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Proteins Separation and Purification by Expanded Bed Chromatography and Simulated Moving Bed Technology

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Abstract

The study focuses on the adsorption kinetics for proteins separation and purification by expanded bed chromatography and salt gradient ion-exchange simulated moving bed technology.

Mathematical models are developed for the protein adsorption kinetics in an expanded bed, and tested against the pilot scale experimental data:

- 1) BSA adsorption in an expanded bed, where a Streamline 50 column was packed either with 300mL Streamline DEAE (classic ion exchanger) or with 300mL Streamline Direct CST I (new type of ion exchanger with the multimodal ligand), carried out in this thesis.
- 2) lysozyme adsorption in an expanded bed, where a Streamline 50 column was packed with 400mL Streamline SP to test 3-zone model, data from the published paper of Bruce and Chase (2001).

Then model parametric sensitivity is analyzed. The effects of protein intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion, solid axial dispersion, particle size axial dispersion and bed voidage axial variation, on breakthrough curves are evaluated for the expanded bed adsorption with Streamline DEAE, Streamline SP and Streamline Direct CST I.

The feasibility of capturing both BSA and myoglobin by an expanded bed adsorption process is addressed, where a Streamline 50 column is packed with 300mL Streamline Direct CST I. Furthermore, two-component proteins (BSA/myoglobin) competitive adsorption and displacement on Streamline Direct CST I are studied based on static batch experiments, frontal analysis and column displacement experiments.

The design of the high-density matrix of adsorbent used in expanded beds can be improved by including a single heavier inert core material in the macroporous crosslinked resin matrix, that is called pellicular adsorbent or inert core adsorbent. The decrease of the intraparticle diffusion resistance by inert core adsorbents is quantitatively estimated by the parameter $1/\Theta$. Furthermore, the theoretical analysis is given to demonstrate the potential application of the inert core adsorbents in the fast, high-performance liquid chromatography (HPLC) for the resolution of biological macromolecules as a result of the decrease of the intraparticle diffusion resistance.

A real gradient SMB model is used to analyze the performance of salt gradient ion-exchange SMB for linear and nonlinear ion-exchange equilibrium isotherm of proteins. Some strategies will be discussed for the selection of salt gradient and the selection of flow rate in each section of salt gradient ion-exchange SMB. The gradient SMB configurations, open loop, closed loop and closed loop with a holding vessel, are compared. Finally, we will present a comparison of the two strategies of modeling, equivalent gradient TMB model and real gradient SMB model, for the prediction of internal concentration profiles in gradient SMB with open loop and closed loop.

RESUMO

Nesta tese é estudada a cinética de adsorção para separação e purificação de proteínas através da cromatografia de leito fluidizado e da cromatografia de troca iónica por gradiente salino em leito móvel simulado (LMS).

Modelos matemáticos são desenvolvidos para prever a cinética de adsorção de proteínas em leito fluidizado e os resultados são comparados aos dados experimentais obtidos em uma unidade piloto:

- 1) Adsorção de albumina de soro bovino (Bovine Serum Albumin-BSA) em leito fluidizado, onde uma coluna *Streamline 50* é empacotada tanto com 300 mL do adsorvente *Streamline DEAE* (resina de troca iónica clássica) como com 300 mL do adsorvente *Streamline Direct CST I* (novo tipo de resina com ligante multimodal elaborado neste trabalho).
- 2) Adsorção de lisozima em leito fluidizado, onde uma coluna *Streamline 50* é empacotada com 400 mL do adsorvente *Streamline SP* para testar o modelo de 3-zonas aos dados reportados por Bruce e Chase (2001).

Analisa-se a sensibilidade paramétrica do modelo. São analisados os efeitos da resistência à difusão intraparticular da proteína, resistência à transferência de massa no filme, dispersão axial tanto na fase líquida como na fase sólida, dispersão axial segundo o tamanho da partícula e porosidade, sobre as curvas de ruptura de adsorção em leito fluidizado com *Streamline DEAE*, *Streamline SP* e *Streamline Direct CST I*.

É avaliada a possibilidade de capturar o BSA e a mioglobina através de um processo de adsorção por leito fluidizado, onde uma coluna *Streamline 50* é empacotada com 300 mL do adsorvente *Streamline Direct CST I*. Ademais, a adsorção competitiva e de deslocamento do sistema binário de proteínas (BSA/mioglobina) são estudadas baseadas sobre experimentos de: batelada, cromatografia de análise frontal e de deslocamento.

O projecto da matriz de alta densidade do adsorvente usado nos leitos fluidizados pode ser melhorado pela inclusão de um material inerte mais pesado na rede macroporosa da matriz da resina, que é chamado adsorvente pelicular ou adsorvente de núcleo inerte. O decréscimo da resistência da difusão intraparticular no adsorvente de núcleo inerte é quantitativamente analisado pelo parâmetro $1/\Theta$. Além disso, análises teóricas são apresentadas para demonstrar o potencial de aplicação dos adsorventes de núcleo inerte na cromatografia líquida de alta performance (*HPLC – high-performance liquid chromatography*) para a resolução de macromoléculas uma vez que tal adsorvente promove um decréscimo da resistência de difusão intraparticular.

Um modelo baseado na concepção intermitente de LMS gradiente é usado para analisar o desempenho da unidade de leito móvel simulado de permuta iónica por gradiente salino para isotermas de equilíbrio de troca iónica lineares e não-lineares das proteínas. Algumas estratégias são discutidas para a selecção do gradiente salino e do caudal em cada secção da unidade de LMS de permuta iónica por gradiente salino. As configurações do LMS gradiente: circuito fechado e aberto, circuito fechado com tanque de retenção, são comparadas. Finalmente, é apresentada uma comparação entre as duas estratégias de modelização – modelo de leito móvel verdadeiro gradiente equivalente e modelo intermitente de leito móvel simulado gradiente – para a previsão dos perfis de concentração no LMS gradiente de circuito aberto e de circuito fechado.

Résumé

Cette étude porte sur les cinétiques d'adsorption de la séparation et la purification des protéines par chromatographie en lit fluidisé et par chromatographie d'échange ionique par gradient salin en lit mobile simulé (LMS).

Les modèles mathématiques ont été développés pour prédire la cinétique d'adsorption de protéines en lit fluidisé et les résultats ont été comparés aux données expérimentales obtenues dans une unité pilote :

- 1) Adsorption de l'albumine de sérum bovin (*Bovine Serum Albumin* - BSA) en lit fluidisé, où une colonne *Streamline 50* est remplie soit avec 300 mL d'adsorbant *Streamline DEAE* (résine d'échange ionique classique) ou soit avec 300 mL de *Streamline Direct CST I* (nouveau type de résine avec ligand multimodal)
- 2) Adsorption de lysozyme en lit fluidisé, où une colonne *Streamline 50* est emballée avec 400 mL d'adsorbant *Streamline SP* pour tester le modèle de 3-zones avec les données reportées par Bruce et Chase (2001).

La sensibilité paramétrique du modèle a été analysée. On a ainsi évalué les effets de différents paramètres sur les courbes de percages de l'adsorption en lit fluidisé avec *Streamline DEAE*, *Streamline SP* et *Streamline Direct CST I* : résistance à la diffusion intraparticulaire de la protéine, résistance du transfert de matière dans le film, dispersion axiale aussi bien dans la phase liquide que dans la phase solide, dispersion axiale au niveau de la particule et enfin la variation axiale des espaces vides du lit.

Il a été confirmé la possibilité de capturer le BSA et la myoglobine à travers un procédé d'adsorption en lit fluidisé, où une colonne *Streamline 50* serait remplie avec 300 mL d'adsorbant *Streamline Direct CST I*. Par ailleurs, l'adsorption compétitive et les déplacements du système binaire de protéines (BSA/myoglobine) ont été étudiés en se reposant sur des d'expériences de type batch, des chromatographies d'analyse frontale et des expériences de déplacements.

Le concept de la matrice de haute densité d'adsorbant utilisé dans les lits fluidisés peut être amélioré par l'intrusion d'un matériau inerte plus lourd dans le réseau macroporeux de la matrice de résine, et appelé adsorbant au noyau inerte ou adsorbant pelliculaire. La diminution de la résistance à la diffusion intraparticulaire par des adsorbants au cœur inerte est quantitativement analysée par le paramètre l/Θ . De plus, des analyses théoriques sont présentées pour démontrer le potentiel d'application des adsorbants aux noyaux inertes en chromatographie liquide de haute performance (HPLC) pour la résolution de macromolécules biologiques par suite de la baisse de la résistance de la diffusion intraparticulaire.

Un modèle basé sur le LMS gradient est utilisé pour analyser la performance d'une unité du lit mobile simulé à échange ionique par gradient salin pour des isothermes d'équilibre d'échange ionique linéaires ou non linéaires des protéines. Quelques stratégies seront mises en évidence et comparées pour le choix du gradient salin et la sélection du débit dans chaque section du LMS à échange ionique par gradient salin. Différentes configurations du LMS gradient ont été comparées : circuit fermé ou ouvert entre autre.

Enfin, nous présenterons une comparaison des deux stratégies de modélisation mis en œuvre – le modèle du lit mobile vrai gradient équivalent et le modèle du lit mobile simulé gradient – pour la prédiction des profils de concentration dans un LMS gradient avec circuit ouvert ou fermé.

摘要

本论文研究膨胀床和盐梯度离子交换模拟移动床技术分离和精制蛋白质。

首先建立了数学模型。该模型用于描述膨胀床吸附蛋白质的动力学，并用下列的实验测定数据验证该数学模型。

1. 实验测定牛血清蛋白在膨胀床内的吸附突破曲线。使用型号为 Streamline50 膨胀床, 柱内分别填充 300 毫升型号为 Streamline DEAE 的第一代吸附剂和型号为 Streamline Direct CST I 的第二代吸附剂。
2. 溶菌酶在膨胀床内柱内吸附突破曲线。该实验数据摘自已发表的科技论文[Bruce and Chase (2001)]。型号为 Streamline50 膨胀床填充 400 毫升型号为 Streamline SP 的第一代吸附剂。

基于模型参数敏感性的分析, 探讨诸因素对膨胀床内突破曲线的影响, 诸因素包括蛋白质在吸附剂内的扩散阻力、吸附剂外膜传质阻力、柱内液相轴向弥散、吸附剂的轴向弥散、吸附剂颗粒轴向分布和床层空隙率轴向变化。

实验研究了牛血清蛋白和肌红蛋白在膨胀床内竞争吸附与解吸。使用型号为 Streamline50 膨胀床, 并填充 300 毫升型号为 Streamline Direct CST I 的第二代吸附剂。同时间歇吸附实验、前锋分析、和柱子取代实验被用于分析血清蛋白和肌红蛋白在型号为 Streamline Direct CST I 的第二代吸附剂上竞争吸附和取代的机理。

使用高密度惰性芯吸附剂能提高膨胀床吸附蛋白质的效率, 因为该设计减少了大分子蛋白质的颗粒内扩散阻力。蛋白质的颗粒内扩散阻力的减少量能够用模型参数 $1/\Theta$ 估算。基于线性拆分因子的导出式, 证明了采用惰性芯吸附剂填充的色谱分离大分子蛋白质效果明显好于普通吸附剂填充的色谱分离效果。此外, 本论文还给出了用于计算惰性芯吸附剂填充色谱的理论板当量[等效]高度线性解析式。

采用梯度 SMB 数学模型分析盐梯度模拟移动床分离和精制蛋白质的性能。对盐梯度选择和流速选择的策略进行了详细讨论。比较了循环与非循环的梯度模拟移动床, 在循环梯度模拟移动床操作中, 加入一个固定体积的混合器可以减少梯度的波动。比较了两种常用的数学模型, 梯度 SMB 模型和等价梯度 TMB 模型, 讨论了简单的等价梯度 TMB 模型的实用性。

Table of Contents

List of Figures	VII
List of Tables	XIII
1. Introduction	1
1.1 Relevance and Motivation	1
1.2 Objectives and Outline	3
References	5
2. Modeling separation of proteins by inert core adsorbent in a batch adsorber	7
2.1 Introduction	8
2.2 Mathematical model and analytical solutions	10
2.2.1 Mathematical model	10
2.2.2 Analytical solution for Henry isotherm	14
2.2.3 Analytical solution for Rectangular isotherm	16
2.3 Results and discussion	16
2.3.1 Effect of the model parameters on the time evolution of bulk concentration and uptake radial profiles	18
2.3.2 Method for estimation of effective pore diffusivity of protein and film mass transfer coefficient from the bulk concentration profile	24
2.4 Conclusions	27
Notation	28
References	30

3. Modeling breakthrough and elution curves in fixed bed of inert core adsorbents: analytical and approximate solutions	35
3.1 Introduction	36
3.2 Mathematical model and analytical and approximate solutions	38
3.2.1 Analytical and approximate solutions for breakthrough curves	42
3.2.1.1 Analytical solution	42
3.2.1.2 Parabolic-profile approximate solution	44
3.2.1.3 Quasi-lognormal distribution approximate solution	45
3.2.2 Analytical and approximate solutions for peak elution curves	47
3.2.2.1 Analytical and approximate solutions for Dirac input	47
3.2.2.2 Analytical and approximate solutions for the generalized sample input mode	47
3.2.3 Derived equation of resolution R_s based on Q-LND approximation	49
3.3 Results and discussion	51
3.3.1 Effect of model parameters on breakthrough curves	51
3.3.2 Effect of model parameters on peak elution curves	55
3.3.3 Resolution of two components by the fixed bed packed with inert core adsorbent	59
3.4 Conclusions	62
Notation	62
References	65
 4. Analytical breakthrough and elution curves for inert core adsorbent with sorption kinetics	 69
4.1 Introduction	70
4.2 Mathematical model and analytical and approximate solutions	71
4.2.1 Analytical solution and parabolic-profile approximate solution for breakthrough curves	74
4.2.1.1 Analytical solution	75

4.2.1.2 Parabolic-profile approximate solution	78
4.2.2 Analytical and approximate solutions for peak elution curves	79
4.2.3 Moments and height equivalent to a theoretical plate	79
4.2.4 Analytical solution for residence time distribution of an inert tracer with Dirac input	80
4.3 Results and discussion	81
4.3.1 Effect of model parameters on breakthrough curves	81
4.3.2 Effect of model parameters on peak elution curves	84
4.3.3 Theoretical plate number and the equivalent height to a theoretical Plate (<i>HETP</i>)	86
4.3.4 Residence time distribution	87
4.4 Conclusions	88
Notation	89
References	90
5. A 3-zone model for protein adsorption kinetics in expanded beds	93
5.1. Introduction	94
5.2 Mathematical model	96
5.2.1 Zone division of the column	96
5.2.2 Model development	97
5.2.3 Model parameters	100
5.2.4 Numerical method	101
5.3 Results and discussion	101
5.3.1 Comparison between the experimental data and simulation results	101
5.3.2 Parametric sensitivity analysis on the breakthrough curves	104
5.3.2.1 Effect of intraparticle diffusion coefficient	105
5.3.2.2 Effect of film mass transfer coefficient	107
5.3.2.3 Effect of liquid axial dispersion	108
5.3.2.4 Effect of adsorbent axial dispersion	110
5.3.3 Effect of adsorbent particle size on the breakthrough curves	111

5.3.4 Modified uniform model by taking account the adsorbent particle size and bed voidage axial dispersions	112
5.4 Conclusions	113
Notation	114
References	116
6. Experimental and modeling study of protein adsorption in expanded bed	119
6.1 Introduction	120
6.2 Experimental	123
6.2.1 Equipment, adsorbents and model protein	123
6.2.2 Batch adsorption experiments	124
6.2.3 Residence time distribution experiments	125
6.2.4 Experimental procedures for the whole expanded bed adsorption process	125
6.3 Mathematical model for the protein adsorption in expanded beds	126
6.4 Results and discussion	130
6.4.1 Adsorption isotherm and BSA effective pore diffusivity	130
6.4.2 Bed expansion and liquid axial dispersion coefficient in expanded bed	133
6.4.3 BSA protein breakthrough behavior in expanded beds packed with Streamline Direct CST I and with Streamline DEAE	136
6.4.4 Comprehensive evaluations on the whole expanded-bed protein adsorption process with Streamline DEAE and with Streamline CST I	144
6.5 Conclusions	149
Notation	151
References	152
Appendix	156
 7. Expanded bed adsorption/desorption of proteins with Streamline	
Direct CST I adsorbent	159
7.1 Introduction	160
7.2 Materials and methods	163

7.2.1 Equipment, Streamline Direct CST I, model proteins	163
7.2.2 Batch adsorption equilibrium isotherm experiments	164
7.2.3 Column displacement experiments	165
7.2.4 Frontal analysis in a fixed bed	165
7.2.5 Experimental procedures for the whole expanded bed adsorption Process	166
7.3 Results and discussion	167
7.3.1 Single- and two-component BSA/myoglobin adsorption isotherm	167
7.3.2 Frontal analysis and column displacement measurements	172
7.3.3 BSA and myoglobin competitive adsorption/desorption in an expanded bed	177
7.4 Conclusions	179
References	179

8. Proteins separation and purification by salt gradient ion-exchange

simulated moving bed	183
8.1 Introduction	184
8.2 Gradient SMB strategies of modeling	190
8.2.1 Formation of salt gradient in ion-exchange SMB chromatography	190
8.2.2 Model equations for the real gradient SMB model	190
8.2.3 Proteins and salt adsorption equilibrium isotherm on ion exchanger	193
8.2.4 Model parameters and numerical method	195
8.3 Results and discussion	196
8.3.1 Proteins separation by salt gradient ion-exchange SMB with linear ion-exchange equilibrium isotherm	196
8.3.2 Comparison of gradient SMB configurations: open loop, closed loop and closed loop with a holding vessel	203
8.3.3 Comparison of the two strategies of modeling, gradient SMB/TMB model	208
8.3.4 Salt gradient ion-exchange SMB with nonlinear ion exchange	

equilibrium isotherm of proteins	210
8.3.4.1 Nonlinear ion exchange equilibrium isotherm of BSA and myoglobin on Q-Sepharose FF anion exchanger	210
8.3.4.2 Proteins separation and purification by salt gradient ion-exchange SMB with nonlinear ion exchange equilibrium isotherm	214
8.4 Conclusions	222
Notation	223
References	224
Appendix	228
9. Conclusions and suggestions for future work	231
9.1 Conclusions	231
9.2 Suggestions for future work	234

List of Figures

Chapter 1

Figure 1.1 Preparation and the Three Step Purification Strategy for the high purify protein production	2
--	---

Chapter 2

Figure 2.1 Batch adsorber and inert core adsorbent	10
Figure 2.2 Langmuir isotherm.	17
Figure 2.3 Effect of r_c on dimensionless bulk concentrations as a function of reduced time at different Bi for constant value of N	19
Figure 2.4 Effect of r_c on dimensionless bulk concentrations as a function of reduced time at constant loading ratio $L = (1 - r_c^3)N$ for $Bi = 100$.	20
Figure 2.5 Uptake radial profiles within the adsorbent shell at various times for $Bi = 100$ and $r_c = 0.6$.	22
Figure 2.6 Comparison of analytical solution of versus for rectangular isotherm and numerical solution for Langmuir isotherm with small values of λ at different Bi for $N = 0.9$	23
Figure 2.7 Verification of the numerical solution by comparison with analytical solution for Henry isotherm.	24
Figure 2.8 Estimation of effective pore diffusivity, D_{pe} , at high Bi value for Langmuir isotherm.	26
Figure 2.9 Estimation of effective pore diffusivity, D_{pe} , at small Bi value for Langmuir isotherm.	27

Chapter 3

Figure 3.1 Scheme of fixed-bed adsorber and inert core adsorbent	39
Figure 3.2 Typical elution curve.	49
Figure 3.3 Effect of ξ_C on $y - \tau$ breakthrough curves.	53
Figure 3.4 Effect of Bi on $y - \tau$ breakthrough curves for the column packed with inert core adsorbent and conventional adsorbent	54
Figure 3.5 Effect of Pe on $y - \tau$ breakthrough curves for the column packed with inert core adsorbent and conventional adsorbent	55
Figure 3.6 Effect of ξ_C on $y - \tau$ elution curves for $\theta = 10^{-1}$	56
Figure 3.7 Effect of Pe on $y - \tau$ elution curves for the column packed with inert core adsorbent and conventional adsorbent	57
Figure 3.8 Effect of Bi on $y - \tau$ elution curves for the column packed with inert core adsorbent and conventional adsorbent	57
Figure 3.9 Elution curves for different input modes at the column packed with inert core adsorbent adsorbent and conventional adsorbent	58
Figure 3.10 Influence of inert core radius on the relative intraparticle diffusion resistance.	60
Figure 3.11 The resolution of two components at the column packed with inert core adsorbent and conventional adsorbent	61

Chapter 4

Figure 4.1 Scheme of fixed-bed adsorber and inert core adsorbent	71
Figure 4.2 Effect of ξ_C on $y - \tau$ breakthrough curves.	82
Figure 4.3 Effects of Pe on $y - \tau$ breakthrough curves for inert core adsorbent.	83
Figure 4.4 Effects of Bi on $y - \tau$ breakthrough curves for inert core adsorbent.	83
Figure 4.5 Effect of ψ on $y - \tau$ breakthrough curves for inert core adsorbent	84

Figure 4.6 Effect of ξ_c on $y - \tau$ breakthrough curves for short column.	84
Figure 4.7 Effect of Pe on $y - \tau$ elution curves for inert core adsorbent.	85
Figure 4.8 Effect of Bi on $y - \tau$ elution curves for inert core adsorbent.	85
Figure 4.9 Effect of ψ on $y - \tau$ elution curves for inert core adsorbent.	86
Figure 4.10 Effect of ξ_c on $HETP^*$ and N_T under restricted adsorption /desorption rate	86
Figure 4.11 Effect of Bi on $y - \tau$ residence time distribution for inert core adsorbent.	87
Figure 4.12 Effect of Pe on $y - \tau$ residence time distribution for inert core adsorbent.	88

Chapter 5

Figure 5.1 Expanded bed with three zones and flow configuration in Bruce and Chase experimental system (2001).	96
Figure 5.2 Comparison among the in-bed experimental breakthrough curves and the simulation results.	102
Figure 5.3 Parametric sensitivity analysis of intraparticle diffusion coefficient to in-bed breakthrough curves in expanded bed.	106
Figure 5.4 Parametric sensitivity analysis of film mass transfer coefficient to in-bed breakthrough curves in expanded bed.	107
Figure 5.5 Parametric sensitivity analysis of liquid axial dispersion coefficient to in-bed breakthrough curves in expanded bed.	109
Figure 5.6 Parametric sensitivity analysis of adsorbent axial dispersion coefficient to in-bed breakthrough curves in expanded bed.	110
Figure 5.7 Effect of adsorbent particle size on in-bed breakthrough curves in expanded bed and comparison between the experimental data and the simulation results predicted by the modified uniform model.	112

Figure 5.8 Streamline SP adsorbent particle size axial distribution and bed voidage axial variation in an expanded bed.	113
---	-----

Chapter 6

Figure 6.1 BSA adsorption isotherms on Streamline DEAE and on Streamline direct CST I at the room temperature.	131
Figure 6.2 Estimation of BSA effective pore diffusivity in Streamline DEAE adsorbents and in Streamline CST I adsorbents in batch adsorber.	132
Figure 6.3 Relationship between bed expansion degree and superficial liquid flow velocity in expanded beds packed with Streamline DEAE and with Streamline CST I.	134
Figure 6.4 Experimental data of RTD curves are fitted by the analytical solution with the dirac input of acetone tracer at expanded beds packed with Streamline DEAE and packed with Streamline direct CST I.	135
Figure 6.5 Experimental and predicted BSA breakthrough curve in expanded bed packed with Streamline direct CST I.	137
Figure 6.6 Contribution of each model parameter to the breakthrough curve in expanded bed packed with Streamline direct CST I.	139
Figure 6.7 Experimental and predicted BSA breakthrough curve in expanded bed packed with Streamline DEAE.	141
Figure 6.8 Effect of the particle size axial distribution and bed voidage axial distribution on the breakthrough curves in expanded beds packed with Streamline DEAE.	143
Figure 6.9 Effluent curves of BSA protein during adsorption, washing, and elution stages in expanded bed packed with Streamline direct CST I.	145
Figure 6.10 Effluent curves of BSA protein during adsorption, washing, and elution stages in expanded bed packed with Streamline DEAE.	145
Figure 6.11 Effect of ionic strength of buffer on BSA adsorption isotherms on Streamline DEAE and on Streamline direct CST I.	147

Figure 6.12 Effect of salt concentration in buffer on BSA adsorption isotherms on Streamline DEAE and on Streamline direct CST I	148
---	-----

Chapter 7

Figure 7.1 Effect of ionic strength, salt concentration and pH value in feedstocks on BSA adsorption isotherm on Streamline Direct CST I.	169
Figure 7.2 Effect of salt concentration on BSA adsorption isotherm on Streamline Direct CST I at 50mM acetate buffer (PH 7).	170
Figure 7.3 Myoglobin adsorption isotherm on streamline Direct CST I in 50mM acetate buffer with different pH value.	170
Figure 7.4 BSA and myoglobin competitive adsorption isotherm on Streamline Direct CST I measured in static batch experiments at 50mM acetate buffer (pH 5).	171
Figure 7.5 Displacement adsorption between BSA and myoglobin in a fixed bed packed with Streamline Direct CST I.	172
Figure 7.6 Breakthrough curves in a fixed bed packed with Streamline Direct CST I for single- and two-component BSA/myoglobin adsorption system.	174
Figure 7.7 Effluent curves of BSA and myoglobin during adsorption, wash and elution stages in expanded bed packed with Streamline direct CST I.	177

Chapter 8

Figure 8.1 Schematic diagram of a four-zone isocratic/gradient SMB with closed loop	186
Figure 8.2 Operation modes for gradient SMB.	189
Figure 8.3 Experimental conditions and configuration in salt gradient ion-exchange SMB with open loop.	196
Figure 8.4 Transient concentration profiles before next switch in salt gradient ion -exchange SMB with open loop in the first full cycle.	198
Figure 8.5 Concentration profiles at half switch time with different cycles in salt	

gradient ion-exchange SMB with open loop.	199
Figure 8.6 Cyclic steady state internal concentration profiles during a switch time interval in salt gradient ion-exchange SMB with open loop.	201
Figure 8.7 Cyclic steady state internal concentration profiles during a switch time interval in salt gradient ion-exchange SMB with open loop ($C_s^D = 0.3M$ and $C_s^F = 0.13M$).	202
Figure 8.8 Simulation conditions and configurations of salt gradient ion-exchange SMB.	204
Figure 8.9 Internal concentration profiles at cycle steady state in salt gradient ion-exchange SMB with open loop.	205
Figure 8.10 Internal concentration profiles at cycle steady state in salt gradient ion-exchange SMB with closed loop.	206
Figure 8.11 Internal concentration profiles at cycle steady state in salt gradient ion-exchange SMB with closed loop and a holding vessel ($V_s=10\text{mL}$).	207
Figure 8.12 Internal concentration profiles at the steady state in salt gradient ion-exchange SMB with open loop and closed loop, respectively, predicted by gradient TMB model.	209
Figure 8.13 Ion exchange equilibrium isotherms of BSA and myoglobin on Q-Sepharose FF resin at room temperature.	211
Figure 8.14 Plots of $\log(q/C)$ versus $\log(C_s)$ in the linear ion exchange equilibrium isotherm.	212
Figure 8.15 BSA and myoglobin breakthrough curves at various NaCl concentration	213
Figure 8.16 Operating conditions and configuration in salt gradient ion-exchange SMB with open loop.	219
Figure 8.17 BSA and myoglobin separation with complete recovery of myoglobin in raffinate stream (Case 1).	220
Figure 8.18 BSA purification from myoglobin impurity without the recovery of myoglobin (Case 2).	221

List of tables

Table 2.1 Langmuir equilibrium parameters for BSA adsorption on CB-6AS inert core adsorbent (data from Zhang and Sun, 2002)	17
Table 5.1 The model parameters used in the simulations based on the experimental data of Bruce and Chase (2001)	104
Table 5.2 Parametric sensitivity analysis to assess the impact of changes in D_p , k_{fk} , D_{Lk} , and D_{Sk} on the breakthrough time at $C/C_0 = 0.05$ in expanded beds.	108
Table 6.1 Experimental conditions and model parameters used for the simulation of the breakthrough curves in expanded beds.	140
Table 7.1 Some properties of Streamline Direct CST I adsorbent.	164
Table 7.2 Some properties of BSA and myoglobin.	164
Table 8.1 Some properties of Q-Sepharose-FF anion exchanger.	190
Table 8.2 Separation factor of BSA to myoglobin by Q-Sepharose FF anion exchanger under linear adsorption equilibrium isotherm.	214
Table 8.3 Some constraints to the net fluxes for BSA and myoglobin separation in salt gradient ion-exchange SMB.	216
Table 8.4 Flow rate ratio range for the separation and purification of BSA and myoglobin in salt gradient ion-exchange SMB with open loop.	217

1. Introduction

1.1 Relevance and Motivation

“Proteins” a word derived from the Greek word “Proteios”, which means “of the first rank” was coined by Berzelius in 1938 to stress the importance of this group of organic compounds. Proteins not only play an important action in biology, but also have large potential applications in pharmaceuticals and therapeutics, food processing, textiles and leather goods, detergents, paper manufacturing.

