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Effect of polar interactions on the nonlinear behavior of phenol and aniline in reversed phase liquid chromatography

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ABSTRACT

The effect of the coverage density and the activity of the unreacted silanol groups of the non-endcapped octadecyl bonded phase on the adsorption properties were investigated. The adsorption of two polar low molecular weight compounds with weak acidic (phenol) and with basic (aniline) character was measured. Adsorption data were acquired by frontal analysis from methanol–water and acetonitrile–water solutions to investigate the influence of the type of the organic modifier on the solute retention mechanism and adsorption under non-linear conditions. The adsorption behavior of phenol changes from Langmuir type (from aqueous mixture of methanol) to BET type (from aqueous mixture of acetonitrile). The adsorption of aniline becomes BET-type, regardless of the mobile phase composition in the tested range of the surface coverage of octadecyl ligands. The nature of the organic modifier significantly affects the retention mechanism and the shape of the overloaded elution bands of the studied compounds.

1. Introduction

The separation of compounds in reversed phase liquid chromatography (RPLC) results from the difference of their distribution between the mobile phase (usually aqueous solution of an organic solvent) and the hydrophobic bonded layer. Solute molecules may adsorb on the surface of the stationary bonded phase. An alternative mechanism of the distribution is the partition of the analyte between the mobile and bonded phases [1,2].

The separation selectivity in reversed-phase chromatography using octadecyl (C_{18}) bonded phase is optimized by the quantitative and qualitative composition of the binary mobile phase [3]. When the organic solvent in the mobile phase is replaced by another one, the properties of the stationary phase also change. The changes of the separation selectivity may be caused by solute interaction with the solvated stationary phase (organic ligands and solvent molecules are extracted into the hydrophobic region of bonded ligands) [4–7]. The quantitative mobile phase composition also has an important effect on the values of all the parameters of the adsorption isotherm and on the retention factor of the solute [1]. The changes of the mobile phase composition affect significantly the properties of the interfacial region the C_{18}-bonded silica surface [8–11]. It is caused by two effects. First, the increase of the organic solvent concentration in the aqueous mobile phase increases the competition for the hydrophobic adsorption sites between the solute and organic modifier. That results in the decrease of the retention factor of the analyte when the concentration of the organic modifier in the mobile phase increases. Second, the structure of the C_{18} chains that define the interphase between the mobile phase and the solid silica is affected because of the preferential adsorption of water and organic modifier on the residual accessible silanols and organic ligands [3,12–14]. At low organic modifier content in the mobile phase, the bonded chains are brought together by the intermolecular C_{18} dispersive interactions while they tend to exclude water. At high organic modifier concentration, the rupture of the octadecyl intermolecular interactions via chain solvation by the modifier and the formation of a 'brush-like' structure is observed [3,14]. The solvation process depends on the type of organic modifier, the length of organic ligands and their coverage density [14–17].

In a previous work [18] we have examined the effect of the nature of the organic solvent on the nonlinear adsorption behavior of phenol on endcapped reversed phase packing materials with different surface coverage of the octadecyl bonded ligands. The endcapping process shields a significant amount of the unreacted residual silanol-groups and make the hydrophobic interactions predominant even at low surface coverage of the C_{18} ligands on the retention process of phenol molecules.

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The goal of our present work was to investigate the effect of the significant amount of polar groups results the lack of the endcapping on the nonlinear retention behavior of two polar test compounds with low molecular weight. The Galushko test [19] is a well-known method to describe the silanol activity of a modified silica column using data of the retention difference between phenol and aniline molecules from the same mobile phase under linear condition. For that reason, we have chosen aniline and phenol as our test compounds to compare their retention behavior under nonlinear conditions.

We give a comprehensive overview of the effects that influence the overloaded elution band shapes under these conditions in reversed phase liquid chromatography. By means of five in-house-made non-endcapped octadecyl bonded phases with different surface coverage, the effect of the residual polar groups, and the change of the surface hydrophobicity was tracked by monitoring the change of the overloaded elution bands of the test molecules.

The overloaded elution bands of phenol and aniline were modeled on the basis of their adsorption isotherm data, the connection between the change of the isotherm parameters with the change of the surface coverage was determined. Furthermore, a numerical method was used to predict the overloaded band shape on columns with any desired surface coverage.

