Batch dynamic adsorption of dipeptides onto reversed-phase silica gel

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ABSTRACT

Batch adsorption kinetics of two dipeptides, N-carbobenzoxy-L-leucyl-glycine and N-carbobenzoxy-glycyl-L-phenylalanine, onto the reversed-phase C_{18} silica gel has been experimentally and theoretically studied in this paper. This dynamic adsorption process was described with a rate-equation model, in which the surface interaction rates is considered to be finite and follows an intrinsic kinetics of Langmuir type. The model is validated with the experimental batch kinetics data. The model parameters, rate constants and pore diffusivity, were estimated by matching the experimental data with the theoretical predictions obtained from numerical solution of the model equations. The dependence of these parameters on various factors including initial solute concentration, ratio of solution volume to adsorbent mass, pH and methanol content were examined.

INTRODUCTION

Reversed-phase liquid chromatography is a powerful technique for the purification of peptides and proteins from natural and synthetic sources. Mathematical modelling to predict the process performance of a full scale column is essential for the design and optimisation of such chromatographic processes. The application of such approach requires model parameters, which characterise the thermodynamics and kinetics of the process. Since the intraparticle mass transfer and surface interaction are independent of operation mode, the parameters such as pore diffusivity and rate constants for surface interaction can be estimated using the batch experimental data [1,2]. Compared with column operation mode, the batch mode taking place in a finite bath has the main advantages of less expensive and less computational effort involved in the parameter estimation.

Most of previous works has been concerned with the retention factor in reversed-phase liquid chromatography [3–5]. However, the use of retention coefficients only addresses the thermodynamics of the process and it is not possible to assess the bandspreading in the column process. Furthermore, slow binding kinetics was observed in reverse phase chromatographic system and the activation energies for binding and dissociation were clearly commensurate with those involved in the rupture of weak chemical bonds [6]. In a column dynamic study, we also found that the bandspreading in a reversed-phase column can be well interpreted with the intrinsic kinetics mechanism [7].

In this work, batch dynamic adsorption of two dipeptides onto reversed-phase C_{18} silica gel was experimentally studied. A rate-equation model, incorporated with the intrinsic adsorption kinetics, was proposed to describe the adsorption process. The experimental data were used to validate the proposed model and to extract the relevant model parameters. The dependence of model parameters, rate constants and pore diffusivity, on various factors has been examined. These factors include the

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initial solute concentration, the ratio of solution volume to sorbent mass, pH and the methanol content in the mixed solvent.

THEORETICAL

Model equation

Under consideration is the batch uptake process taking place in a finite bath, in which porous adsorbent particles with uniform spherical shape and size are suspended in the bath liquid by mechanical means, and the solution is stirred such as the bulk concentration is uniform through the bath. A rate-equation model is developed to describe this batch dynamic adsorption process. This model takes into account external diffusion in the stagnant film around the adsorbent particle, pore diffusion through the void in the particle and the interaction between adsorbates and pore surface.

In formulating this model, we have assumed isothermal behaviour and constant liquid volume in the bath. The film mass transfer coefficient is taken to be constant. The pore diffusivity is also assumed to be independent of solute concentration.

Having made the above assumptions, we can write the mass balance equation for the solute k in the particle phase as follows

$$\varepsilon_m \cdot \frac{\partial C(k)}{\partial t} + (1 - \varepsilon_m) \cdot \frac{\partial C_\mu(k)}{\partial t} =$$

$$\varepsilon_m D_p(k) \frac{1}{r^2} \cdot \frac{\partial}{\partial r} \cdot \left(r^2 \cdot \frac{\partial C_\mu(k)}{\partial r} \right) \quad (1)$$

where $\partial C_\Omega/\partial t$ is governed by the following Langmuir kinetic equation:

$$\frac{\partial C_\mu(k)}{\partial t} = k_a(k)C(k)\left[1 - \sum_{j=1}^{NC} \frac{C_\mu(j)}{C_{\mu s}(j)} \right] - k_d(k)\left[\frac{C_\mu(k)}{C_{\mu s}(k)} \right] \quad (2)$$

where k_a is the adsorption rate constant (1/s); k_d is the desorption rate constant (mmol/ml · s), and $C_{\mu s}$ is the sorbent capacity.