With the development of molecular biology technologies, various kinds of proteins can be prepared from upstream processes and from biological raw materials. However, there exist various proteins and contaminants in these source feedstocks, and the key issue is that proteins can be separated and purified efficiently from the sources materials, in order to reduce the production cost of the high purity protein. The development of techniques and methods for proteins separation and purification has been an essential prerequisite for many of the advancements made in biotechnology.

Most separation and purification protocols require more than one step to achieve the desired level of protein purity. In Figure 1.1, a three step separation and purification strategy is presented by Amersham Biosciences, which included capture, intermediate separation and purification, and final polishing during a downstream protein separation and purification process. In the capture step the objectives are to isolate, concentrate and stabilize the target proteins. During the intermediate separation and purification step the objectives are to remove most of the bulk impurities, such as other proteins and nucleic acids, endotoxins and viruses. And in the polishing step most impurities have already been removed except for trace amounts or closely related substances. The objective is to achieve

final purity of protein.

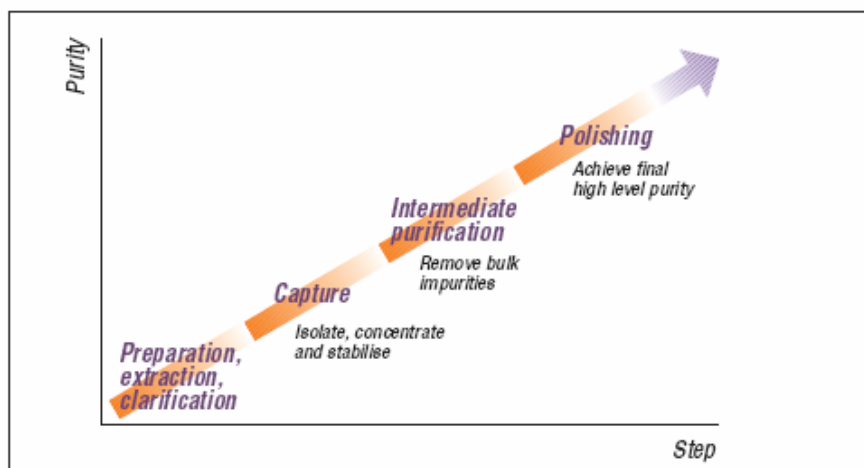


Figure1.1 Preparation and the Three Step Separation and Purification Strategy for the high purify protein production (Protein Purification Handbook from Amersham Biosciences).

In the capture step, as primary recovery of proteins, the expanded bed adsorption (EBA) technology has been widely applied to capture proteins directly from crude unclarified source materials, such as, *E. coli* homogenate, yeast, fermentation, mammalian cell culture, milk, animal tissue extracts (Chase, 1994; Hjorth, 1997). The expanded bed is designed in a way that the suspended adsorbent particles capture target protein molecules while cells, cell debris, particulate matter and contaminants pass through the column unhindered. After loading and washing, the bound proteins can be eluted by elution buffer and be concentrated in a small amount of elution solution, apart from the bulk impurities and contaminants in source materials. In the last decade, various applications of EBA technology have been reported from lab-scale to pilot-plant and large-scale production.

During the intermediate purification and final polishing steps, the techniques of the conventional elution chromatography have been applied successfully. New challenge should be the application of simulated moving bed (SMB) to the separation and purification of proteins. Simulated moving bed (SMB) chromatography is a continuous process, which for preparative purposes can replace the discontinuous regime of elution chromatography.

Furthermore, the countercurrent contact between fluid and solid phase used in SMB chromatography maximizes the mass transfer driving force, leading to a significant reduction in mobile and stationary phase consumption when compared with elution chromatography (Broughton et al., 1961; Juza et al., 2000; Rodrigues et al., 2004; Ruthven et al., 1989). More significant improvement is the development of gradient SMB technology for the separation and purification of proteins (Jensen et al., 2000; Houwing et al., 2002), where a step-wise gradient can be formed by introducing a solvent mixture with a lower strength at the feed inlet port compared to the solvent mixture introduced at the desorbent port, then the adsorbents have a lower binding capacity to proteins in section I and section II to improve the desorption and have a stronger binding capacity in section III, and IV to increase adsorption in SMB chromatography (a four-zone SMB). The solvent consumption by gradient mode can be decreased significantly when compared with isocratic SMB chromatography (Jensen et al., 2000). Moreover, when a given feed is applied to gradient SMB chromatography, the protein obtained from the extract stream can be enriched if protein has a medium or high solubility at the high solvent strength, while the raffinate protein is not diluted at all.

1.2 Objectives and Outline

The objectives of this thesis are the study of proteins adsorption kinetics in expanded bed and gradient SMB chromatography.

With specially designed adsorbents and columns, the adsorption behavior of the expanded bed is comparable to that of the fixed bed (Chase, 1994), but there still exists some differences between the expanded bed and the fixed bed. A mathematical model suitable to expanded bed adsorption process should be developed in order to efficiently optimize and design EBA process (Wright and Glasser, 2001), which is one of our research objectives in this thesis.

Streamline direct CST I is a new type of ion exchanger with multimodal functional group, which not only takes advantage of electrostatic interaction, but also takes advantage of hydrogen bond interaction and hydrophobic interactions to bind proteins in order to get a

high binding capacity even in high ionic strength and salt concentration feedstocks (Johansson et al, 2003). It will be a significant improvement of EBA technology to capture proteins, such as BSA and myoglobin, by an expanded bed packed with Streamline Direct CST I adsorbents. In this thesis, relative experimental researches will be reported.

In an expanded bed, the adsorbents with a high-density matrix are used to form a stable expansion at high feed flow rates. Recently, some authors have improved the design of the high-density matrix of adsorbent, by including a single heavier inert core material in the macroporous crosslinked resin matrix, that is called pellicular adsorbent or inert core adsorbent (Palsson et al., 2000; Theodossion et al., 2002). The inert core adsorbents not only increase the particle density to form stable expansion at high feed flow rate in the expanded bed, but also reduce the protein diffusion resistance inside adsorbent due to shortening of the diffusion path, which made the adsorption behavior in EBA process more efficiently. In addition, the inert core adsorbents also are potential to use in the fast, high-performance liquid chromatography (HPLC) due to reduce the intraparticle diffusion resistance for biological macromolecules (Kirkland et al., 2000; Rodrigues, 1997). In this thesis, some theoretical analysis about the protein adsorption kinetics on the inert core adsorbent will be addressed.

The application of gradient SMB technology to proteins separation and purification is more attractive. Up to now, the research is just underway, because of the expensive experimental research for practical proteins separation and purification by gradient SMB chromatography; in published works, also authors used an equivalent gradient TMB (true moving bed) model to simplify the simulation, and only linear adsorption was dealt with. In this thesis, we will begin our research with simulation, using a real gradient SMB, to study the behavior of the separation of BSA and myoglobin by salt gradient ion exchange SMB chromatography.

Outline of this thesis

Protein adsorption behaviors on inert core adsorbents are studied theoretically in a batch adsorber (in Chapter 2) and in a fixed bed chromatography (in Chapter 3 and in

Chapter 4 where sorption kinetics is included).

Mathematical models of EBA are developed in Chapter 5. In Chapter 6, protein adsorption in EBA is analyzed, and simulated results are tested against the experimental data of BSA adsorption in a Streamline 50 column packed with Streamline DEAE, and Streamline Direct CST I, respectively.

In Chapter 7, BSA and myoglobin competitive adsorption/desorption in an expanded bed packed with streamline Direct CST I is studied experimentally, where Streamline 50 column is packed with 300mL Streamline Direct CST I adsorbents.

In Chapter 8, a detailed simulation, using a real gradient SMB model, is carried out to study the behavior of the separation of BSA and myoglobin by salt gradient ion exchange simulated moving bed chromatography.

Finally, Chapter 9 has the conclusions of this thesis and some suggestions for future work.

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2. Modeling separation of proteins by inert core adsorbent in a batch adsorber^{*}

Adsorption/desorption kinetics of protein on the binding ligand of inert core adsorbent in a batch adsorber is analyzed theoretically for Langmuir isotherm coupled with the intraparticle diffusion and film mass transfer resistances. For the two limiting cases of Langmuir isotherm, there are analytical solutions. New analytical solutions are derived for Henry isotherm, and the analytical solution of shrinking core model is recommended for rectangular isotherm. The effects of the inert core radius, equilibrium constant, intraparticle diffusion and film mass transfer resistances on the time evolution of bulk concentration and particle radial profiles were investigated. The applicable range of the analytical solution with rectangular isotherm is given. A new method to estimate both film mass transfer coefficient, k_f , and effective pore diffusivity, D_{pe} , from a single bulk concentration-time curve in batch adsorber is given and tested with literature data for the adsorption of BSA on CB-6AS inert core adsorbent.

^{*}This chapter is based on the paper by Li, P., Xiu, G. H. and Rodrigues, A. E., "Modeling separation of proteins by inert core adsorbent in a batch adsorbent", *Chemical Engineering Science*, 58, 3361-3371, 2003.

2.1. Introduction

The process in which a target protein is directly captured from the crude feedstock, such as bacteria, yeast, and mammalian cell culture, is becoming important in biotechnology and pharmaceutical industry. Expanded-bed adsorption (EBA) is a single pass operation in which desired proteins are purified directly from crude, particulate containing feedstock, without the need for separate clarification, concentration and initial purification. The bed expansion increases bed voidage, which allows for unhindered passage of cells, cell debris and other particulates during application of crude feed to the column. Many successful processes using EBA have been reported for protein purification directly from *E. coli* homogenate, yeast, fermentation, mammalian cell culture, milk, animal tissue extracts, etc. (Bertrand, Cochet and Cartron, 1998; Anspach et al., 1999; Bruce and Chase, 2001; Ozyurt, Kirdar and Ulgen, 2002; Smith et al., 2002).

Recently, inert core adsorbents were developed to improve the separation performance of proteins in expanded bed (Griffith et al., 1997; Voute et al., 1999; Pai, Gondkar, and Lali, 2000; Palsson, Gustavsson, and Larsson, 2000; Tong and Sun, 2001; Jahanshahi et al., 2002; Theodossiou et al., 2002). For example, UpFront's FastLine adsorbents (UpFront Chromatography A/S, Denmark) are based on highly cross-linked agarose beads with a central core of high density, e.g. glass or stainless steel; and ZSA pellicular adsorbent matrix (Jahanshahi et al., 2002) is a pellicular particle comprising a solid core (zirconia-silica) coated with a porous skin of 4% (w/v) cross-linked agarose. These inert core adsorbents have increased density by the incorporation of heavier inert core and are adequate for stable expansion at high flow rates in expanded bed. In addition, the protein diffusion resistance inside inert core adsorbent is effectively reduced due to the shortening of the diffusion path. In the protein separation processes by elution chromatography, expanded-bed and simulated moving bed, the intraparticle diffusion resistance has significant influence on the operation performance of the process (Rodrigues, 1997; Kirkland et al., 2000); therefore, adopting this kind of binding ligand inert core adsorbent to decrease protein diffusion resistance will be attractive in proteins separation industry. Poroshell 300SB-C18 adsorbent (from Agilent technologies Inc., 2001) is a 5 μm particle with a thin layer of porous silica on a solid inert

core, which has been used for fast, high-performance resolution proteins in liquid-chromatography (Kirkland et al., 2000).

Usually, the adsorption/desorption equilibrium and kinetics for the protein onto the adsorbent are evaluated in the batch absorber (Owen and Chase, 1999; Hunter and Carta, 2000; Zhang and Sun, 2002); the measured model parameters (for example, adsorption equilibrium parameters and the effective intraparticle diffusivity) are then used to predict the performance of industrial chromatography processes, such as elution chromatography, expanded-bed and simulated moving bed. Therefore, modeling separation of proteins by inert core adsorbent in a batch adsorber will be important, which is our objective in this chapter. Although numerous models describing the kinetics of protein adsorption to porous particles in the batch adsorber have been researched, their relevance to the analysis of adsorption to inert core adsorbent is marginal due to their various simplifying assumptions.

For Langmuir isotherm, two limiting cases frequently occur in separation of proteins. One is the high favorable adsorption equilibrium or irreversible adsorption for the affinity adsorption with favorably selective ligand on the adsorbent; the other one is the linear (Henry) adsorption equilibrium for the reversible adsorption of the low concentration protein from the diluted crude feedstock. For adsorption onto inert core adsorbent, analytical solutions were derived and recommended for linear (Henry) adsorption equilibrium and high favorable adsorption equilibrium (irreversible adsorption) where the intraparticle diffusion and film mass transfer resistances were both considered. We also developed the program package for the general mathematical model with orthogonal collocation method. A significant feature of this new model is that it furnishes an analytical solution to the problem for linear and rectangular adsorption isotherms, which is suitable in most cases for capturing proteins in dilute crude feedstocks.

Intraparticle diffusivity measurements are usually performed via batch uptake experiments. The experimental data of the bulk concentration profile are fitted with the simulation results of a suitable transport model to estimate the effective intraparticle diffusivity. Although the stirring speed can be changed in batch adsorption experiment, the film mass transfer resistance is sometimes not negligible in many experimental systems; in

those cases the estimation of the intraparticle diffusivity becomes more difficult. Geankopolis (1983) gave a correlation to estimate *a priori* film mass transfer coefficient in stirred-tank experiments; such procedure was applied by Zhang and Sun (2002) to estimate the intraparticle diffusivity of BAS in CB-6AS inert core adsorbent from the bulk concentration profiles in batch adsorber. Another recommended method to determine both the intraparticle effective diffusivity and the film mass transfer coefficient from a single bulk concentration-time curve in batch adsorber is the error maps technique (Kaguei, One, and Wakao, 1989; Xiu et al., 1993, 1994). In this chapter, a new method is proposed on how to get both the intraparticle effective diffusivity and the film mass transfer coefficient values from a single bulk concentration-time curve in batch adsorber simultaneously from the slope of model dimensionless time τ versus real experimental time t .

2. 2. Mathematical model and analytical solutions

2.2.1 Mathematical model

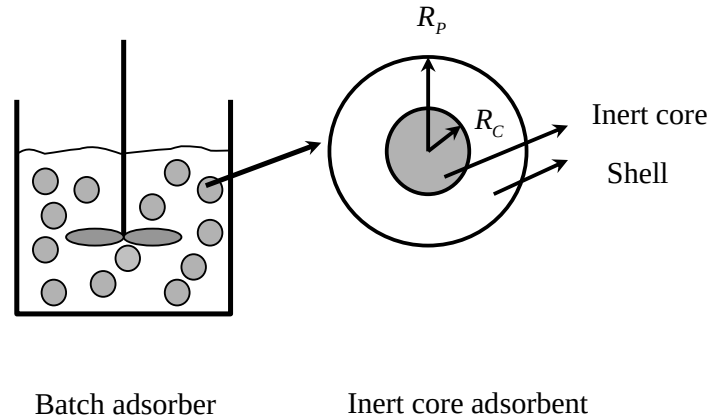


Fig. 2.1. Batch adsorber and inert core adsorbent

The batch adsorber and the inert core adsorbent are shown in Fig. 2.1. There are three steps involved in the protein adsorption from the bulk solution into an adsorbent shell: (i) mass transfer from bulk liquid to the outer surface of the particles (film mass transfer resistance); (ii) diffusion through the pores of the adsorbent shell (intraparticle diffusion resistance) (Horstmann and Chase, 1989), and (iii) the protein binding to the pore-wall

surface (surface-reaction resistance). Adsorption kinetic studies showed that the diffusion of proteins in the adsorbent matrix was the rate-controlling step during the protein adsorption process (Horstmann and Chase, 1989; Skidmore, Horstmann, and Chase, 1990; Champluvier and Kula, 1992).

In this work the liquid phase concentration of protein in the pore was assumed to be in equilibrium with the adsorbed phase concentration of protein at any radial position, and a pore diffusion model was employed to describe protein uptake kinetics. Intraparticle mass transfer occurs by diffusion in liquid-filled pores with a driving force expressed in terms of the pore fluid concentration gradient (Weaver and Carta, 1996; Wright, Muzzio, and Glasser, 1998; Zhang and Sun, 2002); a constant diffusivity was assumed.

The other assumptions and simplifications are (Chanda and Rempel, 1997, 1999; Li, Xiu and Rodrigues, 2003): (1) the adsorbent consists of an inert core, non-permeable to the protein solution; (2) the pore diffusion model will describe the protein transport in the outer shell layer; (3) the outer shell layer undergoes negligible swelling or shrinking during sorption.

Mass balance of adsorbate for the liquid phase in batch adsorber:

$$V_L \frac{dC_i}{dt} + \frac{3W_A}{\bar{\rho}_p R_p} k_f \left[C_i - (C_{pi})_{R=R_p} \right] = 0 \quad (2.1)$$

where V_L is the fluid volume in the adsorber, W_A and $\bar{\rho}_p$ are the mass and the apparent density of the adsorbent particle, respectively, C_i is the adsorbate concentration in bulk phase, C_{pi} is the adsorbate concentration in adsorbent pore, R_p is the radius of the adsorbent particle, R is the radial distance in adsorbent, t is the time, and k_f is the film mass transfer coefficient. $\bar{\rho}_p = [R_C^3 \rho_C + (R_p^3 - R_C^3) \rho_s] / R_p^3$, in which ρ_C is the density of inert core of adsorbent, ρ_s is the density of the adsorbent shell, R_C is the radius of the inert core of adsorbent.

Mass balance in the adsorbent shell:

$$\varepsilon_s \frac{\partial C_{pi}}{\partial t} + \rho_s \frac{\partial \hat{q}_i}{\partial t} = D_{pe} \left(\frac{\partial^2 C_{pi}}{\partial R^2} + \frac{2}{R} \frac{\partial C_{pi}}{\partial R} \right) \quad (R_c \leq R \leq R_p) \quad (2.2)$$

where ε_s is porosity of the adsorbent shell, $\hat{q}_i$ is the amount adsorbed per kilogram of adsorbent shell, D_{pe} is the effective pore diffusivity.

The boundary conditions are:

$$k_f \left[C_i - (C_{pi})_{R=R_p} \right] = D_{pe} \left(\frac{\partial C_{pi}}{\partial R} \right)_{R=R_p} \quad (2.3)$$

$$\left(\frac{\partial C_{pi}}{\partial R} \right)_{R=R_c} = 0 \quad (2.4)$$

The initial conditions are:

$$\begin{cases} C_i = C_{i0}, C_{pi} = 0 & (\text{adsorption}) \\ C_i = 0, \hat{q}_i = \hat{q}(C_{i0}) & (\text{desorption}) \end{cases} \quad (2.5)$$

The adsorption isotherm is assumed to be of a Langmuir type

$$\hat{q}_i = \frac{q_m K C_{pi}}{1 + K C_{pi}} \quad (2.6)$$

where q_m and K are the maximum adsorbent capacity and equilibrium constant, respectively.

The following dimensionless variables are introduced:

$$c_{pi} = \frac{C_{pi}}{C_{i0}}, c_i = \frac{C_i}{C_{i0}} \quad (\text{dimensionless fluid concentrations in pore and bulk phases})$$

$$q_i = \frac{\hat{q}_i}{\hat{q}(C_{i0})} \quad (\text{dimensionless adsorbed concentration in adsorbent shell})$$

$$r = \frac{R}{R_p} \quad (\text{dimensionless radial distance in adsorbent})$$

$$\tau = \frac{D_{pe} t}{(1 + \xi_m) R_p^2 \varepsilon_s} \quad (\text{dimensionless contact time})$$

where $\xi_m = \rho_s \hat{q}(C_{i0}) / \varepsilon_s C_{i0}$ is the capacity factor.

Model Eqs (2.1)-(2.6) in dimensionless form become

$$\frac{dc_i}{d\tau} + 3N \left(\frac{\partial c_{pi}}{\partial r} \right)_{r=1} = 0 \quad (2.7)$$

$$\left(\frac{1 + \xi_m dq_i/dc_{pi}}{1 + \xi_m} \right) \frac{\partial c_{pi}}{\partial \tau} = \frac{\partial^2 c_{pi}}{\partial r^2} + \frac{2}{r} \frac{\partial c_{pi}}{\partial r} \quad (r_C \leq r \leq 1) \quad (2.8)$$

$$\left(\frac{\partial c_{pi}}{\partial r} \right)_{r=1} = Bi [c_i - (c_{pi})_{r=1}] \quad (2.9)$$

$$\left(\frac{\partial c_{pi}}{\partial r} \right)_{r=r_C} = 0 \quad (2.10)$$

$$\begin{cases} c_i = 1, c_{pi} = 0 \text{ (adsorption)} \\ c_i = 0, q_i = 1 \text{ (desorption)} \end{cases} \text{ at } \tau = 0 \quad (2.11)$$

$$q_i = \frac{c_{pi}}{\lambda + (1 - \lambda)c_{pi}} \quad (2.12)$$

where $\lambda = 1/(1 + KC_{i0})$ is the dimensionless constant separation factor.

The other dimensionless model parameters are:

$$Bi = \frac{k_f R}{D_{pe}} \quad (\text{Biot number})$$

$$N = \frac{\varepsilon_s (1 + \xi_m) W_A}{V_L \bar{\rho}_p} \quad (\text{Loading ratio}/(1 - r_C^3))$$

The loading ratio is the ratio of the number of moles retained in the adsorbent particle (pores + adsorbed) and the number of moles present initially in the bulk phase. The value of the capacity factor ξ_m is in the range 10^2 to 10^4 . In this paper, we take $\xi_m = 2.5 \times 10^2$ as an example from the literature (Zhang and Sun, 2002). In fact, when $\xi_m \geq 10^2$, the term $(1 + \xi_m dq_i/dc_{pi})/(1 + \xi_m)$ in Eq. (2.8) can be reduced to dq_i/dc_{pi} .

The model equations will be solved by orthogonal collocation, which transforms the system of partial differential equation (2.8) into a system of ordinary differential equations of initial value type. In this chapter, 10 radial collocation points for adsorbent were selected. These equations were integrated in the time domain using Gear's stiff variable step integration routine.

For the two limiting cases of the Langmuir isotherm, there are analytical solutions.

- (a) *Linear (Henry isotherm; $\lambda = 1$):* This situation is valid only for the adsorption kinetics with $KC_{i0} \ll 1$ or for dilute initial adsorbate concentrations leading to $KC_{i0} \ll 1$, Eq. (2.6) becomes $\hat{q}_i = q_m KC_{pi} = K_H C_{pi}$, new solutions will be given in the later part of this chapter;
- (b) *Rectangular adsorption isotherm ($\lambda = 0$):* This situation is valid for adsorption kinetics with $KC_{i0} \gg 1$ or for high initial concentrations of adsorbate leading to $KC_{i0} \gg 1$. Eq. (2.6) becomes $\hat{q}_i = q_m$, Suzuki and Kawazoe (1974) derived the analytical solution, which will be modified for the inert core adsorbent.

2.2.2 Analytical solutions for Henry isotherm

When $\lambda = 1$, Eq. (2.8) can be rearranged as

$$\frac{\partial c_{pi}}{\partial \tau} = \frac{\partial^2 c_{pi}}{\partial r^2} + \frac{2}{r} \frac{\partial c_{pi}}{\partial r} \quad (r_c \leq r \leq 1) \quad (2.13)$$

The Laplace transform can be used to solve Eq. (2.13) combined with Eqs (2.7), (2.9)~(2.11). The results in the Laplace domain are as follows:

$$\bar{c}_{pi} = \begin{cases} \frac{Bi}{r} \Omega_1 & \text{(adsorption)} \\ \frac{1}{p} - \frac{Bi}{r} \Omega_1 & \text{(desorption)} \end{cases} \quad (2.14)$$

where

$$\Omega_1 = \frac{\sin[i\sqrt{p}(r-r_c)] + i\sqrt{p}r_c \cos[i\sqrt{p}(r-r_c)]}{[pBi + (p + 3NBi)(pr_c - 1)]\sin[i\sqrt{p}(1-r_c)] + i\sqrt{p}[pBir_c + (p + 3NBi)(1-r_c)]\cos[i\sqrt{p}(1-r_c)]} \quad (2.15)$$

where the overhead bar indicates the Laplace transform, p is the Laplace transform parameter. Let

$$-i\sqrt{p} = \sqrt{\gamma} \quad (2.16)$$

Then the following analytical solution can be derived by inverse Laplace transform of Eq.

