlation in liquid chromatography

T. HANAI
International Institute of Technological Analysis, Health Research Foundation, Institute Pasteur de Kyoto 5F, Hyakumanben, Sakyo-ku, Kyoto 606 (Japan)

ABSTRACT

The analysis of retention mechanisms and the prediction of retention time in liquid chromatography were examined by physico-chemical parameters. The basic parameters used for the calculation were the octanol–water partition coefficient (log P), the Van der Waals volume, the dissociation constant and Hammett's sigma constant. The log P values were calculated by Rekker's method and the Van der Waals volumes were calculated by Bondi's method. The dissociation constants were calculated by the modified Hammett's equation. A method for the prediction of retention time in reversed-phase liquid chromatography is proposed and demonstrated from these calculated values and the retention was analysed by the measured enthalpy and proposed selectivity.

CONTENTS

1. INTRODUCTION

The optimization of chromatography and the characterization of solutes under different chromatographic conditions are important in solving purification problems in various fields of chemistry. Chromatographic results indicate the relative differences in the nature of the solutes. If, therefore, solutes can be characterized by physico-chemical parameters in addition to their relative retention times, chromatographic

0021-9673/91/$03.50 © 1991 Elsevier Science Publishers B.V.

conditions may be optimized and the nature of the solutes can be determined from chromatographic retention times.

Retention differences related to chemical structure have been discussed in normal-phase liquid chromatography (adsorption liquid chromatography, including thin-layer chromatography), where hydrogen bonding and π–π interactions are dominant. This approach is extended here to analyse the retention mechanisms in aqueous-phase liquid chromatography, including reversed-phase and ion-exchange liquid chromatography.

The molecular interactions which occur are probably related to solubility. A relative solubility in water expressed as an octanol/water partition coefficient has been proposed mathematically as Hansch's π-constant, and this was later summarized as Rekker's hydrophobic fragmental constant. Rekker's hydrophobic fragmental constant is used to optimize reversed-phase liquid chromatography, and Hammett's equation is used to predict the retention time of ionizable compounds. However, Hansch's π-constant and Rekker's hydrophobic fragmental constant are relative values and do not explain the meaning of solubility properties. To characterize the solutes from a basic knowledge about molecules, their capacity ratios based on their Van der Waals volume (VWV) calculated by Bondi's method is analysed here. The chromatographic behaviour of alkanes is used to provide standard values in both reversed-phase liquid chromatography and gas chromatography. The difference of the logarithm of capacity ratio ($\Delta\log k'$) was found to be related to the delocalization energy of polyaromatic hydrocarbons (PAHs). The value $\Delta\log k'$ was first described as a π-energy effect related to delocalization energy, and was later referred to as a selectivity R^* as a result of a common explanation for the effect of different substitutes. A quantitative structure relationship in liquid chromatography is described as well as solubility properties.

2. SOLUBILITY PROPERTIES AS A FUNCTION OF RETENTION IN LIQUID CHROMATOGRAPHY

The analysis of the retention mechanism and the prediction of retention time have been examined by several methods. Computer-aided analysis is difficult as the retention mechanism is not well understood and the analysis is carried out by trial and error. How are the solutes held on, or in, a support? This must depend on the physico-chemical interaction between the solutes and the stationary phase [1]. When a strong solvent in which the solute dissolves is used, the solute is eluted very quickly from the column. In this case, the forces holding the substance on the stationary phase should be similar to those responsible for dissolution in a solvent. Eight solubility properties are recognized: Van der Waals (London dispersion) forces, dipole–dipole, ion–dipole, Coulombic and repulsion forces, charge transfer complexes, hydrogen bonding and co-ordination bonds [2]. An attempt has been made to explain the retention mechanisms based on solubility properties, and a possible mechanism was analysed by nuclear magnetic resonance (NMR) and electron spectroscopy for chemical analysis (ESCA) [3]. The molecular interactions which are probably involved in retention are summarized in Table 1.

The molecular interactions in liquid chromatography can be explained by the solubility properties listed in Table 1 [4,5]. The retention of a single molecule is not due

TABLE 1

CLASSIFICATION OF LIQUID CHROMATOGRAPHY

Symbols: ● = important; ○ = considerable; △ = considerable on a packing.