Eqns. 1 and 2 are subject to following initial and boundary conditions:

$$t = 0; \ C(k) = 0; \ C_\mu(k) = 0$$

$$t = 0; \frac{\partial C(k)}{\partial r} = 0 \quad (3)$$

$$r = R_0; \ \varepsilon_m D_p(k) \cdot \frac{\partial C(k)}{\partial r} = k_m(k)[C_b(k) - C(k)]$$

The mass balance equation for the finite bath is written as

$$V \cdot \frac{dC_b(k)}{dt} = -\left(\frac{m}{\rho_p}\right)\left(\frac{3}{R_0}\right)\left(\varepsilon_m D_p(k) \cdot \frac{\partial C(k)}{\partial r}\bigg|_{R_0} \right) \quad (4)$$

The initial condition for eqn. 4 is given by

$$t = 0, \ C_b(k) = C_b^0(k) \quad (5)$$

At equilibrium, the following Langmuir isotherm is obtained by setting the righ-hand side of eqn. 2 to zero:

$$C_\mu(k) = \frac{C_{\mu s}(k)b(k)C(k)}{1 + \sum_{j=1}^{NC} b(j)C(j)} \quad (6)$$

where b is the Langmuir constant, which is the ratio of adsorption to the desorption rate constants. The isotherm parameters b and $C_{\mu s}$ were determined by independent batch experiments and details of which can be found in ref. 8.

Solution method

The partial differential equation for particle phase (eqn. 1) was reduced to ordinary differential equations using the orthogonal collocation technique [9]. The resulting equations are in the form of coupled differential and algebraic equations and they were then solved with DASSL, a Differential/Algebraic System Solver [10]. The values of the kinetic parameters and diffusivities were estimated by matching the theoretical predictions of this model with the experimental concentration decay versus time curves. The optimisation method of Powell's conjugated direction method (see ref. 11) and the subroutine PCD [12] were used in this matching procedure.

EXPERIMENTAL

Materials

Two dipeptides, N-Cbz-L-leucyl-glycine (Cbz = carbobenzoxy) (p*I* 5.97) and N-Cbz-glycyl-L-phenylalanine (p*I* 5.72) (Sigma, St. Louis, MO, USA), were used as adsorbates in batch uptake experiments. The choice of the dipeptides is mainly based

on the consideration that these two dipeptides have relatively similar adsorption behavior under the selected conditions. The adsorbent is 5-μm Adsorphere C_{18} silica gel with 80 Å pore size, 12% carbon loaded, 200 m^2/g surface area, which was completely end capped (Alltech, IL, USA). The Na_2HPO_4, $NaH_2PO_4.2H_2O$, H_3PO_4 and HPLC-grade methanol were purchased from BDH (Victoria, Australia). All these chemicals were used as received.

Analytical apparatus

Analytical Beckman System Gold (San Ramon, CA, USA) was employed. It consists of a dual-pump programmable solvent module 126, a rapid scanning dual-wavelength monitoring detector module 167, a Model 210A sample injection valve and an analog module 406. The Beckman Serial Interface card is used to provide the communication between the computer and the high-performance liquid chromatography (HPLC) modules. A 250 × 4.6 mm I.D. stainless-steel column packed with 5-μm reverse phase silica gel C_{18} was employed.

Mobile phase

Both batch dynamic experiments and analysis were carried out using solutions containing 45% to 55% (v/v) HPLC-grade methanol in 75 mM phosphate buffer. The buffer solution was prepared with analytical UNIVAR reagent-grade sodium dihydrogenorthophosphate, disodium hydrogenorthophosphate and Millipore deionised water. The pH of the solutions were adjusted to about 2.98, 3.18, 3.42 and 4.18 with H_3PO_4 in presence of pure methanol. The mobile phase used in chromatographic analysis procedure was filtered by 0.45-μm cellulose acetate filters (Sartorious, Göttingen, Germany) and degassed under vacuum.

