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Adsorption equilibria of proline in hydrophilic interaction chromatography

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ABSTRACT

The adsorption behavior of proline under hydrophilic interaction chromatography conditions was investigated from six aqueous solutions of acetonitrile. Proline adsorption isotherms were recorded at each mobile phase composition by frontal analysis and inverse method. The BET model was found to be the best choice to describe the nonlinear behavior of proline adsorption under hydrophilic interaction chromatography conditions. The adsorption isotherm parameters were derived from two independent parameter estimation methods. The parameters derived from regression analysis of the frontal analysis data and from overloaded elution bands were found to be in good agreement with the excess isotherm of water. The mobile phase composition at which the maximum excess adsorption of water was observed corresponded to the maximum saturation capacity measured for proline.

1. Introduction

Hydrophilic interaction chromatography (HILIC) [1] is the mode for the separation of polar compounds generally from aqueous solution of acetonitrile, with high acetonitrile concentration. With the decrease of the acetonitrile concentration, a range of mobile phase compositions with low sample retention can be observed. When the acetonitrile concentration is below a threshold, the retention of the samples may increase with the decrease of the concentration of acetonitrile (per aqueous liquid chromatography, PALC) [2]. The width of the composition boundary between HILIC and PALC modes depends on the adsorbent and the sample properties.

Significant enrichment of water has to be considered using aqueous solutions as mobile phase on polar adsorbents [3]. The retention of solutes may be due to the combination of adsorption and liquid–liquid partition mechanism, which – together with possible additional effects, such as ion-exchange – control the retention [4]. The water-rich layer above the surface governs the mixed retention of polar compounds under HILIC conditions. The higher the organic modifier concentration the stronger is the repulsion of the polar analytes from the aqueous-organic bulk mobile phase and the retention volumes increase, what results the 'HILIC-half' of the U-shape plot of the retention factor as a function of the organic modifier content [5].

The most accurate method to describe the physical–chemical properties of the adsorbent surface is the frontal analysis (FA) [6]. An alternative method with lower chemical requirement for isotherm determination is the modeling of overloaded elution bands using the inverse method (IM) [7]. Nonlinear chromatography data can give a detailed view of the surface processes in comparison with the data derived from analytical injections. The nonlinear behavior of polar analytes can help optimize the preparative scale separations.

The goal of our study was to determine the effect of the preferential water adsorption on silica surface on the nonlinear behavior of proline as test compound under HILIC conditions from aqueous solution of acetonitrile as mobile phase.

2. Theory

2.1. Measurement of solvent adsorption

In hydrophilic interaction chromatography, an adsorbed layer of water is formed on the surface of the polar stationary phase. The excess amount of water present at the surface can be characterized by measuring its excess isotherm. The determination of the excess isotherms starts with measuring the thermodynamic void volume of the column by the minor disturbance method. The equilibrium between the binary mobile phase and the adsorbed phase is perturbed and the elution volume of the perturbation is recorded at every mobile phase composition from pure water to pure acetonitrile.

For the determination of the thermodynamic void volume of the system, the NA (nothing-is-adsorbed) convention was used [15] to determine the Gibbs dividing plane. The vNA (the concentration is expressed in volume fraction) and nNA (the concentration is in

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molar fraction) conventions were used and the results are compared in our study. The thermodynamic void volume determined by the NA conventions should be equal to the void volume in the chromatographic system if determined with an ideal mixture of noncompressible liquids.

When the composition of the mobile phase is expressed by volume fraction (vNA convention), the thermodynamic void volume of the column is derived as:

$$V_{0/\mathrm{vNA}} = \int_0^1 V_R(\phi)d\phi \tag{1}$$

where ϕ is the volume fraction of water in the mobile phase, and V_R is the retention volume of the disturbance peak. In the case of the nNA convention the equation is:

$$V_{0/\mathrm{nNA}} = \int_0^1 V_R(x)dx \tag{2}$$

where x is the molar fraction of water in the mobile phase.

The excess adsorption isotherm of water can be calculated from the data above as [8]:

$$\Gamma_{\mathrm{vNA}}\ \ (\phi) = \frac{1}{V_{a/\mathrm{vNA}}}\int_0^{\phi}[V_R(\phi') - V_{0/\mathrm{vNA}}]d\phi' \tag{3}$$

$$\Gamma_{\mathrm{nNA}}(x) = \frac{1}{V_{a/\mathrm{nNA}}}\int_0^{x}[V_R(x') - V_{0/\mathrm{nNA}}]dx' \tag{4}$$

where $V_{a/\mathrm{vNA}}$ and $V_{a/\mathrm{nNA}}$ are the volume of the adsorbent in the case of the two respective conventions, and they are given by:

$$V_{a/\mathrm{vNA}} = V_G - V_{0/\mathrm{vNA}} \tag{5}$$

$$V_{a/\mathrm{nNA}} = V_G - V_{0/\mathrm{nNA}} \tag{6}$$

where V_G is the total geometrical volume of the column.

