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Mobile phase effects in reversed-phase and hydrophilic interaction liquid chromatography revisited

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ABSTRACT

Correct adjustment of the mobile phase is equally important as the selection of the appropriate column for the separation of polar compounds in LC. Both solvophobic and selective polar interactions control the retention in the Reversed Phase and Hydrophilic Interaction modes. The retention models describing the effects of the volume fraction of the strong eluent component in binary mobile phases on the sample retention factors apply in a limited mobile phase composition range. We introduced a three-parameter retention model, which provides improved prediction of retention over a broad mobile phase range, under isocratic and gradient elution conditions. The model does not imply any assumptions concerning either adsorption or partition distribution mechanism, but allows estimating retention in pure strong and in pure weak mobile phase components. The experimental retention data for phenolic acids and flavones on several core-shell columns with different types of stationary phases agree with the theory.

Many polar columns with important structural hydrophobic moieties show dual retention mechanism, (Reversed Phase in water rich mobile phases and Hydrophilic Interaction at high acetonitrile concentrations). It is possible to select the mobile phase compositions in each of the two modes for separations of samples containing compounds largely differing in polarity. The three-parameter model describes the retention in each mode, with separately determined best-fit parameters. We applied the two-mode model to the retention data of sulfonamides and benzoic acid related compounds on a new polymethacrylate zwitterionic monolithic micro-column.

1. Introduction

Retention models describe theoretically the effects of the separation conditions on the retention, usually characterized as the retention factor, factor, $k=(t_R/t_0-1)$; t_R and t_0 are the retention time of the analyte and the column hold-up time, respectively. k is defined as the ratio of the number of the solute moles in the stationary and in the mobile phases, respectively: $k=m_S/m_m$. and is proportional to the distribution constant, $k=K_D\,V_S/V_m$. A few retention models characterizing the effects of the mobile phase on separation date from the early days of HPLC, when they were derived assuming either adsorption or partition retention mechanisms [1,2]. Snyder introduced the "Linear Solvent Strength" model (LSS), which is widely used in reversed-phase LC to describe the effects of the volume fraction of the strong (polar organic) solvent, φ, on the retention in binary aqueous-organic mobile phases:

$$\log k = \log k_w - S \cdot \varphi \quad (1)$$

The retention factor, $k=(t_R/t_0-1)$, characterizes the ratio of the number of the solute moles in the stationary phase, m_S, to the number of moles in the mobile phase, m_m; $k=m_S/m_m$; k_w is the solute retention factor in the weak solvent (water in RP LC) and S characterizes the organic solvent elution strength [1]. Snyder and Soczewinski [3] proposed a displacement (S-S) model of retention in normal phase chromatography, which describes the effects of the volume fraction of the strong (more polar) solvent, φ, in a two-component organic mobile phase, on the retention factor, k and can be written, in the simplified form, as [4]:

$$\log k = \log a_0 - m \cdot \log \varphi \quad (2)$$

a_0 is the retention factor in the pure strong (more polar) solvent in a two-component organic mobile phase.

Many polar compounds elute too early in reversed-phase chromatography to allow successful separation. Hydrophilic Interaction Liquid Chromatography (HILIC) with polar columns and aqueous organic mobile phases often provides significant improvement in the retention and separation efficiency [5]. Theoretically, Eq. (2) may apply in HILIC (with φ as the water volume fraction in aqueous – organic mobile phases) [6–8].

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Snyder and Poppe compared the relative merits of the partition and adsorption mechanisms in liquid chromatography [2].The simple two-parameter equations, Eqs. (1) and (2), do not describe satisfactorily experimental data in some separation systems, especially in the HILIC mode [9–12]. Several three- or more- parameter models including second-order terms provide improvement in the data fit [10,11], but the physical meaning of the additional terms often is not clear. Mutlti-parameter equations generally provide better fit to the experimental data than two-parameter linear (or linearized) models, with the quality of the fit improving with any additional parameter, which however often may lack physical meaning [13]. A simple approach to improve the model fit is formal extension of Eqs. (1) or (2) by adding a quadratic φ^2 term [11]. Even a quadratic model usually does not allow describing accurately the RP retention over the full range of aqueous-organic mobile phases [14,15].

To account for possible combination of adsorption and partition effects, Jin et al. [16] presented a three-parameter mixed mode retention model, which combines Eqs. (1) and (2):

$$\ln k = \ln a_0 - b \cdot \ln \varphi_{H_2O} \tag{3}$$

Eq. (3) is more suitable for the description of the HILIC retention than polynomial empirical equations and can account for mixed-mode HILIC-RP mechanism on a single polar column [17]. However, the two-parameter equations, Eqs. (1) and (2) and the three-parameter Eq. (3) fail at very low concentrations of the strong solvent, φ, in the mobile phase.

The retention models principally do not only describe the mobile phase effects under isocratic conditions, but can be adapted also for gradient-elution LC retention [18].