Experimental description

The transient uptake experiments were carried out for both single components and binary mixtures for various pH and methanol contents using 1.5-ml micro test tubes (Eppendorf, Hamburg, Germany). A quantities of pre-weighed silica gel was added to a series of dipeptide solutions of predetermined initial concentrations. The ratio of sorbent mass to solution volume was kept about 42 mg per ml. At the moment of sorbate–sorbent contact, mixing is start-

ed and the tube was gently rotated for a given period of time. At a certain time a small sample was withdrawn and the two dipeptides concentrations were measured by analytical HPLC Beckman System Gold at 238 nm and 254 nm.

In this work, a series of runs were carried out to investigate the effect of initial dipeptide concentrations, the amount of sorbents, pH and methanol concentration in solution. The ratio of initial concentration for the two dipeptides in finite batch is 1:1 and have limited diluted value about 30 mM. The range of methanol concentrations from 45% to 55% and pH ranged from 2.98 to 4.20 were chosen. The small range of methanol concentration is because when the methanol concentration is greater than 55% the two dipeptides have very close affinity, hence separation is not possible. On the other hand, if the methanol concentration is less than 45%, the affinities of the two dipeptides are widely different but too high. As a result, the two peaks are eluted so far apart with problems of wasting carrier fluid and longer cycle time. The similar effects of pH with those of methanol composition on retention time are also observed.

RESULTS AND DISCUSSION

Transient uptake experiments were carried out for a binary mixture of dipeptides except the cases in Fig. 1 where single component systems are studied. The initial adsorbate concentration in finite bath for both dipeptides is about 18 mM. The film mass
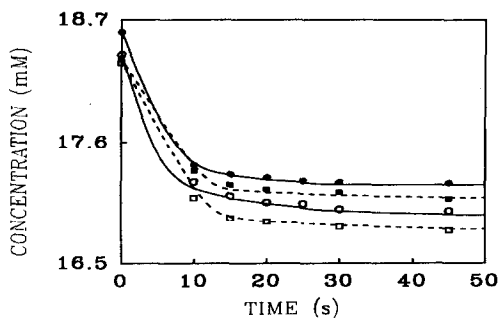

Fig. 1. Comparison between experimental data (symbols) and the finite rate model (dashed lines) to single and binary component batch adsorption systems. Symbols: filled symbols, Cbz-Leu-Gly; open symbols, Cbz-Gly-Phe; ■, □: single component; ●, ○: binary component.

TABLE I

EXPERIMENTAL CONDITIONS

V (ml)	1.2
pH	3.42
m (mg)	42
Methanol content (%)	55

transfer coefficient was evaluated from correlation [13]. Other conditions used in the experiments are given in Table I unless otherwise indicated in figures or figure captions.

In Fig. 1 the proposed model (*i.e.* intrinsic kinetics model) for the description of the batch dynamic adsorption of dipeptides onto reversed-phase silica gel is fitted to the experimental uptake data. The parameter values for the pore diffusivity and the rate constants, as given in Table II, were obtained from the fitting between the model prediction and experimental data. As shown in this figure, the intrinsic kinetics model yields a reasonable fit. The pore diffusivity and sorption rate constants extracted from these batch experiments have been used to successfully predict the column elution profile [7]. It was also found that the intrinsic kinetics could contribute significantly to the bandspreading. An exact explanation for the slow surface interaction is currently not available. However, this could be due to the high activation energy for interaction between the hydrophobic groups of dipeptides and the immobilised hydrocarbonaceous groups.

The data in Table II suggest that the rate constants and pore diffusivity for the single component

systems are quite close to those for the corresponding binary component system. This seems to indicate that the sorption rate constants and pore diffusivity for a given species is basically not influenced by other species. This feature would facilitate the parameter evaluation. That is to say that batch experiment for single component can be used to obtain the kinetic parameters and pore diffusivity for multicomponent system. However, for the system having an adsorption kinetics other than Langmuir type, the kinetic parameters for single component systems may be different from those for binary mixtures because of the possible existence of lateral interaction between adsorbed solutes. The adsorption behavior of the system in this work is well described by the kinetics and isotherm of Langmuir type, and therefore the interaction between adsorbed solutes is not significant.