2.2. Frontal analysis

Frontal analysis measurements were carried out using the step-wise technique [9]. One channel of the multichannel solvent delivery system was used to deliver the sample solution and the other to pump the pure mobile phase. Adsorption isotherms were measured at six acetonitrile–water compositions. Acetonitrile percentage was varied in the range 70–85% (v/v). At each solvent composition, the maximum concentration of proline was fixed at 4 g/dm^3. Breakthrough curves were recorded at 215 nm.

The integral mass balance of breakthrough curves between the equilibrium mobile phase concentrations C_i and C_{i+1} (in g/dm^3 of the solution) shows that the equilibrium concentration of the analyte on the stationary phase is given by [10]:

$$q_{i+1} = q_i + \frac{(C_{i+1} - C_i)(V_{F,i+1} - V_0 - V_{ex})}{V_a} \tag{7}$$

where q_i and q_{i+1} are the adsorbed concentrations of the analyte (in g/dm^3 of the adsorbent), when the stationary phase is in equilibrium with the solute mobile phase concentrations C_i and C_{i+1}, respectively, at the ith and $(i+1)$th step. $V_{F,i+1}$ is the retention volume of the breakthrough curve at the $(i+1)$th step and V_{ex} the extra-column volume. The retention volume of each front was derived using the equal area method [9].

2.3. Modeling overloaded elution bands

In addition to FA, adsorption isotherms were also measured by the IM. The equilibrium dispersive model was used to model the experimental data. This model assumes that all contributions due to the non-equilibrium can be lumped into an apparent axial dispersion term. It represents a reasonable approximation of the real system when the mass transfer in the chromatographic column is controlled only by molecular diffusion across the mobile phase flowing around the packing particles and if the exchange of feed components between the stationary and mobile phase is very fast. The mass-balance equation was solved using the Rouchon backward–forward algorithm [9]. The injection profile (boundary condition of mass-balance equation) was modeled as the convolution between an exponential modified Gaussian function and a rectangular wave [11]. Measured and calculated band profiles were compared by evaluating the following objective function:

$$\min\sum_i (C_i^{\mathrm{calc}} - C_i^{\mathrm{meas}})^2 \tag{8}$$

where C_i^{calc} and C_i^{meas} are the calculated and measured concentrations at point i. At the end of each iterative loop, the isotherm parameters are changed to minimize the objective function using a super-modified simplex algorithm [12]. UV detector calibration was carried out using the plateau absorbances recorded in FA for the different equilibrium mobile phase concentrations of proline. A polynomial equation was used to fit absorbance-concentration data.

3. Experimental

3.1. Instrumentation and chemicals

The measurements were performed using an Agilent (Palo Alto, CA, USA) liquid chromatograph. This instrument includes a 1100 binary-solvent delivery system, a manual injector with a 20-μL sample loop, 1100 series variable wavelength UV detector, column thermostat and a data acquisition station.

Acetonitrile, methanol and proline were purchased from Sigma–Aldrich. Water was purified using Milli-Q system (Millipore, El Paso, TX, USA).

The column employed for the measurement of the water excess isotherm, was a 250 mm × 4.6 mm Waters Atlantis porous silica with 5 μm average particle diameter. The adsorption isotherms of proline were determined on a 150 mm × 4.6 mm Waters Atlantis column packed with the same silica gel. All measurements were carried out with a constant 1.0 cm^3/min flow rate, at 278 K.

4. Results and discussion

4.1. Measurement of hold-up and extra-column volumes

In addition to the thermodynamic determination (Eqs. (1) and (2)), V_0 was also determined by the weight difference method (wd) [13,14]. The column was successively filled with water and organic modifier and $V_{0/\mathrm{wd}}$ calculated as:

$$V_{0/\mathrm{wd}} = \frac{m_{\mathrm{H_2O}} - m_{\mathrm{org}}}{\rho_{\mathrm{H_2O}} - \rho_{\mathrm{org}}} \tag{9}$$

where $m_{\mathrm{H_2O}}$ and m_{org} are the masses of the column filled with water and organic modifier, respectively, and $\rho_{\mathrm{H_2O}}$, and ρ_{org} the corresponding densities. The measurements were carried out with acetonitrile as organic modifier. The resulting values are listed in Table 1. The volume of the adsorbent was calculated as the difference of the geometric column volume and the void volume. The weight difference method gives another opportunity to estimate the thermodynamic void volume of the columns. This method assumes the total volume of the liquid phase in the column as the mobile phase. At this point the void volumes determined by Eqs. (1) and (2) become comparable with the results obtained from the independent weight difference method.