	Van der Waals	Repulsion	London dispersion	Hydrophobic	Dipole–dipole	Charge-transfer	Hydrogen-bonding	Coulomb (ion–ion and ion–dipole)	Ligand formation	Complex formation	Salting-out	Steric effect
Size-exclusion	●	○	○									
Reversed-phase	○	○	○	●	△		△					
Reversed-phase ion-par	○	○	○	●	△			●				
Ion-pair partition	○	○	○				○	●				
Ion-exchange	○	○	○	○	○		△	●			○	
Normal-phase (adsorption)	○	○	○		●	○	●			△		○
Charge-transfer	○	○	○		○	●		○				
Salting-out	○	○	○	○	○			○			●	
Ligand-exchange	○	○	○					○	●			
Chelation	○	○	○			○		○		●		
Affinity	○	○	○	○	○	○	○	○	○	○		●
Chiral separation	○	○	○		○	○	○		○			●

to only one property, but to a combination of several different properties. However, one typical property can usually be determined experimentally. The probable interactions can be estimated from the chemical structure of the analytes and packing materials.

One packing can be used for several different types of liquid chromatography by suitable selection of the eluent components. As an example, a column packed with octadecyl-bonded silica gel was used for size-exclusion liquid chromatography in tetrahydrofuran, for normal-phase liquid chromatography in *n*-hexane and for reversed-phase liquid chromatography in acetonitrile. The chromatograms obtained are shown in Fig. 1.

The elution volumes of polystyrene, decylbenzene and toluene were 1.05, 1.57 and 1.64 ml, respectively (Fig. 1A). This means that size-exclusion liquid chromatography can be achieved with an elution volume between 1.05 and 1.64 ml in this system. The elution volumes of decylbenzene and toluene were 1.70 and 1.84 ml, respectively, in *n*-hexane (Fig. 1B) and the elution volumes of dodecane, heptane, hexane, pentane and butylbenzene were 1.69, 1.73, 1.77, 1.77 and 1.77 ml, respectively. This type of chromatography is called adsorption or non-aqueous reversed-phase liquid chromatography. The elution order of alkylbenzenes was reversed in acetonitrile, as shown in Fig. 1C. The elution volumes of toluene and octylbenzene were 2.18 and 4.66 ml,

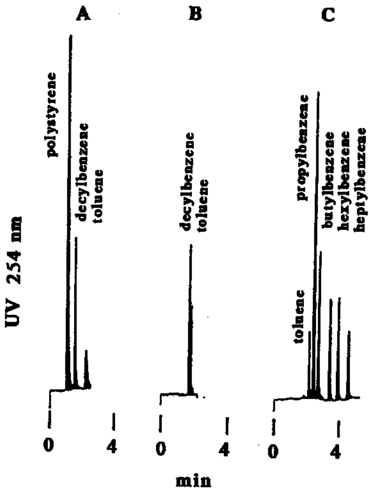


Fig. 1. Chromatograms obtained on one column using different solvents. Column: 15 cm × 4.6 mm I.D., packed with 5-μm Develosil ODS. Eluents: A, tetrahydrofuran; B, *n*-hexane; C, acetonitrile.

respectively. The elution volume increased with the addition of water into the acetonitrile. These elution orders are based on the solubility of solutes, except for size-exclusion liquid chromatography.

The digitization of solubility properties from chemical structures will be required for the further development of the optimum system based on solubility properties.

3. OPTIMIZATION OF REVERSED-PHASE LIQUID CHROMATOGRAPHY BY PARTITION COEFFICIENT

The determination of the solubility of drugs is very important in the field of pharmaceuticals. One method measures this solubility in terms of the partition coefficient between octanol and water. The mathematical calculation of an octanol/water partition coefficient was first proposed by Leo *et al.* [6] as Hansch's π-constant, and was later proposed by Rekker [7] as a hydrophobic fragmental constant (log P). Attempts have been made to measure such an octanol/water partition coefficient by liquid chromatography [8–12]. It is possible to correlate the elution order in reversed-phase liquid chromatography with the octanol/water partition coefficient (log P). The log k' of standard compounds was correlated with log P calculated by Rekker's method [13]. An organic modifier effect on log P in reversed-phase liquid chromatography has been described, and a method for the qualitative analysis of free fatty acids was proposed [13–15]. Hanai *et al.* [16] reported the prediction of retention time in a given eluent from log P for aromatic hydrocarbons. The log k' values of phenols [17] and nitrogen-containing compounds [18] were also related to their log P value, and the calculated log P was used for the qualitative analysis of urinary aromatic acids by reversed-phase liquid chromatography [19,20]. The inclusion of the dissociation constant in the calculation makes it possible to predict the retention times of ionized aromatic acids [21,22]; the dissociation constant was calculated by the method proposed by Perrin *et al.* [23].

