Short communication

Determination of adsorption isotherms of proteins by H-root method: Comparison between open micro-channels and conventional packed columns

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Abstract

This communication compares the accuracy of a micro open parallel plate system (μOPPS) with a conventional packed column for predicting isotherm data by using the H-root method (HRM). HRM is restricted to compounds obeying the Langmuir isotherm model. The performance of the two chromatographic systems was simulated by using comprehensive mathematical models. Operating conditions were varied and their effects on the accuracy of predictions was evaluated. Better accuracy in the isotherm predictions was obtained with the packed column due to its higher efficiency. However, good predictions can be obtained with the μOPPS with the advantage of significantly lower sample consumption.
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1. Introduction

H-root method (HRM) is a dynamic chromatographic method for the prediction of isotherm data. HRM is based on the H-transformation theory (HTT) and is restricted to compounds obeying the Langmuir isotherm model [1]. A very similar approach called the ω-transformation was developed by Rhee et al. [2], both approaches are mathematically equivalent [3]. HRM, originally developed by Chen et al. [4], required detailed chromatograms. Jen and Pinto [5] proposed a modified version of HRM, which required less data. Despite the potential of HRM for predicting isotherm data, its application is not widespread. To the best of our knowledge, only a few papers have been published on this topic [4–8]. A similar methodology has been proposed by Felinger et

al. [9]. The main advantages of HRM are that pure analytes are not required, and a single frontal analysis is enough to determine competitive isotherm coefficients [8]. A limitation of this approach is that it assumes a priori that a Langmuir isotherm model is valid [1,4–8]. It is therefore imperative to follow the estimations of the Langmuir coefficient with probe nonlinear chromatographic experiments to validate the original assumption. In a previous publication [6] we analyzed the application of HRM for predicting isotherm data for a protein-salt system in two micro-chromatographs: the micro open tubular system (μOTS) and the micro open parallel plata system (μOPPS).

Micro-systems have significant advantages over bench-scale systems: faster analysis time, smaller dead volumes and lower sample consumption. The amount of sample required is a critical parameter when studying analytes such as biomolecules or isomers, which are expensive, and in many cases, not commercially available [8]. The present publication compares the performance of the μOPPS to a

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conventional packed column as tools for predicting isotherm data.

2. Theory

2.1. The μOPPS model

A schematic representation of the μOPPS is shown in Fig. 1a. A detailed description of the mathematical model employed to simulate the performance of the μOPPS is described elsewhere [10,11]. Briefly, adsorption of the solute is assumed to take place on the side surfaces of the channel, while the top and bottom surfaces are assumed to be inactive. With these assumptions, the dimensionless mass balance equation for a single solute and the phase ratio (ϕ) in the μOPPS are [6,10]:

$$\frac{\partial C_i}{\partial \tau} - \frac{12}{7} \frac{\partial C_i}{\partial Y} \left(\frac{b^2 + d^2}{d^2} \right) \left(\frac{(X^2 - 1)(Z^2 - 1)}{b^2/d^2(X^2 - 1) + (Z^2 - 1)} \right)$$
$$= \frac{1}{\theta_{X,i}} \frac{\partial^2 C_i}{\partial X2} + \frac{1}{Pe_i} \frac{\partial^2 C_i}{\partial Y^2} + \frac{1}{\theta_{Z,i}} \frac{\partial^2 C_i}{\partial Z2} \tag{1}$$

$$\phi_{OPPS} = \frac{1}{b} \tag{2}$$

where the 12/7 factor in Eq. (1) is the result of adapting the velocity profile of the original publication (which was developed for an elliptical cross section) to a rectangular cross section [12].

