Korean J. Chem. Eng., **23**(6), 997-1002 (2006)

Simulation of protein adsorption in a batchwise affinity chromatography with a modified rate model

Mohammad Reza Aboudzadeh†, Zhu Jiawen* and Wu Bin*

Nuclear Research Center for Agriculture & Medicine, AEOI, P.O. Box 31585-4395, Karaj, Iran
*Chemical Engineering Research Center, East China University of Science and Technology, Shanghai, 200237, P.R. China
(*Received 27 March 2006 • accepted 19 June 2006*)

Abstract−A rate model was adapted to simulate the dynamics of protein adsorption. This model takes axial dispersion and film mass transfer into account where there is a nonlinear adsorption isotherm for protein. The model equations were solved with the application of orthogonal collocation method on finite elements. The model is validated with experimental adsorption of urokinase in a batchwise column chromatographic process. Adsorption kinetics and isotherm were measured in a batchwise operation. With the assumption of back mixing at the column inlet, the effect of the different flow pattern on the concentration change inside the column can be simulated with the rate model.

Key words: Simulation, Protein, Batchwise Chromatography, Affinity Chromatography, Rate Model

INTRODUCTION

Preparative chromatography is often used as a final purification step in the production of many chemicals and biochemicals such as proteins, due to its versatility and requirement for high separation resolution [Joukje et al., 2003; Wei et al., 2003; Kaczmarski et al., 2001].

The present paper focuses on a modeling and simulation approach for a non-linear chromatographic process. There are several reasons why modeling and simulations are used in this study: firstly, to optimize output, it is generally beneficial to know precisely when and at what concentration the preferred product can be drawn. For this purpose, the process should be modeled and accurately described; secondly, with so many components and time-dependent processes occurring within the chromatographic process, the resulting effluent history is usually difficult to predict when feed concentrations fluctuate or when operating concentrations are changed for scale-up; and finally, a biological experimental study is often expensive and complex. Numerical simulation of these complex systems offers an efficient and economical method for scale up and optimization. Experiments are still required, but feedback from numerical simulation can greatly reduce the amount for experimental work.

Batchwise separation processes are extensively used in many industries. The general dynamics of batchwise chromatographic processes are rather complex. Detailed analytical solutions are only available for simple systems, in which there are linear isotherms and there is no competition between solutes for sorbant sites (interference effect) [Dünnebier et al., 1998]. However, in many large-scale separation processes, interference effects between solutes are significant and the absorption/desorption isotherms are usually complex and nonlinear; henceforth, numerical solution is required. To obtain precise simulation of the complex systems, correct models for description of the chromatographic process should be established.

†To whom correspondence should be addressed.
E-mail: Aboudzadeh@yahoo.com

THEORY

1. Models

Based on different assumptions, models for description of chromatographic process can be roughly classified into different groups as:

(1) Local equilibrium models: these models assume that the mobile and the stationary phases are in equilibrium and neglect any hydrodynamic dispersion or film mass transfer effects. Therefore, local equilibrium theory considers only pure thermodynamic effects on the column dynamics [Yu and Wang, 1986].

(2) Stage models: In these models the column is assumed to be divided into many stages [Guiochon and Ghodbabe, 1988]. Each stage is assumed to be independent and mobile and stationary phases are mixed completely. Although they can give reasonable approximations for systems with linear isotherms, they provide little understanding of the dynamics.

(3) Rate equation models: In theoretical equilibrium theory, zone spreading effects are ignored. In the rate models, zone spreading effects are considered. The greatest focus of the rate models lies in the detailed mass transfer mechanisms and the reactions, which might occur within the columns. The rate models also represent the dynamics through mass balance equations [Seidel and Gelbin, 1986; Chen and Hsu, 1987; Aboudzadeh et al., 2006]. Potentially, they can take complex isotherms and mass transfer effects into account and can simulate column dynamics in details [Hritzko et al., 2002].

2. Mathematical Modeling Equations

In the present work a rate model for a chromatographic column with differential mass balances both in the mobile and static phases has been considered, which takes into account convection and important effects of nonidealities, such as axial dispersion, mass transfer, and nonlinear adsorption isotherm.

For simplification, a system based on non-porous particles can also be modeled. It is useful for preliminary simulations. The less computation time comes from assuming a lumped mass transfer

coefficient for movement of solute from the bulk liquid phase to the adsorbed phase.