3.1. Prediction of retention time from the partition coefficients (log P) in reversed-phase liquid chromatography

Various methods have been used for the prediction of retention time in reversed-phase liquid chromatography. The number of carbon atoms of the aliphatic chain was used for alcohols [14] and phenols [24–26], and this method was further improved by the use of molecular connectivity [27].

Another approach is the use of a quantitative structure–activity relationship, where Hansch's π-constant [6] and Rekker's hydrophobic fragmental constant [7] are derived from the octanol/water partition coefficients and the partition coefficient is measured by liquid chromatography. The partition coefficient calculated by Rekker's method has a linear relationship with log k' measured in reversed-phase liquid chromatography [13]:

$$\log k' = y \log P + m \qquad (1)$$

where y and m are constant in a given system. An example of this relationship is given in Fig. 2.

A good correlation coefficient was obtained in 20–80% acetonitrile–water mixtures. The standard compounds used to evaluate the columns were 2-hydroxyacetophenone, coumarin, acetophenone, indole, propiophenone, butyrophenone, isopropylbenzoate, butylbenzoate and isopentylbenzoate. A polynomial regression between the slope (y) and the percentage concentration (x) of acetonitrile in water was calculated from eqn. 2:

$$y = \sum_{i=0}^{i=n} a_i x^i \qquad n = 2\text{–}8 \qquad (2)$$

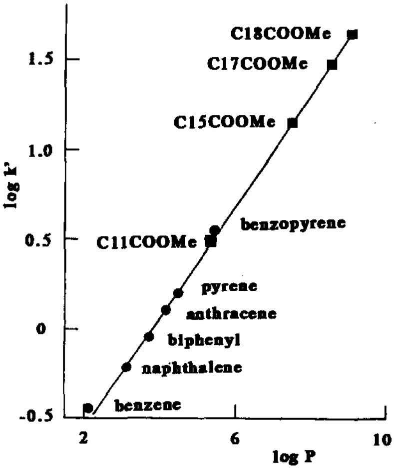


Fig. 2. Relationship between hydrophobicity (log P) and the logarithm of the capacity ratio (log k'). Column: 25 cm × 4.0 mm I.D., packed with Partisil ODS. Eluent: 95% ethanol–water (1:1).

A fourth-degree polynomial was calculated by regression from the log k' values of standard compounds measured in five acetonitrile–water mixtures.

An optimization of the organic modifier concentration for the separation of known compounds has been proposed. When the value of the actual column plate number, the resolution and log P of pairs of compounds a and b are known, the percentage concentration (x) required for their separation is calculated from eqns. 3–5 [15,16]:

$$R_s = (\alpha - 1)\, N_{\text{eff}}^{\frac{1}{2}}/4\alpha \tag{3}$$

$$y = [\log N_{\text{eff}}^{\frac{1}{2}} - \log (N_{\text{eff}}^{\frac{1}{2}} - 4R_s)]/(\log P_a - \log P_b) \tag{4}$$

$$x = \sum_{j=0}^{j=n} b_j y^j \tag{5}$$

The difference between the log k' values measured and predicted by eqns. 1 and 2 from the log P values calculated by Rekker's hydrophobic fragmental constant is within 5%. An example of log P values obtained experimentally compared to those calculated by Rekker's method is shown in Fig. 3.

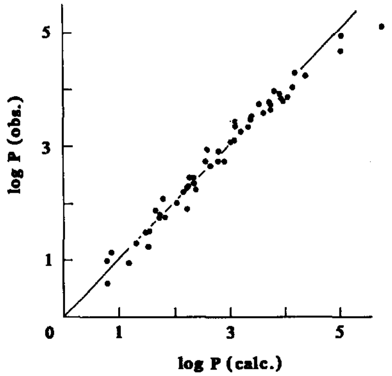


Fig. 3. Comparison of calculated and observed log P values. Column: 25 cm × 4.1 mm I.D., packed with Unisil QC_{18}. Eluent: 60% aqueous acetonitrile. Column temperature: 30°C.