(2.14) based on the residual theorem. The concentration in the adsorbent pore for $r \geq r_C$ is

$$c_{pi} = \begin{cases} c_{i\infty} + \frac{2Bi}{r} \sum_{n=1}^{\infty} \Omega_2 e^{-\gamma_n \tau} & \text{(adsorption)} \\ 1 - c_{i\infty} - \frac{2Bi}{r} \sum_{n=1}^{\infty} \Omega_2 e^{-\gamma_n \tau} & \text{(desorption)} \end{cases} \quad (2.17)$$

where

$$\Omega_2 = \frac{\sin[\sqrt{\gamma_n}(r - r_C)] + \sqrt{\gamma_n} r_C \cos[\sqrt{\gamma_n}(r - r_C)]}{A_n \sin[\sqrt{\gamma_n}(1 - r_C)] + B_n \sqrt{\gamma_n} \cos[\sqrt{\gamma_n}(1 - r_C)]} \quad (2.18)$$

The definition of volume-averaged dimensionless pore concentration $\langle c_{pi} \rangle$ is

$$\langle c_{pi} \rangle = \frac{3}{(1 - r_C^3)} \int_{r_C}^1 c_{pi} r^2 dr. \quad (2.19)$$

Then, one has

$$\langle c_{pi} \rangle = \begin{cases} c_{i\infty} + \frac{6Bi}{(1 - r_C^3)} \sum_{n=1}^{\infty} \Omega_3 e^{-\gamma_n \tau} & \text{(adsorption)} \\ 1 - c_{i\infty} - \frac{6Bi}{(1 - r_C^3)} \sum_{n=1}^{\infty} \Omega_3 e^{-\gamma_n \tau} & \text{(desorption)} \end{cases} \quad (2.20)$$

where

$$\Omega_3 = \left(\frac{1}{\gamma_n} \right) \frac{(1 + \gamma_n r_C) \tan[\sqrt{\gamma_n}(1 - r_C)] - \sqrt{\gamma_n}(1 - r_C)}{A_n \tan[\sqrt{\gamma_n}(1 - r_C)] + B_n \sqrt{\gamma_n}} \quad (2.21)$$

Based on Eq. (2.7), one has the following expression for the dimensionless bulk concentration:

$$c_i = \begin{cases} c_{i\infty} - 6NBi \sum_{n=1}^{\infty} \Omega_3 e^{-\gamma_n \tau} & \text{(adsorption)} \\ 1 - c_{i\infty} + 6NBi \sum_{n=1}^{\infty} \Omega_3 e^{-\gamma_n \tau} & \text{(desorption)} \end{cases} \quad (2.22)$$

where the final equilibrium concentration of adsorbate in the batch adsorber is

$$c_{i\infty} = \frac{1}{1 + (1 - r_C^3)N} \quad (2.22a)$$

The coefficients in Eqs (2.18) to (2.22) can be calculated by

$$A_n = 2(Bi - 1) + (3NBi - \gamma_n) - (Bi + 2)\gamma_n r_C + (Bi\gamma_n + 3NBi - \gamma_n)r_C^2 \quad (2.23)$$

$$B_n = (Bi + 2) + [3NBi - \gamma_n + 2(Bi - 1)]r_C - (3NBi - \gamma_n)r_C^2 \quad (2.24)$$

in which γ_n is the positive roots which is determined by the following transcendental equation that is obtained by letting the denominator of Eq. (2.15) be zero based on Eq. (2.16):

$$\tan[(1 - r_C)\sqrt{\gamma_n}] = \sqrt{\gamma_n} \frac{(1 - r_C)(\gamma_n - 3NBi) + r_C Bi \gamma_n}{(1 + r_C \gamma_n)(\gamma_n - 3NBi) - Bi \gamma_n} \quad (2.25)$$

It should be noted that Chanda and Rempel (1997, 1999) derived the analytical solutions for inert core adsorbent at $Bi \rightarrow \infty$.

2.2.3 Analytical solution for rectangular isotherm

For the pore diffusion model with the rectangular isotherm, Eq. (2.8) is usually modified by the shrinking-core model (Do, 1982). A solution is then given (Suzuki and Kawazoe, 1974; Weber and Chakravoti, 1974) as

$$c_i = \frac{a^3 + r_F^3}{1 + a^3} \quad (r_C \leq r_F \leq 1) \quad (2.26)$$

$$\tau = (1 + a^3) \left\{ \frac{1}{3} \left(1 - \frac{1}{Bi} \right) \ln \left(\frac{r_F^3 + a^3}{1 + a^3} \right) + \frac{1}{6a} \ln \left[\frac{(1 - a + a^2)(r_F + a)^2}{(r_F^2 - ar_F + a^2)(1 + a)} \right] \right. \\ \left. - \frac{1}{a\sqrt{3}} \left[\tan^{-1} \left(\frac{2r_F - a}{a\sqrt{3}} \right) - \tan^{-1} \left(\frac{2 - a}{a\sqrt{3}} \right) \right] \right\} \quad (r_C \leq r_F \leq 1) \quad (2.27)$$

where

$$a = \left(\frac{1}{N} - 1 \right)^{1/3} \quad (2.28)$$

in which $r_F = R_F/R_p$ is the position of the shrinking front, for rectangular isotherm

$$\tau = D_{pe} t / R_p^2 \varepsilon_S \xi_m, \quad N = W_A \varepsilon_S \xi_m / \bar{\rho}_p V_L, \quad \text{and} \quad \xi_m = \rho_S \hat{q}(C_{i0}) / C_{i0} \varepsilon_S.$$

2.3. Results and discussion

Adsorption isotherms of Langmuir type are shown in Fig. 2.2, where $\lambda = 1$ for Henry isotherm and $\lambda = 0$ for rectangular isotherm (irreversible adsorption). When $\lambda \leq 0.01$, the adsorption isotherm approaches rectangular isotherm, a high favorable adsorption isotherm.

The equilibrium constants, q_m and K are usually obtained by separate experiments in a batch adsorber (Hunter and Carta, 2000; Tong and Sun, 2001). The equilibrium adsorption isotherm can be measured from various experiments in which the initial adsorbate concentration or the adsorbent mass is changed. The final equilibrium concentration $c_{i\infty}$ is measured and the adsorbed amount in equilibrium $q_{i\infty}$ is calculated from material balance.

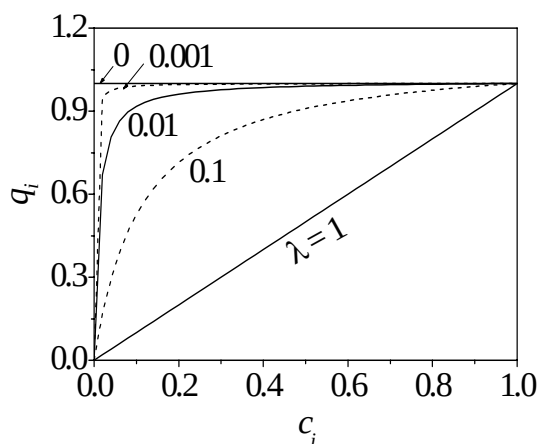


Fig. 2.2. Langmuir isotherm ($0 \leq \lambda \leq 1$). $\lambda = 1$: Henry isotherm; $\lambda = 0$: rectangular isotherm.

Table 2.1. Langmuir equilibrium parameters for BSA adsorption on CB-6AS inert core adsorbent (Zhang and Sun, 2002)

NaCl $\text{kmol} \cdot \text{m}^{-3}$	q_m $\text{kg} \cdot \text{m}^{-3}$	K $\text{m}^{-3} \cdot \text{kg}$	λ at $C_0 = 0.1 \text{ kg} \cdot \text{m}^{-3}$	λ at $C_0 = 1.0 \text{ kg} \cdot \text{m}^{-3}$	λ at $C_0 = 2.0 \text{ kg} \cdot \text{m}^{-3}$
0.05	14.8	8.475	0.541	0.106	0.056
0.10	34.8	7.813	0.561	0.113	0.061
0.20	57.0	5.102	0.662	0.164	0.089
0.30	41.0	27.778	0.265	0.035	0.018
0.50	38.3	0.877	0.919	0.533	0.363

Table 1 reports constant separation factors, λ , for BSA adsorption equilibrium on CB-6AS inert core adsorbent at various initial concentrations, C_0 , based on literature data (Zhang and Sun, 2002). The matrix of CB-6AS adsorbent is a dense pellicular composite matrix prepared by entrapping the steel sphere with agarose gel with the radius of particle

$R_p = 69.05\mu\text{m}$ and the radius of inert core $R_c = 35.6\mu\text{m}$. As shown in Table 2.1, there are situations corresponding to the limiting cases of linear and rectangular isotherms.

2.3.1. Effect of the model parameters on the time evolution of bulk concentration and uptake radial profiles

Due to the inert core included in the adsorbent (for example, $r_c = 0.6$ and 0.8), the total amount of adsorbed protein will decrease for the same value of N when compared with the adsorbed amount in the adsorbent without the inert core ($r_c = 0$). This is shown in Fig.2.3 where the dimensionless bulk concentration c_i is plotted as a function of $\sqrt{\tau}$ for various Biot numbers ($Bi = 1, 10, 100$) at constant N ($N = 2$ for Henry and Langmuir isotherms and $N = 0.9$ for rectangular isotherm).

For Henry and rectangular isotherms, the dimensionless bulk concentration changes with time are, respectively, calculated by the analytical solutions as shown in Figs 2.3a (Henry isotherm; Eq. (2.22)) and 2.3c (rectangular isotherm; Eq. (2.26)); while for Langmuir isotherm with $\lambda = 0.1$, the orthogonal collocation method was used to calculate the dimensionless bulk concentration changes with time as shown in Fig. 2.3b. All results demonstrate that r_c influences the dimensionless bulk concentration changes with time; the final equilibrium concentration $c_{i\infty}$ in the bulk phase will be determined by the following relationships:

- a) $1/[1 + (1 - r_c^3)N]$ for Henry isotherm;
- b) $[1 - (1 - r_c^3)Nq(c_{i\infty})]$ for Langmuir type isotherm and
- c) $[1 - (1 - r_c^3)N]$ for rectangular isotherm.

The adsorption capacity of the adsorbent (kg protein/kg adsorbent) with high value of r_c will be lower than in the case of adsorbents without inert core, and therefore the dimensionless bulk concentration at equilibrium $c_{i\infty}$ will be higher.

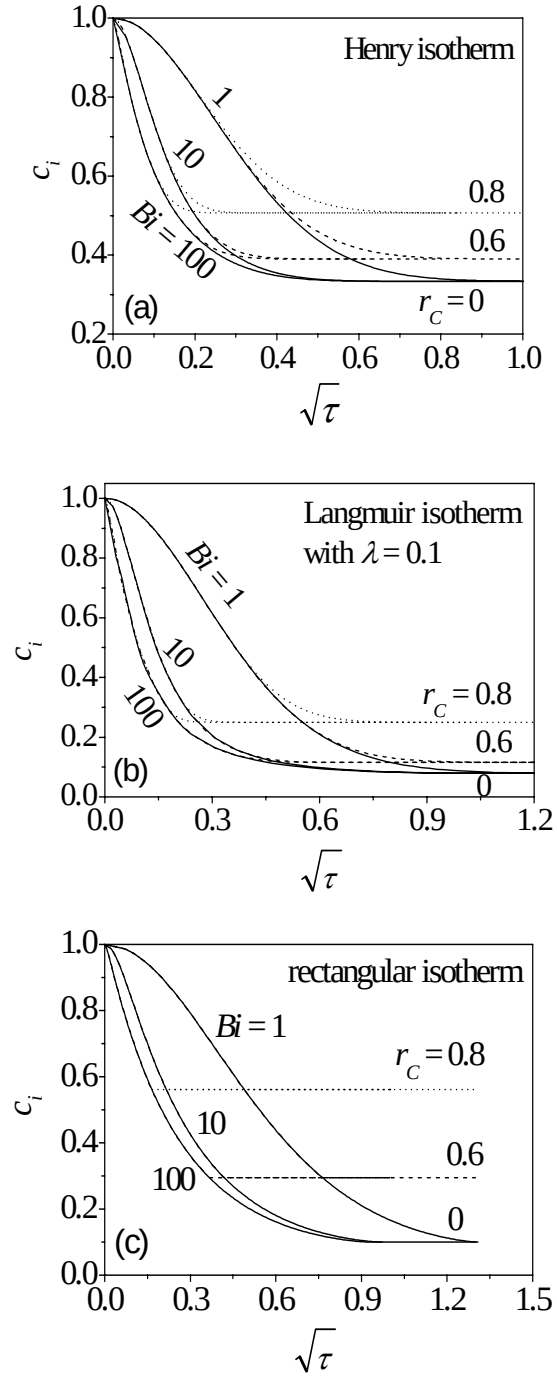


Fig. 2.3. Effect of r_c on dimensionless bulk concentrations c_i as a function of reduced time $\sqrt{\tau}$ at different Bi (1, 10 and 100) for constant value of N : (a) Henry isotherm for $N = 2$; (b) Langmuir isotherm with $\lambda = 0.1$ for $N = 2$; (c) rectangular isotherm for $N = 0.9$.

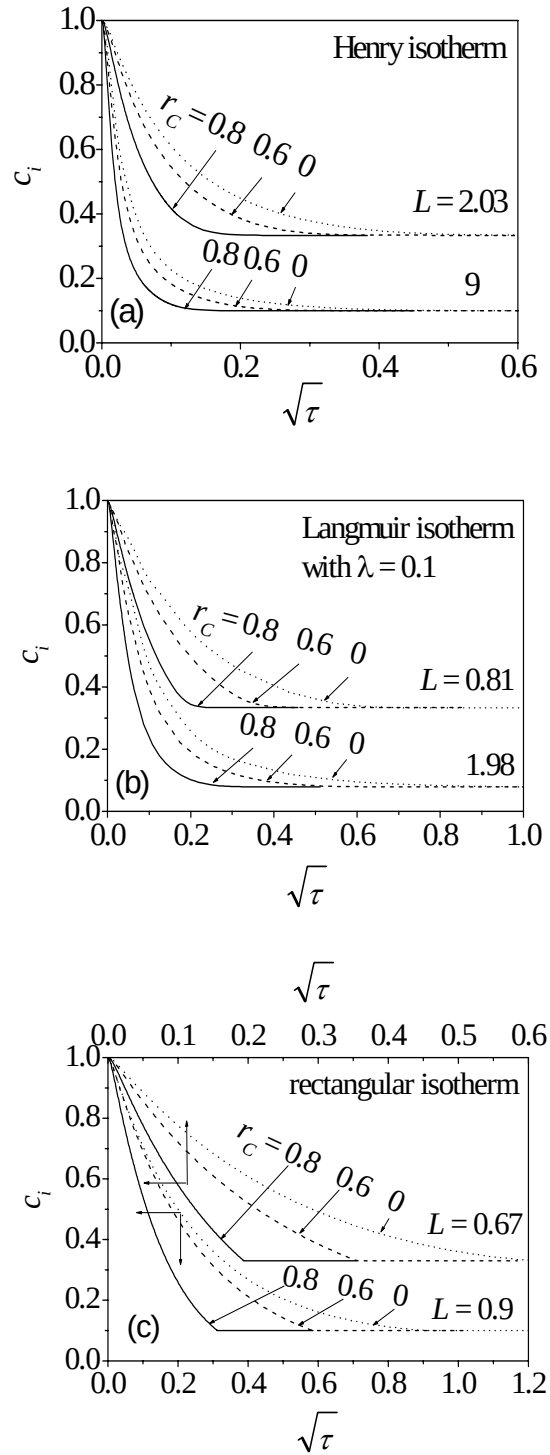


Fig. 2.4. Effect of r_c on dimensionless bulk concentrations c_i as a function of reduced time $\sqrt{\tau}$ at constant loading ratio $L = (1 - r_c^3)N$ for $Bi = 100$: (a) Henry isotherm for $L = 2.03$ and 9 ; (b) Langmuir isotherm with $\lambda = 0.1$ for $L = 0.81$ and 1.98 ; (c) rectangular isotherm for $L = 0.67$ and 0.9 .

Fig. 2.4 shows the effect of r_c on the dimensionless bulk concentration changes with time at constant loading ratio $L = (1 - r_c^3)N$ (moles in the adsorbent/moles in bulk fluid) for Henry isotherm ($\lambda = 1$; Fig. 2.4a), Langmuir isotherm ($\lambda = 0.1$; Fig. 2.4b) and rectangular isotherm ($\lambda = 0$; Fig. 2.4c). When the loading ratio $L = (1 - r_c^3)N$ is kept constant (comparing adsorbents with different core radius and different adsorbent shell capacity but with the same loading ratio), the dimensionless bulk concentrations approach the same equilibrium value, but the time to approach equilibrium is evidently shortened for higher value of r_c . Higher value of r_c means shorter diffusion path within the adsorbent, resulting in lower intraparticle diffusion resistance. Due to an inert core included in the adsorbent, the adsorbed amount in the shell layer is increased by the factor $(1 - r_c^3)$, but the time to reach the same equilibrium state is only about one third for $r_c = 0.6$ and one tenth for $r_c = 0.8$ compared with the case of $r_c = 0$. In fact, it is very difficult for protein to diffuse into the inner part of the adsorbent. For example, with $120\mu\text{m}$ SP Sepharose FF adsorbent, it takes 6h for diffusion of BSA into the inner region of the adsorbent (Linden et al., 2002). Therefore, thin shell adsorbent will effectively decrease the diffusion resistance, and shorten the operating time.

Uptake radial profiles in the adsorbent shell are shown in Fig. 2.5 with Henry isotherm ($\lambda = 1$; Fig. 2.5a), Langmuir type ($\lambda = 0.1$; Fig. 2.5b) and rectangular isotherm ($\lambda = 0$; Fig. 2.5c). The pattern of the uptake radial profiles in the adsorbent shell is related with the dimensionless constant separation factor λ . The parameter λ is influenced by both the adsorption equilibrium constant K and the initial bulk concentration of the protein C_{i0} . When $\lambda = 1$ (Henry isotherm), the shell of adsorbent will fill up with adsorbate molecules from the particle surface to the inner part of adsorbent, and the uptake profiles change gradually (Fig. 2.5a); for lower values of λ , the pattern of the uptake profiles becomes sharper (Fig. 2.5b). Finally, when λ approaches zero (rectangular isotherm), the pattern of the uptake profiles is consistent with the shrinking core model (Fig. 2.5c).

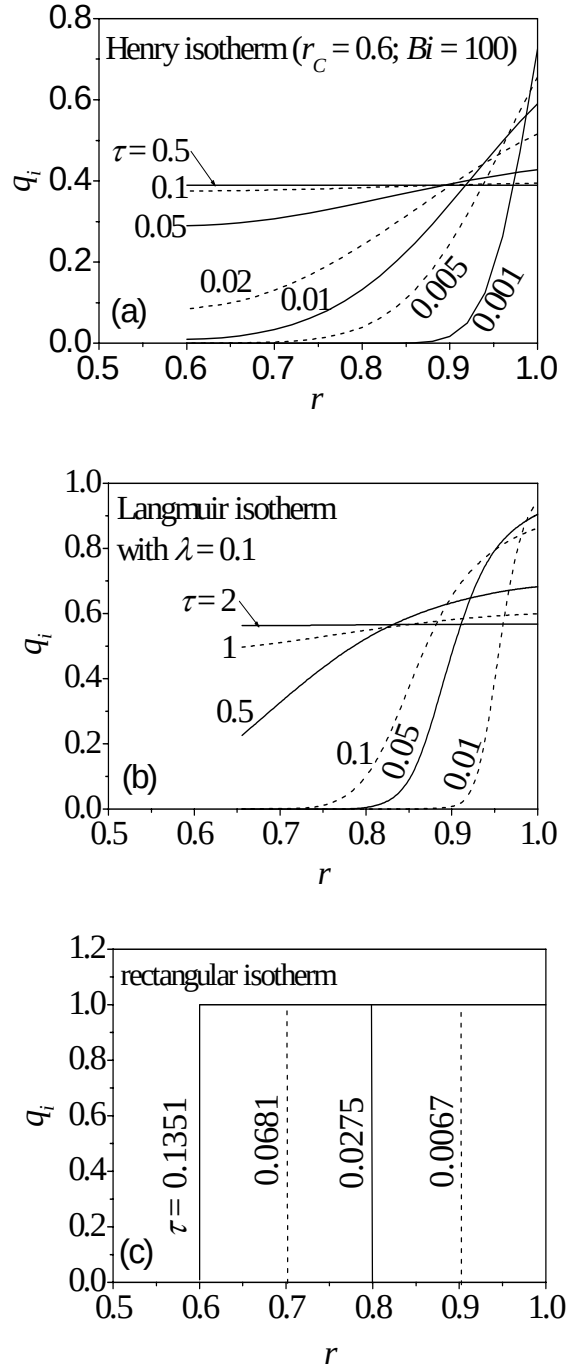


Fig. 2.5. Uptake radial profiles within the adsorbent shell at various times for $Bi = 100$ and $r_c = 0.6$: (a) Henry isotherm for $N = 2$; (b) Langmuir isotherm with $\lambda = 0.1$ for $N = 2$; (c) rectangular isotherm for $N = 0.9$.

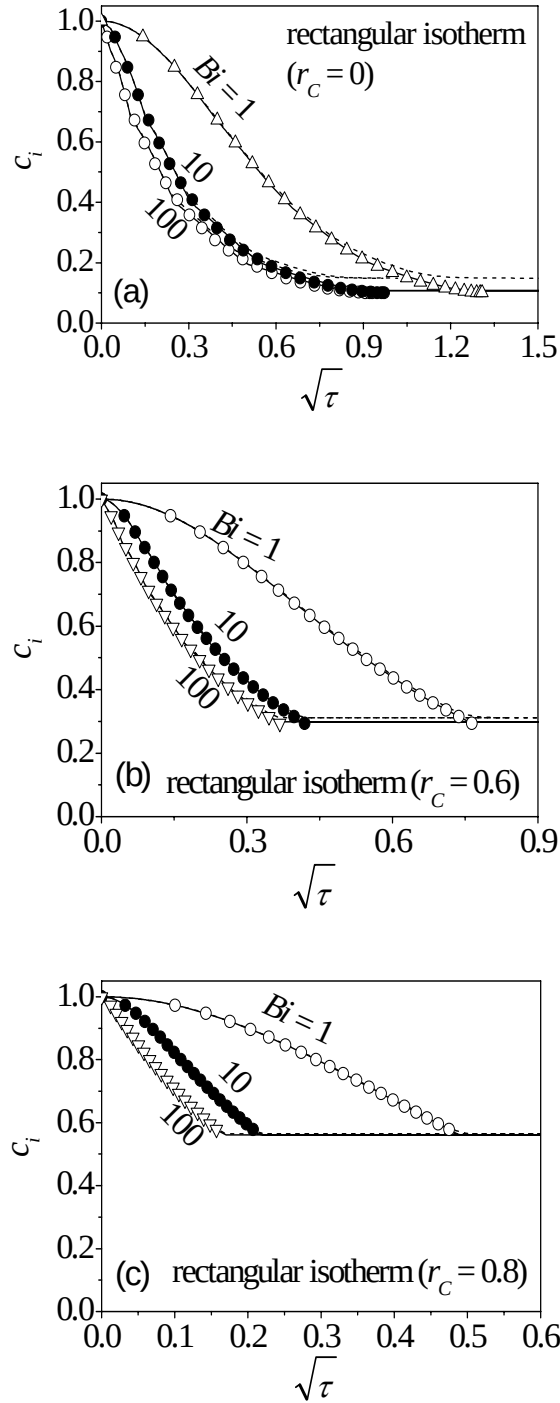


Fig. 2.6. Comparison of analytical solution of c_i versus $\sqrt{\tau}$ for rectangular isotherm and numerical solution for Langmuir isotherm with small values of λ at different Bi for $N = 0.9$: (a) $r_c = 0$; (b) $r_c = 0.6$; (c) $r_c = 0.8$. Points: analytical solution (Eq. (2.26)); dashed lines: numerical solution with $\lambda = 0.01$; solid lines: numerical solution with $\lambda = 0.001$.

When the protein concentration in the crude feedstock is very low, the adsorption isotherm can be in some cases in the linear region if the adsorption mechanism is reversible; in this case, the analytical solution (Eq. (2.22)) can be used to model and optimize the performance of the adsorption of proteins in a batch adsorber. When the adsorbent has high affinity to the adsorbate, the adsorption equilibrium is close to the rectangular isotherm. However, by comparing the analytical solution (Eq. (2.26)) with rectangular isotherm and the numerical solution with Langmuir type for low values of λ (for example $\lambda = 0.01$ and $\lambda = 0.001$), we found that for equilibrium adsorption isotherm with $\lambda < 0.01$, the adsorption kinetics in batch systems can be modeled and optimized with the analytical solution (Eq. (26)), as shown in Fig. 2.6 where $r_c = 0$ (Fig. 2.6a), $r_c = 0.6$ (Fig. 2.6b), and $r_c = 0.8$ (Fig. 2.6c). In addition, the accuracy of the numerical solution is verified very well by comparing the numerical solution with analytical solution (Eq. (2.22)) under the Henry isotherm, as shown in Fig. 2.7.

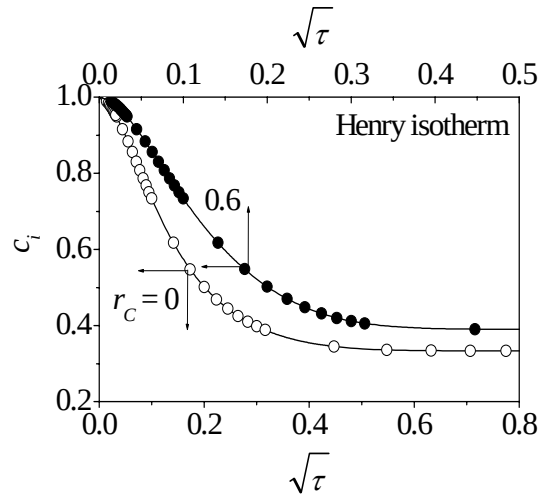


Fig. 2.7. Verification of the numerical solution by comparison with analytical solution for Henry isotherm ($Bi = 10$ and $N = 2$). Solid lines: numerical solution; circle points: analytical solution (Eq. (2.22)).

2.3.2. Method for estimation of effective pore diffusivity of protein and film mass transfer coefficient from the bulk concentration profile

In this chapter a method is presented to determine both values of k_f and D_{pe} from

model dimensionless time τ versus real time t from experiment corresponding to the same level of bulk concentration. The experimental data are selected from literature (Zhang and Sun, 2002) concerning BSA adsorption on CB-6AS inert core adsorbent at $0.3 \text{ kmol}\cdot\text{m}^{-3}$ NaCl concentration. The dimensionless model parameters for the experimental system are $\lambda = 0.035$, $r_C = 0.516$, $\xi_m = 232.8$ and $N = 0.795$.

Fig. 2.8a shows the experimental data of the bulk concentration profile of BSA as a function of time; the simulation results of the bulk concentration profile of BSA at the same experimental conditions for various Bi are shown in Fig. 2.8b. Then at each $c_{\text{exp}} = c_{\text{cal}}$ value, we read the corresponding values of real time t and model dimensionless time τ for various Bi , and plot the $\tau \sim t$ curves for various Bi as shown in Fig. 2.8c. Since the other model parameters, λ , r_C , ξ_m and N , are the same for the experimental data and simulation results, the correct Bi value is that which leads to a straight-line $\tau \sim t$, with slope $D_{pe}/[(1+\xi_m)R_p^2\epsilon_s]$. The effective pore diffusivity D_{pe} can be estimated based on the above slope and then the film mass transfer coefficient k_f can be calculated from the correct Bi . Fig. 2.8c shows that when film mass transfer is negligible, that is $Bi \geq 100$ ($Bi = 492.8$ in Zhang and Sun, 2002), a straight-line $\tau \sim t$ is obtained with slope $1.161 \times 10^{-5} \text{ s}^{-1}$, and the estimated effective pore diffusivity is $D_{pe} = 2.20 \times 10^{-12} \text{ m}^2 \cdot \text{s}^{-1}$. With known D_{pe} and $Bi = 492.8$, the film mass transfer coefficient is calculated as $k_f = 1.57 \times 10^{-5} \text{ m}^2 \cdot \text{s}^{-1}$. With the estimated D_{pe} and k_f values, the simulation results (solid line in Fig. 2.8a) are in very good agreement with the experimental data. In this case the effect of the film mass transfer resistance can be neglected. From Fig. 2.8a, it is also obvious that the diffusion rate of protein BSA in CB-6AS adsorbent shell is very slow; it takes about 4 hours to reach the final equilibrium concentration.