2.2. The conventional packed column model

A schematic representation of the conventional packed column is shown in Fig. 1b. A numerical model was developed to simulate the performance of a conventional packed column using the algorithm proposed by Phillips et al. [13]. The reader is referred to the original publication for details. The dimensionless mass balance equations for a single solute within the packed column are:

$$\frac{\partial C_i}{\partial \tau} + \phi_{Packed} \frac{\partial q_i}{\partial \tau} + \frac{\partial C_i}{\partial Y} - Pe_i \frac{\partial^2 C_i}{\partial Y^2} = 0 \tag{3}$$
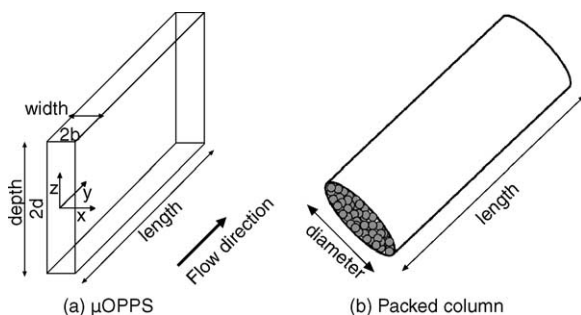

Fig. 1. Schematic representation of μOPPS (a) and conventional packed column (b).

$$\frac{\partial q_i}{\partial \tau} = St_i(q_i^* - q_i) \tag{4}$$

In order to use the same values of the Langmuir parameters for the simulations of the μOPPS and packed column, ϕ_{Packed} was defined as follows:

$$\phi_{Packed} = \frac{\rho_b a_s}{\varepsilon} \tag{5}$$

2.3. H-root method equations

For detailed information about HRM or the HTT the reader is referred to the literature [1,3–6,14–16]. Briefly, HRM calculates the Langmuir parameters (Eq. (6)) of the analytes from their chromatographic response.

$$q_i^* = \frac{V_{m_i} K_{m_i} C_i}{1 + \sum_{j=1}^{n_c} K_{m_j} C_j} = \frac{a_i C_i}{1 + \sum_{j=1}^{n_c} K_{m_j} C_j} \tag{6}$$

HRM consists of two main parts: linear elution experiments to calculate the linear isotherm coefficient a_i (Eq. (6)), and nonlinear frontal experiments to calculate the competitive interference parameter K_{m_i} (Eq. (6)). The HRM equations for the conventional packed column and the μOPPS were developed previously [4–6]. The linear isotherm coefficient, a_i, is calculated by employing the retention time (T_R) of the linear elution peaks:

$$a_i = \left(\frac{T_{R,i}}{T_0} - 1 \right) \frac{1}{\phi} \tag{7}$$

The coefficients K_{m_i} are calculated by employing the frontal column capacity factor (K_i) and the linear elution capacity factor (k_i) [6,10]. Sub-indices 1 to n represent the analytes in order of their decreasing column retention. A system of n equations, formed by Eqs. (8) and (9), is solved in order to calculate the interference parameters K_{m_i} [5].

$$\sum_{i=1}^{n} \left(\frac{K_{m_i} CF_i}{(k_i/K_n) - 1} \right) - 1 = 0 \tag{8}$$

$$\sum_{i=1}^{n} \left(\frac{K_{m_i} CF_i}{K_{j+1} k_i/(K_j k_{j+1}) - 1} \right) - 1 = 0,$$
$$(1 \le j \le n - 1) \tag{9}$$

3. Numerical simulations

Simulations were performed in order to test the potential of the μOPPS and conventional packed column with the HRM for predicting isotherm data under different operating conditions. The flow chart for obtaining a_i and K_{m_i} by employing the numerical simulators is included elsewhere [6]. The protein-salt system selected was Conalbumin (CON) and NaCl, exchanging on PAE resin (a polyethyleneimine

Table 1
Parameters used for isotherm predictions

Solute	D (cm^2/s)[a]	MW (g/mol)[a]	K_m (cm^3/μmol)[b]	a (cm)[b]
NaCl	2.0×10^{-5}	58.43	70.8	7.08×10^{-3}
Conalbumin	5.94×10^{-7}	80,000	228.8	1.92×10^{-2}

[a] Obtained form Tyn and Gusek [17].
[b] Obtained from Raje [18].