The values of log P obtained from eqn. 1 using measured log k' values of 54 compounds, including alkyl alcohols, alkyl phenones, alkyl benzoates, PAHs and halogenated benzenes, were related to the log P values calculated according to Rekker [7]. The average difference was within 0.16 log P unit. The correlation coefficient was 0.981 [16].

3.2. Prediction of retention time from the partition coefficients and dissociation constants in reversed-phase liquid chromatography

The retention time of the molecular form of compounds can be predicted by the above calculation method, but the retention times of ionized compounds is also very important. The dissociation constant (pK_a) was therefore added to the calculation to predict the retention time of aromatic acids [21]:

$$k' = \frac{k'_m + k'_i(K_a/[H^+])}{1 + K_a/[H^+]} \tag{6}$$

where k'_m is the capacity ratio of molecular form of the acid and can be obtained from log P, and k_i is the capacity ratio of the 100% ionized acid. Unfortunately, k'_i cannot be obtained mathematically at present, and the value is close to zero.

Eqn. 6 was further modified to improve the precision at low pH, where the strong acid used for pH control eliminates the retention of weak acids from the hydrophobic surface of packings [22]. The modified eqn. 7 is given in the following form, and a slight modification of constant A improves the precision:

$$k' = A\,\frac{k'_m - k'_i}{2}\tanh(\mathrm{p}K_a - \mathrm{pH}) + \frac{k'_m + k'_i}{2} \tag{7}$$

The dissociation constant can be mathematically calculated by Hammett's equation [23], and the organic solvent effect on pK_a was also examined [22]. The modified values are: benzoic acids, 4.20–1.00$\Sigma\sigma$; 2-hydroxybenzoic acids, 4.20–1.13$\Sigma\sigma$; phenylacetic acids, 4.30–0.35$\Sigma\sigma$; cinnamic acids, 4.38–0.66$\Sigma\sigma$; and mandelic acids, 3.38–0.454$\Sigma\sigma$, where σ = Hammett's sigma constant.

An example of the relationship between the predicted and observed log k' values at pH 4.26 is shown in Fig. 4.

The agreement between the observed and predicted k' values of aromatic acids is within 10%. An error greater than 10% for 3-hydroxy-2-naphthoic acid [3(OH)NA] and 2-hydroxybenzoic acid [2(OH)BA] was attributed mainly to the error in K_a [21]. In Fig. 4, IPA, CA, 3MeOCA and NA are indole-3-propionic acid, cinnamic acid, 3-methoxycinnamic acid and 2-naphthoic acid, respectively. The partition coefficient, log P, and the dissociation constant, pK_a, can be obtained by simple calculation, and the retention time can be predicted from reversed-phase liquid chromatography.

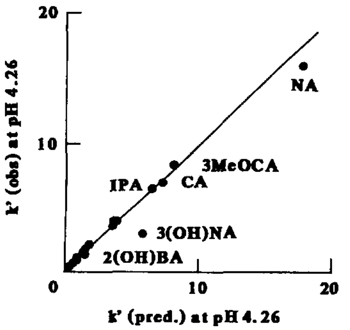


Fig. 4. Relationship between observed and predicted capacity ratio at pH 4.26. Column: 15 cm × 4.1 mm I.D., packed with 5-μm polystyrene gel (Hitachi 3013). Eluent: 30% aqueous acetonitrile with 0.05 M sodium phosphate. Column temperature: 55°C.

However, each group of compounds requires individual standard compounds to obtain the constants for the equations which were used for the calculation of retention time, resolution and concentration of the acetonitrile organic modifier. This means that if a compound had two or more different types of substituted groups, then the retention time predicted from one set of equations was often far from the observed value. The system based on the partition coefficient between octanol and water was therefore not adequate for producing an optimized system for mixtures of different types of compounds. Log P is a relative quantity obtained by experiment; it is not a solubility property. A further optimization of liquid chromatography was therefore attempted using a basic physico-chemical property, the VWV, calculated by Bondi's method [28].