The following assumptions of column chromatography, which have been mentioned by Whitley and Wang [Whitley et al., 1993], are also adopted in this work: (1) the column is packed with particles that are spherical and uniform in size; (2) local equilibrium exists between the mobile and solid phases and the chromatographic process is isothermal; (3) the concentration gradient in the radial direction of the column and in the angular direction of the particle are negligible; (4) mass transfer coefficients are constant and independent of every components; and (5) the dispersion coefficients are constant.

Based on these assumptions, two mass balance equations can be derived, one for the mobile phase percolating through the bed of particles, the other for the solid phase particles. The equations used in the rate model are the following:

Mass balance in the mobile phase:

$$\frac{\partial C}{\partial t} = D_L\frac{\partial^2 C}{dx^2} - v\frac{\partial C}{dx} - k_f\frac{6}{d_p}\frac{1-\varepsilon_b}{\varepsilon_b}(q^* - q) \tag{1}$$

Mass balance in the solid phase:

$$\frac{\partial q}{\partial t} = k_f\frac{6}{d_p}(q^* - q) \tag{2}$$

Initial conditions: since there are two partial differential equations, they need to have two initial conditions, so:

$$t=0, \qquad C=0 \ \& \ q=0 \tag{3}$$

Boundary conditions: there are two boundary conditions for the mobile phase, one at the column inlet and other at the column exit:

$$x=0, \qquad D_L\frac{\partial C}{\partial x} = \nu(C - C^0) \tag{4}$$

$$x=L, \qquad \frac{\partial C}{\partial x} = 0 \tag{5}$$

Adsorption isotherm: The classical and simple model, Langmuir adsorption isotherm (Eq. (6)) is used to describe the nonlinearity in this study:

$$q^* = \frac{aC}{1+bC} \tag{6}$$

The resulting system of coupled partial differential equations should be solved by a suitable numerical method.

In the simulation, the Chung and Wen correlation [Chung and Wen, 1968] is used to calculate the axial dispersion coefficient in bulk mobile phase:

$$Pe_b = \frac{L}{2\varepsilon_b R}(0.2 + 0.011Re^{0.48}) \tag{7}$$

The film mass transfer coefficient between adsorbent and bulk mobile phase is calculated according to the Wilson and Geankoplis correlation [Wilson and Geankoplis, 1966]:

$$J \equiv \left[\frac{k_f}{u_0\varepsilon_b}\right]Sc^{2/3} = \frac{1.09}{\varepsilon_b}Re^{-2/3} \tag{8}$$

3. Numerical Methodology

Most numerical solutions of the general models of chromatography use finite difference methods. In all cases, an error is introduced when the differentials are replaced with finite differences. This error is a function of the size of the space and time increments used for the integration. The formulation of the finite method permits the generation of optimally ordered finite difference relations, which are accurate [Baker, 1983]. Thus, the orthogonal collocation method on finite elements was used to obtain the solution of this model.

The solution is constructed by dividing the space coordinate into N_E intervals, with N_E+1 nodes, with coordinates S_i, such that:

$$0 = S_1 < S_2 < \ldots < S_i < \ldots < S_{N_E} < S_{N_{E+1}} = L \tag{9}$$

Orthogonal collocation is then applied to each element and the solution on each of them is approximated by interpolation. The space variable on each element is:

$$s = \frac{x - S_i}{S_{i+1} + S_i}, \qquad s \in [0, 1] \tag{10}$$

On each element i a number N_p of interior collocation points are chosen as the roots of an N_p-th degree orthogonal polynomial [Villadsen and Michelsen, 1978]. The first and second-order differentials with respect to space are then approximated by using two matrices A and B, which are obtained by solving the Gaussian-Jacobi polynomial [Baker, 1983; Villadsen and Michelsen, 1978]. So, we have at the *k*-th collocation point on the *i*-th element:

$$\left.\frac{\partial C}{\partial s}\right|_{C=C_{ij}} = \sum_{k=1}^{N_p+2} A_{jk}C_{ki}, \qquad j=1, 2, \ldots, N_p+2 \tag{11}$$

$$\left.\frac{\partial^2 C}{\partial s^2}\right|_{C=C_{ij}} = \sum_{k=1}^{N_p+2} B_{jk}C_{ki}, \qquad j=1, 2, \ldots, N_p+2 \tag{12}$$

At the internal element boundaries, continuity of concentration and flux is imposed. At s=0 and s=1, the boundary conditions (Eqs. (4)-(5)) are used.

