

## Application of the UNIFAC method for assessment of retention in reversed-phase liquid chromatography

ĽUBOMÍR DAŠKO<sup>a</sup>

Polymer Institute SAS, Dúbravská 10, Bratislava (Czechoslovakia)

(First received October 29th, 1990; revised manuscript received January 14th, 1991)

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### ABSTRACT

A method for the qualitative assessment of the retention of analytes in reversed-phase liquid chromatography is suggested. The separation is based on the partitioning of analyte molecules between the stationary and mobile phases. The stationary phase is represented by hexane, cumene and 1-octanol and the mobile phase is composed of mixtures of acetonitrile and water or methanol and water. With the help of the UNIFAC group contribution method, the activity coefficients of analytes in both phases were determined. Subsequently the partition coefficients were calculated and used for the assessment of retention. The theoretical results were compared with experimental data and reasonable agreement was achieved.

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### INTRODUCTION

The development of a reversed-phase liquid chromatographic (RPLC) separation method for a new analyte is usually a time-consuming process. It is preferable to choose one of the many procedures available in the literature for retention prediction. A review devoted to theory and methodology of LC was published recently by Dorsey *et al.* [1].

The mechanism of separation in RPLC is often considered to be based on the partitioning of molecules of analytes between the stationary and mobile phases. The value of the partition coefficient can be used for the assessment of retention because the logarithm of the capacity factor is closely related to the logarithm of the partition coefficient [2].

In the case of pure liquid-liquid (L-L) partitioning, the exact compositions of the stationary and mobile phases must be defined for the calculation of partition coefficients. The composition of the mobile phase is well known, but that of the stationary phase is more problematic.

A pure L-L partition mechanism for the separation of analytes in RPLC is unlikely. Horváth *et al.* [2] took into account hydrophobic interactions of the hydrophobic part of an analyte molecule with a bonded alkyl ligand. Martire and

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<sup>a</sup> Address for correspondence: Kovpakova 12, 851 01 Bratislava, Czechoslovakia.

Boehm [3] applied statistical thermodynamics for the calculation of chemical potentials of analytes in both phases with respect to the density of coverage of the silica gel surface with alkyl ligands and conformational changes in bonded ligands depending on the composition of the eluent (breathing of bonded phases).

These two models are an excellent introduction to the understanding of the mechanism of separation in RPLC. Their practical utilization is complicated because of problems with the determination of parameters for calculation.

A pure L–L partition model has also been developed. Grünbauer and Tomlinson [4] modelled the stationary phase with cyclohexane. For the calculation of the logarithm of the partition coefficient they applied Wilson's concept of local composition. The binary interaction parameters were calculated by a curve-fitting procedure from experimental information.

The partition coefficient can be calculated from known values of the activity coefficients of the substance of interest in both phases. An interesting and simple means for the assessment of activity coefficients is the UNIFAC group contribution method [5], where the parameters for calculation are tabulated for immediate reference. This method has been utilized for retention prediction in gas–liquid chromatography [6,7]. Petrovic *et al.* [8] have used the UNIFAC method in RPLC, but they did not calculate directly the activity coefficients of analytes in the stationary phase.

In this paper a method is described for the assessment of the retention of analytes by applying the UNIFAC group contribution method. A pure L–L partition model is adopted. Direct calculation of activity coefficients of analytes in model stationary and mobile phases was performed. By comparing calculated and experimental results and taking into account the effort required to obtain theoretical results according to this model, the acceptability of this strong approximation is confirmed.

#### DESCRIPTION OF THE MODEL

This model is similar to that of Grünbauer and Tomlinson [4]. In this case stationary phase is represented by a liquid (hexane, cumene and 1-octanol). It is assumed that molecules of the stationary phase cannot diffuse into the mobile phase. The mobile phase is composed of mixtures of methanol and water or acetonitrile and water. The same precondition as for stationary phase is valid for the mobile phase, *i.e.*, molecules of the mobile phase cannot penetrate into the stationary phase. Molecules of analytes are partitioned between the stationary and mobile phase. Model analytes used were 1-alcohols, alkylbenzenes, phenol, benzene and chlorobenzene.