Neue and Kuss [10] presented a more complex three-parameter empirical model for reversed-phase LC, which, unlike most other three-parameter or more complex models [20], allows direct analytical prediction of gradient retention times. The prediction accuracy of the particular retention models does not bring significant advantages over the simple LSS model for most low- and high-molecular compounds tested in reversed-phase LC [13,18,19,21]. Recent studies compare the accuracy of various models for the prediction of retention in HILIC systems. The LSS, the quadratic and the Neue Kuss models, provided similar prediction errors for the isocratic and gradient retention of small molecules, peptides and proteins, but the non-linear models could be applied only in a limited mobile phase range [7,19,22–24].

In hydro-organic mobile phases, both polar and hydrophobic adsorbent groups may cause preferential sorption of either acetonitrile or water, depending on the mobile phase composition [25]. The adsorbed liquid, especially water, changes the properties of the stationary phase, and becomes a part of it. Alpert [5] originally assumed that partition between a stagnant water-rich layer adsorbed on the polar sorbent, and the water-poor bulk mobile phase, controls the retention in HILIC systems. However, the chemistry of the solid phase strongly affects both the water adsorption and the retention and selectivity of HILIC separations, which significantly differs between bare silica and polar chemically bonded phases [26]. Columns with bonded hydroxyl, diol and zwitterionic ligands show stronger water adsorption in comparison to bare silica. On the other hand, the hydrosilated silica materials show low affinity to water. At full column saturation, the excess adsorbed water, V_{ex}, fills up to 45.3% of the pore volume of normal silica-based columns, but only 2.6–5.5% of the pore volume of hydrosilated silica columns [27].

Eq. (2) does not account for possible retention in mobile phases with high concentrations of the strong solvent (water). Introducing an additional term, *b*, into Eq. (2) corrects for possible retention in pure weak solvent, $k_0 = 1/(b)^m$, in Eq. (4) [4,28]:

$$k' = (b + a \cdot \varphi)^{-m'} \tag{4}$$

The parameter *m*' is proportional to the area of the solid surface occupied by one molecule of the solvent B. Recently, we observed improved data fit for the three-parameter Eq. (4) in the reversed-phase LC on core-shell columns, in comparison to the two-parameter Eqs. (1) and (2). The two-parameter equations show some positive and negative deviations from linearity [9].

The quality of the data fit alone unfortunately does not prove the correctness of the underlying mathematical model. Sometimes, the improved fit of a multi-parameter model equations may be due just to including the data subject to systematical or random errors. It should be kept in mind that fitting the model equation parameters by regression analysis is based on the assumption of the normal statistical error distribution for the whole data set. This may apply more or less for the experimental retention times, but the model equations employ retention factors, *k*, which are affected by combined errors in the determination of the solute's retention time, t_R, and in the experimental column hold-up time, t_0. Hence the random error probablilty increases for weakly retained compounds, not to speak about the effect of possible gross errors in the determination of t_0. Hence, some problems with the data fit may be due to the quality of the experimental data rather than to the failure of the theoretical model. For this reason, fitting more than three-parameter model equations can hardly be practical, taking into account that some parameters may either lack physical meaning, or accurate model physical parameters are not available and often must be corrected by empirical factors.

In gradient reversed-phase liquid chromatography, the volume fraction φ of a strong solvent (acetonitrile, methanol) in water increases proportionally to the volume of the mobile phase, *V*, that has flowed through the column from the starting concentration, $\varphi = A$, to the final concentration, φ_G at the end of the gradient, where $V = V_G$ (the gradient volume), [29]:

$$\varphi = A + B \cdot V \tag{5}$$

The parameter $B = (\varphi_G - A)/V_G$ is the gradient ramp (steepness). The precision of the retention time prediction in gradient HPLC is important for compound identification, for setting peak integration time windows and in fundamental studies of retention [30]. Different models (LSS, S-S, or ABM) characterize in different ways the effects of the gradient program on the retention. For gradients with different ramps (*B*) and ranges ($\varphi_G - A$), the elution volumes, $V_{R(g)}$, can be predicted from the parameters of the isocratic model equations: Eq. (6) for the LSS model [18,31] and Eq. (7) for the S-S model [4]. The three-parameter ABM model, Eq. (4), can be adapted to describe the retention under the linear gradient conditions by Eq. (8) [28]:

$$V_{R(g)} = \frac{1}{S \cdot B} \log\left[2.31 \cdot S \cdot B \cdot \left(V_m \cdot 10^{(\log k_w - S \cdot A)} - V_D\right) + 1\right] + V_m + V_D \tag{6}$$

$$V_{R(g)} = \frac{1}{B}\left[(m+1) B \cdot (k_0 \cdot V_m + V_D \cdot A^m) + A^{(m+1)}\right]^{\frac{1}{m+1}} - \frac{A}{B} + V_m + V_D \tag{7}$$

$$V_{R(g)} = \frac{1}{a \cdot B}\left[(m' + 1) \cdot a \cdot B \cdot [V_m - V_D(b + a \cdot A)^{m'}] + (b + A \cdot a)^{(m'+1)}\right]^{\frac{1}{m'+1}} - \frac{b + a \cdot A}{a \cdot B} + V_m + V_D \tag{8}$$