In brief, the above technique discretizes Eqs. (1), (4) & (5) into the following system:

$$\frac{\partial C}{\partial t} = D_L\sum_{k=1}^{N_p+2} B_{jk}C_{ki} - v\sum_{k=1}^{N_p+2} A_{jk}C_{ki} - k_f\frac{6}{d_P}\frac{1-\varepsilon_b}{\varepsilon_b}(q^* - q) \tag{13}$$

$$s = 0 \rightarrow D_L\sum_{k=1}^{N_p+2} A_{jk}C_{ki} = \nu(C - C^0) \tag{14}$$

$$s = S_k \rightarrow \lfloor C_{(N_p+2)} \rfloor_k = [C_1]_{k+1} \tag{15}$$

$$s = 1 \rightarrow \sum_{k=1}^{N_p+2} A_{jk}C_{ki} = 0 \tag{16}$$

And

$$\left.\frac{\partial q}{\partial t}\right|_{q=q_{ji}} = k_f\frac{6}{d_p}(q_{ji}^* - q_{ji}) \tag{17}$$

After the model equations have been converted to ordinary differential and algebraic form, the system can be solved conveniently by an integration method [Petzold, 1982]. In the present work, the discretized model equations (Eqs. (13)-(17)) have been numerically solved by time integration with the fourth order Runge-Kutta method.

EXPERIMENTS

Urokinase (EC 3.4.99.26), which is used as a plasminogen for

therapeutic thrombolysis, can be obtained from human urine and purified by different kinds of separation methods. Chromatography is usually used to yield a high purity product of urokinase. The purification of urokinase has been conducted by affinity chromatography in the authors' laboratory.

In the experiment, the affinity gel was prepared with epichlorohydrin activated agarose (Sepharose 4B) as the matrix and p-aminobenzamidine as a ligand. In preparation of the affinity gel, agarose gel was firstly treated with epichlorohydrin to form epoxy-activated gel; amination of the activated gel with concentrated ammonia solution was conducted to form amino-terminals; the amino-terminals were then converted to carboxyl-terminals in acylation reaction of succinic anhydride and the gel; and finally, the affinity gel was prepared by coupling the p-aminobenzamidine with the carboxy-gel. The ligand density in the prepared affinity gel was 55 mol/g wet gel.

In a batch column chromatographic purification of urokinase, the affinity gel was packed in a φ10×150 mm glass chromatography column with a final bed height of 120 mm. The packed column was pre-equilibrated with phosphate buffer. The solution of crude urokinase (with specific activity 500 IU/mg protein) was fed into the column and washed with phosphate buffer. And finally, elution was performed with an acetate buffer contain sodium chloride. After affinity chromatography, purified urokinase with specific activity 68400 IU/mg protein was obtained with a recovery yield of 92.2%.

The adsorption of urokinase on the affinity gel in a batch chromatography was numerically simulated and compared with the experimental result. The isotherm parameters used in the simulation were also obtained from experiments. The change of urokinase concentration during a batchwise adsorption experiment is shown in Fig. 1, with the isotherm being shown in Fig. 2. The adsorption isotherm of urokinase on the prepared affinity gel can be expressed in the form of the Langmuir isotherm (solid line):

$$q=7899C/(2146+C) \tag{18}$$

In which q is the adsorbed quantity of urokinase per mL of wet gel (IU/mL wet gel); C is the enzyme concentration in the supernatant (IU/mL). The details of experimental methods, assay methods and materials have been reported in prior [Cao et al., 1997].

