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## Note

### Application of local-composition theory to reversed-phase liquid chromatography

H. J. M. GRÜNBAUER and E. TOMLINSON\*

*Physical Pharmacy Group, Department of Pharmacy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam (The Netherlands)*

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The extrapolation of chromatographic retention determined in modified aqueous eluents to zero modifier conditions is an important tool in many applications of reversed-phase high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). In practice, extrapolations of this type are always performed by extension of the usually observed linear relationship between  $\log k'$  or  $R_M$  and the volume fraction of water or modifier in the eluent used for their measurement. A theoretical basis for this method has been presented by Soczewinski and Wachtmeister<sup>1</sup> who showed that the application of regular solution theory<sup>2</sup> gives retention as a linear function of eluent composition, (expressed as a volume fraction). However, the work of these authors, as well as the closely related study of Kemula *et al.*<sup>3</sup> on liquid-liquid distribution coefficients, refers to mixed organic mobile phases such as chloroform-hexane<sup>1</sup> or isooctane-hexadecane<sup>3</sup>. In these cases, it is probably justified to consider the organic phase as a regular solution<sup>2</sup>, whilst the aqueous phase does not need to be taken into account. However, the opposite situation is encountered in reversed-phase chromatography where strongly non-regular aqueous mixtures of, for example, acetone and methanol have to be considered, and for these cases the reliability of quantitative results obtained using regular solution theory is questionable<sup>4,5</sup>. As a consequence, it appears useful to investigate more appropriate theories of non-ideal aqueous mixtures for their prediction of retention-composition relationships. In a previous paper<sup>6</sup>, the application of a "local-composition theory" has been suggested for this purpose. This type of theory has been shown<sup>7</sup> to reproduce the molar excess free energies of significantly non-ideal mixtures, and even the occurrence of phase separations in partially miscible mixtures can be correctly predicted<sup>7,8</sup>. In our opinion, the application of local-composition theory to reversed-phase chromatographic systems could be of interest in helping to elucidate not only the validity of linear extrapolations, but also the general applicability of liquid-liquid distribution models to reversed-phase HPLC.

The essential feature of any local-composition theory is the assumption that deviations from thermodynamic ideality in a multicomponent liquid mixture can be attributed to the existence of so-called local compositions around a central molecule which differ from the macroscopic composition of the mixture. Together with the presumed pairwise additivity of nearest-neighbour interactions, this assumption per-

mits the modelling of thermodynamic excess functions in terms of parameter pairs characteristic of a given binary combination. In the version of local composition theory to be employed in the present study<sup>8</sup>, the characteristic parameters  $A_{ij}$  and  $A_{ji}$  for a particular combination of molecules  $i$  and  $j$  are given by

$$A_{ij} = \rho_{ij} \cdot \exp -[(\lambda_{ij} - \lambda_{jj})/RT] \quad (1)$$

such that

$$\rho_{ij} = V_i/V_j \quad (2)$$

where the molar volumes of species  $i$  and  $j$  are represented by  $V_i$  and  $V_j$ , respectively. Interaction energies between an adjacent pair of molecules  $i$  and  $j$ , or two molecules  $j$ , are accounted for by the parameters  $\lambda_{ij}$  and  $\lambda_{jj}$ , respectively. The exponential term in eqn. 1 can be thus viewed as a Boltzmann factor which serves to correct the overall compositions for specific local effects. It follows immediately from theory that, in principle, for a multicomponent mixture, once reliable estimates for the characteristic parameters of its constituent binary combinations are known, all thermodynamic excess properties can be computed. For example, it has been shown previously that the distribution coefficient,  $K_d$ , of a solute in a partially miscible solvent-water system can be calculated using  $A$  values derived from independent literature data on

TABLE I

NUMERICAL VALUES OF CHARACTERISTIC BINARY PARAMETERS USED FOR THE  $\log k' + C$  VERSUS  $\phi_w$  CURVES OF FIG. 1

Binary (1)-(2)	$\rho_{12}$ (25°C)	$A_{12}$ (25°C)	$A_{21}$ (25°C)
Water-methanol	0.4435	1.3549	0.5563
Water-cyclohexane	0.1661	0.0008	0.0038
Water- <i>n</i> -hexane	0.1373	0.0004	0.0050
Water-benzene	0.2021	0.0107	0.0106
Water-diethyl ether	0.1725	0.5689	0.1013
Water-ethyl acetate	0.1834	0.4285	0.2759
Water- <i>n</i> -decanol	0.0939	4.0286	1.4213
Water- <i>n</i> -butanol	0.1965	0.0986	1.7568
Water-isobutanol	0.1945	0.1892	1.3497
Cyclohexane-methanol	2.6699	0.0559	0.4832
Cyclohexane- <i>n</i> -hexane	0.8263	0.9751	0.9292
Cyclohexane-benzene	1.2164	0.7007	0.8752
Cyclohexane-diethyl ether	1.0382	0.8221	0.8182
Cyclohexane-ethyl acetate	1.1041	0.5258	0.6004
Cyclohexane- <i>n</i> -decanol	0.5653	0.0803	1.3941
Cyclohexane- <i>n</i> -butanol	1.1825	0.0476	0.4462
Cyclohexane-isobutanol	1.1705	0.0127	0.8538
Methanol- <i>n</i> -hexane	0.3096	0.2854	0.0362
Methanol-benzene	0.4556	0.5293	0.0903
Methanol-diethyl ether	0.3889	1.0199	0.2791
Methanol-ethyl acetate	0.4136	1.0788	0.3286
Methanol- <i>n</i> -decanol	0.2118	4.9082	0.0270
Methanol- <i>n</i> -butanol	0.4429	1.9172	0.5217
Methanol-isobutanol	0.4384	1.6430	0.7150