4. USE OF VAN DER WAALS VOLUME AS A BASIC VALUE IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

A system was examined in which only the size and the π-energy of the molecules may be involved in their retention by reversed-phase liquid chromatography using octadecyl-bonded silica gels as the packing. If the hydrogen bonding and Coulombic forces are negligible, the retention of the molecules depends upon their size, and the presence of π-electrons enhances the selectivity. The molecular size, *i.e.*, th VWV, was calculated by Bondi's method [28]. The delocalization energy was obtained from the literature as a property of the π-electrons [29–31]. An example of the relationship between VWV and log k' is shown in Fig. 5.

The maximum log k' values were obtained from the relationship between the retention of n-alkanes and the VWVs [32]. Values for Δlog k' were calculated as the difference between this maximum log k' and the observed log k' values of PAHs. The results for PAHs were applied to other compounds, the standard compounds used being benzene, naphthalene, phenanthrene, chrysene and tetrasene. The selectivity was derived from the relationship between the Δlog k' values and the delocalization energy. The retention is given by the following equation:

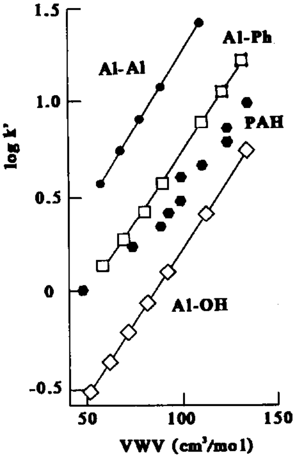
$$\log k'(\mathrm{PAH}) = \log k'(\mathrm{VWV}) - \Delta\log k' \qquad (8)$$

where log k'(VWV) is related to molecular size.

The selectivity obtained on some octadecyl-bonded silica gels in acetonitrile–water mixtures was very similar, so an optimization in reversed-phase liquid chromatography can be carried out from the VWV and selectivity. This approach was also applied to alkyl alcohols whose Δlog k' values were also related to the delocalization energy, even if they did not have a double bond in their structure, and the values were therefore referred to as a hydrogen bonding effect [32]. The retention of alcohols is given by eqn. 9:

$$\log k'(\mathrm{alcohol}) = \log k'(\mathrm{VWV}) - \log k'(\mathrm{hydrogen\ bonding}) \qquad (9)$$

This hydrogen bonding effect was later renamed as selectivity. This prediction system for the retention time in reversed-phase liquid chromatography was applied to evaluate the selectivity of a phenyl-bonded silica gel in acetonitrile–water and



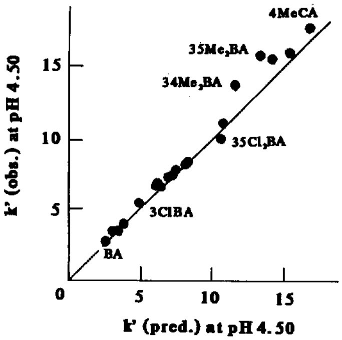


Fig. 5. Relationship between Van der Waals volume (VWV) and the logarithm of the capacity ratio (log k'). Column: 15 cm × 4.6 mm I.D., packed with 5-μm Develosil ODS. Eluent: 80% aqueous acetonitrile. Column temperature: 30°C. Al-Al = Alkanes (pentane–decane); Al-Ph = alkylbenzenes (toluene–octylbenzene); PAH = polyaromatic hydrocarbons (benzene–benzopyrene); Al-OH = alkyl alcohols (butyl alcohol–dodecyl alcohol).

Fig. 6. Predicted and observed capacity ratios (k') of aromatic acids at pH 4.50. Column: 15 cm × 6.0 mm I.D., ERC-1000 (ODS). Eluent: 25% aqueous acetonitrile with 0.05 M sodium phosphate. Column temperature: 40°C. BA = Benzoic acid; 3ClBA = 3-chlorobenzoic acid; 35Cl$_2$BA = 3,5-dichlorobenzoic acid; 34Me$_2$BA = 3,4-dimethylbenzoic acid; 35Me$_2$BA = 3,5-dimethylbenzoic acid; 4MeCA = 4-methylcinnamic acid.