coated, silica-based weak anion exchanger). The experimental Langmuir parameters, diffusion coefficients and molecular weights are summarized in Table 1. Linear elution and nonlinear frontal simulations were carried out for CON using NaCl as the modulating salt. Simulations were performed for the μOPPS and the packed column by varying the parameters Pe_i and ϕ. The simulation parameters used are shown in Table 2, unless otherwise stated. From Table 2 it can be seen that there is a difference of 5–6 orders of magnitude in sample consumption (V_F) between the μOPPS and the packed column. It has to be noted that due to the dissimilarity between these two chromatographic systems, the *standard* operating conditions (i.e. those used in practice) for each system are significantly different. Additionally, it can be seen from Table 2 that the sample concentration and sample volumes used for the linear elution simulations (in both the packed column and the μOPPS) are much lower than those used for the frontal nonlinear simulations.

4. Results and discussion

4.1. Prediction of linear isotherms

The coefficient a_i is calculated from data obtained directly from the elution chromatograms. Peclet number (Pe) and phase ratio (ϕ) are parameters expected to affect the accuracy of the estimation of a_i in both chromatographic systems. Pe is directly related to band spreading and ϕ measures the column capacity. Higher deviations in predicting a_i are expected at higher Pe [6] or lower ϕ [4,5]. Shown in Fig. 2a are the experimental and the predicted linear isotherms obtained as a function of Pe and ϕ in the μOPPS. Pe was varied by varying v_{avg}, and ϕ_{OPPS} was varied by varying b. From the figure it can be observed that the isotherms are accurately predicted; the average deviations in predicted a_{CON} for the variations studied were only 4%. The packed column has a higher efficiency than that of the μOPPS, due to it much higher ϕ. Shown in Fig. 2b are experimental and predicted linear isotherms obtained in the packed column as functions of Pe and ϕ: the deviations in predicting a_{CON} were very close to 0%. Pe was changed by varying v_{avg}, and ϕ_{Packed} by varying a_s. While the packed column was found to give more accurate predictions than the μOPPS, it is noted that the isotherm predictions obtained with the μOPPS are sufficiently accurate considering that they are within the usual error bar of most experimental measurements of isotherms. Also, notably this prediction is obtained in the μOPPS with a sample size that is approximately 5 orders of magnitude (Table 2) lower than the amount consumed in the packed column.

Table 2
Simulation parameters used for the linear elution and nonlinear frontal simulations.

| Parameter	Packed column		μOPPS	
Cross section	r_C (cm)	0.23	b (μm)	10–30
			d (μm)	10–30
Length (cm)		25.0		3.0
Packing parameters	ε (dimensionless)	0.60	NA	
	a_s (cm^2/g)	1×10^4–1×10^5		
	ρ_b (g/cm^3)	0.45		
C_F (μmol/cm^3)	Linear simulations	0.0001	Linear simulations	0.0001
	Nonlinear simulations	0.05	Nonlinear simulations	0.05
V_F (cm^3)	Linear simulations	0.125	Linear simulations	1.2×10^{-7}–1.1×10^{-6}
	Nonlinear simulations	12.5	Nonlinear simulations	3.6×10^{-5}–3.2×10^{-4}
v_{avg} (cm/s)		0.25–1.50		0.001–0.015
kc_{NaCl} (s^{-1})[a]		0.1010		NA
kc_{CON} (s^{-1})[a]		0.1515		NA