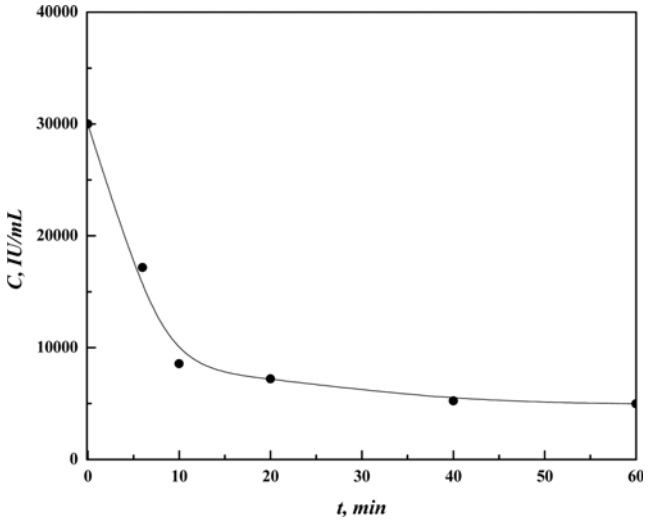

Fig. 1. Batchwise adsorption of urokinase.

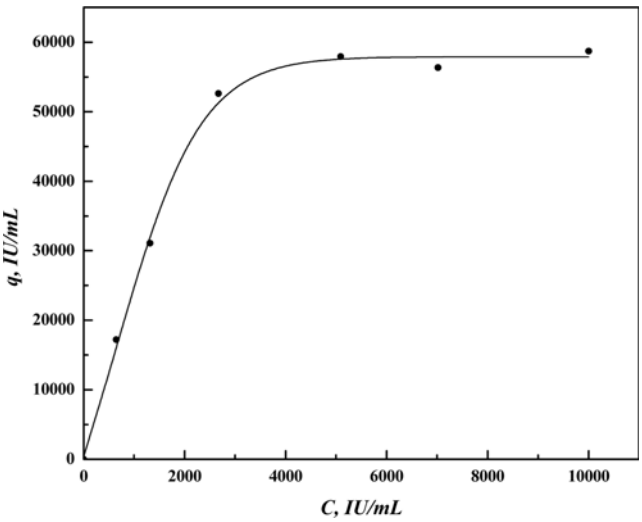

Fig. 2. Isotherm for adsorption of urokinase on affinity gel.

THE PROCESS SIMULATION

The basic principles of the present simulation process have been described in Eqs. (1)-(6).

To simulate liquid chromatography for a particular system, the partial differential equations were converted to ordinary differential and algebraic form and then numerically solved. A full quantitative description of the chromatographic process and values for necessary parameters available from experiments, the published literature calculation using suitable equations should be included in the data file. The simulated concentration profile in the column and the effluent history information are included in the output file.

Adjustment has been made in consideration of special features of the experimental setup in this simulation. The bed height was 120 mm and the glass column's height was 150 mm, which caused a dead volume of 2.36 mL above the bed. Such a large dead volume cannot be neglected in simulation, as this causes a time delay in breakthrough simulation and may change the input profile coming into the bed. In the present simulation, both plug flow and fully mixed flow patterns in the void part of the column (dead volume) are taken into account.

To include the dead volume effect in the model (Eqs. (1)-(6)), it is necessary to define the changes in the bed inlet concentration. Therefore, the dead volume above the bed is taken as one continuous stirred tank reactor (CSTR). Assuming the inlet concentration to be constant, the following equation can be derived from the law of mass conservation:

$$C_b=C_f(1-e^{-t/\tau_d}) \tag{19}$$

Also, the change of concentration at the inlet side of the bed can be simplified as:

$$C=C_1+[(C_2-C_1)/2t_{dead}]*\Delta t, \text{ for } \Delta t\le 2t_{dead} \tag{20}$$

Fig. 3 shows the effect of time delay on time required for the bed inlet concentration to reach the column inlet concentration.