Fig. 2.9 shows cases where film mass transfer resistance is important. In Fig. 2.9a, the experimental data are shown for the case where $\lambda = 0.035$, $r_C = 0.516$, $\xi_m = 232.8$ and $N = 0.795$. Combining Fig. 2.9a and model results from Fig. 2.9b, a straight-line $\tau \sim t$ is

only obtained if $Bi = 10$. The slope of that line is $1.161 \times 10^{-5} \text{ s}^{-1}$, and the effective pore diffusivity is estimated as $D_{pe} = 2.20 \times 10^{-12} \text{ m}^2 \cdot \text{s}^{-1}$; the film mass transfer coefficient is then $k_f = 3.186 \times 10^{-7} \text{ m}^2 \cdot \text{s}^{-1}$. The simulation results (solid line in Fig. 2.9a) with $D_{pe} = 2.20 \times 10^{-12} \text{ m}^2 \cdot \text{s}^{-1}$ and $k_f = 3.186 \times 10^{-7} \text{ m}^2 \cdot \text{s}^{-1}$ describe very well the experimental data.

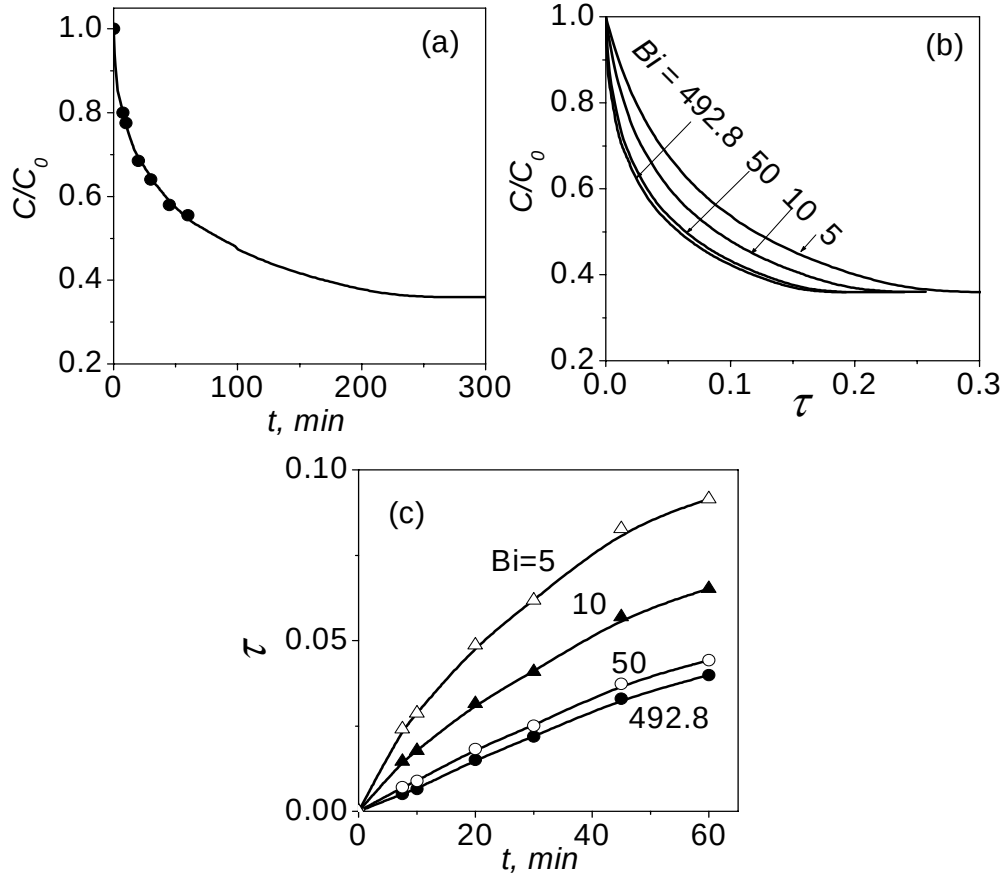


Fig. 2.8. Estimation of effective pore diffusivity, D_{pe} , at high Bi value for Langmuir isotherm ($\lambda = 0.035$). (a) Experimental data of the bulk concentration c_i as a function of time for BSA adsorption on CB-6AS adsorbent (data from Zhang and Sun, 2002) at $\lambda = 0.035$, $r_C = 0.516$, $\xi_m = 232.8$ and $N = 0.795$. Points: experimental data, solid line: simulation results with $k_f = 1.57 \times 10^{-5} \text{ m}^2 \cdot \text{s}^{-1}$ and $D_{pe} = 2.20 \times 10^{-12} \text{ m}^2 \cdot \text{s}^{-1}$. (b) Model results of the bulk concentration profiles concentrations c_i as a function of dimensionless time τ for various Bi values at $\lambda = 0.035$, $r_C = 0.516$, $\xi_m = 232.8$ and $N = 0.795$. (c) $\tau \sim t$ curves for various Bi obtained by reading the corresponding τ and t values at each $c_{\text{exp}} = c_{\text{cal}}$ point.

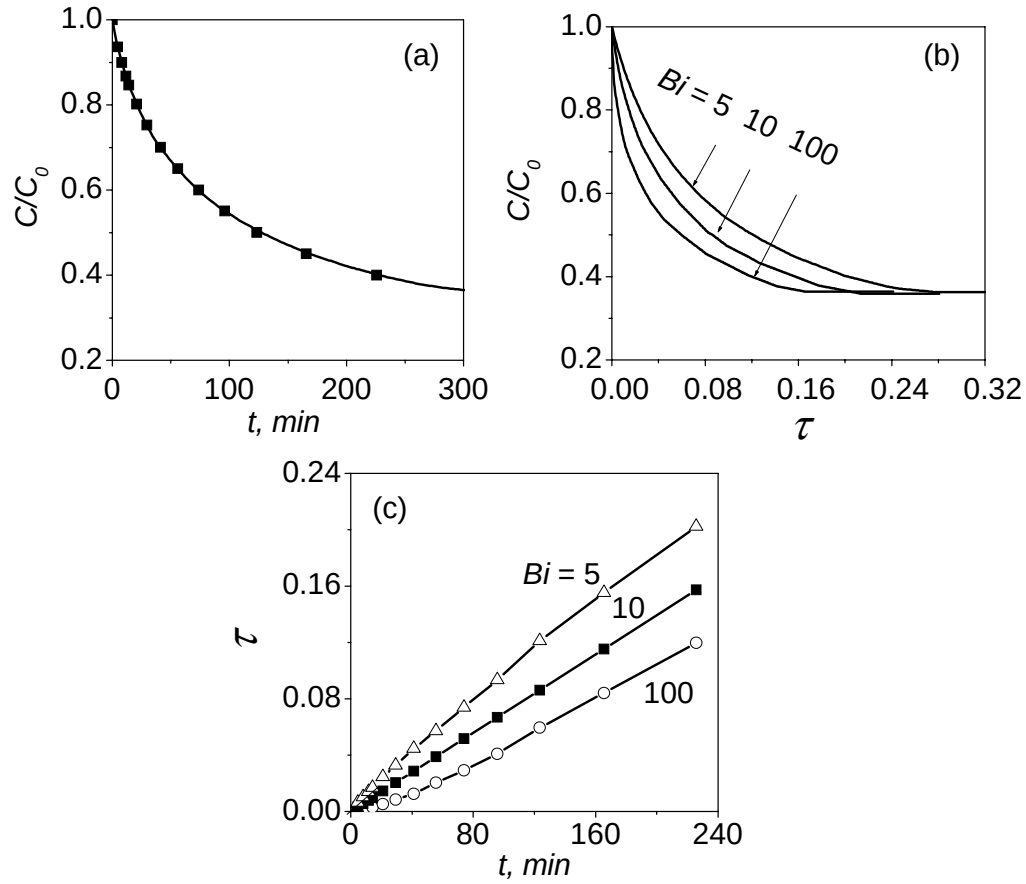


Fig. 2.9. Estimation of effective pore diffusivity, D_{pe} , at small Bi value for Langmuir isotherm ($\lambda = 0.035$). (a) Bulk concentration c_i as a function of time for BSA adsorption on CB-6AS adsorbent at $\lambda = 0.035$, $r_C = 0.516$, $\xi_m = 232.8$ and $N = 0.795$. Points: experimental data; solid line: simulation results with $k_f = 3.186 \times 10^{-7} \text{ m}^2 \cdot \text{s}^{-1}$ and $D_{pe} = 2.20 \times 10^{-12} \text{ m}^2 \cdot \text{s}^{-1}$. (b) Model results of the bulk concentration profiles concentrations c_i as a function of dimensionless time τ for various Bi values at $\lambda = 0.035$, $r_C = 0.516$, $\xi_m = 232.8$ and $N = 0.795$. (c) $\tau \sim t$ curves for various Bi obtained by reading the corresponding τ and t values at each $c_{\text{exp}} = c_{\text{cal}}$ point.

2.4 Conclusions

For inert core adsorbents with the same type of shell layer and different inert core radii, the adsorbent capacity is decreased by the factor $(1 - r_C^3)$. The dimensionless equilibrium concentration $c_{i\infty}$ in the bulk phase in a batch adsorber will increase with r_C ; the

corresponding values of $c_{i\infty}$ are $1/[1 + (1 - r_C^3)N]$ for Henry isotherm, $[1 - (1 - r_C^3)Nq(c_{i\infty})]$ for Langmuir type, and $[1 - (1 - r_C^3)N]$ for rectangular isotherm. When the loading ratio $(1 - r_C^3)N$ (number of moles in the adsorbent/number of moles in the bulk liquid) is constant, the dimensionless bulk concentrations approach the same equilibrium value, but the time to approach equilibrium is evidently shortened for the adsorbent with higher value of the inert core radius r_C . Higher r_C means shorter diffusion path within the adsorbent, resulting in lower intraparticle diffusion resistance.

The pattern of the uptake profiles in the shell of the adsorbent strongly depends on the dimensionless constant separation factor λ . When λ approaches unit, the uptake profile changes gradually from the particle surface to the inner region; when λ decreases, the pattern of the uptake profile becomes sharper, and when λ approaches zero, the uptake profile pattern is consistent with the shrinking core model.

For high favorable equilibrium isotherm ($\lambda < 0.01$), the adsorption kinetics can be described by the analytical solution for rectangular isotherm.

The effective pore diffusivity, D_{pe} , and external film mass transfer coefficient, k_f , both can be estimated simultaneously from $\tau \sim t$ curve which was obtained by comparing the experimental data of the bulk concentration-time profile and the simulation results of pore diffusion model at same experimental conditions (λ , r_C , ξ_m and N).

Notation

Bi	Biot number $(= k_f R / D_{pe})$
c_i	dimensionless adsorbate concentration in bulk phase
c_{pi}	dimensionless adsorbate concentration in adsorbent pore
C_i	adsorbate concentration in bulk phase, $\text{kg} \cdot \text{m}^{-3}$
C_{i0}	initial adsorbate concentration, $\text{kg} \cdot \text{m}^{-3}$

C_{pi}	adsorbate concentration in adsorbent pore, $\text{kg}\cdot\text{m}^{-3}$
D_{pe}	effective pore diffusivity, $\text{m}^2\cdot\text{s}^{-1}$
K	equilibrium constant, $\text{m}^3\cdot\text{kg}^{-1}$
K_H	Henry equilibrium constant, $\text{m}^3\cdot\text{kg}^{-1}$
k_f	film mass transfer coefficient, $\text{m}\cdot\text{s}^{-1}$
L	Loading ratio (number of moles in adsorbent/number of moles in bulk liquid)
N	Loading ratio $/(1 - r_C^3)$
p	Laplace transform parameter
q_i	dimensionless adsorbed concentration in adsorbent shell
q_m	equilibrium constant, $\text{kg}\cdot\text{kg}^{-1}$
$\hat{q}_i$	adsorbed concentration in adsorbent shell, $\text{kg}\cdot\text{kg}^{-1}$
r	dimensionless radial distance
r_C	dimensionless radius of inert core of adsorbent
r_F	dimensionless radial position of the adsorption front in the particle
R	radial distance in adsorbent, m
R_C	radius of core of adsorbent, m
R_F	radial position of the adsorption front in the particle
R_p	radius of adsorbent, m
t	time, s
V_L	volume of bulk phase, m^3
W_A	mass of adsorbent, kg

Greek letters

γ_n	roots of transcendental Eq. (2.25).
ε_s	porosity of adsorbent shell
λ	dimensionless constant separation factor $[= 1/(1 + KC_{i0})]$
ξ_m	capacity factor $[= \rho_s \hat{q}(C_{i0})/(\varepsilon_s C_{i0})]$
ρ_C	density of adsorbent core, $\text{kg}\cdot\text{m}^{-3}$
$\bar{\rho}_p$	apparent density of adsorbent particle, $\text{kg}\cdot\text{m}^{-3}$
ρ_s	density of adsorbent shell, $\text{kg}\cdot\text{m}^{-3}$
τ	dimensionless contact time

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3. Modeling breakthrough and elution curves in fixed bed of inert core adsorbents: Analytical and approximate solutions ^{*}

A mathematical model is developed for fixed-beds packed with inert core adsorbents. New analytical solutions to predict breakthrough and elution curves are derived for linear adsorption systems coupled with axial dispersion, film mass transfer and intraparticle mass transfer. New approximate solutions are also obtained based on the assumptions of parabolic concentration profile in the adsorbent shell and the quasi-lognormal distribution for the impulse response in order to predict breakthrough and elution curves. The applicability of these approximate solutions is suggested by comparison with the new analytical solutions. The effects of the size of inert core, sample input mode, axial dispersion, film mass transfer resistance and intraparticle diffusion resistance, on the breakthrough and elution curves are discussed. The decrease of the intraparticle mass transfer resistance by using inert core adsorbents is quantitatively analyzed by introducing the parameter $1/\Theta$. Furthermore, an analytical expression for the resolution of two components is derived based on the quasi-lognormal distribution approximate solution; the resolution of two components is improved with the inert core adsorbent when compared with the conventional adsorbent, especially for biomacromolecules where the intraparticle diffusion rate is slow.

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3.1. Introduction

Inert core adsorbents have been recently used in expanded bed to capture bio-macromolecules directly from crude particle-containing feedstock (Griffith et al., 1997; Voute et al., 1999; Pai, Gondkar, and Lali, 2000; Palsson, Gustavsson, and Larsson, 2000; Tong and Sun, 2001; Jahanshahi et al., 2002; Theodossiou et al., 2002). These inert core adsorbents have increased density by the incorporation of heavier inert core and are adequate for stable expansion at high flow rates in expanded bed. For example, UpFront's FastLine adsorbents (UpFront Chromatography A/S, Denmark) are based on highly cross-linked agarose beads with a central core of high density, e.g. glass or stainless steel; and ZSA pellicular adsorbent matrix (Jahanshahi et al., 2002) is a pellicular particle comprising a solid core (zirconia-silica) coated with a porous skin of 4% (w/v) cross-linked agarose. Meanwhile, when operating at high flow rate in expanded bed, the slow diffusion rate of protein into the pore of the adsorbent will reduce the column efficiency, and cause early breakthrough. By adopting these dense inert core adsorbents, the protein diffusion resistance inside adsorbent is effectively reduced due to shortening of the diffusion path, resulting in enhanced column efficiency to ensure fast, high performance capturing proteins from the particulate containing feedstock by expanded bed.

In fact, intraparticle diffusion resistance for biological macromolecules is often quite large for commercial available adsorbent particles (Bruce and Chase, 2001; Hunter and Carta, 2000; Lewus and Carta, 1999; Linden et al., 2002; Hubbuch et al., 2002). Several improved approaches have been developed to reduce the effect of slow diffusion, namely the use of large-pore supports in chromatographic processes (Rodrigues, 1997), and non-porous beads to eliminate mass transfer resistance within the pore completely (Lee, 1997). It should be emphasized that Kirkland and co-workers (Kirkland, 1992; Kirkland et al., 2000) developed "Poroshell" adsorbent. Poroshell 300SB-C18 (from Agilent technologies Inc.) is a 5 μm particle with a thin layer of porous silica on a solid inert core, which has been used successfully for fast, high-performance resolution proteins in liquid-chromatography. Although the loading capacity of the columns packed with inert core adsorbents is relative low, the ability to quickly recover proteins with high biological activity overcomes this shortcoming; the latter is very important for the production of proteins in the pharmaceutical and biotechnology industry.

Mathematical models are required to predict breakthrough and elution curves in adsorption columns for optimizing the design and operating conditions. Considerable attention has been paid to the kinetics of mass transfer in chromatographic columns under linear conditions, because many analytical applications of chromatography are carried out under conditions where the sample size is small, the concentration is low, and thus, the equilibrium isotherm is linear, and the study of the kinetic contributions to band broadening in nonlinear chromatography is simplified by the application of the results obtained in linear chromatography (Cen and Yang, 1986; Haynes, 1975; Rasmuson and Neretnieks, 1980; Rodrigues et al., 1991; Rodrigues et al., 1992; Rosen, 1952; Xiu and Li, 1999; Li, Xiu and Rodrigues, 2003a). Recently, the process in which a target protein is directly captured from the crude feedstock, such as bacteria, yeast, mammalian cell culture, became important to the biotechnological and pharmaceutical industry. In this practical separation process of proteins, linear adsorption equilibrium occurs for very low protein concentration in the diluted crude feedstock.

For conventional adsorbents without inert core, there have been many attempts to find analytical solutions for breakthrough and elution curves for linear adsorption isotherm (Rosen, 1952; Rasmuson and Neretnieks, 1980; Haynes, 1975; Xiu, 1996; Xiu and Li, 1999); however, those solutions are not valid to predict breakthrough and elution curves for inert core adsorbent systems. Shams (2001) modeled the transient behavior of adsorption/desorption from a fixed-bed packed with thin-film-coated spherical particles/hollow spheres when axial dispersion and film mass-transfer resistance are negligible.

Our first objective is to derive new analytical solutions to predict breakthrough and elution curves for fixed-bed columns packed with inert core adsorbents where the axial dispersion, film mass transfer and intraparticle diffusion all are considered.

The mathematical models become more complex when axial dispersion is coupled with film mass transfer resistance and intraparticle diffusion resistance; analytical solutions can be obtained for linear adsorption isotherms, but they are often expressed in terms of slowly convergent series, or contain transcendental functions that have to be evaluated numerically via proper subroutines. Generally, two methods can be used to simplify the mathematical model

for fixed-bed adsorber: (i) one is to assume the adsorbate concentration profile in the adsorbent in advance (Liaw et al., 1979; Rice, 1982; Cen and Yang, 1986; Do and Rice, 1986; Xiu et al., 1997) and (ii) the other is to simulate the impulse response, and get the breakthrough curve using the convolution theorem (Wiedemann et al., 1978; Linek and Dudukovic, 1982; Wang and Lin, 1986; Xiu and Li, 1999).

The second objective of this work is to derive the approximate solutions for inert core adsorbent system to conveniently predict the breakthrough and elution curves.

Usually, the column efficiency is expressed by the theoretical plate number and the height equivalent to a theoretical plate (HETP), which is obtained from the first absolute moment and the second central moment of the impulse response for linear systems (Suzuki and Smith, 1971; Ruthven, 1984). From the analytical expression of *HETP*, the contribution of axial dispersion, intraparticle diffusion resistance and film mass transfer resistance can be evaluated separately. A parameter, $1/\Theta$, is introduced to quantitatively evaluate the decrease of the intraparticle diffusion resistance by using inert core adsorbents. As a result of lower intraparticle diffusion resistance, the elution curves are sharper and narrower, which will favor the resolution in the chromatographic separation of two components in columns packed with inert core adsorbent. Based on the quasi-lognormal distribution approximate solution for elution curves, an analytical expression for the resolution is derived for linear chromatography in packed columns with inert core adsorbents where axial dispersion, film mass transfer and intraparticle diffusion are considered.

3.2. Mathematical model and analytical and approximate solutions

Here we consider an isothermal adsorption column packed with inert core particles shown in Fig. 3.1. At time zero, a step change or impulse change in the concentration of an adsorbate was introduced in a flowing stream. The adsorption column was subjected to axial dispersion, film mass transfer resistance and intraparticle diffusion resistance. The following assumptions are presumed in the analysis:

1. Fick's Law governs axial dispersion in the bulk fluid phase within the column,
2. The adsorption isotherm is linear,

3. Fick's Law of diffusion governs transport within the adsorbent shell,
4. The axial fluid velocity in the column is constant,
5. The adsorbent consists of a core of uniform thickness on a spherical particle that is inert and impenetrable to solution, and the outer layer undergoes negligible swelling or shrinking during sorption (Chanda and Rempel, 1999).

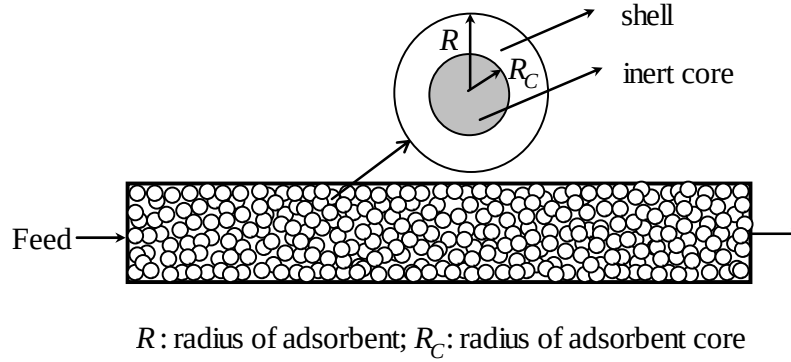


Fig. 3.1. Scheme of fixed-bed adsorber and inert core adsorbent.

Based on the above assumptions, the fixed-bed adsorber can be described by the following set of equations. The material balance equation for the adsorbate in a volume element of the bed is:

$$D_L \frac{\partial^2 C}{\partial Z^2} - \frac{u}{\varepsilon_B} \frac{\partial C}{\partial Z} - \frac{\partial C}{\partial t} - \frac{(1 - \varepsilon_B)(R^3 - R_C^3)}{R^3} \rho_s \frac{\partial q_{av}}{\partial t} = 0 \quad (3.1)$$

where D_L is the axial dispersion coefficient, C is the concentration in the fluid phase, u denotes superficial velocity, q_{av} is the volume-averaged adsorbed concentration over the adsorbent shell, ε_B denotes the bed porosity, hence $(1 - \varepsilon_B)$ denotes the fractional volume taken up by the solid phase, Z is the axial distance from column entrance. $(R^3 - R_C^3)/R^3$ denotes the fractional volume taken up by the shell for inert core adsorbent, in which R_C is the

radius of inert core, and R is the radius of the adsorbent, ρ_s is the density of adsorbent shell.

The mass balance in the adsorbent shell is

$$\frac{\partial q}{\partial t} = D_s \left(\frac{\partial^2 q}{\partial r^2} + \frac{2}{r} \frac{\partial q}{\partial r} \right) \quad (R_c \leq r \leq R) \quad (3.2)$$

where q is the adsorbed concentration in adsorbent shell, D_s is the surface diffusivity, r is the radial distance in adsorbent.

The initial and boundary conditions for Eqs (3.1) and (3.2) are

$$C(Z, 0) = 0 \quad (3.3)$$

$$C(0, t = 0) = C^0 t_c \delta(t) \quad (\text{Dirac input}) \quad (3.4a)$$

where the reference concentration is the ratio between the amount injected and the fluid volume in the column, $C^0 = n / \varepsilon_B V$ and $t_c = \varepsilon_B L / u$ is the space time.

$$C(0, t) = C_0 \quad (\text{step input}) \quad (3.4b)$$

$$C(\infty, t) \text{ is limited} \quad (3.5)$$

$$q(r, Z, 0) = 0 \quad (3.6)$$

$$\left(\frac{\partial q}{\partial r} \right)_{r=R_c} = 0 \quad (3.7)$$

$$\frac{(R^3 - R_c^3)}{R^3} \rho_s \frac{\partial q_{av}}{\partial t} = \frac{3}{R} k_f \left(C - \frac{q_s}{K} \right) \quad (3.8)$$

where the adsorption equilibrium isotherm is:

$$q_s(Z, t) = q(R, Z, t) = K C_s \quad (3.9)$$

and the average adsorbed concentration is:

$$q_{av} = \frac{3}{(R^3 - R_c^3)} \int_{R_c}^R q(r, Z, t) r^2 dr \quad (3.10)$$

Introducing dimensionless variables for the adsorbed concentration and bulk phase concentration, $x = \frac{q}{K C_0}$ and $y = \frac{C}{C_0}$ for step input, and $y = \frac{C}{C^0 \tau_c}$ for Dirac input

($\tau_c = \frac{t_c D_s}{R^2}$), respectively, axial bed coordinate, $\zeta = \frac{Z}{L}$, radial particle coordinate, $\xi = \frac{r}{R}$ and

time, $\tau = \frac{t D_s}{R^2}$, model equations become:

$$\frac{1}{Pe} \frac{\partial^2 y}{\partial \zeta^2} - \frac{\partial y}{\partial \zeta} - \theta \frac{\partial y}{\partial \tau} - \theta \delta_m (1 - \xi_C^3) \frac{\partial x_{av}}{\partial \tau} = 0 \quad (3.11)$$

$$\frac{\partial x}{\partial \tau} = \frac{\partial^2 x}{\partial \xi^2} + \frac{2}{\xi} \frac{\partial x}{\partial \xi} \quad (\xi_C \leq \xi \leq 1) \quad (3.12)$$

$$y(\zeta, 0) = 0 \quad (3.13)$$

$$y(0, \tau) = \delta(\tau) \quad (\text{Dirac input}) \quad (3.14a)$$

$$y(0, \tau) = 1 \quad (\text{Step input}) \quad (3.14b)$$

$$y(\infty, \tau) \text{ is limited} \quad (3.15)$$

$$x(\xi, \zeta, 0) = 0 \quad (3.16)$$

$$\left(\frac{\partial x}{\partial \xi} \right)_{\xi=\xi_C} = 0 \quad (3.17)$$

$$(1 - \xi_C^3) \frac{\partial x_{av}}{\partial \tau} = 3Bi(y - x_s) \quad (3.18)$$

$$x_s(\zeta, \tau) = x(1, \zeta, \tau) \quad (3.19)$$

$$x_{av} = \frac{3}{(1 - \xi_C^3)} \int_{\xi_C}^1 x(\xi, \zeta, \tau) \xi^2 d\xi \quad (3.20)$$

The model parameters are

$$\delta_m = \left(\frac{1 - \varepsilon_B}{\varepsilon_B} \right) K \rho_s \quad (\text{distribution ratio based on adsorbent shell})$$

$$Bi = \frac{k_f R}{K \rho_s D_s} \quad (\text{modified Biot number})$$

$$\theta = \frac{\varepsilon_B L D_s}{u R^2} \quad (\text{ratio of space time and intraparticle diffusion time})$$

$$Pe = \frac{uL}{\varepsilon_B D_L} \quad (\text{Pelect number based on adsorber length})$$

3.2.1. Analytical and approximate solutions for breakthrough curves

3.2.1.1. Analytical solution

The solution of Eq. (3.12) for the special case where $x_s = 1$, denoted by $U(\xi, \tau)$, is (Li, Xiu, and Rodrigues, 2003b):

$$U(\xi, \tau) = 1 + \frac{2}{\xi} \sum_{n=1}^{\infty} \frac{\sin[\sqrt{\gamma_n}(\xi - \xi_C)] + \sqrt{\gamma_n} \xi_C \cos[\sqrt{\gamma_n}(\xi - \xi_C)]}{(1 + \gamma_n \xi_C^2 - \gamma_n \xi_C^3) \sqrt{\gamma_n} \cos[\sqrt{\gamma_n}(1 - \xi_C)]} \exp(-\gamma_n \tau) \quad (3.21)$$

in which γ_n are the positive roots of

$$\tan[(1 - \xi_C) \sqrt{\gamma_n}] = -\xi_C \sqrt{\gamma_n} \quad (3.22)$$

Thus, we have

$$\frac{dU(\xi, \tau)}{d\tau} = -2 \sum_{n=1}^{\infty} \left(\frac{\gamma_n}{\xi} \right) \frac{\sin[\sqrt{\gamma_n}(\xi - \xi_C)] + \sqrt{\gamma_n} \xi_C \cos[\sqrt{\gamma_n}(\xi - \xi_C)]}{(1 + \gamma_n \xi_C^2 - \gamma_n \xi_C^3) \sqrt{\gamma_n} \cos[\sqrt{\gamma_n}(1 - \xi_C)]} \exp(-\gamma_n \tau) \quad (3.23)$$

Applying Duhamel's theorem, the volume-averaged, $x_{av}(\zeta, \tau)$, is then given in terms of the surface concentration of $x_s(\zeta, \tau)$ (Rosen, 1952):

$$x_{av}(\zeta, \tau) = \frac{6}{(1 - \xi_C^3)} \sum_{n=1}^{\infty} \left(\frac{1 + \gamma_n \xi_C^2}{1 + \gamma_n \xi_C^2 - \gamma_n \xi_C^3} \right) \int_0^{\tau} x_s(\zeta, \phi) \exp[-\gamma_n(\tau - \phi)] d\phi \quad (3.24)$$

Taking into account Eq. (3.16), i.e., $x_s(\zeta, 0) = 0$, we have

$$\frac{\partial x_{av}(\zeta, \tau)}{\partial \tau} = \frac{6}{(1 - \xi_C^3)} \sum_{n=1}^{\infty} \left(\frac{1 + \gamma_n \xi_C^2}{1 + \gamma_n \xi_C^2 - \gamma_n \xi_C^3} \right) \int_0^{\tau} \frac{\partial x_s(\zeta, \phi)}{\partial \phi} \exp[-\gamma_n(\tau - \phi)] d\phi \quad (3.25)$$

The Laplace transform of Eq. (3.25) is obtained by using the convolution theorem for the right-hand side of equation, i.e.

$$p \bar{x}_{av} = \frac{1}{(1 - \xi_C^3)} Y_D \bar{x}_s \quad (3.26)$$

where

$$Y_D = 6 \sum_{n=1}^{\infty} \left[\left(\frac{1 + \gamma_n \xi_C^2}{1 + \gamma_n \xi_C^2 - \gamma_n \xi_C^3} \right) \frac{p}{p + \gamma_n} \right] \quad (3.27)$$

in which the overhead bar indicates the Laplace transform, p is the Laplace transform parameter.