The partition process is assumed to be an equilibrium process and the following equation is valid [9]:

$$\mu_a^0 + RT \ln x_a^s \gamma_a^s = \mu_a^0 + RT \ln x_a^m \gamma_a^m \quad (1)$$

where  $\mu_a^0$  denotes to the chemical potential of the analyte in the standard state,  $x_a^s$ ,  $\gamma_a^s$ ,  $x_a^m$  and  $\gamma_a^m$  are the mole fraction and activity coefficients of the analyte in the stationary and mobile phase, respectively,  $T$  is absolute temperature and  $R$  is the gas constant.

In the case of the same standard state for both phases, it follows for the partition coefficient [9]:

$$\ln K = \ln \left( \frac{x_a^s}{x_a^m} \right) = \ln \left( \frac{\gamma_a^m}{\gamma_a^s} \right) \quad (2)$$

According to eqn. 2 one can calculate the logarithm of the partition coefficient of an analyte on a mole fraction basis.

The UNIFAC group contribution method was applied for the calculation of the activity coefficients of analytes of interest. The group contribution method transforms a solution of molecules into a solution of groups. In the original paper [5] it is stated that "The number of distinct groups must remain small, but not so small as to neglect significant effects of molecular structure on physical properties".

The UNIFAC method is closely related to UNIQUAC [10]. The logarithm of the activity coefficient of component  $a$  ( $\gamma_a$ ) is the sum of combinatorial ( $\gamma_a^C$ ) and residual ( $\gamma_a^R$ ) parts [5]:

$$\ln \gamma_a = \ln \gamma_a^C + \ln \gamma_a^R \quad (3)$$

In the UNIFAC method the definition equation for the combinatorial part is the same as in UNIQUAC:

$$\ln \gamma_a^C = \ln \left( \frac{\phi_a}{x_a} \right) + \frac{z}{2} \cdot q_a \ln \left( \frac{\theta_a}{\phi_a} \right) + l_a - \left( \frac{\phi_a}{x_a} \right) \sum_p x_p l_p \quad (4)$$

where  $x_a$  is the mole fraction of component (analyte)  $a$ , the summation is over all components,  $\theta_a$  is the area fraction and  $\phi_a$  is the segment fraction. The definition equations are

$$\theta_a = \frac{q_a x_a}{\sum_p \phi_p x_p} \quad (5)$$

$$\phi_a = \frac{r_a x_a}{\sum_p r_p x_p} \quad (6)$$

The parameters  $r_a$  and  $q_a$  are defined by

$$r_a = \sum_k v_k^{(a)} R_k \quad (7)$$

$$q_a = \sum_k v_k^{(a)} Q_k \quad (8)$$

where  $v_k^{(a)}$  is the number of groups of type  $k$  in molecule  $a$ . The parameters  $R_k$  and  $Q_k$  represent the Van der Waals group volume and surface area, respectively.

The last parameter for calculation of the combinatorial part is  $l_a$ , for which the following equation is valid:

$$l_a = \frac{z}{2} (r_a - q_a) - (r_a - 1) \quad (9)$$

$$z = 10 \quad (10)$$

The difference between the UNIFAC and UNIQUAC methods is in the expressions for calculation of the residual part. The residual part in UNIFAC can be calculated according to the following equation [5]:

$$\ln \gamma_a^R = \sum_k v_k^{(a)} [\ln \Gamma_k - \ln \Gamma_k^{(a)}] \quad (11)$$

where  $\Gamma_k$  is the group residual activity coefficient and  $\Gamma_k^{(a)}$  is the residual activity coefficient of group  $k$  in a reference solution of molecules  $a$  only.  $\Gamma_k$  and  $\Gamma_k^{(a)}$  can be calculated by the same equation [5]:

$$\ln \Gamma_k = Q_k \left[ 1 - \ln \left( \sum_m \varepsilon_m \psi_{mk} \right) - \sum_m \left( \frac{\varepsilon_m \psi_{km}}{\sum_n \varepsilon_n \psi_{nm}} \right) \right] \quad (12)$$

where  $\varepsilon_m$  is the area fraction of group  $m$ :

$$\varepsilon_m = \frac{Q_m X_m}{\sum_n Q_n X_n} \quad (13)$$

and  $X_m$  is the mole fraction of group  $m$  in the mixture. Sums are over all different groups.  $\psi_{mn}$  is the group interaction parameter, given by

$$\psi_{mn} = \exp - \left[ \frac{U_{mn} - U_{nn}}{RT} \right] = \exp - \left[ \frac{A_{mn}}{T} \right] \quad (14)$$

where  $U_{mn}$  is the measure of the interaction energy between groups  $m$  and  $n$ . Parameters  $A_{mn}$  must be evaluated from experimental phase equilibrium data.

$$A_{mn} \neq A_{nm} \quad (15)$$

All known group interaction parameters are tabulated together with other parameters which are necessary for calculation [11]. Of course, the table of interaction parameters is not completely comprehensive [11]. Some parameter are missing because of deviations from experimental results after calculations [5]. On the other hand, very good correlations were obtained in various mixtures [5,12].