With time held constant, decrease in the dead volume will lead

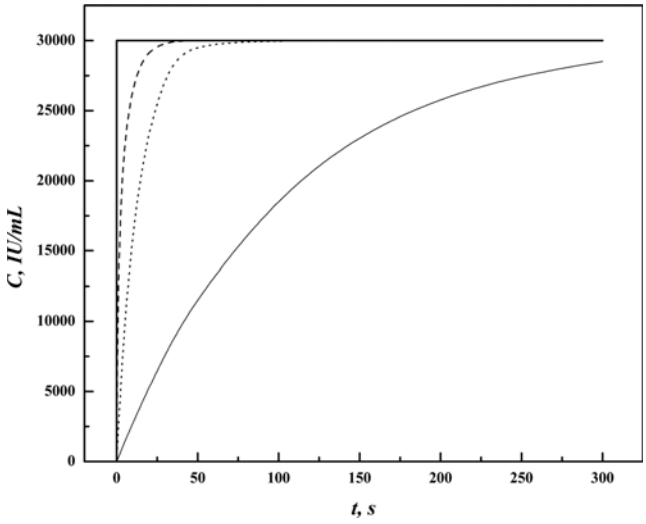

Fig. 3. Effect of time delay on the bed inlet concentration.
——t_{dead}=0 s; — — t_{dead}=1 s; ----- t_{dead}=10 s; —— t_{dead}= 100 s; C_1=0 & C_2=30,000 IU/mL.

to decrease in the exponent in Eq. (19). If the dead volume can be decreased to zero, then the inlet bed concentration will reach the column inlet concentration immediately. Therefore, conditions as close as possible to the ideal situation are recommended.

RESULT AND DISCUSSION

In our study, the chromatography process of urokinase in a batchwise chromatographic process was simulated. The simulation was compared with experimental data.

The breakthrough curves resulting from the simulation are shown in Fig. 4. Bullets in the figure are experimental data. In the breakthrough calculation, we used the Chung and Wen correlation [Chung and Wen, 1968] to estimate the axial mixing in the mobile phase and the Wilson and Geankoplis equation [Wilson and Geankoplis, 1966] to calculate the film mass transfer coefficients. Isotherm and kinetic constants were measured in experiments. So, the value of parameters at this study has been determined based either on the independent experiments or available empirical correlations as outlined in Table 1.

In Fig. 4a, one could find the simulation result agrees with the experimental data well both in retention time and in shape of the breakthrough curve. In this figure there is a little discrepancy between simulation results and experimental points that can be caused by some simple assumptions that have been already mentioned at this modeling. A probable reason can be due to the fact that the system

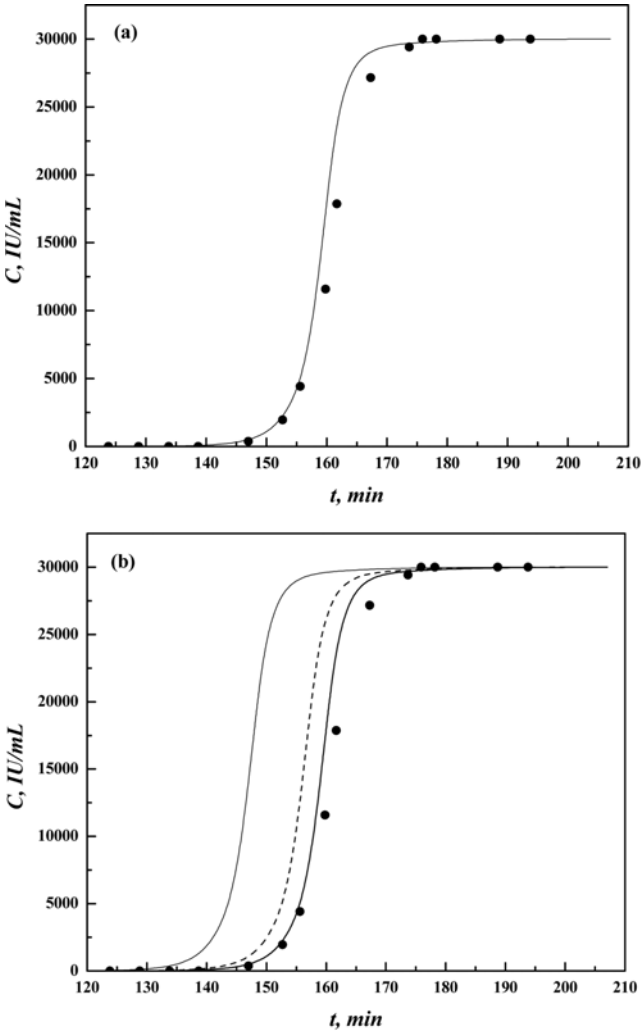

Fig. 4. Breakthrough curve of urokinase in a batchwise chromatographic column.
(a) ● experimental data; ——simulation adsorption results (b) ● experimental data; —— plug flow without dead volume; plug flow in dead volume; — — CSTR in dead volume: batchwise chromatographic column (D_i=10 mm): packed with prepared affinity gel (p-aminobenzamidine - Sepharose 4B) with a bed height of 120 mm, volume of the voidage above the bed: 2.36 mL; feed solution flow rate: 0.2 mL/min, concentration of urokinase: C=30,000 IU/mL; phosphate buffer: 0.05 mol/L, pH 7.2 (containing 0.5 mol/L sodium chloride); T=281 K

has been modeled for non-porous particles; also, the model is a concept of equilibrium isotherms. This assumption cannot be always