tetrahydrofuran–water mixtures. The selectivity of the phenyl-bonded silica gel was clearly observed in the chromatographic behaviour of chlorobenzenes as a selectivity difference [33]. This approach was applied to the analysis of the liquid chromatographic behaviour of phenols [34] and aromatic acids [35]. The retention of the molecular form of phenols and aromatic acids was predicted from eqns. 10 and 11:

$$\log k'(\mathrm{ArOH}) = \log k'(\mathrm{VWV}) - \log k'(\mathrm{Ph}) - \log k'(\mathrm{OH}) \tag{10}$$

$$\log k'(\mathrm{ArCOOH}) = \log k'(\mathrm{VWV}) - \log k'(\mathrm{Ph}) - \log k'(\mathrm{COOH}) \tag{11}$$

where log k'(ArOH) and log k'(ArCOOH) are the capacity ratios of a substituted phenol and aromatic acid, respectively. Log k'(VWV) is obtained from their VWV, and log k'(Ph) is the selectivity of the phenyl group. Log k'(OH) and log k'(COOH) are the selectivity of a hydroxyl and carboxyl group conjugated with phenyl, respectively. The retention times of ionized aromatic acids were also predicted from their VWVs, selectivity and dissociation constants by eqn. 6 where the maximum capacity ratios (k'_m) were calculated from their VWVs and selectivity instead of from their log P values [35]. An example is shown in Fig. 6.

The difference between the predicted and observed capacity ratio was within 10%. The error for 3,4- and 3,5-dimethylbenzoic acids (3,4-Me$_2$BA and 2,5-Me$_2$BA) was more than 10%, but the error for 3,4-Me$_2$BA should depend on the value of selectivity, but not the dissociation constant. The correlation coefficient was 0.990 (n = 21).

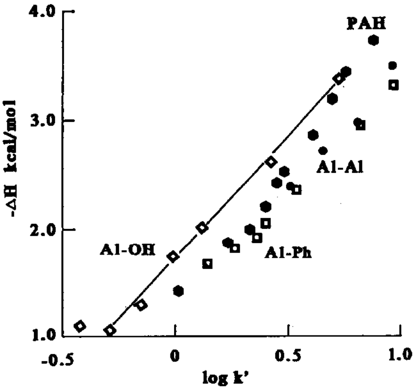
5. ENTHALPY RELATED TO RETENTION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

The higher the enthalpy (ΔH), the longer the retention time, and log k' has a linear correlation to enthalpy [36]. As seen in Fig. 7, each group of compounds exhibits such a linear relationship between enthalpy and log k', although it is not perfect. The ΔH values are also independent of the VWVs as seen in Fig. 8.

The $\Delta\Delta H$ value of a methylene unit of alkyl alcohols is fairly constant, and the hydrogen-bonding energy can therefore also be considered as constant [33]. Substituted benzenes with longer alkyl groups have a larger selectivity constant. This result seems unreasonable because alkylbenzenes with a larger alkyl group must lose their aromaticity even if their selectivity difference becomes smaller. The $\Delta\Delta H$ value of a methylene unit alkylbenzenes is, however, not constant and larger molecules have a higher $\Delta\Delta H$ value than the average $\Delta\Delta H$ value of a methylene unit. The larger $\Delta\Delta H$ value may be due to dimerization between solutes, as described by Ben-Neim [37], and/or to the strong direct interaction between alkylbenzene and the octadecyl group of the packing.

In contrast, the isomeric effect of the $\Delta\Delta H$ value of substitutes of chlorobenzenes is very small. This means that the molecular size of isomers is almost the same under these conditions. The $\Delta\pi$ values of isomers are very large, the difference in the log k' values of the isomers being due mainly to the selectivity and not to the enthalpy.

The selectivity of PAHs is about twice the ΔH of these compounds. This result supports the assumption that retention is mainly due to selectivity. Furthermore, the Δlog k' values of tetrasene and chrysene, which are four-ring PAHs, can be related to their ΔH values but not to their selectivity. This means that, although the calculated molecular size obtained using Bondi's method is the same, the actual size is not the same under these chromatographic conditions [38].



