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Impact of an error in the column hold-up time for correct adsorption isotherm determination in chromatography
II. Can a wrong column porosity lead to a correct prediction of overloaded elution profiles?

Jörgen Samuelsson[a], Jia Zang[b], Anne Murunga[b], Torgny Fornstedt[a,*], Peter Sajonz[b,*]

[a] Department of Physical and Analytical Chemistry, BMC Box 577, SE-751 23 Uppsala, Sweden
[b] Merck Research Laboratories, P.O. Box 2000, Separation and Purification Center of Excellence, Rahway, NJ 07065, USA

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ABSTRACT

The adsorption isotherm was determined for phenol in methanol/water on a C-8 stationary phase using frontal analysis in staircase mode, assuming different total column porosities, from 1 to 87%. Each set of adsorption isotherm data, with a certain column porosity, was fitted to various adsorption models and the generated parameters were used to calculate overloaded elution band profiles that were compared with experiments. It was found that the bi-Langmuir model had an optimum fit for a porosity that corresponds well with the value found experimentally. The adsorption energy distribution (AED) calculations and error analysis confirmed a bimodal energy distribution. It was also found that band profiles can be accurately predicted with a quite arbitrary chosen porosity, under prerequisite that a wrong but flexible adsorption model is chosen instead of the correct one. The latter result is very useful for quick optimizations of preparative separations where the exact value of the column porosity is not available.

1. Introduction

In preparative chromatography the hold-up time (or volume) is an essential parameter for computer-assisted optimizations of the experimental conditions to get maximal throughput and product yield. However, the determination of the column hold-up volume is not trivial and its proper estimation has remained an important issue for many years [1–3]. More recently Gritti et al. made a more stringent definition of the hold-up volume [4]. All methods for determination of the hold-up volume contain sources of errors; therefore the value obtained from the hold-up volume will be different depending on which method was used. For example, recently a 14% difference was found between the unretained marker thiourea and the pyconometry method for determining the hold-up volume in the same chromatographic system [5].

In a recent study [6] it was found based on computer-generated data that an error in the hold-up volume results in serious errors in the adsorption isotherm coefficients and that the error increases for larger degrees of nonlinearity of the chromatographic system. This result was later confirmed with experimental data [5]. Seidel-Morgenstern showed that a wrong porosity could predict quite satisfactorily peak profiles, at least for low concentrations [7]; however, as this was not the main topic of the paper, the effect was not systematically investigated. In a more recent study we investigated the importance of a small error in the hold-up time on the choice of adsorption isotherm model that can fit to computer-generated adsorption isotherm data [8]. Our results showed that data from a true Langmuir or a true bi-Langmuir model used with an underestimated hold-up time have a better fit to a more heterogeneous model while for an overestimated hold-up time models describing false adsorption processes such as multi-layer adsorption or solute–solute interactions are assumed. Scatchard plots and calculations of the adsorption energy distribution (AED) confirmed the deviations from the Langmuir behavior [8].

In this paper we present experimental adsorption isotherm data determined by frontal analysis in the staircase mode for phenol on a C-8 stationary phase and methanol/water as mobile phase. The purpose of the study is twofold. Firstly, we will systematically investigate if wrong column porosities can still lead to correct predictions

* Corresponding authors. Tel.: +1 732 5948430; fax: +1 732 5943887.
E-mail addresses: Torgny.Fornstedt@ytbioteknik.uu.se (T. Fornstedt), Peter_Sajonz@Merck.Com (P. Sajonz).

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of overloaded elution profiles. Secondly, we will examine if it is possible to estimate the columns porosity only from the measurement of equilibrium isotherm data, assuming that the true adsorption isotherm model is known.

2. Experimental

2.1. Chemicals and materials

The water used was distilled and purified by a Hydro System purchased from Hydro Service & Supplies Inc. (Garfield, NJ, USA). HPLC grade methanol and phenol were obtained from Fluka (Buchs, Switzerland). Thiourea was purchased from Aldrich (St. Louis, MO, USA).

2.2. Instrumentation and HPLC method conditions

An Agilent 1100 Series HPLC system from Agilent Technologies (Palo Alto, CA, USA) was used for all experiments. This system is equipped with an auto injector containing a sample tray cooler, a multi-solvent delivery system and a temperature controlled column compartment set at 25.0 °C. The detector-wavelengths monitored were 254, 292 and 260 nm, respectively. The flow rate used for all experiments was 1.0 mL/min. The mobile phase used was 40/60 (v/v) methanol/water. The mobile phase was also used as diluent for sample preparations. The column was an Advantage ARMOR ADV5218 C-8 (nominal particle size: 5 μm; 4.6 mm × 250 mm I.D.) obtained from Analytical Sales and Services (Pompton Plains, NJ, USA).