The Laplace transform of Eq. (3.11), combined with Eqs (3.18) and (3.26) is:

$$\frac{1}{Pe} \frac{\partial^2 \bar{y}}{\partial \zeta^2} - \frac{\partial \bar{y}}{\partial \zeta} - \theta(p + \delta_m Y_T) \bar{y} = 0 \quad (3.28)$$

where

$$Y_T = \frac{Y_D}{\left(1 + \frac{Y_D}{3Bi}\right)} \quad (3.29)$$

The solution of Eq. (3.28) in Laplace domain is

$$\bar{y} = \frac{1}{p} \exp(r_1 \zeta) \quad (3.30)$$

where

$$r_1 = \frac{Pe}{2} - \frac{1}{2} \sqrt{Pe^2 + 4Pe(p + \delta_m Y_T)\theta} \quad (3.31)$$

Finally, the analytical solution in time-domain is:

$$y_B(\tau) = \frac{1}{2} + \frac{1}{\pi} \int_0^{\infty} \exp\left(\frac{Pe\zeta}{2} - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} + a}{2}}\right) \sin\left(\beta\tau - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} - a}{2}}\right) \frac{d\beta}{\beta} \quad (3.32)$$

where

$$a = Pe \left(\frac{Pe}{4} + 3\theta\delta_m I_1 \right) \quad (3.33)$$

$$b = \theta Pe (\beta + 3\delta_m I_2) \quad (3.34)$$

$$I_1 = \frac{Bi^2 I_3 + Bi(I_3^2 + I_4^2)}{(Bi + I_3)^2 + I_4^2} \quad (3.35)$$

$$I_2 = \frac{Bi^2 I_4}{(Bi + I_3)^2 + I_4^2} \quad (3.36)$$

$$I_3 = 2 \sum_{n=1}^{\infty} \left[\left(\frac{1 + \gamma_n \xi_C^2}{1 + \gamma_n \xi_C^2 - \gamma_n \xi_C^3} \right) \frac{\beta^2}{\beta^2 + \gamma_n^2} \right] \quad (3.37)$$

$$I_4 = 2 \sum_{n=1}^{\infty} \left[\left(\frac{1 + \gamma_n \xi_C^2}{1 + \gamma_n \xi_C^2 - \gamma_n \xi_C^3} \right) \frac{\beta \gamma_n}{\beta^2 + \gamma_n^2} \right] \quad (3.38)$$

When $\xi_C \rightarrow 0$, the above solutions Eqs (3.32)-(3.38) take the form given by Rasmuson and Neretnieks (1980) and Xiu et al. (1997).

3.2.1.2. Parabolic-profile approximate solution

In order to reduce the complexity of the analytical solution, parabolic concentration profiles in the adsorbent without inert core have been assumed in some previous studies of breakthrough curves (Liaw et al., 1979; Rice, 1982; Cen and Yang, 1986; Xiu et al., 1997). In the present work, we use the following parabolic-profile assumption in the adsorbent shell

$$x(\xi, \zeta, \tau) = a(\zeta, \tau) + b(\zeta, \tau) \xi^2 \quad (\xi_C \leq \xi \leq 1) \quad (3.39)$$

The solution of Eq. (3.12) for the special case that $x_s = 1$ under the parabolic-profile assumption, denoted by $U(\xi, \tau)$, is

$$U(\xi, \tau) = 1 - \frac{(1 - \xi_C^3)(1 - \xi^2)}{2H_1} \exp\left(-\frac{3}{H_1} \tau\right) \quad (3.40)$$

where

$$H_1 = \frac{1}{2}(1 - \xi_C^3) - \frac{3}{10}(1 - \xi_C^5) \quad (3.41)$$

The volume-averaged, $x_{av}(\zeta, \tau)$, is given in terms of the surface concentration of $x_s(\zeta, \tau)$ as shown for the parabolic-profile approximation:

$$x_{av}(\zeta, \tau) = \frac{3}{H_1} \int_0^{\tau} x_s(\zeta, \phi) \exp\left[-\frac{3}{H_1}(\tau - \phi)\right] d\phi \quad (3.42)$$

Thus, it is not difficult to obtain the following expression in Laplace domain:

$$Y_D = \frac{\frac{3}{H_1} p}{\frac{3}{H_1} + p} \quad (3.43)$$

The time domain solution for parabolic-profile assumption is the same as Eqs (3.32)-(3.34), in which

$$I_1 = \frac{H_2(1 - \xi_c^3)\beta^2}{9 + H_2^2\beta^2} \quad (3.44)$$

$$I_2 = \frac{3(1 - \xi_c^3)\beta}{9 + H_2^2\beta^2} \quad (3.45)$$

where

$$H_2 = H_1 + \frac{(1 - \xi_c^3)}{Bi} \quad (3.46)$$

3.2.1.3. Quasi-lognormal distribution approximate solution

Wiedemann et al. (1978), and Linek and Dukukovic (1982) approximated the breakthrough curves using the moment of the impulse response in terms of an orthogonal polynomial series expansion. Wang and Lin (1986) assumed that the quasi-lognormal probability density function, $y_\delta(\tau)$, can be used to represent the impulse response of the system, where $y_\delta(\tau)$ is the product of the lognormal probability density function and the zeroth moment of the impulse response of the system, μ_0 , that is,

$$y_\delta(\tau) = \frac{\mu_0}{\sqrt{2\pi}\sigma\tau} \exp\left[-\frac{(\ln \tau - \mu)^2}{2\sigma^2}\right] \quad (3.47)$$

The first absolute moment, μ_1 , and the second central moment, μ_2' of Eq. (3.47) in the τ -domain are given as follows

$$\mu_1 = \exp\left(\mu + \frac{\sigma^2}{2}\right) \quad (3.48)$$

$$\mu_2' = \exp(2\mu + \sigma^2) [\exp(\sigma^2) - 1] \quad (3.49)$$

The solution in the Laplace domain of the system of equations of the general kinetic model can also provide equations relating the first absolute moment and the section central moment of elution bands to the characteristics of the retention equilibrium and the mass transfer kinetics, respectively. The expressions of μ_0 , the first absolute moment, μ_1 , and the second central

moment, μ_2' , for the response to a Dirac input can be obtained from model Eqs (3.11)-(3.20) as:

$$\mu_0 = 1 \quad (3.50)$$

$$\mu_1 = \zeta\theta[1 + \delta_m(1 - \xi_C^3)] \quad (3.51)$$

$$\mu_2' = \mu_2 - \mu_1^2 = \frac{2\zeta\theta\delta_m(1 - \xi_C^3)^2}{3} \left(\frac{1}{\Theta} + \frac{1}{Bi} \right) + \frac{2\zeta\theta^2}{Pe} [1 + \delta_m(1 - \xi_C^3)]^2 \quad (3.52)$$

where

$$\frac{1}{\Theta} = \frac{(1 - \xi_C)^2}{(1 - \xi_C^3)} \left[\xi_C + \frac{(1 - \xi_C)^3}{5(1 - \xi_C^3)} \right] \quad (3.53)$$

The parameter, $1/\Theta$ represents the decrease of the intraparticle diffusion resistance when using inert core adsorbent relative to the case of conventional adsorbent. It is obvious that $\Theta = 5$ if $\xi_C \rightarrow 0$, which is the same as spherical particle without core (Xiu et al., 1997).

If $y_\delta(\tau)$ represents the impulse response of the system, the parameters of the Q-LND are obtained from Eqs (3.48) and (3.49) as:

$$\mu = \ln \mu_1 - \frac{1}{2} \ln \left(1 + \frac{\mu_2'}{\mu_1^2} \right) \quad (3.54)$$

$$\sigma = \left[\ln \left(1 + \frac{\mu_2'}{\mu_1^2} \right) \right]^{1/2} \quad (3.55)$$

The approximation of a step response, that is, the breakthrough curve, $y_B(\tau)$, is simply the integral of the impulse response, $y_\delta(\tau)$, or,

$$y_B(\tau) = \int_0^\tau y_\delta(\tau) d\tau \quad (3.56)$$

By combining Eq. (3.56) with Eq. (3.47) and introducing $\beta = \frac{\ln \tau - \mu}{\sigma}$, the analytical expression of the Q-LND approximation, in which only three moments of the impulse response appear, can be obtained as follows:

$$y_B(\tau) = \mu_0 \int_{-\infty}^{(\ln \tau - \mu)/\sigma} \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{\beta^2}{2}\right) d\beta \quad (3.57)$$

3.2.2. Analytical and approximate solutions for peak elution curves

3.2.2.1. Analytical and approximate solutions for Dirac input

The analytical solutions for Dirac input, that is, the elution curves $y_\delta(\tau)$, is obtained by using the differential of the breakthrough curves, $y_B(\tau)$, that is

$$y_\delta(\tau) = \frac{dy_B(\tau)}{d\tau} \quad (3.58)$$

Based on Leibnitz's rule for differentiation of integrals, we have the analytical solution and the parabolic-profile approximate solution for elution curve $y_\delta(\tau)$ as

$$y_\delta(\tau) = \frac{1}{\pi} \int_0^\infty \exp\left[\frac{Pe\zeta}{2} - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} + a}{2}}\right] \cos\left(\beta\tau - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} - a}{2}}\right) d\beta \quad (3.59)$$

The above equation also can be obtained based on the Laplace transform method (Haynes, 1975).

The quasi-lognormal distribution approximate solution for elution curves is given by above-mentioned Eq. (3.47).

3.2.2.2. Analytical and approximate solutions for the generalized sample input mode

The sample-input mode has great influence on the behavior of elution curves for a loss of column efficiency due to the band broadening. Now we introduce more generalized sample-input mode to see what effects the input modes have on the elution curves.

Case I: for preparative chromatography. The input is a square pulse of injection time τ_{inj} , i.e., $H(\tau) - H(\tau - \tau_{inj})$, where $H(\tau)$ is Heaviside's unit function.

Case II: for analytical chromatography. The input is $[H(\tau) - H(\tau - \tau_{inj})]/\tau_{inj}$, which means that the amount injected is unit.

The response curves can be derived by using the superposition principle for linear systems as:

$$y_{U,I}(\tau) = y_B(\tau) - H(\tau - \tau_{inj})y_B(\tau - \tau_{inj}) \quad (Case I) \quad (3.60)$$

$$y_{U,II}(\tau) = \frac{1}{\tau_{inj}} [y_B(\tau) - H(\tau - \tau_{inj})y_B(\tau - \tau_{inj})] \quad (Case II) \quad (3.61)$$

It is evident that $y_{U,I}(\tau) = y_B(\tau)$ (i.e., breakthrough curve for step input) if $\tau_{inj} \rightarrow \infty$ for Case I, and $y_{U,II}(\tau) = y_\delta(\tau)$ (i.e., the peak elution curve for Dirac input) if $\tau_{inj} \rightarrow 0$ for Case II.

Combining Eq. (3.32) with Eqs (3.60) and (3.61), respectively, the analytical solution and parabolic-profile approximate solution of the elution curves for the generalized sample-input mode in the time-domain are

$$y_{U,I}(\tau) = \frac{1}{\pi} \int_0^\infty \exp \Omega_1 \{ \sin(\beta\tau - \Omega_2) - H(\tau - \tau_{inj}) \sin[\beta(\tau - \tau_{inj}) - \Omega_2] \} \frac{d\beta}{\beta} \quad (Case I) \quad (3.62)$$

$$y_{U,II}(\tau) = \frac{1}{\tau_{inj}\pi} \int_0^\infty \exp \Omega_1 \{ \sin(\beta\tau - \Omega_2) - H(\tau - \tau_{inj}) \sin[\beta(\tau - \tau_{inj}) - \Omega_2] \} \frac{d\beta}{\beta} \quad (Case II) \quad (3.63)$$

where

$$\Omega_1 = \frac{Pe\zeta}{2} - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} + a}{2}} \quad (3.64)$$

$$\Omega_2 = \zeta \sqrt{\frac{\sqrt{a^2 + b^2} - a}{2}} \quad (3.65)$$

For the generalized-input mode, based on the Q-LND approximation, we have

$$y_{U-QLND,I}(\tau) = \mu_0 \left[\int_{-\infty}^{\frac{\ln \tau - \mu}{\sigma}} \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{\beta^2}{2}\right) d\beta - H(\tau - \tau_{inj}) \int_{-\infty}^{\frac{\ln(\tau - \tau_{inj}) - \mu}{\sigma}} \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{\beta^2}{2}\right) d\beta \right] \quad (Case I) \quad (3.66)$$

$$y_{U-QLND,II}(\tau) = \frac{\mu_0}{\tau_{inj}} \left[\int_{-\infty}^{\frac{\ln \tau - \mu}{\sigma}} \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{\beta^2}{2}\right) d\beta - H(\tau - \tau_{inj}) \int_{-\infty}^{\frac{\ln(\tau - \tau_{inj}) - \mu}{\sigma}} \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{\beta^2}{2}\right) d\beta \right] \quad (Case II) \quad (3.67)$$

3.2.3. Derived equation of resolution R_s based on Q-LND approximation

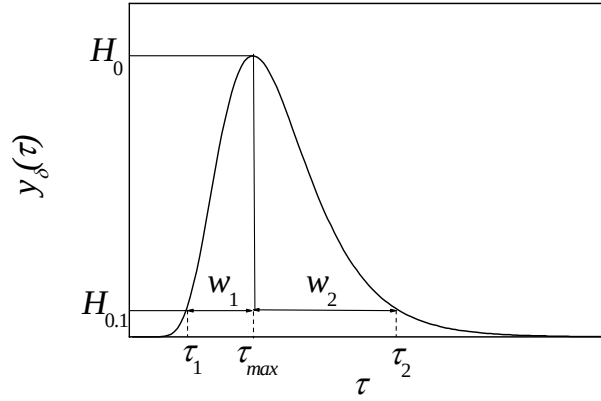


Fig. 3.2. Typical elution curve.

A graphic representation of typical elution curve for Dirac input is shown in Fig. 3.2. Based on the Q-LND approximate solution, Eq. (3.47), the time $\tau_{\max}$ corresponding to the peak maximum H_0 is

$$\tau_{\max} = \exp(\mu - \sigma^2) \quad (3.68)$$

and

$$H_0 = \frac{\mu_0}{\sqrt{2\pi}\sigma} \exp\left(\frac{\sigma^2}{2} - \mu\right) \quad (3.69)$$

The asymmetry factor A is obtained as

$$A = \frac{w_1}{w_2} = \frac{\tau_2 - \tau_{\max}}{\tau_{\max} - \tau_1} = \exp(\sigma\sqrt{2\ln 10}) = \exp(2.146\sigma) \quad (3.70)$$

which w_1 and w_2 are the bandwidths of the left-hand and the right-hand sides at one tenth of the peak height H_0 , respectively, and, τ_1 and τ_2 are the corresponding times, as

$$\tau_1 = \exp[(\mu - \sigma^2) - 2.146\sigma] \quad (3.71)$$

$$\tau_2 = \exp[(\mu - \sigma^2) + 2.146\sigma] \quad (3.72)$$

For Gaussian function $A=1$.

The bandwidth at one tenth of the peak height, $w_{0.1}$, is

$$w_{0.1} = w_1 + w_2 = 2 \exp(\mu - \sigma^2) \sinh(2.146\sigma) \quad (3.73)$$

From the plate theory, the theoretical plate number N_T can be computed using the first absolute moment μ_1 and the second central moment μ_2' , that is

$$N_T = \frac{\mu_1^2}{\mu_2'} \quad (3.74)$$

Thus, we have the following expressions in terms of N_T :

$$\sigma = \sqrt{\ln\left(1 + \frac{1}{N_T}\right)} \quad (3.75)$$

$$\tau_{\max} = \mu_1 \left(1 + \frac{1}{N_T}\right)^{-\frac{3}{2}} \quad (3.76)$$

$$w_{0.1} = 2\mu_1 \left(1 + \frac{1}{N_T}\right)^{-\frac{3}{2}} \sinh\left[2.146 \sqrt{\ln\left(1 + \frac{1}{N_T}\right)}\right] \quad (3.77)$$

The height equivalent to a theoretical plate (*HETP*) is obtained as

$$HETP = \frac{L}{N_T} \quad (3.78)$$

The dimensionless *HETP*^{*} is defined as

$$HETP^* = \frac{HETP}{L} = \frac{1}{N_T} = \frac{\mu_2'}{\mu_1^2} \quad (3.79)$$

In order for a separation to occur, components must have different distributions between the phases. This difference is expressed in the thermodynamically controlled separation factor α as

$$\alpha = \frac{\delta_{m2}}{\delta_{m1}} = \frac{K_2}{K_1} \quad (3.80)$$

The separation factor α is a measurement of the specificity, or, separation ability, of a column. For an effective separation, α must be greater than 1. As α increases, separations become easier and require fewer theoretical plates.

Resolution R_s is a measure of the amount of overlap between two neighboring peaks that indicates the success of a separation. Resolution, which is a dimensionless ratio, is given by the ratio of the distance between the peaks of the two solute bands, and the effective zone width, i.e.,

$$R_s = \frac{(\tau_{\max})_2 - (\tau_{\max})_1}{0.5[(w_{0.1})_2 + (w_{0.1})_1]} \quad (3.81)$$

Written in terms of $HETP^*$, we have

$$R_s = \frac{\left(\frac{\mu_{1,2}}{\mu_{1,1}}\right) \left(\frac{1 + HETP_1^*}{1 + HETP_2^*}\right)^{\frac{3}{2}} - 1}{\left(\frac{\mu_{1,2}}{\mu_{1,1}}\right) \left(\frac{1 + HETP_1^*}{1 + HETP_2^*}\right)^{\frac{3}{2}} \sinh\left[2.146\sqrt{\ln(1 + HETP_2^*)}\right] + \sinh\left[2.146\sqrt{\ln(1 + HETP_1^*)}\right]} \quad (3.82)$$

The integrand of Eqs (3.32), (3.59), (3.62), and (3.63) for both the analytical solution and the parabolic-profile approximate solution are the product of an exponential decaying function and a periodic sine or cosine function. Integration should be performed over each half-period of the sine or cosine wave that was developed by Rasmuson (1985). In addition, during the calculation of the analytical solution for the breakthrough and elution curves, we must get the roots of γ_n from the Eq. (3.22) in advance; about 100 roots of γ_n were considered in the sums of Eqs (3.37) and (3.38). The calculation of Eqs (3.57), (3.66) and (3.67) for Q-LND approximate solution is performed with a polynomial approximation (Zelen and Severo, 1970).

3.3. Results and discussion

3.3.1. Effect of model parameters on breakthrough curves

The effect of model parameters ξ_C , θ , Pe , and Bi , characterizing the size of inert core, intraparticle diffusion resistance, axial dispersion, film mass transfer resistance, on the breakthrough curves are shown in Figs 3.3-3.5, respectively. Predictions based on the analytical solution (solid line, Eq. (3.32)) with Eqs (3.33)-(3.38)), the parabolic-profile approximation (bold dot line, Eq. (3.32) with Eqs (3.33), (3.34) and (3.44)-(3.46)), and the Q-LND approximation (dashed dot line, Eq. (3.57)) are compared. Usually, the dimensionless

separation parameter, δ_m , changes from 10^2 to 10^4 ; in the following discussion, we selected $\delta_m = 10^3$ as an example.

The parameter ξ_C influences both the breakthrough time and the shape of the breakthrough curves. With ξ_C increase, the residence time of the fluid approximately decreases by a factor $(1 - \xi_C^3)$ due to the decrease of the loading capacity of the column according to Eq. (3.51). For small values of θ where the diffusion rate is very slow and fluid retention time is very short, as shown in Fig. 3.3a ($\theta = 10^{-2}$) and Fig. 3.3b ($\theta = 10^{-3}$), the tendency is that the breakthrough time for conventional adsorbents becomes closer to that of inert core adsorbents due to the limitation of the intraparticle diffusion resistance. The zone spreading time means the time from the breakthrough to the saturation of the fixed bed. From the Fig. 3.3b, the zone spreading time for the breakthrough curves can be decreased effectively if using the inert core adsorbent. The decrease of the zone spreading time is important for desorption process, especially for biomacromolecule separation. In Fig. 3.3c, the breakthrough curves are compared for columns packed with the inert core adsorbent ($\xi_C = 0.9$) and with the conventional adsorbent ($\xi_C = 0$) at constant volume of active adsorbent (same stoichiometric point). In order to keep a constant volume of active adsorbent, the length of the column is increased by the factor of $1/(1 - \xi_C^3)$ for the inert core adsorbent, the corresponding model parameters, Pe and θ increased by the factor of $1/(1 - \xi_C^3)$. From Fig. 3.3c, it is apparent the zone spreading time for the breakthrough curves can be decreased effectively by using the inert core adsorbent due to the decrease of the intraparticle diffusion resistance.

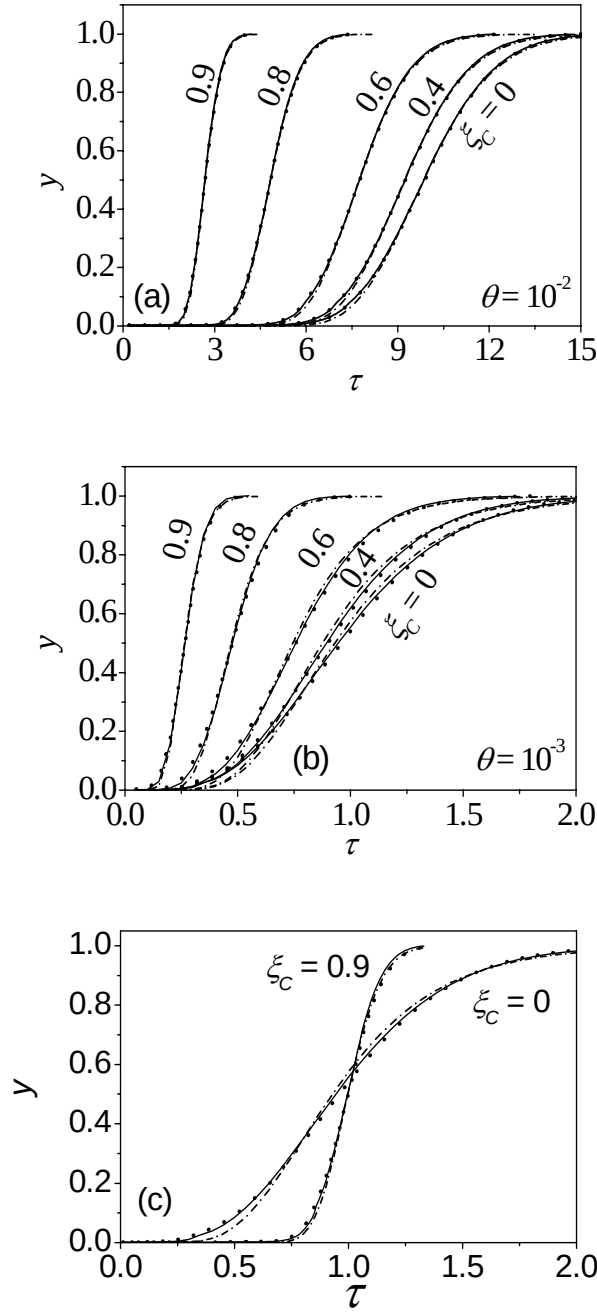


Fig. 3.3. Effect of ξ_C on $y - \tau$ breakthrough curves at $Bi = 10^2$ and $\delta_m = 10^3$: (a) at $\theta = 10^{-2}$ and $Pe = 10^2$, (b) at $\theta = 10^{-3}$ and $Pe = 10^2$, (c) at constant volume of active adsorbent (same stoichiometric point). $\theta = 10^{-3}$ and $Pe = 10^2$ for $\xi_C = 0$; $\theta = 10^{-3} / (1 - \xi_C^3)$ and $Pe = 10^2 / (1 - \xi_C^3)$ for $\xi_C = 0.9$; Solid lines: Analytical solution (Eq. (3.32) with Eqs (3.33)-(3.38)); bold dot lines: Parabolic-profile approximate solution (Eq. (3.32) with Eqs (3.33), (3.34) and (3.44)-(3.46)); dash dot lines: Q-LND approximate solution (Eq. (3.57)).