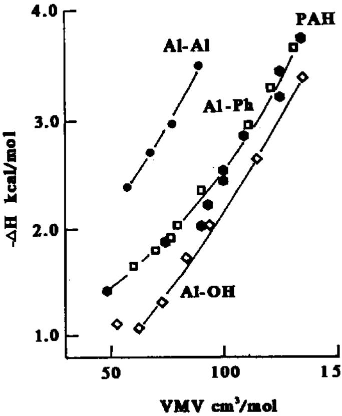


Fig. 7. Relationship between enthalpy ($-\Delta H$) and logarithm of capacity ratio (log k'). Column: 10 cm × 6.0 mm I.D., ERC-ODS. Eluent: 80% aqueous acetonitrile. Al-Al = Alkanes (pentane–octane); Al-Ph = alkylbenzenes (toluene–octylbenzene); PAH = polyaromatic hydrocarbons (benzene–benzopyrene); Al-OH = alkyl alcohols (butyl alcohol–dodecyl alcohol).

Fig. 8. Relationship between enthalpy ($-\Delta H$) and Van der Waals volume (VWV). Experimental conditions as in Fig. 7.

The selectivity (R^*, former energy effect) of alkyl- and chlorophenols varied from 7.9 to 10.1 and from 7.9 to 7.4, respectively. The enthalpy of methylphenols was about 2.0 kcal/mol, and that of chlorophenols varied from 2.0 to 2.4 kcal/mol in the case of pentachlorophenol. This means that the retention difference depended not on the size but on the π-electron density [39]. A similar result was obtained for alkylated and halogenated aromatic acids whose enthalpies were nearly equal, and their selectivity values varied [40]. The ΔH values may depend on the type of packing used and the water content of an eluent [41].

Carbon loading and the end-capping effect of octadecyl-bonded silica gels were studied. The enthalpy was highest on non-end-capped high-carbon octadecyl-bonded silica gels, and slightly higher on high-carbon end-capped gels than low-carbon end-capped gels. There was, however, a selectivity difference in the retention between alkyl alcohols and other hydrophobic compounds depending on the end-capping treatment applied [42]. The order of enthalpies of phenols showed no direct relationship to the capacity ratio or the hydrophobicity of octadecyl-bonded silica gels with a different carbon content [43]. In addition, enthalpy was not directly related to the log k' values nor to the alkyl chain length of bonded-silica gels, even if the retention behaviour of one group of compounds, such as PAHs, and especially the retention of smaller size polar compounds, appeared to indicate this [44].

6. CONCLUSIONS

The qualitative analysis of retention behaviour in liquid chromatography is now well established. However, quantitative analysis is still difficult, *i.e.*, the prediction of retention time and the optimization of separation conditions from physico-chemical properties is not completely successful. One reason is lack of an ideal packing material. The packing has to be stable as part of an instrument, and this is very difficult to achieve in normal-phase liquid chromatography because the humidity of the organic solvent ages the silica gel.

Several packings have been synthesized for reversed-phase liquid chromatography. One possible material is a vinyl alcohol co-polymer gel. This packing is fairly polar and very stable, however, it demonstrates a strong retention capacity for PAHs [45–49]. Although silica gel-based stable octadecyl- and octyl-bonded packings have been synthesized from pure silica gel [50], such an optimization system has not yet been built. Further experiments are required to elucidate the retention mechanism and to systematize it within the context of instrumentation.

REFERENCES

1 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
2 K. Shinoda, *Yoeki To Yokaido*, Maruzen, Tokyo, 1969.
3 W. Funasaka and T. Hanai, presented at the *Annual Autumn Symposium of Chubu Kagaku Karenkyokai, October 1974*, paper IE04.
4 T. Hanai and K. Fujimura, *J. Chromatogr. Sci.*, 14 (1976) 140.
5 T. Hanai and H. Hatano (Editors), *Shin. Khosoku Ekitai Kuromatogurafi (New HPLC)*, Nankodo, Tokyo, 1978, p. 35 (in Japanese).
6 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
7 R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977.