Fig. 2 shows the effect of initial solute concentration on the transient uptake of the binary mixture of dipeptide. The experimental data and predicted curves from the intrinsic kinetics model are displayed in this figure. The best fit values of the parameters for various initial solute concentrations are given in Table III. It is seen from this table that the initial solute concentration appears to have no effects on the parameter values. Small difference may be attributed to the experimental error. Therefore, the assumption that the pore diffusivity is independent of solute concentration is justified. Due to the solubility of the dipeptides, experiments with higher initial concentration are not possible.

The transient uptake under different ratio of bath solution volume to sorbent amount (V/m) is shown

TABLE II

PARAMETERS FOR CASES IN FIG. 1

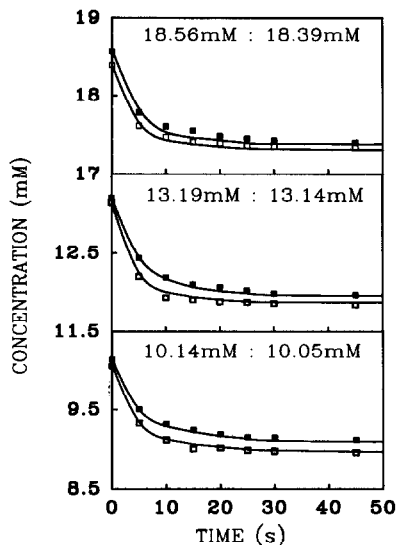|		Intrinsic kinetics			Local equilibrium	Isotherm	
		$D_p \times 10^7$ (cm/s)	k_a (l/s)	$k_d \times 10^2$ (mmol/ml · s)	$D_p \times 10^7$ (cm/s)	$C_{\mu s}$	b (ml/mmol)
Single solute	Leu-Gly	1.18	0.689	3.042	1.020	0.183	22.96
	Gly-Phe	1.69	1.043	2.893	1.351	0.254	36.08
Binary mixture	Leu-Gly	0.995	0.634	2.871	0.992	0.166	22.08
	Gly-Phe	1.48	0.991	2.713	1.415	0.235	36.58

Fig. 2. Effect of initial solute concentrations with fixed concentration ratio Cbz-Leu-Gly/Cbz-Gly-Phe = 1:1. Symbols: ■ = Cbz-Leu-Gly; □ = Cbz-Gly-Phe.
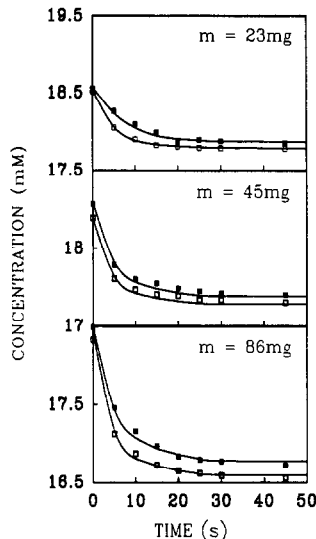

Fig. 3. Effect of ratio V/m on transient uptake. Symbols as in Fig. 2.

in Fig. 3. The parameter values extracted from these experimental data are given in Table IV. The data in Table IV indicate that the effect of V/m is negligible.

In order to elucidate the dependence of binding kinetics on the pH and organic modifier content, transient uptake experiments were carried out for a range of pH values and methanol contents. Representative uptake curves are shown in Figs. 4 and 5. For given pH value and methanol content, the adsorption isotherm of the binary mixture was measured experimentally and equilibrium parameters were extracted for the Langmuir isotherm

equation [8]. The batch dynamic data were then fitted using the intrinsic kinetics model proposed in this paper to obtain the parameter estimates for rate constants k_a, k_d and pore diffusivity D_p.

Generally, the variation of the kinetic parameters due to the change of pH and organic modifier content in solution is not clearly known. Some reasons for this trend could be explained by: (1) the change of physico-chemical properties of solute molecules (e.g. net charge, reconformation, aggregation change, pI and polar state), and (2) surface diffusion may be involved in the overall process.