solute-water, solute-solvent and solvent-water systems<sup>6</sup>. The present study extends this latter work by including a fourth component, the modifier, in order to investigate the dependence of  $K_d$  on the modifier content of a simulated reversed-phase liquid chromatography system. The shapes of the resulting  $\log K_d$  versus composition curves are immediately comparable to those of corresponding  $\log k'$  or  $R_M$  versus composition curves, since the latter quantities differ from  $\log K_d$  by only a constant factor,  $C^9$ .

The ternary mixture water-methanol-cyclohexane has been studied as a model for a typical reversed-phase system. The very small mutual miscibilities of water and cyclohexane, and those of methanol and cyclohexane, have been neglected in this model. The methods used for the calculation of  $\log K_d$  values (indicated in chromatographic terms as  $\log k' + C$ ) and the determination of the  $A$  parameters have been published previously<sup>6,10</sup>. Table I gives those parameters necessary (eqns. 1 and 2) for the calculation of  $\log k' + C$  at differing eluent compositions for seven organic solutes of varying physicochemical properties. Fig. 1 shows the computed relationships for these solutes. In general,  $\log k' + C$  is found to increase with increasing aqueous volume fraction,  $\phi_w$ , although a strictly linear curve is observed only for diethyl ether and ethyl acetate. The remaining curves are approximately linear for only part of the composition range, *i.e.*, for benzene and *n*-hexane where  $\phi_w \leq 0.8$ , and for the three alcohols where  $0.2 \leq \phi_w \leq 0.8$ . These findings are in agreement with the available evidence that, although linear relationships are a very useful first-order approximation for retention-composition behaviour<sup>11</sup>, there exist non-linear dependencies of  $\log k'$  on  $\phi_w$ <sup>4,12-14</sup>. Both types of non-linear behaviour given in Fig.

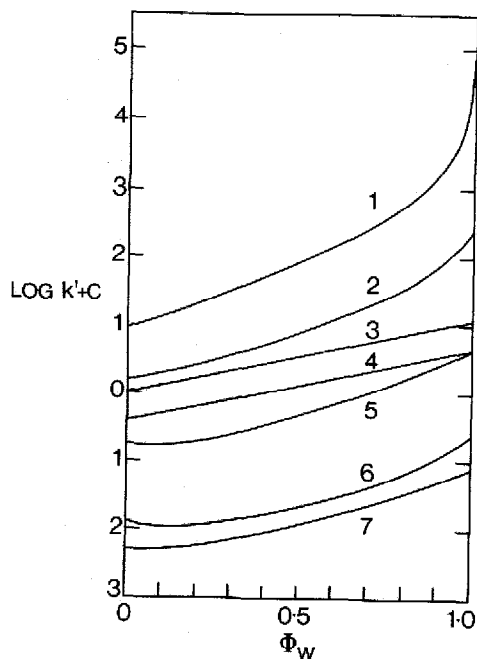


Fig. 1. Calculated values for  $\log k' + C$  in the reversed-phase LC system water-methanol-cyclohexane versus the volume fraction of water,  $\phi_w$ . Parameter values used are listed in Table I. Key: 1 = *n*-hexane; 2 = benzene; 3 = diethyl ether; 4 = ethyl acetate; 5 = *n*-decanol; 6 = *n*-butanol and 7 = isobutanol.

1 have been reported in the literature. For example, non-linear relationships similar to those computed by us for benzene and *n*-hexane have been observed for penicillins in reversed-phase TLC<sup>13</sup> as well as for a large variety of solutes in reversed-phase HPLC<sup>12,14</sup>. Also, Schoenmakers *et al.*<sup>14</sup> have recently published the retention-composition relationship for phenol in a reversed-phase system using aqueous propanol as eluent, which shows a minimum in the water-poor region, an effect very similar to that exhibited in Fig. 1 by the three aliphatic alcohols.

It can be concluded that both linear and non-linear retention-composition relationships in reversed-phase chromatography may be explained by a liquid-liquid distribution model, provided that a local-composition theory is employed to describe deviations from thermodynamic ideality in the mobile phase. In our opinion, this type of approach is much more promising for reliable quantitative predictions than some form of an extended regular solution theory. We are therefore presently engaged in the further development of local-composition theory in order to test its reliability for the prediction of chromatographic retentions and solubilities<sup>15</sup> in mixed aqueous solvents.

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