Table 1. Correlation for estimating model parameters

Parameters	Correlations	Data source/ref.
Isotherm	$q=\frac{aC}{1+bC}$	Experiment (present work)
Mobile-phase axial dispersion coefficient, D_L	$Pe_b=\frac{L}{2\varepsilon_b R}(0.2+0.011Re^{0.48})$	Chung and Wen, 1968
Film mass transfer coefficient, k_f	$J\equiv\left[\frac{k_f}{u_0\varepsilon_b}\right]Sc^{2/3}=\frac{0.19}{\varepsilon_b}Re^{-2/3}$	Wilson and Geankoplis, 1966

valid [Anspach et al., 1990]. Sometimes proteins and nucleic acids bind with adsorbent at different sites [Andrade, 1985; Mazsaroff et al., 1988; Whitely et al., 1989] and may change conformation upon binding [Lin and Karger, 1990]. In these cases, adsorption is slow relative to mass transfer in a separation system and then adsorption kinetics might need to be considered [Whitley et al., 1993]. Adsorption of urokinase on the affinity gel might be non-equilibrium. However, the results show this modified rate model can be acceptable with considering the simple assumptions that have be embedded in this model and have enough accuracy to study this system. This model may be more accurate if one non-equilibrium adsorption isotherm for urokinase is taken and also the porosity is considered in particle. This subject is still under study and the final results will be presented in other literature in the near future.

Fig. 4b shows the two different treatments for the void part of the column (dead volume): plug flow without the void (fine solid line) and fully mixing in the dead volume (thick solid line). The breakthrough curve with plug flow in the dead volume is shown as a dashed line in Fig. 4b, which exhibits a delay of 12 min compared with the fine solid line (plug flow without the dead volume). We found that the full mixing assumption is much closer to the experimental results with the retention time being reproduced correctly and this is reasonable because the flow rate is very slow (0.2 mL/min). Therefore, the actual flow inside the column can be assumed to be divided into two parts: (1) a dead volume at the inlet of the column where inlet flow is thoroughly mixed and the bulk liquid and solutes are evenly distributed inside the dead volume; and (2) a tightly packed bed in which neither channeling nor circulatory motion occur. So, as explained previously, in a simulation of the chromatographic process, the dead volume can be treated as a CSTR without leading to obvious deviation from the actual situation.

We used the two previously proposed patterns of inlet concentration change (Eqs. (19)-(20)) to study the effect of inlet concentration on the simulation of a particular breakthrough curve. The history of inlet concentration under different patterns of change in inlet concentration is shown as in Fig. 5, while numerical simulation results are shown in Fig. 6. We found little difference between the results obtained by using these two patterns; therefore, in the simulation of a chromatographic process with a CSTR at the inlet of the column, the simplified treatment of the inlet concentration which is represented as Eq. (20) can be used in place of the more complicated non-linear function in Eq. (19).

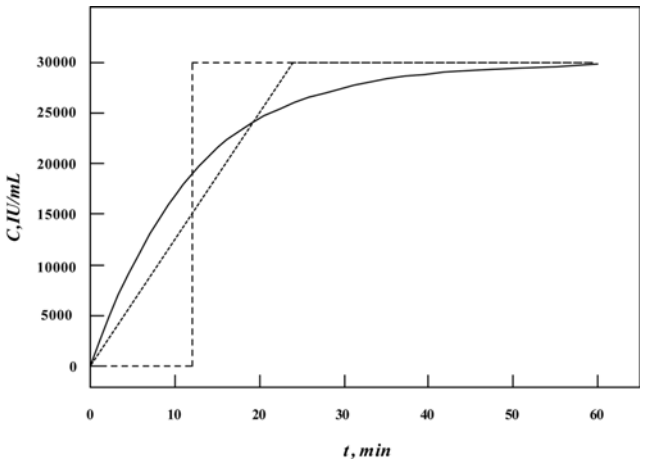

Fig. 5. Flow pattern inside the column and inlet concentration history.
----- plug flow; -------- CSTR linear approach treatment (Eq. (20)); —— CSTR.