2.3. Procedures

The retention volumes of the peaks were determined at the maximum peak height. The retention data for the elution experiments were corrected for the extra-column volume, determined to 0.06 mL, by replacing the column with a zero dead volume union and injecting small volumes (5 μL) of thiourea. The column plate number was determined to $n = 9508$ from the width at half-height of the peak resulting from a 5 μL injection of a diluted phenol solution. For this experiment the column was placed as close to the injection valve as possible, to minimize the extra-column volume. The column plate number of phenol was used later for the numerical calculation of overloaded elution profiles.

The concentration steps generating the frontal chromatograms were obtained in a stepwise manner using one solvent channel with mobile phase and another with a bulk concentration of phenol (80.96 g/L) in mobile phase. The retention times of the frontal chromatograms were calculated at the half-heights of each step in the staircase. We validated the retention times from the half-height method with the area method as reference. The average difference between the two methods was only 0.4% which confirmed that it is acceptable to acquire the adsorption data by the half-height method for our experimental system. The so-called gradient delay volume, *i.e.* the extra-column volume for the frontal analysis experiments, was determined to 1.08 mL by performing a concentration step with thiourea as sample. The retention times for the breakthrough curve experiments were corrected for this delay volume. It was tested if this gradient delay volume had an effect on the retention times of the breakthrough curves, in the following way. With the inert marker thiourea, the column hold-up time was calculated by either performing conventional injections or by performing small steps with the pump and then correct for the gradient delay volume. The value of the hold-up time varied less then 2% by using these two ways. For this reason we can conclude that the gradient delay volume is not too big for the purpose of acquiring accurate retention times of the breakthrough curves.

It has to be noted that even if the experimental setup that is used in this study is suitable for the purpose of accurate isotherm measurements it is not useful for the measurement of kinetic data. The reason for this is because band broadening effects occur due to the large extra-column volume which compared to the column void volume is quite significant. For the accurate measurement of kinetic data (and also isotherm data) a system that uses two large injection loops with two sample switching valves that are connected close to the column inlet (as described in Jacobson et al. [9]) should be used. Such an experimental setup not only minimizes the extra-column volume and thus the band broadening effects, but also minimizes the amount of sample that is required for a complete set of frontal analysis experiments.

The plateaus of the frontal analysis experiments data were used to calculate a fourth degree polynomial calibration curve converting the absorbance units from the detector to concentration units. All calculations were performed using Matlab version 7.0 (MathWorks Inc., Natick, MA, USA).

In this study the column porosity (ε) has been used rather than the hold-up time because it is a dimensionless parameter (defined as $\varepsilon = V_0/V_g$, where V_g is the geometrical column volume and V_0 is the hold-up volume). A porosity of 0% represents a column completely filled with stationary phase and 100% is an empty column. In reality, the column porosity will vary within narrower limits [10].

The raw adsorption isotherm data were fitted to the *n*-Langmuir isotherm equation using a nonlinear fitting procedure and the Marquardt-Levenberg algorithm with at least 1000 different initial guesses selected randomly over all possible solutions. An *n*-Langmuir adsorption isotherm model describes the relationship between the solute concentration in the stationary and mobile phases. The equation for the model is written as

$$q = \sum_{i=1}^{n} \frac{a_i C}{1 + K_i C} \tag{1}$$

where a_i and K_i are numerical coefficients. Three different models were considered in this study, *i.e.* $n = 1$ (Langmuir), $n = 2$ (bi-Langmuir), and $n = 3$ (tri-Langmuir). The ratios a_i/K_i represent the monolayer solute saturation capacities $q_{s,i}$ of each individual adsorption site in the column.

The AEDs were calculated from the raw adsorption isotherm data [11,12]. The Langmuir model was used for the local adsorption isotherm and the AED integral was solved with an iterative algorithm (expectation-maximization method) [13]. All calculations were carried out by expanding the integration limits between $0.1/C_{max}$ to $10/C_{min}$, where C_{min} and C_{max} are the minimum and maximum concentration used in determining the adsorption isotherm. The expansion is necessary to promote conversion of adsorption sites with energy near the integration limits.

3. Results and discussion

3.1. Calculation of adsorption isotherm data from frontal analysis experiments

Frontal analysis experiments for the purpose of adsorption isotherm determination are carried out by a series of concentration step experiments. These experiments can be a series of steps from 0 to C_i, or successive steps from 0 to C_1, then C_1 to C_2 *etc.* The first method is known as frontal analysis in the step-series mode, the second one as frontal analysis in the staircase mode [14]. The advantage of the staircase method is that the column does not have to be re-equilibrated to $C = 0$ after reaching the plateau of each