

## The model of retention in HPLC with binary mobile phase

Sergei N. Lanin, Yuri S. Nikitin

Department of Chemistry, M.V.Lomonosov State University  
of Moscow, 119899, Moscow, U.S.S.R.

Abstract - The retention model in HPLC is proposed, which is based on quasicheical equilibria of the components of the chromatographic system. This model provides a better understanding of the retention mechanism in HPLC and it can be applied for quantitative description of the dependence of retention on the nature and composition of the mobile phase.

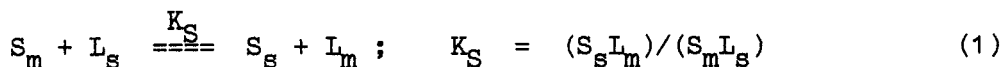
High performance liquid chromatography (HPLC) is known to be one of the most effective methods for analysis the substance mixtures of complex composition. A great number of various organic substances of different nature and properties required various technical approach and instrumentations to separate complex mixtures, identify and determined their components. The choice of the appropriate chromatographic techniques required their optimization based on understanding the retention mechanisms and molecular interactions of the chromatographic system components.

In HPLC, along with the sorbate-sorbent molecular interactions, of great importance are also the sorbate-mobile phase, sorbent-mobile phase interactions, in particular, the interactions between the components of binary, ternary and other mobile phases. At present, it is often impossible to compute quantitatively the thermodynamic characteristics of retention in these complicated systems. Further development of the HPLC theory is largely contributed by the determination of correlation dependences between the retention parameters and various properties of sorbates (S) (ref. 1-5), mobile (ref. 3-8) and stationary phases (ref. 9-12).

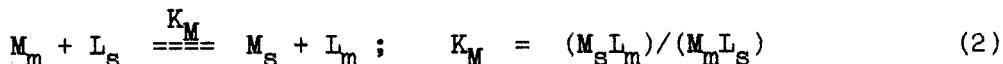
To describe the retention mechanism of substances in most often used variants of HPLC, namely, normal-phase (NP HPLC) and reverse-phase (RP HPLC), it must be used the common approach based on the distribution of substances (sorbates) in dynamic equilibrium between the mobile and stationary phases. This approach provides a common standing for studying these variants of HPLC.

Now consider the system most frequently used in HPLC: the stationary phase (SP), sorbate (S) and the two-component mobile phase (MP) consisting of the solvent (L) as the basic component and the modifying additive (M). As a rule, RP HPLC includes: L - water, M - organic solvent (methanol, acetonitrile, tetrahydrofuran and other) and NP HPLC includes: L - alkane, M - weak polar or polar organic solvent (methylenechloride, chloroform, alcohols, etc.). In this case, sorption (distribution) from the three-component solution (L,M,S) is exhibited, and  $L \ll M \gg S$ .

Now we take into account the following assumptions: the sorbent surface is chemically and geometrically homogeneous and, consequently, the sorption energy of the MP components is the same on any part of the surface; sorption is of exchange (competitive) character; the absence of molecules associations in the adsorption layer; the surface and volume solutions are ideal. Considering the adsorption as the quasiochemical reversible reaction of exchange (ref. 13-15) (in the simplest case, the areas occupied by molecules S, M and L in the surface layer being equal), we have equilibria for the sorbate.

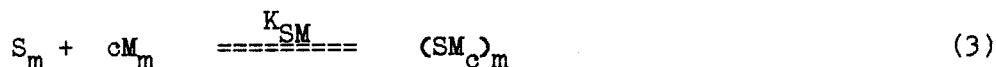


and for the modifier:



where  $K_S$  and  $K_M$  are equilibrium constants and  $S_s$  and  $S_m$ ,  $M_s$  and  $M_m$  and  $L_s$  and  $L_m$  are mole fractions of the solute, modifier and solvent in the stationary phase (s) and mobile phase (m).

Solvation of the solute molecules by the modifier molecules proceeds in the mobile phase:

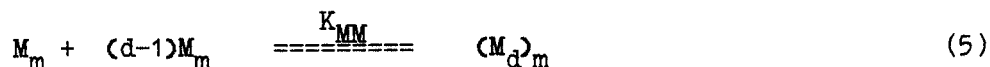


for  $c = 1, 2, 3, \dots, C$  with the equilibrium constant

$$K_{SM} = (SM_c)_m / (S_m M_m^c) , \quad (4)$$

where  $(SM_c)_m$  is the mole fraction of  $(C + 1)$ -molecular associates in the mobile phase.