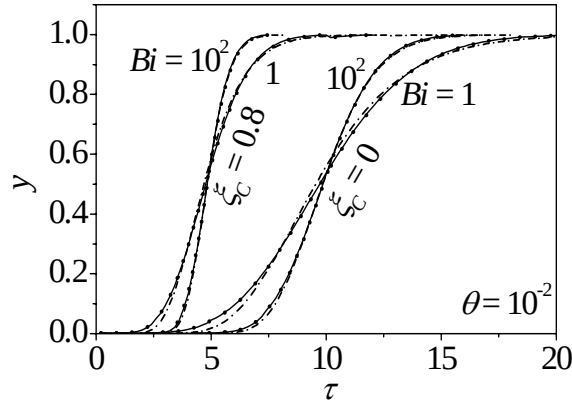


Fig. 3.4. Effect of Bi on $y - \tau$ breakthrough curves for the column packed with inert core adsorbent ($\xi_c = 0.8$) and conventional adsorbent ($\xi_c = 0$) at $Pe = 10^2$, $\theta = 10^{-2}$ and $\delta_m = 10^3$. Solid lines: Analytical solution (Eq. (3.32) with Eqs (3.33)-(3.38)); bold dot lines: Parabolic-profile approximate solution (Eq. (3.32) with Eqs (3.33), (3.34) and (3.44)-(3.46)); dash dot lines: Q-LND approximate solution (Eq. (3.57)).

When the model parameters, Pe and Bi , are below 10^3 and 10^2 , respectively, the effect of axial dispersion and film mass transfer resistance, on the breakthrough curves should be taken into account for conventional or inert core adsorbent packed in the fixed bed, as shown in Figs 3.4 and 3.5.

Figs 3.3-3.5 also show the accuracy of the approximate solutions by comparison of the breakthrough curves with the analytical solution. The breakthrough curves predicted by the analytical solution and approximate solutions are similar for a wide range of the model parameters. Deviations occur for very low value of θ , as shown in Fig. 3.3b, and the prediction based on the parabolic-profile approximate solution will become weaker when film mass transfer resistance is important; in contrast, for the Q-LND approximation, the deviation is more important when Bi is also small, as shown in Fig. 3.4. Although the accuracy of parabolic-profile approximate solution is better than that of the Q-LND approximate solution, the solution of the parabolic profile approximation involves the integration of the product of an exponential decaying function and a periodic sine function, as does the analytical solution; the calculation of the Q-LND approximation is simple and handy, which best fit the need of the industrial application for the design of the column packed by the inert core adsorbent.

According to the Q-LND approximation, Eq. (3.57), the breakthrough curves are

determined by three momentum parameters, μ_0 , μ_1 and μ_2'/μ_1^2 . The term μ_2'/μ_1^2 involves the effect of the intraparticle diffusion, film mass transfer and axial dispersion and is the dimensionless $HETP^*$. Based on Eqs (3.51) and (3.52), this term can be represented as,

$$\frac{\mu_2'}{\mu_1^2} \approx \frac{2}{3\theta\delta_m} \left[\frac{1}{\Theta} + \frac{1}{Bi} \right] + \frac{2}{Pe} = \frac{2uR^2}{3\delta_m\epsilon_B L} \left[\frac{1}{D_s\Theta} + \frac{K\rho_s}{k_f R} \right] + \frac{2\epsilon_B D_L}{uL} \quad (3.83)$$

in which we assumed $\delta_m(1 - \xi_C^3) \gg 1$. The first term represents the effect of the intraparticle diffusion resistance, the second term represents the effect of the film mass transfer resistance, and the third term represents the effect of the axial dispersion. By comparing the values of those three terms for a practical system, one can evaluate a priori which resistance will have a significant impact on the breakthrough curves.

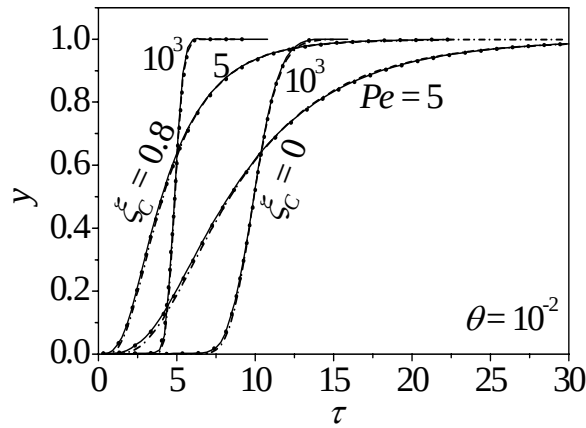


Fig. 3.5. Effect of Pe on $y - \tau$ breakthrough curves for the column packed with inert core adsorbent ($\xi_C = 0.8$) and conventional adsorbent ($\xi_C = 0$) at $Bi = 10^2$, $\theta = 10^{-2}$ and $\delta_m = 10^3$. Solid lines: Analytical solution (Eq. (3.32) with Eqs (3.33)-(3.38)); bold dot lines: Parabolic-profile approximate solution (Eq. (3.32) with Eqs (3.33), (3.34) and (3.44)-(3.46)); dash dot lines: Q-LND approximate solution (Eq. (3.57)).

3.3.2. Effect of model parameters on peak elution curves

The elution curves of chromatography columns packed with inert core adsorbent ($\xi_C > 0$) and conventional adsorbent ($\xi_C = 0$) under the same operating conditions, are compared in Fig. 3.6; the elution curves are predicted by the analytical solution Eq. (3.59) and the simple Q-LND approximate solution Eq. (3.47), respectively. It is obvious that when the

chromatographic column is packed with the inert core adsorbent, the retention time corresponding to the peak maximum is shortened, and the peak becomes sharper due to the low loading capacity of the chromatographic column and the short diffusion path in the adsorbent shell. The column efficiency, measured by the plate number N_T , is enhanced effectively by using the inert core adsorbent; for example, from Fig. 3.6 it can be seen that the plate number increased from 166.7 ($\xi_C = 0$) to 264.8 ($\xi_C = 0.9$).

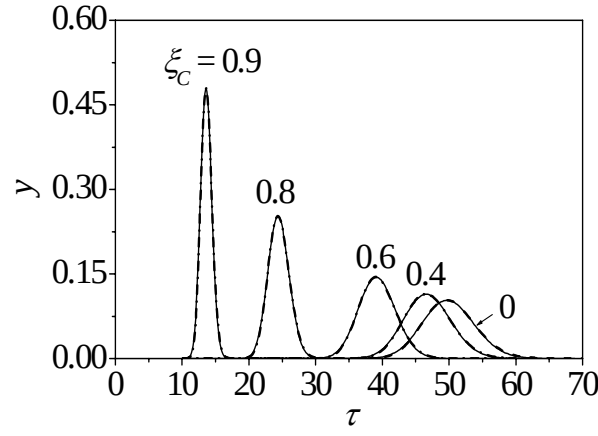


Fig. 3.6. Effect of ξ_C on $y-\tau$ elution curves for $\theta=10^{-1}$ at $Pe=10^3$, $Bi=10$ and $\delta_m=5\times 10^2$. Solid lines: analytical solution (Eq. (3.59)); dash dot lines: Q-LND approximate solution (Eq. (3.47)).

The effect of model parameters Pe and Bi , on the elution curves is shown in Figs 3.7 and 3.8, respectively, for the column packed with the inert core adsorbent, where the elution curves are respectively calculated by the analytical solution Eq. (3.59) and the Q-LND approximate solution Eq. (3.47). It is apparent that if axial dispersion or film mass transfer resistance is important, the peak becomes wider and the time corresponding to the peak maximum is slightly lower. The column efficiency, N_T , decreases when Pe and Bi decrease. In fact, the plate number decreases from 141.7 ($\xi_C = 0.8$) and 62.5 ($\xi_C = 0$) at $Pe = 10^3$ to 4.9 ($\xi_C = 0.8$) and 4.7 ($\xi_C = 0$) at $Pe = 10$, respectively, as extracted from Fig. 7; the column efficiency decreases from 141.7 ($\xi_C = 0.8$) and 62.5 ($\xi_C = 0$) at $Bi = 10^2$ to 13.7 ($\xi_C = 0.8$) and 12.2

($\xi_c = 0$) at $Bi = 1$, respectively, as extracted from Fig. 3.8. Again, we found that even there exists axial dispersion or film mass transfer resistance, the retention time corresponding to the maximum peak becomes short and the peak becomes steep when the chromatographic column packed with the inert core adsorbent, as shown in Fig. 3.7 and Fig. 3.8, by comparing the elution curves between $\xi_c = 0.8$ and $\xi_c = 0$, but the column efficiency is enhanced a little when Pe and Bi values are small.

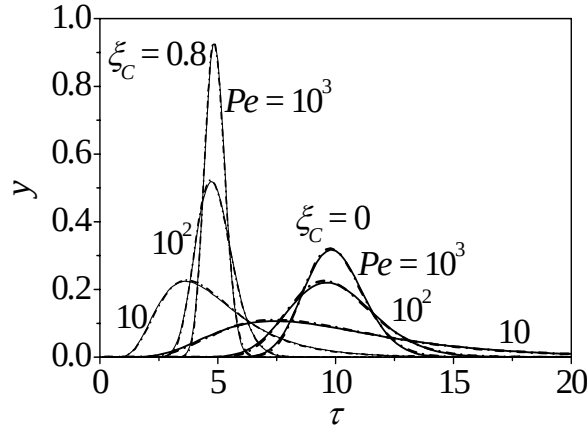


Fig. 3.7. Effect of Pe on $y - \tau$ elution curves for the column packed with inert core adsorbent ($\xi_c = 0.8$) and conventional adsorbent ($\xi_c = 0$) at $Bi = 10^2$, $\theta = 10^{-2}$ and $\delta_m = 10^3$. Solid lines: analytical solution (Eq. (3.59)); dash dot lines: Q-LND approximate solution (Eq. (3.47)).

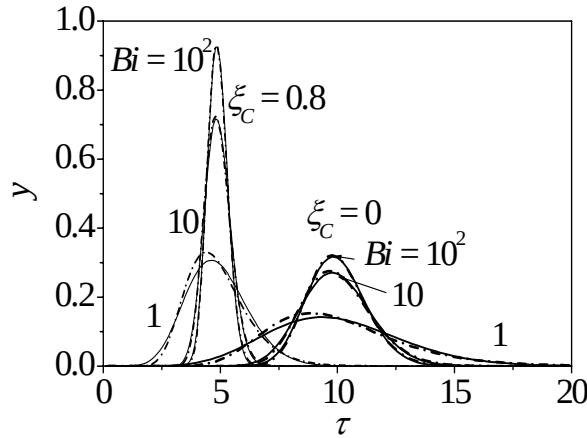


Fig. 3.8. Effect of Bi on $y - \tau$ elution curves for the column packed with inert core adsorbent ($\xi_c = 0.8$) and conventional adsorbent ($\xi_c = 0$) at $\theta = 10^{-2}$, $Pe = 10^3$ and $\delta_m = 10^3$. Solid lines: analytical solution (Eq. (3.59)); dash dot lines: Q-LND approximate solution (Eq. (3.47)).

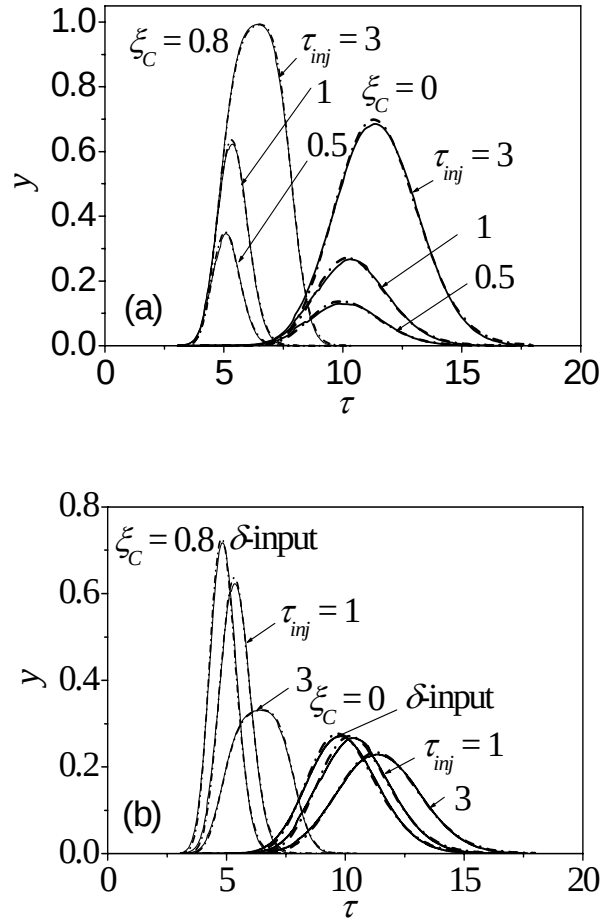


Fig. 3.9. Elution curves for different input modes at the column packed with inert core adsorbent ($\xi_C = 0.8$) and conventional adsorbent ($\xi_C = 0$) at $\theta = 10^{-2}$, $Pe = 10^3$, $Bi = 10$ and $\delta_m = 10^3$: (a) Case I. Solid lines: analytical solution (Eq. (3.62)); dash dot lines: Q-LND approximate solution (Eq. (3.66)), (b) Case II. Solid lines: analytical solution (Eq. (3.63)); dash dot lines: Q-LND approximate solution (Eq. (3.67)).

The effects of the sample-input mode and the sample size on the elution curves are demonstrated in Fig. 3.9, where the elution curves are calculated by the analytical solution Eq. (3.62) and Q-LND approximate solution Eq. (3.66) for the operation mode of preparative chromatography packed with inert core adsorbent, as shown in Fig. 9a, and those are calculated by the analytical solution Eq. (3.63) and the Q-LND approximate solution Eq. (3.67) for the operation mode of analytical chromatography packed with inert core adsorbent, as shown in

Fig. 3.9b. For the operation mode of the preparative chromatography with wide rectangular injection pulse (long injection time τ_{inj}), the peak becomes high and wide. On the contrary, for the operation mode of the analytical chromatography with narrow injection pulse, when the time of injection τ_{inj} is longer, the peak becomes short and wide.

In Figs 3.6-3.9, we compare the elution profiles predicted by the analytical solution and by the Q-LND approximation; the difference between the two solutions is negligible for the chromatography column packed with the inert core adsorbent from high column efficiency to low column efficiency. It is interesting to note that the Q-LND approximate solution can represent the elution curves fairly well when Pe is small, as shown in Fig. 3.7 ($Pe = 10$).

3.3.3. Resolution of two components by the fixed bed packed with inert core adsorbent.

For low asymmetry ($A \leq 1.2$), the elution curves calculated by the Q-LND probability density function are similar to those of the Gaussian function.

Combining Eqs (3.70) and (3.75), we have

$$A = \exp \left[2.146 \sqrt{\ln \left(1 + \frac{1}{N_T} \right)} \right] \quad (3.84)$$

For $A=1.2$ we get $N_T = 138$, which is the lower limit for the Gaussian function to simulate the elution curves.

Based on the above discussion, we conclude that the efficiency of a chromatographic column packed with inert core adsorbent is enhanced, especially for systems where the intraparticle-diffusion rate is slow. This is the case of biological macromolecule separation by liquid chromatography where biomacromolecules slowly diffuse in the adsorbent pores.

The parameter, $1/\Theta$, defined in Eq. (3.53), represent the decrease of the intraparticle diffusion resistance when using inert core adsorbent relative to the case of conventional adsorbent. For conventional adsorbent ($\xi_C = 0$), $1/\Theta = 0.2$, with the increase of ξ_C , the value of $1/\Theta$ drops quickly, as shown in Fig. 3.10. Therefore, when using the inert core adsorbent, the intraparticle diffusion resistance can be reduced effectively, and the elution curves become sharper and narrower, which will favor the resolution of multi-component mixture.

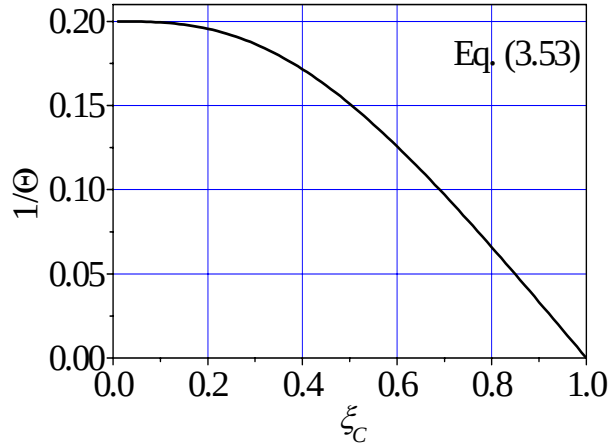


Fig. 3.10. Influence of inert core radius on the relative intraparticle diffusion resistance.

Fig. 3.11 shows the resolution of two components with separation factor $\alpha = 1.3$ in a chromatographic column with inert core adsorbent. The operating conditions (column length, fluid velocity, radius of the adsorbent all) were kept constant; only the inert core adsorbent size, ξ_c was changed. If the film mass transfer resistance is smaller, for example $Bi \geq 50$, with the increase of ξ_c , the resolution is enhanced quickly as shown in Fig. 3.11a. At $Bi = 50$, for the adsorbent without the inert core ($\xi_c = 0$), the resolution R_s is only 0.779, the two components can not be separated very well, as shown in Fig. 3.11b; however, if using the inert core adsorbent (for example, $\xi_c = 0.9$), the resolution R_s is up to be 1.233, the two components can be separated easily, the corresponding elution curves are shown in Fig. 3.11c.

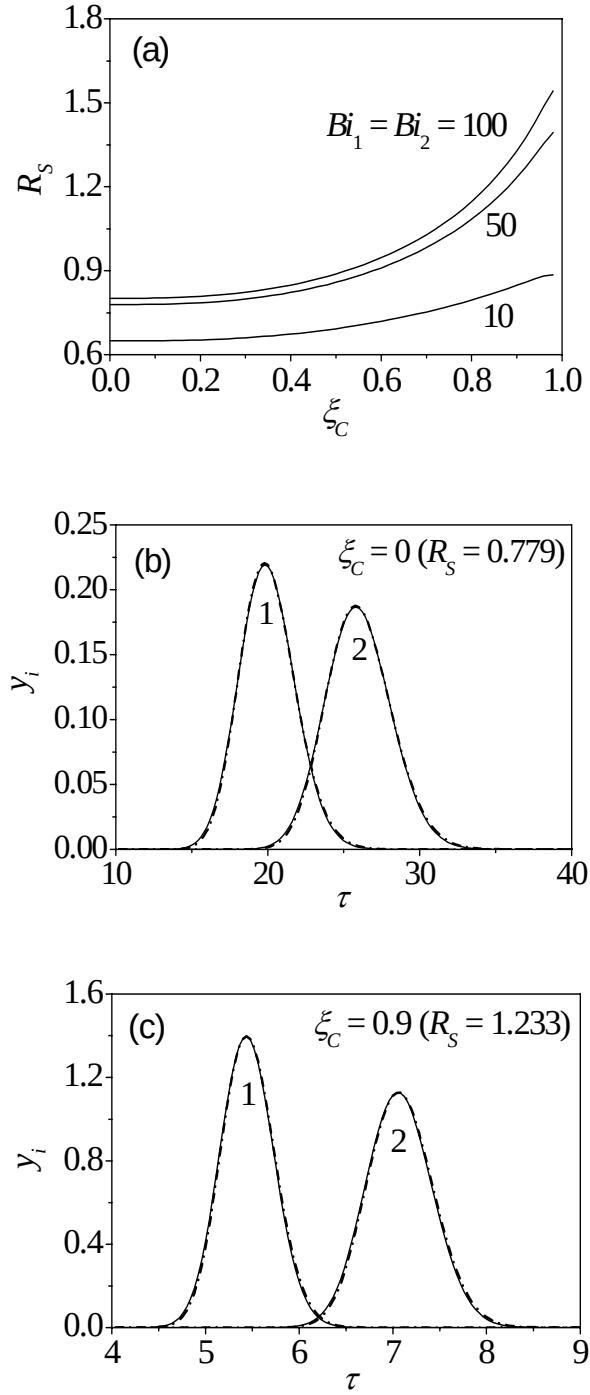


Fig. 3.11. The resolution of two components at the column packed with inert core adsorbent and conventional adsorbent ($\xi_c = 0$) at $\theta_1 = \theta_2 = 4 \times 10^{-2}$, $Pe_1 = Pe_2 = 2 \times 10^3$, $\alpha = 1.3$ and $\delta_{m1} = 5 \times 10^2$. (a) $R_s - \xi_c$ at $Bi_1 = Bi_2 = 100, 50$, and 10 (Eq. (3.82)); (b) $y_i - \tau$ elution curves at $\xi_c = 0$ ($R_s = 0.779$); (c) $y_i - \tau$ elution curves at $\xi_c = 0.9$ ($R_s = 1.233$). Solid lines: analytical solution (Eq. (3.59)); dash dot lines: Q-LND approximate solution (Eq. (3.47)).

3.4 Conclusions

The breakthrough and elution curves for fixed bed columns packed with inert core adsorbent can be predicted by using analytical solution, parabolic-profile approximate solution and Q-LND approximate solution for various input modes when the linear adsorption isotherm is coupled with axial dispersion, film mass transfer and intraparticle diffusion. The accuracy of the parabolic-profile approximation and the Q-LND approximate solution are acceptable over a wide range of operating conditions. However, it should be emphasized that the calculation of the Q-LND approximate solution is simple and handy.

The intraparticle diffusion resistance, film mass transfer resistance and the axial dispersion will influence the breakthrough curves and elution curves. The importance of each resistance can be evaluated a priori by using Eq. (3.83). The reduced amount of the intraparticle diffusion resistance by using inert core can be evaluated quantitatively by parameter, $1/\Theta$, Eq. (3.53).

When the fixed bed is packed with inert core adsorbent, the column separation efficiency will be enhanced due to the shortened diffusion path in the adsorbent, the peak of the elution curves become sharper and narrower, which will be very significant for the biological macromolecule fast separation by chromatography. Based on the Q-LND approximate solution, an analytical expression for the resolution of two components is derived for the linear chromatography packed with the inert core adsorbent and taking into account the axial dispersion, film mass transfer resistance and intraparticle diffusion resistance. Simulated results show that good resolution of two components can be obtained by chromatographic column packed with inert core adsorbent rather than with conventional adsorbent if the rate of the intraparticle diffusion is very slow.

Notation

Bi	Biot number $(= k_f R / K \rho_s D_s)$
C	concentration in bulk phase, $\text{kg}\cdot\text{m}^{-3}$
C_0	inlet concentration, $\text{kg}\cdot\text{m}^{-3}$
C_s	equilibrium liquid concentration with q_s , $\text{kg}\cdot\text{m}^{-3}$

D_L	axial dispersion coefficient, $\text{m}^2 \cdot \text{s}^{-1}$
D_S	intraparticle diffusivity, $\text{m}^2 \cdot \text{s}^{-1}$
K	Henry equilibrium constant, $\text{m}^3 \cdot \text{kg}^{-1}$
k_f	film mass transfer coefficient, $\text{m} \cdot \text{s}^{-1}$
L	column length, m
n	amount of sample injected, kg
p	Laplace transform parameter
Pe	Peclet number based on adsorber length $(= uL / \varepsilon_B D_L)$
q	adsorbed concentration in adsorbent shell, $\text{kg} \cdot \text{kg}^{-1}$
q_{av}	volume-averaged adsorbed concentration in adsorbent shell, $\text{kg} \cdot \text{kg}^{-1}$
q_s	adsorbed concentration in adsorbent shell surface, $\text{kg} \cdot \text{kg}^{-1}$
r	radial distance of adsorbent, m
R	radius of adsorbent, m
R_C	radius of adsorbent core, m
t	time, s
t_c	space time, s
u	superficial velocity, $\text{m} \cdot \text{s}^{-1}$
V	column volume, m^3
x	dimensionless adsorbed concentration $(= q / KC_0)$
x_{av}	dimensionless volume-averaged adsorbed concentration in adsorbent shell $(= q_{av} / KC_0)$
x_s	dimensionless adsorbed concentration in adsorbent shell surface, $(= q_s / KC_0)$
y	dimensionless fluid concentration $(= C / C_0)$
$y_B(\tau)$	dimensionless outlet concentration, y , for step input

$y_{\delta}(\tau)$	dimensionless outlet concentration, y , for Dirac input
$y_{U,I}(\tau)$	dimensionless outlet concentration, y , for <i>Case I</i> input mode
$y_{U,II}(\tau)$	dimensionless outlet concentrations, y , for <i>Case II</i> input mode
$y_{U-QLND,I}(\tau)$	dimensionless outlet concentration, y , for <i>Case I</i> input mode, for Q-LND approximate solution
$y_{U-QLND,II}(\tau)$	dimensionless outlet concentrations, y , for <i>Case II</i> input mode, for Q-LND approximate solution
Z	axial distance from column entrance, m

Greek letters

α	separation factor
β	integration parameter
δ_m	distribution ratio based on adsorbent shell $[= (1 - \varepsilon_B)K\rho_s / \varepsilon_B]$
ε_B	bed porosity
μ	parameter, defined in Eq. (3.47)
μ_0	the zeroth moment
μ_1	the first absolute moment
μ_2'	the second central moment
θ	ratio of space time and intraparticle diffusion time $(= \varepsilon_B LD_s / uR^2)$
ρ_s	density of adsorbent shell, $\text{kg}\cdot\text{m}^{-3}$
ξ	dimensionless radial distance of adsorbent $(= r / R)$
ξ_C	dimensionless radius of adsorbent inert core $(= R_C / R)$
σ	parameter, defined in Eq. (3.47)

τ	dimensionless time $\left[= (tD_s/R^2)\right]$
τ_c	dimensionless space time $\left[= (t_c D_s/R^2)\right]$
ζ	dimensionless axial variable $(= Z/L)$
Θ	dimensionless parameter, defined in Eq. (3.53)

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4. Analytical breakthrough and elution curves for inert core adsorbent with sorption kinetics ^{*}

This chapter is an extension of theoretical investigation in chapter 3. In this chapter, we develop the general kinetic model to the fixed-bed column packed with inert core adsorbents, in which intraparticle diffusion, film mass transfer, liquid axial dispersion and the adsorption-desorption rate were taken into account. Analytical solutions and parabolic-profile approximate solutions are derived under the linear adsorption kinetics to predict the breakthrough and elution curves for the inert core adsorbents, and the analytical expression for Height Equivalent to a Theoretical Plate (*HETP*) is given under the linear adsorption kinetics, by which one can evaluate the independent contribution of liquid axial dispersion, film mass transfer resistance, intraparticle diffusion resistance and the adsorption-desorption rate on *HETP* in the chromatography column packed with inert core adsorbents. In addition, the reduced form of the analytical solution of elution curve represents the residence time distribution of an inert tracer in the chromatography column; with which we can estimate easily intraparticle diffusivity or liquid axial dispersion coefficient by RTD measurement method in chromatography column.

^{*}This chapter is partly based on the paper by Li, P., Xiu, G. H. and Rodrigues, A. E., “Analytical breakthrough curves for inert core adsorbent with sorption kinetics”, *AIChE Journal*, 49, 2974-2979, 2004

4.1. Introduction

From the kinetics viewpoint, the general kinetic model is the most general model of fixed-bed adsorber. In this model, liquid axial dispersion and all the mass transfer resistances are taken into account, namely (1) the external mass transfer of adsorbate from the bulk phase to the external surface of the adsorbent (film mass transfer); (2) the diffusion transport through the pores of adsorbent (intraparticle diffusion); and (3) the adsorption-desorption kinetics at the active sites of adsorbent. The effects of various mass-transfer resistances on the breakthrough and elution curves can be predicted by the general kinetic model. The time domain exact solution of the general kinetic model under the linear adsorption kinetics have been derived to predict the breakthrough curves where the fixed-bed adsorber packed with the conventional spherical/cylinder adsorbent (Rasmuson, 1981; Xiu, 1996) based on the works of Rosen (1952) and Massaldi and Gottifredi (1972). The above-mentioned solutions are invalid to predict the breakthrough and elution curves in the case of inert core adsorbent.