8 W. J. Haggerty and E. A. Murrill, *Res. Dev.*, 25 (1974) 30.
9 R. M. Calson, *J. Chromatogr.*, 107 (1975) 219.
10 J. M. McCall, *J. Med. Chem.*, 18 (1975) 549.
11 M. S. Mirrless, S. T. Moulton, C. T. Murphy and P. J. Taylar, *J. Med. Chem.*, 19 (1976) 615.
12 K. Miyake and H. Terada. *J. Chromatogr.*, 157 (1978) 386.
13 T. Hanai, *Chromatographia*, 12 (1979) 77.
14 M. D'Amboise and T. Hanai, *J. Liq. Chromatogr.*, 5 (1982) 229.
15 T. Hanai, S. Hara, S. Mori and T. Hanai (Editors), *Kuromatogurafi, Bunri Shisutemu (Chromatography, the Separation System)*, Maruzen, Tokyo, 1981, p. 138 (in Japanese).
16 T. Hanai, K. C. Tran and J. Hubert, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 454.
17 T. Hanai and J. Hubert, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 20.
18 T. Hanai and J. Hubert, *J. Liq. Chromatogr.*, 8 (1985) 2463.
19 T. Hanai and J. Hubert, *J. Chromatogr.*, 239 (1982) 527.
20 T. Hanai and J. Hubert, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 500.
21 T. Hanai, K. C. Tran and J. Hubert, *J. Chromatogr.*, 239 (1982) 385.
22 T. Hanai and J. Hubert, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 524.
23 D. D. Perrin, B. Dempsey and E. P. Serjeant, *pKa Prediction for Organic Acids and Bases*, Chapman and Hall, London, 1981.
24 K. Calmmer, L.-E. Edholm and B. E. F. Smith, *J. Chromatogr.*, 136 (1977) 45.
25 J. F. Schabron, R. J. Hurtubise and H. F. Silver, *Anal. Chem.*, 50 (1980) 1911.
26 N. Nomura, *Toyama Univ. Kyoikugakubu-Kiyo*, 27 (1979) 1.
27 B. L. Karger, J. R. Gant, A. Harkopt and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.
28 A. Bondi, *J. Phys. Chem.*, 68 (1964) 441.
29 C. A. Streuli and M. Orloff, *J. Chromatogr.*, 62 (1971) 73.
30 W. Ecknig, B. Trung, R. Radeglia and U. Gross, *Chromatographia*, 16 (1982) 178.
31 A. Streitwieser, Jr., *Molecular Orbital Theory for Organic Chemistry*, Wiley, New York, London, 1961, p. 241.
32 T. Hanai and J. Hubert, *J. Chromatogr.*, 290 (1984) 197.
33 T. Hanai and J. Hubert, *J. Chromatogr.*, 291 (1984) 81.
34 T. Hanai and J. Hubert, *J. Chromatogr.*, 302 (1984) 89.
35 T. Hanai, *J. Chromatogr.*, 332 (1985) 189.
36 W. Melander, D. E. Campbell and Cs. Horváth, *J. Chromatogr.*, 158 (1978) 215.
37 A. Ben-Neim, *Hydrophobic Interaction*, Plenum Press, New York, London, 1980.
38 T. Hanai, A. Jukurogi and J. Hubert, *Chromatographia*, 19 (1984) 266.
39 Y. Arai, M. Hirukawa and T. Hanai, *J. Chromatogr.*, 384 (1987) 279.
40 Y. Arai, J. Yamaguchi and T. Hanai, *J. Chromatogr.*, 400 (1987) 21.
41 H. Colin, J. C. Diez-Mesa, G. Guiochon, T. Czaikowska and I. Miedziak, *J. Chromatogr.*, 167 (1978) 41.
42 J. Yamaguchi, T. Hanai and H. Cai, *J. Chromatogr.*, 441 (1988) 183.
43 J. Yamaguchi and T. Hanai, *J. Chromatogr. Sci.*, 27 (1989) 710.
44 J. Yamaguchi and T. Hanai, *Chromatographia*, 27 (1989) 371.
45 T. Hanai, Y. Arai, M. Hirukawa, K. Noguchi and Y. Yanagihara, *J. Chromatogr.*, 349 (1985) 323.
46 Y. Arai, M. Hirukawa and T. Hanai, *Nihon Kagaku Kaishi*, (1986) 969.
47 Y. Arai, M. Hirukawa and T. Hanai, *J. Liq. Chromatogr.*, 10 (1987) 635.
48 M. Hirukawa, Y. Arai and T. Hanai, *J. Chromatogr.*, 395 (1987) 481.
49 Y. Arai, M. Hirukawa and T. Hanai, *J. Chromatogr.*, 400 (1987) 27.
50 T. Hanai, M. Ohhira and T. Tamura, *LC · GC*, 6 (1988) 922.