Simultaneously, association of the modifier molecules occurs in the mobile phase:



for  $d = 2, 3, 4, \dots, D$  with the equilibrium constant

$$K_{MM} = (M_d)_m / M_m^d , \quad (6)$$

where  $(M_d)_m$  is the mole fraction of  $d$ -molecular associates in the mobile phase. In eqns. 3-6 has been made the assumption that the association constants of molecules in the solution are equal. In molecular chromatography the energies of sequential formation of associates are to be close when uncharged molecules interact. For instance, in RP HPLC the association of organic molecules in aqueous solution proceeds at the expense of non-specific dispersion forces (hydrophobic interactions) and each of the added molecules makes nearly the same contribution to the formation energy of associates.

As no associates appear on the sorbent

$$S_s = [S_s^0] ; \quad L_s = [L_s^0] ; \quad M_s = [M_s^0] \quad (7)$$

and assuming that  $S_m \ll M_m, L_m$  ;  $S_s \ll M_s, L_s$  we have

$$M_s + L_s = 1 , \quad (8)$$

and in the mobile phase

$$[M_m^0] + L_m = 1 \quad , \quad (9)$$

where  $[M_m^0]$  are the total concentration of the different form of modifier in the mobile phase. On the other hand

$$[S_m^0] = S_m \left( 1 + \sum_{c=1}^C K_{SM} M_m^c \right) \quad (10)$$

$$[M_m^0] = M_m \left( 1 + K_{SM} S_m \sum_{c=1}^C c M_m^{c-1} + K_{MM} \sum_{d=2}^D d M_m^{d-1} \right) \quad (11)$$

It follows from eqns. 1 and 2 that

$$K_S = (S_s / S_m) (K_M M_m / M_s) \quad (12)$$

From eqns. 2, 8, and 9 we have

$$K_M = M_s (1 - [M_m^0]) / [M_m (1 - M_s)] \quad (13)$$

Introducing eqn. 11 into eqn. 12, we obtain

$$M_s = K_M M_m / \left[ 1 + M_m (K_M - 1) - K_{SM} S_m \sum_{c=1}^C c M_m^c + K_{MM} \sum_{d=2}^D d M_m^d \right] \quad (14)$$

Introducing eqn. 14 into eqn. 12, we obtain

$$K_S = (S_s / S_m) \left[ 1 + (K_M - 1) M_m - K_{SM} S_m \sum_{c=1}^C c M_m^c + K_{MM} \sum_{d=2}^D d M_m^d \right] \quad (15)$$

It is known (ref. 13) that

$$k' = \Phi (S_s / [S_m^0]) = \Phi K \quad , \quad (16)$$

where  $k'$  is the capacity factor,  $\Phi$  is the phase ratio and  $K$  is the constant of the sorption equilibrium of the solute, then introducing eqn. 10 into eqn. 16, we obtain

$$k' = \Phi (S_s / S_m) \left( 1 + K_{SM} \sum_{c=1}^C M_m^c \right)^{-1} \quad (17)$$

Introducing volume  $S_s / S_m$  from eqn.15 into eqn.17, we obtain

$$\begin{aligned} 1/k' = & (1/ \Phi K_S) \left( 1 + K_{SM} \sum_{c=1}^C M_m^c \right) \left[ 1 + (K_M - 1) M_m - \right. \\ & \left. - K_{SM} S_m \sum_{c=1}^C c M_m^c - K_{MM} \sum_{d=2}^D d M_m^d \right] \end{aligned} \quad (18)$$

Sums  $\sum_{c=1}^C M_m^c$ ,  $\sum_{c=1}^C c M_m^c$  and  $\sum_{d=2}^D d M_m^d$  from eqn.18 are the converging power series (according to Dalamber's criterion, because  $M_m < 1$  (ref. 16)) and therefore for the great values  $C$  and  $D$  we have

$$\begin{aligned} 1/k' = & (1/ \Phi K_S) \left( 1 + K_{SM} M_m / L \right) \left[ 1 + (K_M - 1) M_m - \right. \\ & \left. - K_{SM} S_m M_m / L^2 - K_{MM} M_m (1/L^2 - 1) \right] \end{aligned} \quad (19)$$