Binding ligand inert core adsorbents are being used for bioseparations, e.g. the expanded bed for proteins separation (Bertrand et al., 1998; Owen and Chase, 1999; Ozyurt et al., 2002), and it is potential to use in the fast, high-performance liquid chromatography (HPLC) because of short diffusion path resulting in low intraparticle diffusion resistance for biomacromolecules separation (Lee, 1997; Rodrigues, 1997; Hunter and Carta, 2000). One example of such adsorbents is Poroshell 5 μm particle with a thin layer of porous silica on a solid core used for fast, high resolution protein separation (Agilent Technologies, 2001; Kirkland et al., 2000). Although the loading capacity of the columns packed with the inert core adsorbent is relative low, the ability of quick recovery of biomacromolecules with high biological activity overcomes this shortcoming; the latter is very importance for the production of biomacromolecules in the pharmaceutical and biotechnology industry. Furthermore, for the rapid separation of biomacromolecules, the slow adsorption-desorption rate at the active sites caused by steric hindrance of biomacromolecules (Corsel et al., 1986; Cramer and Subramanian, 1990; Whitley et al., 1991; Frey and Vilfan, 2002) will affect significantly the column behavior; therefore, the general rate model will be more effective to model the rapid separation process of biomacromolecules by chromatography.

In this paper, we extend the general kinetic model to the fixed-bed packed with inert core adsorbents, where intraparticle diffusion, film mass transfer, liquid axial dispersion and the adsorption-desorption rate were taken into account. Under the linear adsorption kinetics, analytical solutions are derived to predict the breakthrough and elution curves, and Height Equivalent to a Theoretical Plate (*HETP*). Moreover, an analytical solution to represent the residence time distribution (RTD) of an inert tracer in the chromatography column is recommended also.

4.2 Mathematical model and analytical and approximate solutions

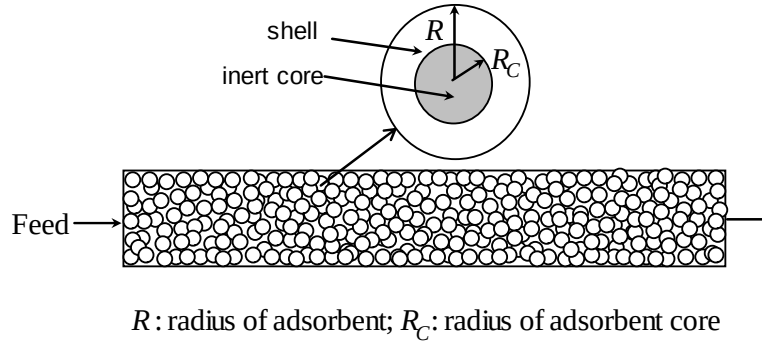


Figure 4.1. Scheme of fixed-bed adsorber and inert core adsorbent.

The schematic diagram of the fixed-bed and the inert core adsorbent is shown in Fig. 4.1. Here we consider an isothermal adsorption column packed with porous inert core particles. At time zero, either a step change or a Dirac input in the concentration of an adsorbate was introduced to a flowing stream. The adsorption column was subjected to liquid axial dispersion, film mass transfer, intraparticle diffusion, and the restricted adsorption-desorption rate. The following assumptions are made: (1) Fick's Law governs both the axial dispersion in the bulk fluid phase within the column and the transport within the adsorbent particles; (2) the axial fluid velocity in the column is constant; (3) the adsorbent consists of a core of uniform thickness on a spherical particle that is inert and impenetrable to solution, and the outer layer undergoes negligible swelling or shrinking

during sorption (Chanda and Rempel, 1999, 2001).

Based on the preceding assumptions, the fixed-bed adsorber can be described by the following set of equations. The material balance equation for bulk phase is

$$D_L \frac{\partial^2 C}{\partial Z^2} - \frac{u}{\varepsilon_B} \frac{\partial C}{\partial Z} - \frac{\partial C}{\partial t} - \frac{(1 - \varepsilon_B)}{\varepsilon_B} \frac{3}{R} \varepsilon_s D_p \left(\frac{\partial c}{\partial r} \right)_{r=R} = 0 \quad (4.1)$$

where D_L is the axial dispersion coefficient, C is the concentration in the fluid phase, u denotes superficial velocity, c is the concentration in the adsorbent pore, D_p is pore diffusivity of adsorbate, ε_B denotes the fraction void volume in the column, hence $(1 - \varepsilon_B)$ denotes the fractional volume taken up by the solid phase, ε_s is the shell porosity, Z is the axial distance from column entrance, r is the radial distance of the adsorbent, R is the radius of the adsorbent, and t is the time.

The mass balance in the adsorbent shell is

$$\varepsilon_s \frac{\partial c}{\partial t} + \rho_s \frac{\partial q}{\partial t} = \varepsilon_s D_p \left(\frac{\partial^2 c}{\partial r^2} + \frac{2}{r} \frac{\partial c}{\partial r} \right) \quad (R_c \leq r \leq R) \quad (4.2)$$

where q is the concentration in adsorbed phase, ρ_s is the density of adsorbent shell, and R_c is the radius of adsorbent inert core.

For reversible adsorption, the linear adsorption kinetics is assumed as:

$$\rho_s \frac{\partial q}{\partial t} = k_{ads} \left(c - \frac{q}{K} \right) \quad (4.3)$$

where k_{ads} is the kinetic constant of adsorption, and K is the Henry Equilibrium constant.

Equation (4.3) is a standard Langmuir adsorption/desorption kinetic expression written with the additional assumption that solute loading is sufficiently low at all times so that the number of available sites for adsorption does not change as the solute moves through the column.

The initial and boundary conditions for Eqs (4.1) and (4.2) are

$$C(Z, 0) = 0 \quad (4.4)$$

$$C(0, t = 0) = C^0 t_c \delta(t) \quad (\text{Dirac input}) \quad (4.5a)$$

where the reference concentration is the ratio between the amount injected and the fluid volume in the column, $C^0 = n / \varepsilon_B V$ and $t_c = \varepsilon_B L / u$ is the space time.

$$C(0, t) = C_0 \quad (\text{step input}) \quad (4.5b)$$

$$C(\infty, t) \text{ is limited} \quad (4.6)$$

$$c(r, Z, 0) = 0 \quad (4.7)$$

$$q(r, Z, 0) = 0 \quad (4.8)$$

$$\left(\frac{\partial c}{\partial r} \right)_{r=R_c} = 0 \quad (4.9)$$

$$\varepsilon_s D_p \left(\frac{\partial c}{\partial r} \right)_{r=R} = k_f [C - (c)_{r=R}] \quad (4.10)$$

Rearrange the above Eqs. (4.1)-(4.10) in dimensionless form as

$$\frac{1}{Pe} \frac{\partial^2 y}{\partial \zeta^2} - \frac{\partial y}{\partial \zeta} - \theta \frac{\partial y}{\partial \tau} - 3\theta v \left(\frac{\partial x}{\partial \xi} \right)_{\xi=1} = 0 \quad (4.11)$$

$$\frac{\partial x}{\partial \tau} + \xi_m \frac{\partial w}{\partial \tau} = \frac{\partial^2 x}{\partial \xi^2} + \frac{2}{\xi} \frac{\partial x}{\partial \xi} \quad (\xi_c \leq \xi \leq 1) \quad (4.12)$$

$$\frac{\partial w}{\partial \tau} = \psi(x - w) \quad (4.13)$$

$$y(\zeta, 0) = 0 \quad (4.14)$$

$$y(0, \tau) = \delta(\tau) \quad (\text{Dirac input}) \quad (4.15a)$$

$$y(0, \tau) = 1 \quad (\text{Step input}) \quad (4.15b)$$

$$y(\infty, \tau) \text{ is limited} \quad (4.16)$$

$$x(\xi, \zeta, 0) = 0 \quad (4.17)$$

$$w(\xi, \zeta, 0) = 0 \quad (4.18)$$

$$\left(\frac{\partial x}{\partial \xi} \right)_{\xi=\xi_c} = 0 \quad (4.19)$$

$$\left(\frac{\partial x}{\partial \xi} \right)_{\xi=1} = Bi [y - (x)_{\xi=1}] \quad (4.20)$$

The dimensionless variables are

$$x = \frac{c}{C_0}, w = \frac{q}{KC_0}, y = \frac{C}{C_0} \text{ for step input, and } y = \frac{C}{C_0 \tau_c} \text{ for Dirac input } (\tau_c = t_c D_p / R^2)$$

(dimensionless concentrations in adsorbent pore, adsorbed phase and bulk fluid phase)

$$\xi = \frac{r}{R} \quad (\text{dimensionless radial distance})$$

$$\zeta = \frac{Z}{L} \quad (\text{dimensionless axial distance})$$

and the model parameters are

$$\nu = \left(\frac{1 - \varepsilon_B}{\varepsilon_B} \right) \varepsilon_s \quad (\text{porosity ratio})$$

$$\xi_m = \frac{K \rho_s}{\varepsilon_s} \quad (\text{capacity factor})$$

$$Bi = \frac{k_f R}{\varepsilon_s D_p} \quad (\text{Biot number})$$

$$\theta = \frac{\varepsilon_B L D_p}{u R^2} \quad (\text{ratio of time constants for convection in outer fluid and pore diffusion})$$

$$Pe = \frac{u L}{\varepsilon_B D_L} \quad (\text{Peclet number based on adsorber length})$$

$$\tau = \frac{D_p t}{R^2} \quad (\text{dimensionless contact time})$$

$$\psi = \frac{k_{ads} R^2}{D_p K \rho_s} \quad (\text{dimensionless adsorption rate constant})$$

4.2.1 Analytical solution and parabolic-profile approximate solution for breakthrough curves

4.2.1.1. Analytical solution

According to Eq. (4.13), in Laplace-domain we have

$$\bar{w} = \frac{\psi \bar{x}}{p + \psi} \quad (4.21)$$

in which the overhead bar indicates the Laplace transform, p is the Laplace transform parameter.

Combined with Eq. (4.12)

$$\frac{\partial^2 \bar{x}}{\partial \xi^2} + \frac{2}{\xi} \frac{\partial \bar{x}}{\partial \xi} - \lambda^2 \bar{x} = 0 \quad (\xi_c \leq \xi \leq 1) \quad (4.22)$$

where

$$\lambda = \sqrt{\frac{\xi_m \psi p}{\psi + p} + p} \quad (4.23)$$

The solution of Eq. (4.22), taking into account the boundary condition, Eq. (4.19), is

$$\bar{x} = \frac{\sinh[\lambda(\xi - \xi_c)] + \lambda \xi_c \cosh[\lambda(\xi - \xi_c)]}{[\cosh(\lambda \xi_c) - \lambda \xi_c \sinh(\lambda \xi_c)]} \left(\frac{m_1}{\lambda \xi} \right) \quad (4.24)$$

where the integration constant m_1 is calculated from Eq. (4.20) as

$$m_1 = Bi \bar{y} \frac{\lambda [\cosh(\lambda \xi_c) - \lambda \xi_c \sinh(\lambda \xi_c)]}{\lambda (1 + Bi \xi_c - \xi_c) \cosh[\lambda(1 - \xi_c)] + (\lambda^2 \xi_c + Bi - 1) \sinh[\lambda(1 - \xi_c)]} \quad (4.25)$$

Thus,

$$\left(\frac{\partial \bar{x}}{\partial \xi} \right)_{\xi=1} = \frac{\lambda(1 - \xi_c) \coth[\lambda(1 - \xi_c)] + (\lambda^2 \xi_c - 1)}{\lambda(1 + Bi \xi_c - \xi_c) \coth[\lambda(1 - \xi_c)] + (\lambda^2 \xi_c + Bi - 1)} Bi \bar{y} \quad (4.26)$$

Inserting Eq. (4.26) into Eq. (4.11), we have the following equation in Laplace domain:

$$\frac{1}{Pe} \frac{\partial^2 \bar{y}}{\partial \zeta^2} - \frac{\partial \bar{y}}{\partial \zeta} - \theta [p + 3\nu G(p)] \bar{y} = 0 \quad (4.27)$$

where

$$G(p) = \frac{Bi Y_D(p)}{Bi + Y_D(p)} \quad (4.28)$$

$$Y_D(p) = \frac{\lambda \coth[\lambda(1 - \xi_c)] + \lambda^2 \xi_c}{\lambda \xi_c \coth[\lambda(1 - \xi_c)] + 1} - 1 \quad (4.29)$$

in which $Y_D(p)$ is the particle transfer function.

The solution in Laplace-domain is

$$\bar{y} = \frac{1}{p} \exp(r_1 \zeta) \quad (4.30)$$

where the transfer function of the system relating outlet and inlet adsorbate concentration in the Laplace domain is

$$r_1 = \frac{Pe}{2} - \sqrt{\frac{1}{4} Pe^2 + \theta Pe[p + 3vG(p)]} \quad (4.31)$$

Finally, we have the analytical solution to predict the breakthrough curve as follows

$$y_B(\tau) = \frac{1}{2} + \frac{1}{\pi} \int_0^\infty \exp\left(\frac{Pe\zeta}{2} - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} + a}{2}}\right) \sin\left(\beta\tau - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} - a}{2}}\right) \frac{d\beta}{\beta} \quad (4.32)$$

with

$$a = Pe\left(\frac{Pe}{4} + 3\theta v I_1\right) \quad (4.33)$$

$$b = \theta Pe(\beta + 3v I_2) \quad (4.34)$$

$$I_1 = \frac{Bi^2 I_3 + Bi(I_3^2 + I_4^2)}{(Bi + I_3)^2 + I_4^2} \quad (4.35)$$

$$I_2 = \frac{Bi^2 I_4}{(Bi + I_3)^2 + I_4^2} \quad (4.36)$$

$$I_3 = \frac{\xi_C(\phi_1^2 + \phi_2^2)[(H_1^2 + H_2^2) + \xi_C(\phi_1 H_1 - \phi_2 H_2)] + \xi_C(\phi_1^2 - \phi_2^2) + (\phi_1 H_1 + \phi_2 H_2)}{\xi_C^2(\phi_1^2 + \phi_2^2)(H_1^2 + H_2^2) + 2\xi_C(\phi_1 H_1 + \phi_2 H_2) + 1} - 1 \quad (4.37)$$

$$I_4 = \frac{\xi_C^2(\phi_1^2 + \phi_2^2)(\phi_2 H_1 + \phi_1 H_2) + (\phi_2 H_1 - \phi_1 H_2) + 2\xi_C \phi_1 \phi_2}{\xi_C^2(\phi_1^2 + \phi_2^2)(H_1^2 + H_2^2) + 2\xi_C(\phi_1 H_1 + \phi_2 H_2) + 1} \quad (4.38)$$

$$H_1 = \frac{\sinh[2\phi_1(1 - \xi_C)]}{\cosh[2\phi_1(1 - \xi_C)] - \cos[2\phi_2(1 - \xi_C)]} \quad (4.39)$$

$$H_2 = \frac{\sin[2\phi_2(1 - \xi_C)]}{\cosh[2\phi_1(1 - \xi_C)] - \cos[2\phi_2(1 - \xi_C)]} \quad (4.40)$$

$$\phi_1 = \sqrt{\frac{\sqrt{c_1^2 + c_2^2} + c_1}{2}} \quad (4.41)$$

$$\phi_2 = \sqrt{\frac{\sqrt{c_1^2 + c_2^2} - c_1}{2}} \quad (4.42)$$

$$c_1 = \frac{\xi_m \psi \beta^2}{\psi^2 + \beta^2} \quad (4.43)$$

$$c_2 = \beta + \frac{\xi_m \psi^2 \beta}{\psi^2 + \beta^2} \quad (4.44)$$

When $\xi_C \rightarrow 0$, the above solutions, Eqs (4.32)-(4.44), become those given by Rasmuson (1981).

If the adsorption rate is much faster than the diffusion rate, i.e., ψ is so large that equilibrium exists at all local positions in the adsorbent, adsorption equilibrium may be assumed, i.e., $x = w$ (Lloyd and Warner, 1990). In this case, Eqs (4.32)-(4.36) are still valid for the exist solution provided that Eqs (4.37)-(4.44) are replaced by the following Equations:

$$I_3 = \phi \frac{2\xi_C \phi [(H_1^2 + H_2^2) + \xi_C \phi (H_1 - H_2)] + (H_1 + H_2)}{2\xi_C^2 \phi^2 (H_1^2 + H_2^2) + 2\xi_C \phi (H_1 + H_2) + 1} - 1 \quad (4.45)$$

$$I_4 = \phi \frac{2\xi_C^2 \phi^2 (H_1 + H_2) + (H_1 - H_2) + 2\xi_C \phi}{2\xi_C^2 \phi^2 (H_1^2 + H_2^2) + 2\xi_C \phi (H_1 + H_2) + 1} \quad (4.46)$$

$$H_1 = \frac{\sinh[2\phi(1 - \xi_C)]}{\cosh[2\phi(1 - \xi_C)] - \cos[2\phi(1 - \xi_C)]} \quad (4.47)$$

$$H_2 = \frac{\sin[2\phi(1 - \xi_C)]}{\cosh[2\phi(1 - \xi_C)] - \cos[2\phi(1 - \xi_C)]} \quad (4.48)$$

$$\phi = \sqrt{\frac{(1 + \xi_m)\beta}{2}} \quad (4.49)$$

4.2.1.2. Parabolic-profile approximate solution

In order to reduce the complexity of the exact analytical solution, parabolic concentration

profile in the adsorbent has been assumed (Liaw et al., 1979; Rice, 1982; Xiu, 1996; Xiu et al., 1997). In this work, a possible approximation for the parabolic profile in the adsorbent shell is

$$x(\xi, \zeta, \tau) = d_1(\zeta, \tau) + d_2(\zeta, \tau)\xi^2 \quad (\xi_C \leq \xi \leq 1) \quad (4.50)$$

Applying the boundary conditions, along with the definition of volume-averaged x , $(x)_{av}$, one obtains

$$\left(\frac{\partial x}{\partial \xi} \right)_{\xi=1} = \frac{1}{I_5} \left[y - \frac{(x)_{av}}{(1 - \xi_C^3)} \right] \quad (4.51)$$

where

$$I_5 = \frac{5(1 - \xi_C^3) - 3(1 - \xi_C^5)}{10(1 - \xi_C^3)} + \frac{1}{Bi} \quad (4.52)$$

When the governing diffusion Equation (4.12) is volume-averaged, we have

$$\frac{\partial (x)_{av}}{\partial \tau} + \xi_m \frac{\partial (w)_{av}}{\partial \tau} = \frac{3}{I_5} \left[y - \frac{(x)_{av}}{(1 - \xi_C^3)} \right] \quad (\xi_C \leq \xi \leq 1) \quad (4.53)$$

Combined with Eq. (4.13) in Laplace domain, finally, we have the same solution as Eqs (4.30) and (4.31), in which

$$G(p) = \frac{1}{I_5} \left[1 - \frac{1}{1 + \left(\frac{\xi_m \psi p}{\psi + p} + p \right) \frac{(1 - \xi_C^3) I_5}{3}} \right] \quad (4.54)$$

The time-domain solution has the same form as the general solution, Eqs (4.32)-(4.34), in which I_1 , I_2 , I_3 and I_4 should be replaced by the following Equations:

$$I_1 = \frac{1}{I_5} \left(1 - \frac{I_3}{I_3^2 + I_4^2} \right) \quad (4.55)$$

$$I_2 = \frac{1}{I_5} \left(\frac{I_4}{I_3^2 + I_4^2} \right) \quad (4.56)$$

$$I_3 = 1 + \frac{(1 - \xi_C^3) I_5}{3} \left(\frac{\xi_m \psi \beta^2}{\psi^2 + \beta^2} \right) \quad (4.57)$$

$$I_4 = \frac{(1 - \xi_C^3)I_5}{3} \left(\beta + \frac{\xi_m \psi^2 \beta}{\psi^2 + \beta^2} \right) \quad (4.58)$$

4.2.2. Analytical and approximate solutions for peak elution curves

The analytical solution for Dirac input mode, i.e., the elution curve $y_\delta(\tau)$, is obtained by using the differential of the analytical solution for the breakthrough curve, $y_B(\tau)$, as

$$y_\delta(\tau) = \frac{dy_B(\tau)}{d\tau} \quad (4.59)$$

Based on Leibnitz's rule for differential of integrals, we have the analytical solution and the parabolic-profile approximate solution for peak elution curve $y_\delta(\tau)$ as

$$y_\delta(\tau) = \frac{1}{\pi} \int_0^\infty \exp \left[\frac{Pe\zeta}{2} - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} + a}{2}} \right] \cos \left(\beta\tau - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} - a}{2}} \right) d\beta \quad (4.60)$$

where a and b calculated by Eq. (4.33)-(4.44) for analytical solution and a and b calculated by Eq. (4.33), (4.34), (4.55)-(4.58), and (4.52) for parabolic-profile approximate solution .

This analytical solution also can be obtained based on the Laplace transform method (Haynes, 1975).

4.2.3 Moments and height equivalent to a theoretical plate (HETP)

The solution in Laplace domain of the system of equations of the general kinetic model, Eqs (4.11)-(4.20), provides equations relating to the first absolute moment and the second central moment of elution curves at the exit (where $\zeta = 1$) to characterize the retention equilibrium and the mass transfer kinetics, respectively. In this work, the expressions of the first absolute moment, μ_1 , and the second central moment, μ_2' , for Dirac input mode can be obtained as

$$\mu_1 = \theta \left[1 + \nu (1 - \xi_C^3) (1 + \xi_m) \right] \quad (4.61)$$

$$\mu_2' = 2\theta\nu(1-\xi_C^3) \left[\frac{(1+\xi_m)^2(1-\xi_C^3)}{3} \left(\frac{1}{\Theta} + \frac{1}{Bi} \right) + \frac{\xi_m}{\psi} \right] + \frac{2\theta^2 [1 + \nu(1-\xi_C^3)(1+\xi_m)]^2}{Pe} \quad (4.62)$$

in which

$$\frac{1}{\Theta} = \frac{(1-\xi_C)^2}{(1-\xi_C^3)} \left[\xi_C + \frac{(1-\xi_C)^3}{5(1-\xi_C^3)} \right] \quad (4.63)$$

The parameter, $1/\Theta$, represents the decrease of the intraparticle diffusion resistance when using inert core adsorbent relative to the case of conventional adsorbent.

The theoretical plate number N_T can be calculated using the first absolute moment μ_1 and the second central moment μ_2' ,

$$N_T = \frac{\mu_1^2}{\mu_2'} \quad (4.64)$$

and height equivalent to a theoretical plate ($HETP$) is defined as

$$HETP = \frac{L}{N_T} \quad (4.65)$$

Here, we use the dimensionless height equivalent to a theoretical plate ($HETP^*$) as

$$HETP^* = \frac{HETP}{L} = \frac{2\nu(1-\xi_C^3)}{\theta[1 + \nu(1-\xi_C^3)(1+\xi_m)]^2} \left[\frac{(1+\xi_m)^2(1-\xi_C^3)}{3} \left(\frac{1}{\Theta} + \frac{1}{Bi} \right) + \frac{\xi_m}{\psi} \right] + \frac{2}{Pe} \quad (4.66)$$

$$HETP^* = \frac{2\nu(1-\xi_C^3)}{[1 + \nu(1-\xi_C^3)(1+\xi_m)]^2} \left(\frac{\varepsilon_s u R^2}{\varepsilon_B L} \right) \left[\frac{(1+\xi_m)^2(1-\xi_C^3)}{3} \left(\frac{1}{\Theta} \frac{1}{\varepsilon_s D_p} + \frac{1}{k_f R} \right) + \frac{\xi_m^2}{R^2} \frac{1}{k_{ads}} \right] + \frac{2\varepsilon_B D_L}{uL} \quad (4.66a)$$

Equation (4.66a) shows that $HETP^*$ is the sum of the independent contributions of liquid axial dispersion, film mass transfer resistance, pore diffusion resistance, and the restricted adsorption-desorption rate.

4.2.4 Analytical solution for residence time distribution of an inert tracer with Dirac input

Residence time distribution (RTD) method usually is used to estimate liquid axial

dispersion coefficient in packed column, also intraparticle diffusivity can be measured by RTD method (Boyer and Hsu, 1992). For an inert tracer, there exists no adsorption on porous particles, so the previously analytical solution for Dirac input mode may be reduced as Eq. (4.60) with Eq. (4.33)-(4.36) and (4.45)-(4.48), but Eq. (4.67) instead of Eq. (4.49), to predict the RTD curve of an inert tracer with Dirac input mode in fixed beds.

$$\phi = \sqrt{\frac{\beta}{2}} \quad (4.67)$$

4.3. Results and Discussion

The integrand of Eq. (4.32) and Eq. (4.60) for both the analytical solution and the parabolic-profile approximate solution is the product of an exponential decaying function and a periodic sine function. Integration should be performed over each half-period of the sine wave that was developed by Rasmuson (1985). In the following discussion, we selected $\xi_m = 10^3$, $\nu = 0.5$ and $\zeta = 1$ (the point at which the breakthrough and elution curves are measured) as cases.

4.3.1. Effect of model parameters on breakthrough curves

The analytical solution, Eq. (4.32) with Eqs (4.33)-(4.44), is a general solution by which the breakthrough curves for inert core adsorbent ($\xi_c > 0$) and conventional adsorbent ($\xi_c = 0$) can be predicted under the linear adsorption kinetics. The effect of the parameter ξ_c (characterizing the inert core adsorbent) on the breakthrough curves is shown in Fig. 4.2. The breakthrough points for the column packed with the inert core adsorbent occur earlier than that packed with the conventional adsorbent. The shape of the breakthrough curves for the adsorbent with inert core will become sharper.

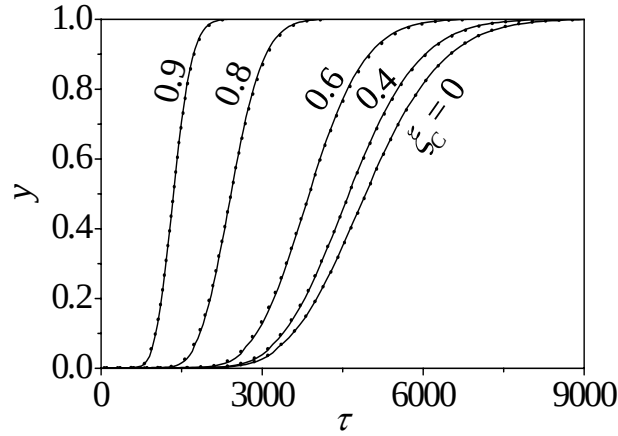


Fig. 4.2. Effect of ξ_c on $y-\tau$ breakthrough curves at $\theta=10$, $Pe=10^2$, $Bi=10^2$, and $\psi=10^{-1}$. Solid lines: Analytical solution (Eq. (4.32) with Eqs (4.33)-(4.44)); dot lines: Parabolic approximation (Eq. (4.32) with Eqs (4.33), (4.34) and (4.55)-(4.58)).