[a] Obtained from Raje and Pinto [19].
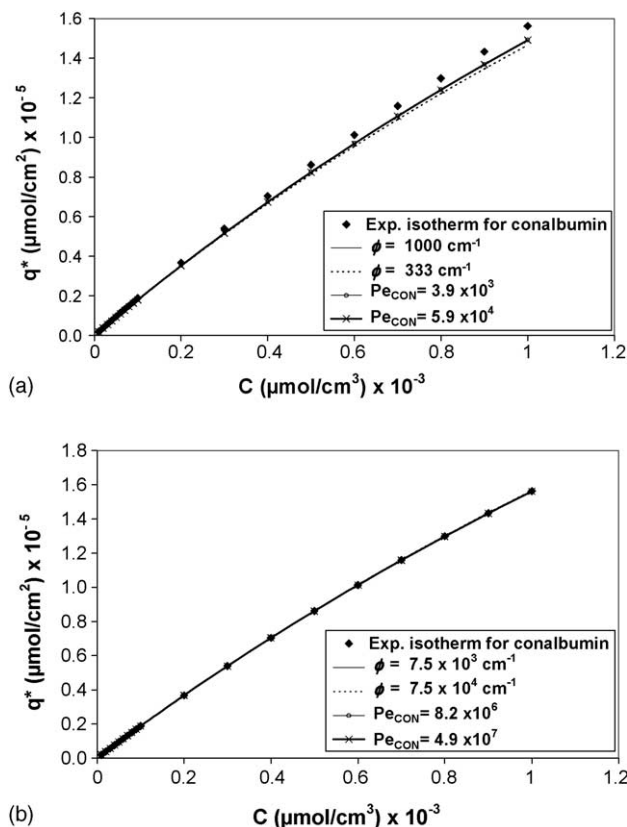
Fig. 2. Experimental and predicted linear isotherms for the protein Conalbumin as functions of Pe and ϕ: (a) in the μOPPS; (b) in the packed column.

Fig. 3. Experimental and predicted nonlinear isotherms for the protein Conalbumin as functions of Pe and ϕ: (a) in the μOPPS; (b) in the packed column.

4.2. Prediction of nonlinear isotherms

In order to predict the interference coefficient (K_{m_i}), it was necessary to simulate frontal injections of the protein at the salt concentration of interest. It is noted that the selection of operating conditions is crucial in the prediction of K_{m_i} [4–6]. Compared in Fig. 3a are the experimental and predicted nonlinear isotherms obtained with the μOPPS as functions of Pe and ϕ_{OPPS}. Within the range of operating conditions studied, both parameters had only a small effect on the accuracy of the predictions. The average deviations in predicting $K_{m_{CON}}$ as a function of Pe and ϕ_{OPPS} were 3% and 6%, respectively. When a short column length ($L = 3$ cm), equal to that of the μOPPS, was utilized for a conventional packed column, the isotherm coefficients were predicted with lower accuracy than with the μOPPS [10]. However, when the packed column length is increased the accuracy improves. Shown in Fig. 3b is a comparison of the predicted and experimental isotherms in a 25 cm column. Excellent results are obtained, and the average deviations in predicting $K_{m_{CON}}$ were only 1%. In conclusion, when high accuracy is required in estimating the nonlinear coefficient, the packed column is superior. However, if available sample volume is a limitation, the μOPPS can be used to great advantage for estimations.
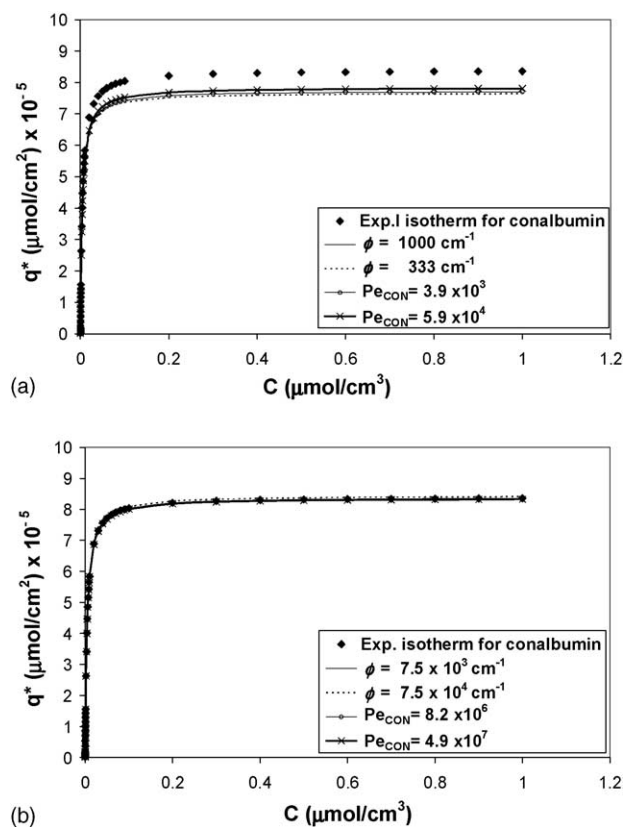
5. Conclusions