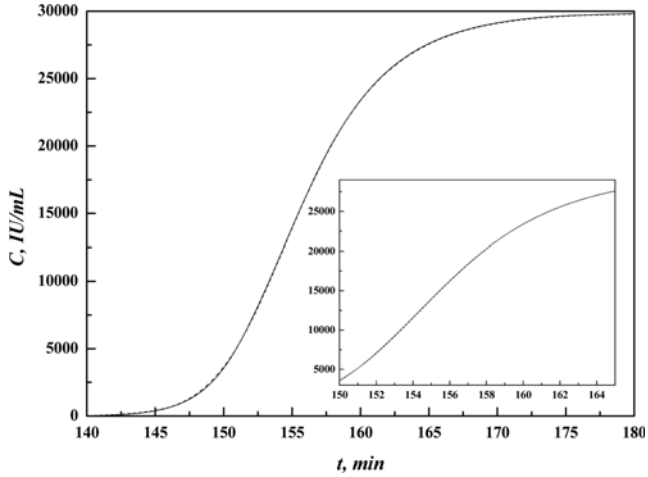

Fig. 6. Effect of change inlet gradient on urokinase breakthrough curve in the same time delay, ——Eq. (19); - - - - - Eq. (20).

CONCLUSION

Protein chromatography in a batchwise operation was simulated with the rate model in this article. The validity of the rate model in the description of a batchwise liquid chromatography process was evaluated by comparison of the simulation results with the actual behavior of the adsorption of urokinase in a batchwise chromatography column. We found that:

1. The rate model was modified by considering the dead volume in the present batchwise chromatographic column as an important factor, which is the reason for deviation from the ideal state of plug flow. An assumption of a fully mixed CSTR at the inlet void section of the column was added to the model in the analysis of the dead volume effect, and gave results that approached the experimental data very closely. That means, in a well-packed column with greatly reduced dead volume, little back-mixing would occur and high resolution for chromatographic separation would be attained.
2. The model can be used to simulate the adsorption of urokinase on the affinity gel with satisfactory accuracy.

ACKNOWLEDGMENT

The authors are grateful to the National Natural Science Foundation of China for its fund support to our research work by the project No.29976014. The State Key Laboratory of Bioreactor Engineering (China) also supported the work.

NOMENCLATURE

A : first-order discretization matrix

A_{ij} : element ij of A
a, b : constants in Eq. (6)
B : second-order discretization matrix
B_{ij} : element ij of B
C : fluid phase concentration
C^0 : inlet fluid phase concentration
C_f : column inlet concentration
C_b : bed inlet concentration
C_1, C_2: protein concentration at the inlet of column before and after the concentration step changing, respectively
D_L : axial dispersion coefficient [m^2/s]
D^∞ : Brownian diffusivity [m^2/s]
J : defined by Eq. (8)
k_f : film mass transfer coefficient [m/s]
L : column length [m]
N_E : number of elements
N_P : number of interior collocation points
Pe : Peclet number [$=u_0L/D_L$]
q : adsorbed quantity in particle phase
q^0 : equilibrium concentration in particle phase
Re : Reynolds number [$=2R\rho u_0\varepsilon_b/\mu$]
R : adsorbent particle radius [m]
s : dimensionless space coordinate
S_k : element nodes
Sc : Schmidt number [$=\mu/\rho D^\infty$]
t : time [s]
u_0 : interstitial fluid velocity [m/s]
v_f : volume flow rate into column
V_d : volume of the void section of the column [m^3]
x : dimensionless column position

Greek Letters

ε_b : interparticle void fraction
ρ : density [kg/m^3]
τ_d : retention time in the dead volume ($=V_d/v_f$) [min]
μ : viscosity [kg/(m s)]

REFERENCES

Aboudzadeh, M. R., Zhu, J. W. and Wu, B., "Modeling of protein adsorption to DEAE sepharose FF: Comparison of data with model simulation," *Korean J. Chem. Eng.*, **23**, 124(2006).