Calculated results were transformed to a volume fraction scale.

The concentration of analyte is usually very low in both phases, which is why the concept of infinitesimal dissolution was applied.

## EXPERIMENTAL

Chromatographic measurements were made using a VCM-300 membrane pump, a six-port injection valve, and a UVM-4 UV-VIS detector (all from Development Works CSAS, Prague, Czechoslovakia). An RIDK 101 refractive index detector (Laboratory Works, Prague, Czechoslovakia) was used for detection of alcohols. For both detectors a TZ-4200 chart recorder was used (Laboratory Works).

Eluents were prepared from acetonitrile (UCB, Brussels, Belgium), methanol (Lachema, Brno, Czechoslovakia) and doubly distilled water. The analytes were 1-alcohols, alkylbenzenes, benzene, chlorobenzene and phenol from Fluka (Buchs, Switzerland) and were used as received.

The separation column (150 × 3.3 mm I.D.) was filled with Separon Six C<sub>18</sub> (Tessek, Prague, Czechoslovakia). The dead volume was determined using sodium nitrate.

Calculations were performed on a TI-56 calculator (Texas Instruments, Dallas, TX, U.S.A.). All parameters necessary for calculation are available in the literature [11].

## RESULTS AND DISCUSSION

Activity coefficients were calculated using the UNIFAC group contribution method [5]. Subsequently partition coefficients ( $K$ ) were calculated according to eqn. 2. Plots of  $\ln K$  versus volume fraction of water ( $\varphi_w$ ) in the mobile phase were constructed. In fig. 1a, b and c the stationary phase is represented by hexane, cumene and 1-octanol, respectively. A homologous series of 1-alcohols were used as analytes. The mobile phase was acetonitrile-water. Experimental results are represented by a plot of logarithm of capacity factor ( $k'$ ) versus volume fraction of water in Fig. 1d.

Fig. 1a-d show a fairly good agreement from the qualitative point of view regardless of the stationary phase selected. Plots of both  $\ln k'$  and  $\ln K$  versus volume fraction of water are markedly curved within the composition range 0-0.4, probably owing to interactions of free silanols groups with the hydroxyl group of the alcohol. The changes in the composition of the mobile phase could induce conformational changes in the alcohol molecules too. Conformational changes are closely related to the hydrophobic surface of the molecules of alcohols. These effects together can contribute to the total behaviour of alcohols in RPLC.

Using the same procedure as for alcohols, the values of  $\ln K$  were calculated for alkylbenzenes and the results are shown in Fig. 2a, b and c, where stationary phases are represented by hexane, cumene and 1-octanol, respectively. The experimental results are shown in Fig. 2d. In contrast to the plots for alcohols, plots of  $\ln k'$  and  $\ln K$  versus volume fraction of water are represented by straight lines for alkylbenzenes. In this instance there is no chance of silanophilic interactions. Conformational changes in short alkyl chains are improbable; if they do occur the changes in the surface are not significant.

From Figs. 1 and 2, one cannot draw any conclusion about the best choice for the stationary phase from the suggested liquids. This is why one had to take into account another analytes, *i.e.*, chlorobenzene and phenol. The mobile phase had to be changed to methanol-water, because not all the necessary parameters for the