When no associates appear in solution,  $K_{SM}=0$  and  $K_{MM}=0$ , the dependence of  $1/k'$  on  $M_m$  is linear. In case of the associates formation between the molecules of the modifier and the sorbate  $K_{SM}>0$  and in the absence of the modifier molecules association,  $K_{MM}=0$ , the value  $1/k'$  increases faster (with increasing content of the modifier  $M_m$  in the mobile phase) than in the first power of  $M_m$ . The formation of associates of modifier molecules between themselves, on the contrary, should decrease  $1/k'$  with respect to the linear dependence. Thus it follows that in the general case eqn. 19 represents a complex curvilinear dependence of sorbate retention on the modifier concentration, the character of this dependence being determined by the ratio of constants  $K_{SM}$ ,  $K_{MM}$ ,  $K_M$  and  $K_S$  corresponding to various types of intermolecular processes evolving in the chromatographic system.

Specific and nonspecific molecular interactions in the mobile phase are particularly exhibited in RP HPLC, which is seen in Fig. 1a, since the dependence of  $1/k'$  on  $M_m$  is nonlinear. This character of the dependence shows that in RP HPLC the contribution of specific and nonspecific interactions of phenols molecules with the molecules of the mobile phase is comparable with the contribution of nonspecific interactions of the sorbate molecules with the hydrophobic surface of the stationary phase.

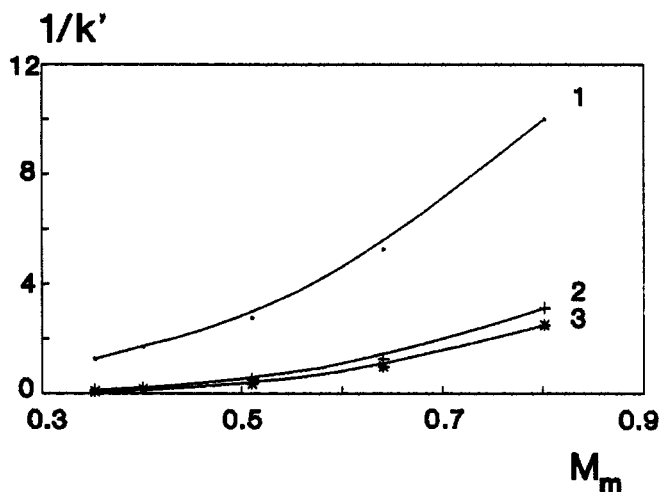


Fig.1a.  
Dependence of the reciprocal value of the capacity factor ( $1/k'$ ) of phenols on the mole fraction ( $M_m$ ) methanol in the binary water-organic mobile phase. Adsorbent LiChrosorb RP-18, 7  $\mu$ m.  
1 - Phenol,  
2 - 3-tert-butylphenol,  
3 - 2-tert-butylphenol.

In the extremal case when adsorption of the modifier is negligible ( $K_M=1$ ) and much less than the interactions of the sorbate with the modifier ( $K_{SM}\gg 1$ ) and  $K_{MM}\ll 1$ , the retention is described by the equation

$$1/k' = (1/\Phi K_S)(1 + K_{SM}M_m/L) \quad (20)$$

As seen from Fig.1b, this dependence is practically linear in comparison with that in Fig.1a.

Taking  $K_M = K_{SM}$  and  $S_m \ll M_m$ , we get

$$1/k' = (K_{SM}K_M/K_S\Phi)(M_m^2/L) \quad (21)$$

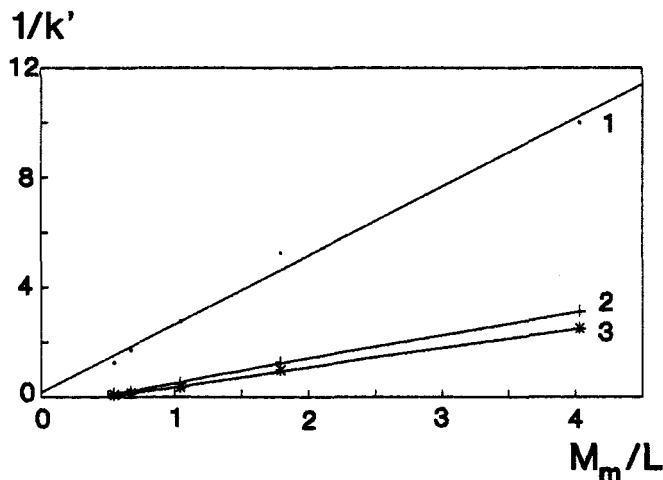


Fig.1b.  
Dependence of the reciprocal value of the capacity factor ( $1/k'$ ) of phenols on the binary water-methanol mobile phase composition, expressed as ( $M_m/L$ ). Adsorbent LiChrosorb RP-18, 7  $\mu$ m.