TABLE III

EFFECT OF INITIAL CONCENTRATION (C_b^0) ON PARAMETERS

	Leu-Gly			Gly-Phe		
	C_b^0 (mM)			C_b^0 (mM)		
	18.56	13.19	10.14	18.39	13.14	10.05
$D_p \times 10^7$ (cm/s)	1.94	1.85	1.75	2.74	2.69	2.65
k_a (l/s)	0.671	0.679	0.681	0.532	0.541	0.543
$k_d \times 10^2$ (mmol/ml · s)	3.86	3.83	3.82	3.22	3.12	3.10

TABLE IV

EFFECT OF V/m ON PARAMETERS

	Leu-Gly			Gly-Phe		
	V/m (ml/mg)			V/m (ml/mg)		
	0.052	0.027	0.014	0.052	0.027	0.014
$D_p \times 10^7$ (cm/s)	1.89	1.94	1.98	2.69	2.74	2.81
k_a (l/s)	0.675	0.671	0.671	0.534	0.532	0.531
$k_d \times 10^2$ (mmol/ml·s)	3.89	3.85	3.85	3.20	3.22	3.21

In Figs. 6 and 7, the adsorption rate constant, k_a and desorption rate constant, k_d are plotted as a function of pH for the different methanol content. It is noted that k_a decreases linearly with increasing pH. The predominant effect of increasing pH is to increase the degree of ionisation of dipeptide molecules, and thus the dipeptide molecules carry more associated ions. In such instance, the frequency for the efficient collision between solute molecules and immobilised hydrocarbonaceous groups is reduced. The adsorption rate constant is, therefore, decreased with increasing pH. At a given pH value, k_a is also decreased with an increase in the methanol content.

This is because the dipeptide molecules are less excluded from the solute at a higher organic solvent content.

The effect of pH and methanol content on the desorption rate constant (k_d) is shown in Fig. 7. It can be seen from this figure that k_d is decreased with increasing pH except the case for Cbz-Gly-Phe at the methanol content of 45%. It is believed that ionised solutes may bind to the hydrocarnaceous group bound surface with the charges oriented away from the surface. The net charge at low pH value may favour the desorption. As a consequence, the k_d is higher at lower pH. However, raising the methanol
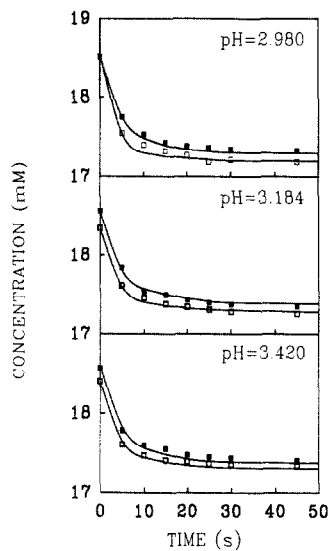

Fig. 4. Effect of pH values on transient uptake. Symbols as in Fig. 2.
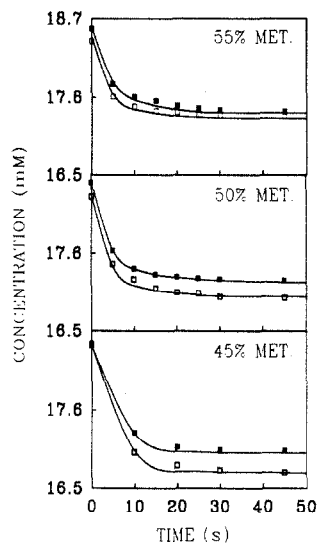
Fig. 5. Effect of methanol (MET.) contents on transient uptake. Symbols as in Fig. 2.
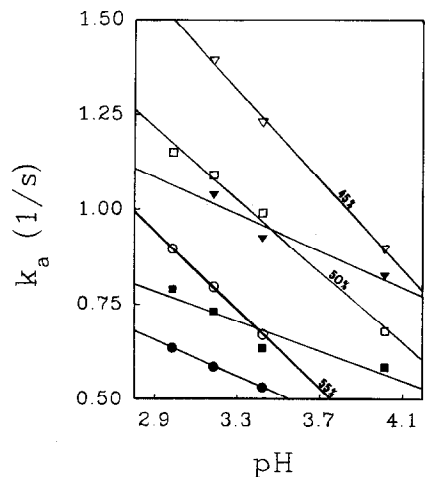
Fig. 6. Adsorption constant *versus* solution pH for various methanol contents (55, 50 and 45%). Symbols: ▽, □, ○ = Cbz-Gly-Phe; ▼, ■, ● = Cbz-Leu-Gly.
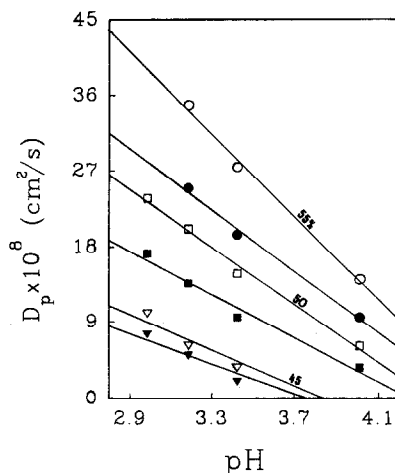