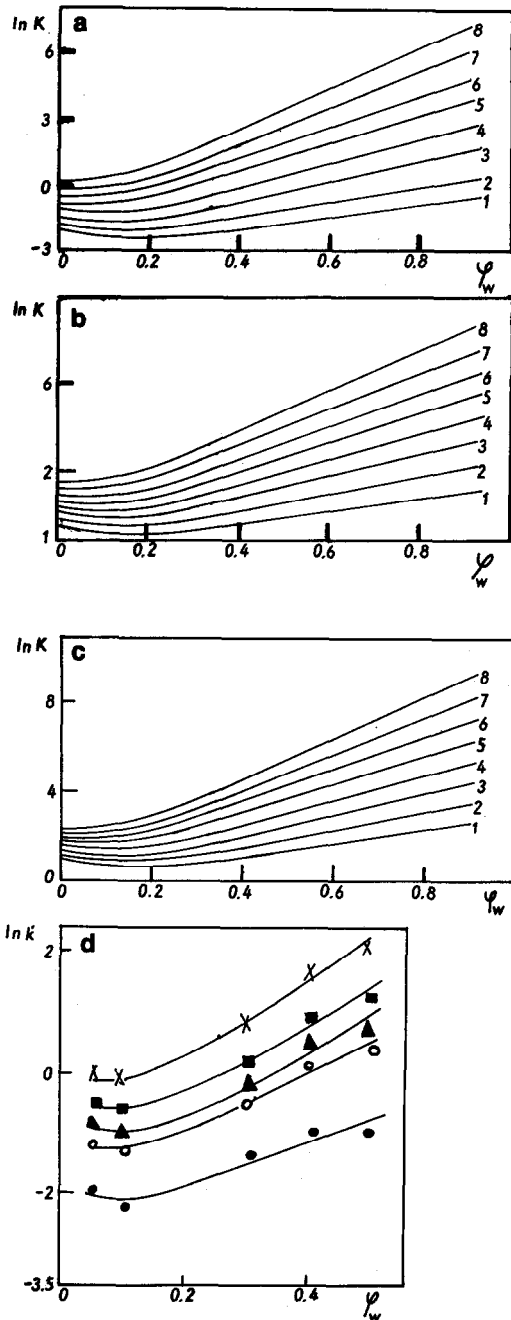


Fig. 1. (a-c) Calculated plots of  $\ln K$  versus volume fraction of water in acetonitrile-water mobile phase. Stationary phase: (a) hexane; (b) cumene; (c) 1-octanol. 1 = 1-Propanol; 2 = 1-butanol; 3 = 1-pentanol; 4 = 1-hexanol; 5 = 1-heptanol; 6 = 1-octanol; 7 = 1-nonanol; 8 = 1-decanol. (d) Experimental results for selected 1-alcohols: plots of  $\ln k'$  versus volume fraction of water in acetonitrile-water mobile phase. ● = 1-Propanol; ○ = 1-hexanol; ▲ = 1-heptanol; ■ = 1-octanol; × = 1-decanol.