Andrade, J. D., *Principles of protein adsorption. Surface and interfacial aspect of biomedical polymers, Vol (2), protein adsorption*, Plenum press, New York (1985).

Anspach, F. B., Johnsoton, A., Wirth, H.-J., Unger, K. K. and Hearn, M. T. W., "High-performance liquid chromatography of amino acids, peptides, and proteins: XCV," *J. Chromatogr.*, **499**, 103 (1990).

Baker, J., *Finite element computational fluid mechanics*, McGraw-hill, New York (1983).

Cao, X. J., Zhu, J. W., Wang, D. W. and Dai, G. C., "Affinity chromatography purification of Urokinase with epichlorohydrin activated agarose matrix," *Chinese J. Chem. Eng.*, **5**(1), 69 (1997).

Chen, T. L. and Hsu, J. T., "Prediction of breakthrough curves by the application of fast Fourier transform," *AIChE J.*, **33**, 1387 (1987).

Chung, S. F. and Wen, C. Y., "Longitudinal dispersion of liquid flowing through fixed and fluidized beds," *AIChE J.*, **14**, 857 (1968).

Dünnebier, G., Engell, S., Klatt, K.-U., Schmidt-Traub, H., Strube, J. and Weirich, I., "Modeling of simulated moving bed chromatographic process with regard to process control design," *Compu. Chem. Eng.*, **22**, S855 (1998).

Guiochon, G. and Ghodbabe, S., "Computer simulation of the separation of a two-component mixture in preparative scale liquid chromatography," *J. Phys. Chem.*, **92**, 3682 (1988).

Hritzko, B. J., Wooley, R. and Wang, N.-H. L., "Standing-Wave design of tandem SMB for linear multicomponent systems," *AIChE J.*, **48**, 2769 (2002).

Kaczmarski, K., Antons, D., Sajonz, H., Sajonz, P. and Guiochon, G., "Comparative modeling of breakthrough curves of bovine serum albumin in anion-exchange chromatography," *J. Chromatogr.*, **925**, 1 (2001).

Joukje, H., Hugo, A. H., Luuk, A. M. and van der Wielen, "Mass-transfer effects during separation of protein in SMB by size exclusion," *AIChE J.*, **49**, 1158 (2003).

Lin, S. and Karger, B. L., "Reversed-phase chromatographic behavior of proteins in different unfolded states," *J. Chromatogr.*, **499**, 89 (1990).

Mazsaroff, I., Cook, S. and Regnier, F. E., "Molecular orientation of immunoglobulin G at high concentration on an ion-exchange sorbent," *J. Chromatogr.*, **443**, 119 (1988).

Petzold, L. R., *DASSL: A differential/algebraic system solver*, Lawrence Livermore National Laboratory Livermore, CA (1982).

Seidel, A. and Gelbin, D., "Breakthrough curves for single solutes in beds of activated carbon with board pore-size distribution-I. Mathematical models of breakthrough curves in beds of activated carbon," *Chem. Engng. Sci.*, **41**, 541 (1986).

Villadsen, J. V. and Michelsen, M. L., *Solution of differential equation model by polynomial approximation*, Prentice-Hall, Englewood Cliffs, New Jersey (1978).

Wei, D. C., Xiao, Y. D., Shu, B. and Yan, S., "Dependence of pore diffusivity of protein on adsorption density in anion-exchange adsorbent," *Biochem. Eng. J.*, **14**, 45 (2003).

Wilson, E. J. and Geankoplis, C. J., "Liquid mass transfer at very low Reynolds numbers in packed beds," *Ind. Eng. Chem. Fundam.*, **5**, 9 (1966).

Whitley, R. D., Van Cott, K. E. and Wang, N.-H. L., "Analysis of non-equilibrium adsorption/desorption kinetics and implication for analytical and preparative chromatography," *Ind. Eng. Chem. Res.*, **32**, 149 (1993).

Whitely, R. D., Wachter, R., Liu, F. and Wang, N.-H. L., "Ion-exchange equilibrium of lysozem, myoglobin, and bovine serum albumin: effective valence and exchanger capacity," *J. Chromatogr.*, **465**, 137 (1989).

Yu, Q. and Wang, N.-H. L., "Multi component interference phenomena in ion exchange columns," *Separation and purification method*, **15**, 127 (1986).