- 1 - Phenol,
- 2 - 3-tert-butylphenol,
- 3 - 2-tert-butylphenol.

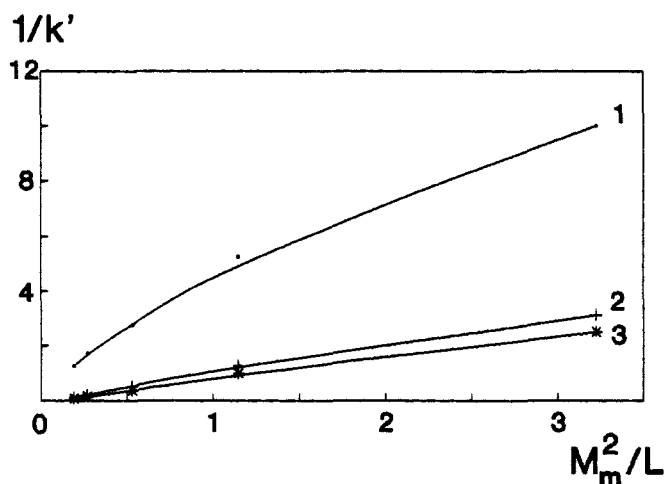


Fig.1c.  
Dependence of the reciprocal value of the capacity factor ( $1/k'$ ) of phenols on the binary water-methanol mobile phase composition, expressed as ( $M_m^2/L$ ). Adsorbent LiChrosorb RP-18, 7  $\mu$ m.

- 1 - Phenol,
- 2 - 3-tert-butylphenol,
- 3 - 2-tert-butylphenol.

Fig.1c shows that the character of this dependence is much closer to linear than that in Fig.1a.

The correctness of suppositions is provided by the fact that the value  $1/k'$  is close to zero when  $M \rightarrow 0$  (Fig.1b,c).

So deviation from linearity (Fig.1a) is caused by sorbate solvation in mobile phase.

The proposed model based on quasicheical equilibria of the components of the chromatographic system provides a better understanding of the retention mechanism in HPLC. This model can be applied for quantitative description of the dependence of retention on the nature and composition of the mobile phase.

#### REFERENCES

1. Kalisz R. Quantitative structure-chromatographic retention relationships. John Wiley, New York, 1987, 318 p.
2. Lanin S.N., Nikitin Yu.S. Chromatographia, 1988. V.25. No.4. P.272.

3. Sadek P.C., Carr P.W., Doherty R.M., Kamlet M.J., R.W.Taft, Abraham M.H. Anal.Chem., 1985. V.57. P.2971.
4. Park J.H., Carr P.W. J.Chromatogr. 1989. V.465. P.123.
5. Lanin S.N., Nikitin Yu.S. Pure & Appl.Chem. 1989. V.61. No.11. P.2027.
6. Horvath Cs., Melander W., Molnar J. J.Chromatogr. 1976. V.125. No.1. P.129.
7. Jandera P., Churasek J. J.Chromatogr. 1974. V.91. P.207.
8. Murakami F. J.Chromatogr. 1979. V.178. No.2. P.393.
9. Colin H., Krstulovic A.M., Connord M.F. et.al. Chromatographia. 1983. V.17. No.1. P.9.
10. Scott R.P.W., Kucera P. J.Chromatogr. 1977. V.142. P.213.
11. Tanaka N., Tocuda Y., Iwaguchi K., Araki M. J.Chromatogr. 1982. V.239. No.2. P.761.
12. Lanin S.N., Nikitin Yu.S., Pyatigin A.A., Staroverov S.M. Chromatographia, 1989. V.27. No.2/3. P.147.
13. Snyder L.R. Principles of Adsorption Chromatography, Marcel Dekker, New York, 1968. 413 p.
14. Dgigit O.M., Kiselev A.V., Terechova M.G., Sherbakova K.D. Zh.Fiz.Khim. 1948. V.22. No.1. P.107.
15. Everett D.H. Trans.Faraday Soc., 1965. V.61. P.2478.
16. Korn G.A., Korn T.M. Mathematical handbook. McGraw-Hill Book Company, New York, 1968.