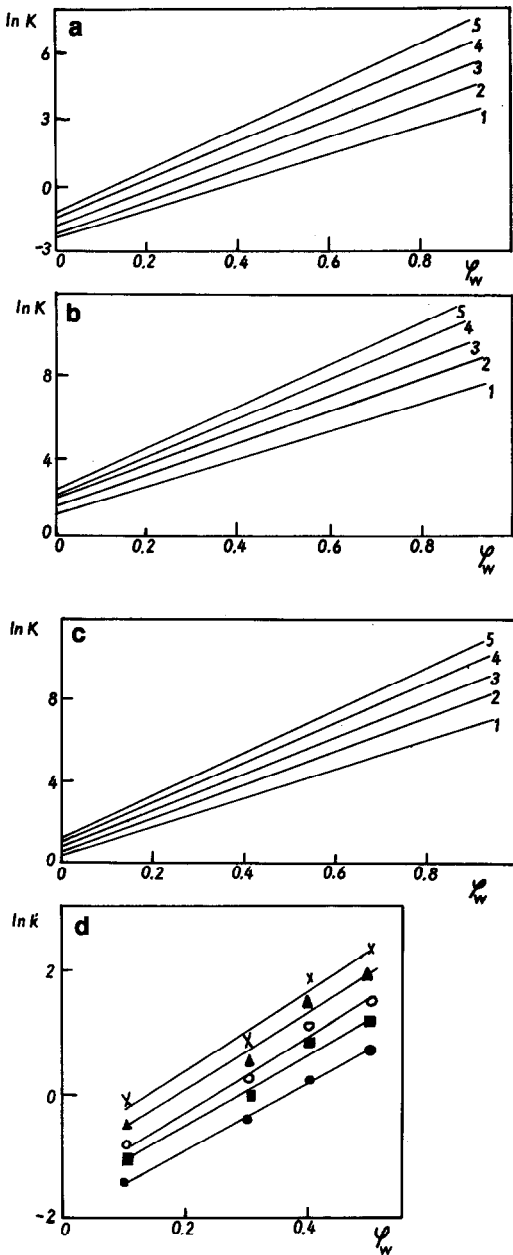


Fig. 2. (a-c) Calculated plots of  $\ln K$  versus volume fraction of water in acetonitrile-water mobile phase. Stationary phase: (a) hexane; (b) cumene; (c) 1-octanol. 1 = Benzene; 2 = toluene; 3 = ethylbenzene; 4 = propylbenzene; 5 = butylbenzene. (d) Experimental plots of  $\ln K'$  versus volume fraction of water in acetonitrile-water mobile phase. ● = Benzene; ■ = toluene; ○ = ethylbenzene; ▲ = propylbenzene; × = butylbenzene.

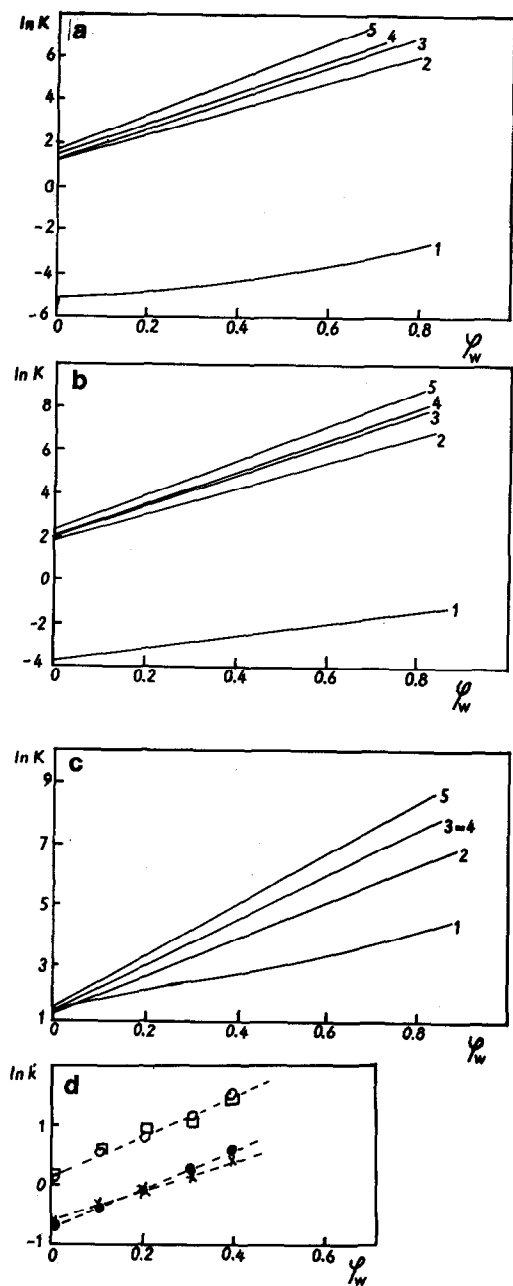


Fig. 3. (a-c) Calculated plots of  $\ln K$  versus volume fraction of water for methanol-water mobile phase. Stationary phase: (a) hexane; (b) cumene; (c) 1-octanol. 1 = Phenol; 2 = benzene; 3 = toluene; 4 = chlorobenzene; 5 = ethylbenzene. (d) Experimental plots of  $\ln k'$  versus volume fraction of water in methanol-water mobile phase.  $\times$  = Phenol;  $\bullet$  = benzene;  $\circ$  = toluene;  $\square$  = chlorobenzene.



acetonitrile–phenol and acetonitrile–chlorobenzene pairs in the UNIFAC group contribution method are known [11].

Calculated plots of  $\ln K$  versus volume fraction of water are shown in Fig. 3a, b and c, where the stationary phase is represented by hexane, cumene and 1-octanol, respectively. Comparing the calculated results with the experimental results (see Fig. 3D), one can see that the best choice for the stationary phase is 1-octanol.

## CONCLUSIONS

A simple method for the qualitative prediction of the retention of analytes in RPLC has been presented. It has been shown that the UNIFAC group contribution method is suitable for calculating activity coefficients and consequently partition coefficients of small molecules between model stationary and mobile phases. The best choice for the stationary phase is 1-octanol. Fairly good assessments of the retention of analytes in RPLC can be obtained in a reasonable time.

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