Fig. 8. Diffusivity value *versus* pH for various methanol contents (55, 50 and 45%). Symbols as in Fig. 6.

content enhances the hydrophobicity of the mixed solvent. This effect reduces the energy needed for a solute to desorb from the surface. Therefore, the desorption rate constant is increased with increasing methanol content.

The pore diffusivity (D_p) determined from the batch uptake measurement for various pH values and methanol contents are shown in Fig. 8. It is seen that the D_p linearly decreases with an increase in pH.
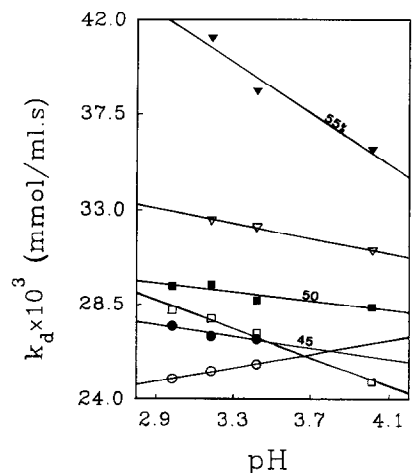

Fig. 7. Desorption rate constant *versus* solution pH for various methanol contents (55, 50 and 45%). Symbols as in Fig. 6.

This observation could be explained again by the ionisation of dipeptide molecule. With the pH increased from 3 to 4, the degree of ionisation of the solutes is enhanced. Thus, more counter-ions are associated with the dipeptide molecules, and this results in a larger resistance for the solute molecules to diffuse in the pore liquid phase. The methanol content also has profound effect on the pore diffusivity. As shown in this figure, D_p is increased with increasing methanol content. Such variation of D_p is unlikely resulted from the reduction of the viscosity, since the viscosity only slightly changes in the methanol content range of 45–55% [14]. One of the possible explanations is that the higher methanol content reduces the electrostatic interaction between the solutes and solvent, and consequently the dipeptide molecules have a higher mobility. As already shown, the pore diffusivity is sensitivity to the variation of both pH and methanol content. The implication of this is that when modelling the reversed-phase liquid chromatographic process under any gradient elution mode, the dependence of D_p on pH and organic modifier content must be taken into account.

CONCLUSIONS

The batch dynamic adsorption of a binary mix-

ture of dipeptides has been experimentally studied. The results obtained from these experiments were fitted well by the proposed model which is incorporated with an intrinsic adsorption kinetics of Langmuir type. For the system under consideration, the pore diffusivity is independent of solute concentration. It is also found that rate constants (k_a, k_d) and pore diffusivity (D_p) for a multicomponent system can be approxiated by those from the corresponding single component systems. These extracted parameters, k_a, k_d and D_p exhibit a linear dependence on the pH value of the solution and methanol content in the mixed solvent.

SYMBOLS

b	Langmuir affinity constant
C	concentration in pore fluid phase
C_b	concentration in bulk fluid of finite bath
C_b^0	initial concentration in bulk fluid of finite bath
C_μ	concentration in adsorbed phase
$C_{\mu s}$	sorbent capacity
D_p	pore diffusivity
j	solute j
k	solute k
k_a	adsorption rate constant
k_d	desorption rate constant
k_m	film mass transfer coefficient
m	sorbent mass in finite bath
NC	number of solute
r	radial coordinate
R_0	particle radius
t	time
V	solution volume in finite bath

Greek symbols

ε_m	particle porosity
ρ_p	particle density

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