The chromatography based H-root method was utilized to predict adsorption isotherms (Type I, Langmuir) for the protein Conalbumin using the salt NaCl as modulator. The performance of the μOPPS and a conventional packed column were simulated. The accuracy of the isotherm predictions obtained with both chromatographic systems was evaluated as a function of Pe and ϕ. The results show that it is possible to predict accurate isotherm data with both, micro and bench-scale systems. In general, better accuracy in isotherm predictions was obtained with the packed column, due to its much higher ϕ. Nevertheless, under the appropriate operating conditions (low Pe, high ϕ), good isotherm predictions can be obtained with the μOPPS as well. A significant advantage of the μOPPS is much lower sample consumption, approximately 5–6 orders of magnitude lower. Thus, when sample volumes are limited, the μOPPS provides an attractive alternative for obtaining good estimates of isotherms parameters.

6. Nomenclature

List of symbols

a Langmuir affinity coefficient (linear isotherm coefficient) (cm), $a_i = V_{m_i} K_{m_i}$

a_s packing surface area per unit mass (cm^2/g)

b microchannel half width (cm)

C solute concentration in the mobile phase (μmol/cm^3)

C_F feed concentration (μmol/cm^3)

d microchannel half depth (cm)

D diffusion/dispersion coefficient (cm^2/s)

k linear elution column capacity factor (dimensionless), $k_i = (T_{R,i} - T_0)/T_0$

K frontal capacity factor (dimensionless), $K_i = (T_{RF,i} - T_0)/T_0$

kc   overall effective mass-transfer coefficient (s^{-1})

K_m Langmuir competitive interference coefficient (cm^3/μmol)

L column length (cm)

n_c number of components

Pe Peclet number (dimensionless), $Pe_i = (v_{avg}L)/D_i$

q average concentration of solute in the stationary phase (μmol/cm^2)

q_* equilibrium concentration of solute in the stationary phase (μmol/cm^2)

r_C packed column radius (cm)

St Stanton number (dimensionless) $St_i = (kc_iL)/v_{avg}$

t time (s)

T_0 holdup time (s)

T_R retention time (s)

T_{RF} breakthrough time (s)

v_{avg} average linear velocity along the length (cm/s)

V_F feed volume (cm^3)

V_m Langmuir parameter (μmol/cm^2)

X dimensionless position along the microchannel width, $X = x/b$

x position along the microchannel width (cm)

Y dimensionless position along the microchannel length, $Y = y/L$

y position along the microchannel length (cm)

Z dimensionless position along the microchannel depth, $Z = z/d$

z position along the microchannel depth (cm)

Greek symbols

ε bed porosity (dimensionless)

ϕ_{OPPS} phase ratio in the μOPPS (cm^{-1}) $\phi_{OPPS} = 1/b$

ϕ_{Packed} phase ratio in the packed column (cm^{-1}) $\phi_{Packed} = \rho_b a_s/\varepsilon$

θ_X dimensionless time along the microchannel width, $\theta_{X,i} = (v_{avg}b^2)/D_iL$

θ_Z dimensionless time along the microchannel depth, $\theta_{Z,i} = (v_{avg}d^2)/D_iL$

ρ_b bed density (g/cm^3)

τ dimensionless time along the microchannel length, $\tau = (v_{avg}t)/L$

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