2. Theory

2.1. Frontal analysis for isotherm determination

Frontal analysis is widely used and the most accurate method to characterize the physico-chemical properties of chromatographic adsorbent beds. Two main variations of the method exist to perform frontal analysis in liquid chromatography: the single step, and the stepwise technique. Detailed methodology of the methods can be found in the literature [20–22]. Since the two techniques are equivalent, to save chemicals and to avoid the error results from the re-equilibration steps with the pure mobile phase between each breakthrough curve, we applied the stepwise method in our work. The principle of the stepwise technique is to perform consecutive and abrupt concentration increases of the compound studied in the mobile phase pumped into the column. After the concentration change in the mobile phase, the column has to be washed with the new eluent for sufficient time to reach the equilibrium between the mobile and the stationary phase. The equilibrium is indicated by the formation of a plateau on the frontal chromatogram between two breakthrough fronts. The calculation of the solute concentration on the stationary phase q at a given mobile phase concentration C, in the case of the stepwise method is given by the following equation [23]:

$$q_{i+1} = q_i + \frac{(C_{i+1} - C_i)(V_{F,i+1} - V_0)}{V_a} \tag{1}$$

where q_i and q_{i+1} are the adsorbed concentration of the analyte when the stationary phase is in equilibrium with the solute mobile phase concentrations C_i and C_{i+1} at the ith and $(i+1)$th step, respectively. If the observed fronts are symmetrical with sharp boundaries $V_{F,i+1}$, the retention volume of the front can be calculated at the volume corresponding to the maximum numerical value of the first derivative of the breakthrough curve of the chromatogram (the inflexion point) at the $(i+1)$th step, V_0 is the void volume of the system include all the volumes contribute in the band broadening, and V_a is the volume of the adsorbent.

2.2. Isotherm models

The Langmuir isotherm [24] describes ideal adsorption on homogeneous surfaces.

$$q = \frac{aC}{1 + b_sC} = \frac{q_sb_sC}{1 + b_sC} \tag{2}$$

where a is the Henry constant of the adsorption – the initial slope of the isotherm – given by

$$a = q_sb_s \tag{3}$$

where q_s is the mono-layer saturation capacity of the adsorbent, and b_s is the related adsorption equilibrium constant.

In several cases, the adsorption of the analyte is not ideal. The interaction between the adsorbed molecules is significant, and multilayer adsorption takes place. Brunauer, Emett, and Teller described a widely used model for non-ideal adsorption on homogeneous surfaces [25]. The equation extended to liquid–solid equilibria is written as:

$$q = \frac{q_sb_sC}{(1 - b_LC)(1 - b_LC + b_sC)} \tag{4}$$

where q_s is the monolayer saturation capacity, with the equilibrium constants for the adsorption on the surface b_s, and b_L is the equilibrium constants of the interaction between the adsorbed molecules. If the interaction between analyte molecules is strong, b_L becomes larger; if there is no interaction between adsorbed molecules, $b_L = 0$ and the equation reduces to a simple Langmuir equation.

2.3. Equilibrium dispersive model of chromatography

The equilibrium dispersive model of chromatography assumes constant equilibrium between the stationary and mobile phases, and uses an apparent dispersion term to account for the band broadening effects. The mass balance equation of the chromatographic system can be written as [20]:

$$\frac{\partial C}{\partial t} + F\frac{\partial q}{\partial t} + u\frac{\partial C}{\partial z} = D_a\frac{\partial^2 C}{\partial z^2} \tag{5}$$

where z is the length, t the time, u the mobile phase linear velocity, F is the phase ratio. When the molecular weight of the studied compound is small and its polarity is moderate, an instantaneous equilibrium between the stationary and the mobile phases can be assumed and the above model can be used with good accuracy. The apparent dispersion coefficient D_a defined as:

$$D_a = \frac{Hu}{2} \tag{6}$$

where H is the height equivalent to a theoretical plate. The isotherm parameters derived from the frontal analysis were used to predict overloaded elution band of the given compound. The overloaded elution bands were modeled using the Martin–Synge algorithm. Detailed method description can be found elsewhere [26].

3. Experimental

3.1. Instruments

The frontal analysis and overloaded band profile measurements were all acquired using an Agilent (Palo Alto, CA, USA) 1100 liquid chromatograph. This instrument includes a binary solvent delivery system, an auto-sampler with a 100 μL sample loop, a diode-array UV-detector, a column thermostat and a data acquisition station.