The effects of the axial dispersion, external mass-transfer resistance, intraparticle diffusion resistance and the adsorption-desorption rate on the breakthrough curves for the inert core adsorbent can be predicted by the exact analytical solution; the typical results are demonstrated in Figs 4.3 to 4.5. The model parameter Pe is usually below 10^3 in fixed-bed chromatography; the effect of the axial dispersion on the breakthrough curves is not negligible as shown in Fig. 4.3. The parameter Bi stands for the ratio of the internal resistance to the external resistance for mass transfer in the adsorbent; the effect of Bi on the breakthrough curves is also not negligible at the given conditions as shown in Fig. 4.4. The global adsorption rate in adsorbents is controlled by the combined effects of diffusion through the shell pore along with the local adsorption rate. The parameter ψ stands for the relative importance of the rate of adsorption and the rate of the pore diffusion. The effect of ψ on the breakthrough curves is shown in Fig. 4.5; it is evident that the effect of ψ can be neglected only when $\psi \geq 10^{-1}$.

Figs 4.2 to 4.5 also present the breakthrough curves predicted by the simple parabolic approximate solution; the accuracy of the approximate solution is acceptable, except for very short column where the intraparticle diffusion resistance is dominant, the deviation between

the analytical solution and the parabolic-profile approximate solution becomes significant, as shown in Fig. 4.6.

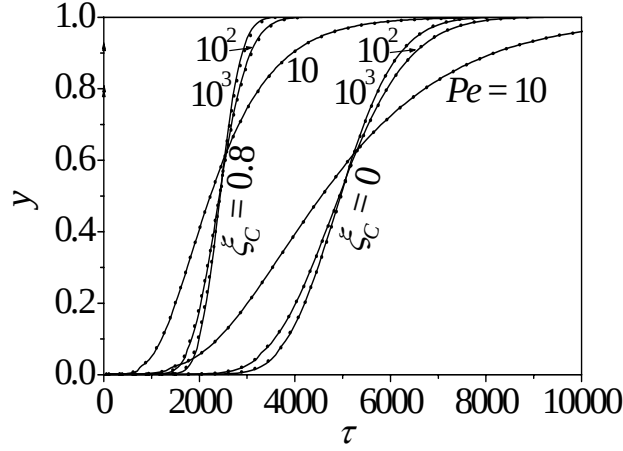


Fig. 4.3. Effects of Pe on $y-\tau$ breakthrough curves for inert core adsorbent at $\theta = 10$, $Bi = 10^2$ and $\psi = 10^{-1}$. Solid lines: Analytical solution (Eq. (4.32) with Eqs (4.33)-(4.44)); dot lines: Parabolic approximation (Eq. (4.32) with Eqs (4.33), (4.34) and (4.55)-(4.58)).

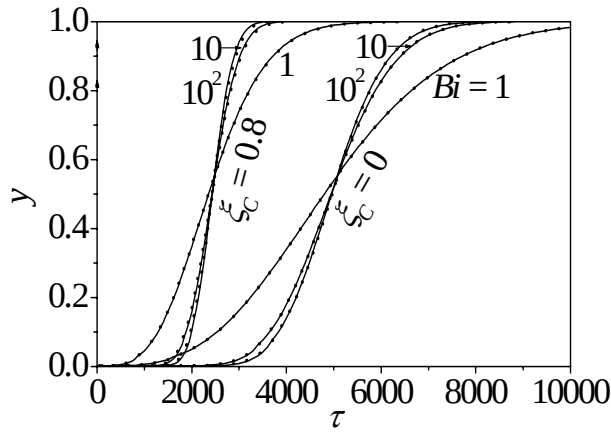


Fig. 4.4. Effects of Bi on $y-\tau$ breakthrough curves for inert core adsorbent at $\theta = 10$, $Pe = 10^3$ and $\psi = 10^{-1}$. Solid lines: Analytical solution (Eq. (4.32) with Eqs (4.33)-(4.44)); dot lines: Parabolic approximation (Eq. (4.32) with Eqs (4.33), (4.34) and (4.55)-(4.58)).

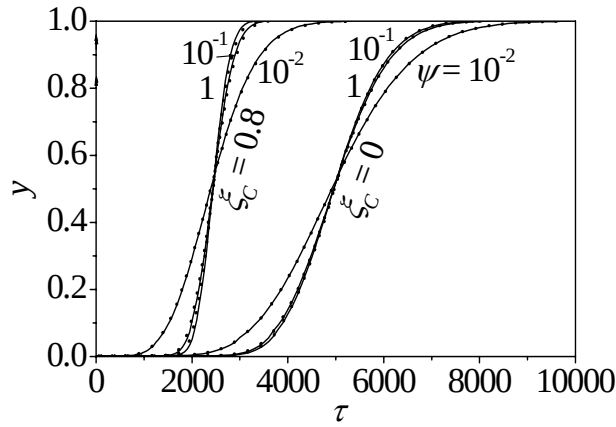


Fig. 4.5. Effect of ψ on $y-\tau$ breakthrough curves for inert core adsorbent at $\theta=10$, $Pe=10^3$ and $Bi=10^2$. Solid lines: Analytical solution (Eq. (4.32) with Eqs (4.33)-(4.44)); dot lines: Parabolic approximation (Eq. (4.32) with Eqs (4.33), (4.34) and (4.55)-(4.58)).

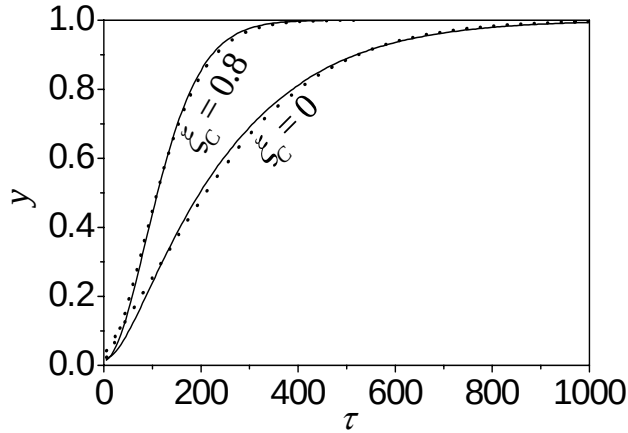


Fig. 4.6. Effect of ξ_C on $y-\tau$ breakthrough curves for short column ($\theta=0.5$) at $Pe=10^3$, $Bi=10^2$ and $\psi=10^{-1}$. Solid lines: Analytical solution (Eq. (4.32) with Eqs (4.33)-(4.44)); dot lines: Parabolic approximation (Eq. (4.32) with Eqs (4.33), (4.34) and (4.55)-(4.58)).

4.3.2. Effect of model parameters on peak elution curves

Figs. 4.7-4.9 demonstrate typical elution curves of chromatography columns packed with inert core adsorbent ($\xi_C = 0.8$) and conventional adsorbent ($\xi_C = 0$) under the restricted adsorption-desorption rate, calculated by analytical solution Eq. (4.60) with Eqs (4.33)-(4.44). It is evident that elution curves for inert core adsorbent are very different from those for conventional adsorbent, the retention time occurs early and the peak is sharper for

the inert core adsorbent as a result of the less loading capacity and the shorter path of intraparticle diffusion, respectively. The model parameters, Pe , Bi and ψ , have significant effects on elution curves, the peak height, peak width and peak asymmetry vary significantly for various Pe , Bi and ψ values.

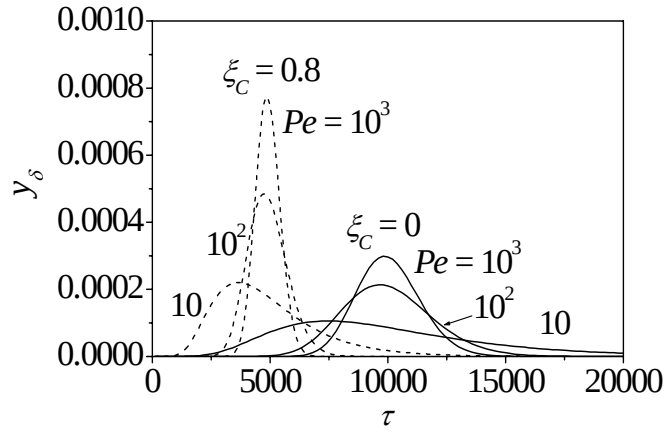


Fig. 4.7. Effect of Pe on $y-\tau$ elution curves for $\theta = 20$, $Bi = 10^2$ and $\psi = 0.1$. Solid lines: $\xi_C = 0$; dashed lines: $\xi_C = 0.8$; calculated by analytical solution Eq. (4.60) with Eqs (4.33)-(4.44).

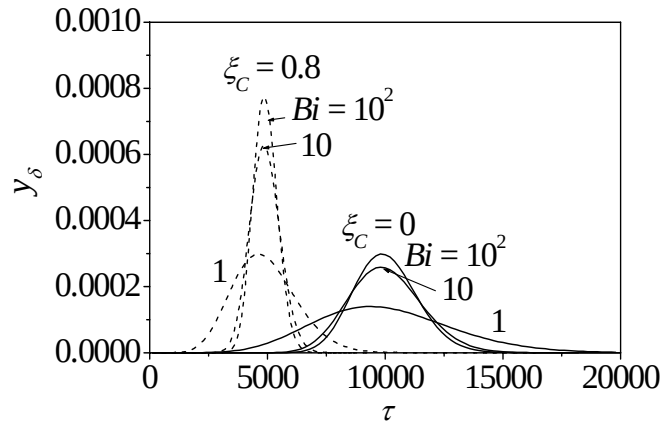


Fig. 4.8. Effect of Bi on $y-\tau$ elution curves for $\theta = 20$, $Pe = 10^3$ and $\psi = 0.1$. Solid lines: $\xi_C = 0$; dashed lines: $\xi_C = 0.8$; calculated by analytical solution Eq. (4.60) with Eqs (4.33)-(4.44).

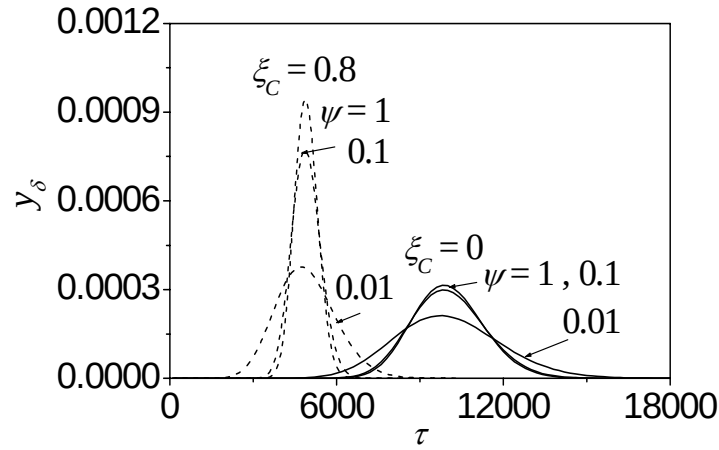


Fig. 4.9. Effect of ψ on $y-\tau$ elution curves for $\theta = 20$, $Bi = 10^2$ and $Pe = 10^3$. Solid lines: $\xi_C = 0$; dashed lines: $\xi_C = 0.8$; calculated by analytical solution Eq. (4.60) with Eqs (4.33)-(4.44).

4.3.3. Theoretical plate number and the equivalent height to a theoretical plate

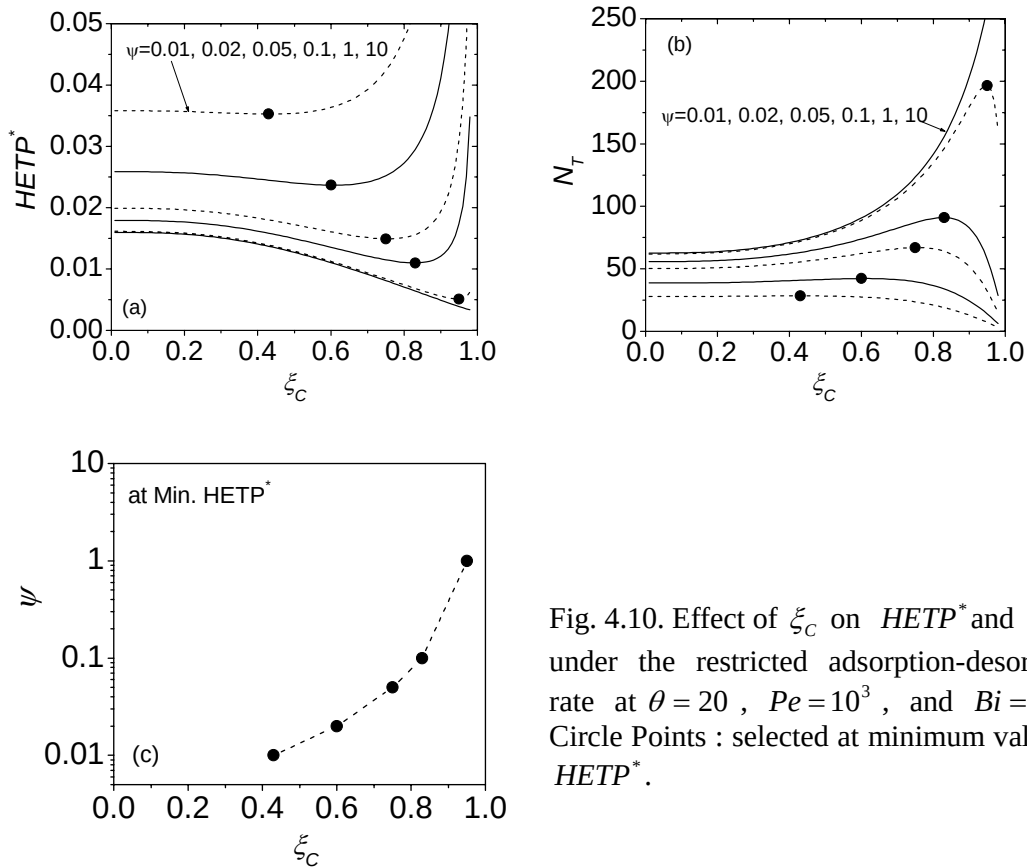


Fig. 4.10. Effect of ξ_C on $HETP^*$ and N_T under the restricted adsorption-desorption rate at $\theta = 20$, $Pe = 10^3$, and $Bi = 10^2$. Circle Points : selected at minimum value of $HETP^*$.

When the adsorption rate is very fast, such as $\psi = 10$, local adsorption equilibrium can be attained immediately on the adsorbed surface of adsorbent; so with the increase of inert core size in adsorbent, the intraparticle diffusion resistance is significantly decreased a result of the shorten of the diffusion path in adsorbents, which will enhance the column efficiency, HETP will decrease also, as shown in Fig 4.10. When the adsorption rate is slow, for small ψ values, instantaneous adsorption equilibrium on the adsorbed surface of adsorbents is not attained. Although the intraparticle diffusion resistance can be decreased significantly with the increase of inert core size, the decrease of the effectively adsorbed surface area in adsorbents (shell volume decrease) will not favor the adsorption of adsorbate with the slow adsorption rate. Therefore, at a given adsorption rate, there exists an optimum inert core size; at this point HETP is minimum and column operation is more efficient, as shown in Figure 4.10.

4.3.4. Residence time distribution

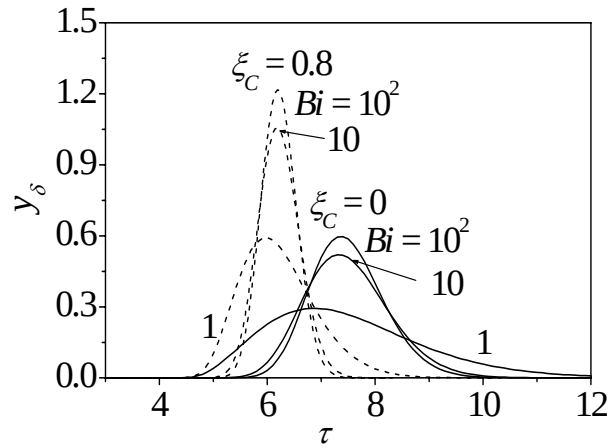


Fig. 4.11. Effect of Bi on $y - \tau$ residence time distribution for $\theta = 5$ and $Pe = 10^3$. Solid lines: $\xi_c = 0$; dashed lines: $\xi_c = 0.8$; calculated by analytical solution (Eq. (4.60) with Eqs (4.33)-(4.36), (4.45)-(4.48) and (4.67)).

Figs 4.11 and 4.12 show the calculated results for the residence time distribution of an inert tracer with Dirac input mode, where liquid axial dispersion, film mass transfer resistance and intraparticle diffusion resistance all are taken into account. The analytical

solution (Eq. (4.60) with Eqs (4.33)-(4.36), (4.45)-(4.48) and (4.67)) can predict RTD curves very well for the chromatography columns packed with inert core adsorbent ($\xi_C = 0.8$) and conventional adsorbent ($\xi_C = 0$). By comparing with experimental RTD curves, either liquid axial dispersion coefficient or intraparticle diffusivity can be estimated by RTD measurement method.

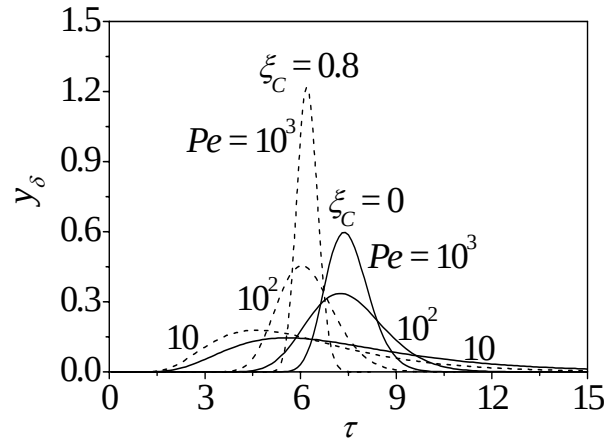


Fig. 4.12. Effect of Pe on $y - \tau$ residence time distribution for $\theta = 5$ and $Bi = 10^2$. Solid lines: $\xi_C = 0$; dashed lines: $\xi_C = 0.8$; calculated by analytical solution (Eq. (4.60) with Eqs (4.33)-(4.36), (4.45)-(4.48) and (4.67)).

4.4. Conclusions

The analytical solution derived based on the general kinetic model can predict the breakthrough and elution curves for the inert core adsorbent where the linear adsorption kinetics coupled with the axial dispersion, external film diffusion resistance, and intraparticle diffusion resistance. The simple parabolic approximate solution is acceptable for prediction of the breakthrough and elution curves.

The equivalent height to a theoretical plate can be expressed as the sum of the independent contributions of liquid axial dispersion, film mass-transfer resistance, intraparticle diffusion resistance and the adsorption-desorption rate at linear adsorption kinetics. The parameter, $1/\Theta$, represents the decrease of the intraparticle diffusion

resistance when using inert core adsorbent relative to the case of conventional adsorbent. At a given adsorption-desorption rate, there exists an optimum inert core size; at this point *HETP* is minimum and column operation is more efficient.

The analytical solution (Eq. (4.60) with Eqs (4.33)-(4.36), (4.45)-(4.48) and (4.67)), in which the liquid axial dispersion, intraparticle diffusion and film mass transfer were taken into account, can predict the RTD curve of an inert tracer with Dirac input mode in the chromatography column packed with inert core adsorbent.

Notation

Bi	Biot number $(= k_f R / \varepsilon_s D_p)$
C	concentration in bulk phase, $\text{kg}\cdot\text{m}^{-3}$
C_0	inlet concentration, $\text{kg}\cdot\text{m}^{-3}$
D_L	longitudinal dispersion coefficient, m^2/s
D_m	molecular diffusivity in mobile phase, m^2/s
D_p	effective pore diffusivity, $\text{m}^2\cdot\text{s}^{-1}$
K	Henry equilibrium constant, $\text{m}^3\cdot\text{kg}^{-1}$
k_{ads}	kinetic constant for adsorption, s^{-1}
k_f	external mass transfer coefficient, $\text{m}\cdot\text{s}^{-1}$
L	length of column, m
N_T	theoretical plate number
p	Laplace transform parameter
Pe	Peclet number based on adsorber length $(= uL / \varepsilon_B D_L)$
q	adsorbed concentration in adsorbent shell, $\text{kg}\cdot\text{kg}^{-1}$
r	radial distance, m
R	radius of supports, m

R_C	radius of core, m
t	time, s
u_0	superficial velocity in the interparticle space in the column, m/s
w	dimensionless amount adsorbed $(= q / KC_0)$
x	dimensionless concentrations $(= c / C_0)$
y	dimensionless concentrations $(= C / C_0)$
Z	axial distance from column entrance, m

Greek letters

β	integration parameter
ϕ	$= \sqrt{\beta/2}$
γ	tortuosity factor
λ	factor in eddy diffusivity
μ_1	the first absolute moment
μ_2'	the second central moment
θ	ratio of time constants for convection in outer fluid and pore diffusion $(= LD_p \varepsilon_B / uR^2)$
ρ_s	density of adsorbent shell, $\text{kg} \cdot \text{m}^{-3}$
ξ	dimensionless radial distance $(= r / R)$
ξ_C	dimensionless radius of adsorbent inert core $(= R_C / R)$
ξ_m	adsorption capacity factor $(= K\rho_s / \varepsilon_s)$
τ	dimensionless contact time $(= tD_p / R^2)$
ν	distribution ratio $[= (1 - \varepsilon_B) \varepsilon_s / \varepsilon_B]$

- ψ dimensionless adsorption rate constant $(= k_{ads} R^2 / D_p K \rho_s)$
- ζ dimensionless axial distance from column entrance $(= Z/L)$

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5. A 3-zone model for protein adsorption kinetics in expanded beds ^{*}

The adsorption behavior of expanded beds is more complex than that of fixed beds, since the adsorbent particle size, local bed voidage and liquid axial dispersion will vary axially with expanded height. Models applicable to fixed beds maybe not adequately describe the hydrodynamic and adsorption behavior in expanded beds. In this chapter, a 3-zone model is developed, in which the model equations are written for the bottom zone, middle zone, and top zone of the column, respectively, and the model parameters, such as the adsorbent particle diameter, bed voidage and liquid axial dispersion coefficient, are zonal values. In-bed breakthrough curves are predicted by the 3-zone model, and tested against literature data for lysozyme adsorption on Streamline SP in an expanded bed.

Model parametric sensitivity is analyzed. The effects of film mass transfer resistance, liquid axial dispersion and adsorbent axial dispersion on the breakthrough curves are weaker than that of protein intraparticle diffusion resistance for stable expanded beds. Adsorbent particle size axial distribution and bed voidage axial variation significantly affect in-bed breakthrough curves, therefore, model parameters should not be assigned uniform values over the whole column; instead the model should account for the adsorbent particle size axial distribution and bed voidage axial variation.

^{*}This chapter is based on the paper by Li, P., Xiu, G. H. and Rodrigues, A. E., “A 3-zone model for protein adsorption kinetics in expanded beds”, *Chemical Engineering Science*, 59, 3837-3847, 2004.

5.1. Introduction

Expanded bed adsorption technology has been widely applied to capture proteins directly from crude feedstocks, such as, *E. coli* homogenate, yeast, fermentation, mammalian cell culture, milk, animal tissue extracts, and other unclarified feedstocks, and various applications have been reported from lab-scale to pilot-plant and large-scale production, (Chase, 1994; Hjorth, 1997; Thommes, 1996; Ujam et al., 2003; Clemmitt and Chase, 2002; Smith et al., 2002; Bai and Glatz, 2003; Anspach et al., 1999). The understanding of hydrodynamic characteristics and adsorption kinetics in expanded beds plays an important role in the design, scale-up, control, and optimization of an expanded bed adsorption unit.

With specially designed for adsorbents and columns, the adsorption behavior of the expanded bed is comparable to that of the fixed bed (Chase, 1994), but there still exists some differences between the expanded bed and the fixed bed, namely: i) the specially designed adsorbent particles have a wide size distribution (the smaller and lighter particles move to positions at the top of the expanded bed, the larger and heavier particles to the bottom, resulting in a stable expansion); ii) bed voidage also varies axially along the length of the expanded bed (as more adsorbent is present at the bottom of the column, a low bed voidage zone is formed; by contrast, at the top zone of the column there is a smaller amount of adsorbent, and a higher bed voidage zone is formed); iii) the liquid axial dispersion in expanded beds is significantly higher than in fixed beds, especially at the bottom of the expanded bed column; and iv) due to the fluidized nature of the expanded bed, adsorbent axial dispersion occurs. Therefore, the hydrodynamics and adsorption kinetics in expanded beds are more complex than in fixed beds, and models available for fixed beds may be not adequate to describe the hydrodynamic and adsorption behavior in expanded beds.

In order to obtain a better understanding of hydrodynamic and adsorption kinetics in expanded beds, in-bed monitoring technology was developed so that samples of liquid or adsorbent particles could be withdrawn and measured from the column at various heights and radial positions (Bruce and Chase, 2001; Willoughby et al., 2000a, b; Yun et al, 2004). These authors showed that adsorbent particle size decreases along the axial height. Using residence time distribution studies, they also showed that bed voidage increases with increased axial

height in column and observed considerable liquid axial dispersion. More significantly, when in-bed breakthrough curves were measured at the bottom, the middle and the top of the column, broader breakthrough curves were observed at the bottom of the column, whereas, at the top of the column, steeper breakthrough curves had formed (Bruce et al. 1999; Bruce and Chase, 2001). Usually, breakthrough curves are considered to characterize the adsorption behavior in expanded beds, accounting for the effects of the adsorption equilibrium, intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion, adsorbent particle axial dispersion, particle size and bed voidage. Therefore, by analyzing in-bed breakthrough curves, one can acquire a good understanding about adsorption kinetics in expanded beds.

Wright and Glasser (2001) developed a mathematical model to predict the breakthrough curve at the exit of the column for protein adsorption in a fluidized bed, where intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion and adsorbent particle axial dispersion were all taken into account; the particle size, bed voidage and liquid axial dispersion coefficient values were averaged over the whole column. Recently, Chen et al. (2003) and Tong et al. (2002) used this model for expanded bed adsorption to predict the breakthrough curves at the exit of the column. Tong et al. (2003) modified this model by taking into account the axial particle size distribution. Following their experimental research using in-bed monitoring in expanded beds, Bruce and Chase (2002) predicted the in-bed breakthrough curves using the mathematical model for fixed beds suggested by Wiblin et al. (1995). In the simulation, they modified the model by taking into account the bed voidage and liquid axial dispersion coefficient axial variation, but the effect of the adsorbent particle axial distribution on in-bed breakthrough curves was neglected.

When capturing proteins in an expanded bed with a high flow velocity, the slow diffusion rate of proteins results in high intraparticle diffusion resistance, significantly affecting the breakthrough curves. It is argued that, in this case, the particle size, characterizing the diffusion path in the adsorbent particles, should have a significant effect on the breakthrough curves (Karau et al., 1997). Therefore, simulation results should be improved when the particle size axial distribution, together with bed voidage and the liquid dispersion axial variations all are taken into account in the mathematical model.

In this chapter, we derive a 3-zone mathematical model (bottom zone, middle zone, and top zone of the column), and adopt zonal parameters for adsorbent particle size, bed voidage and liquid axial dispersion coefficient. In-bed breakthrough curves from literature (Bruce and Chase, 2001) are compared to those predicted by the 3-zone model. The effects of intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion and adsorbent axial dispersion on in-bed breakthrough curves are then analyzed by parametric sensitivity.

5.2. Mathematical model

5.2.1 Zone division of the column

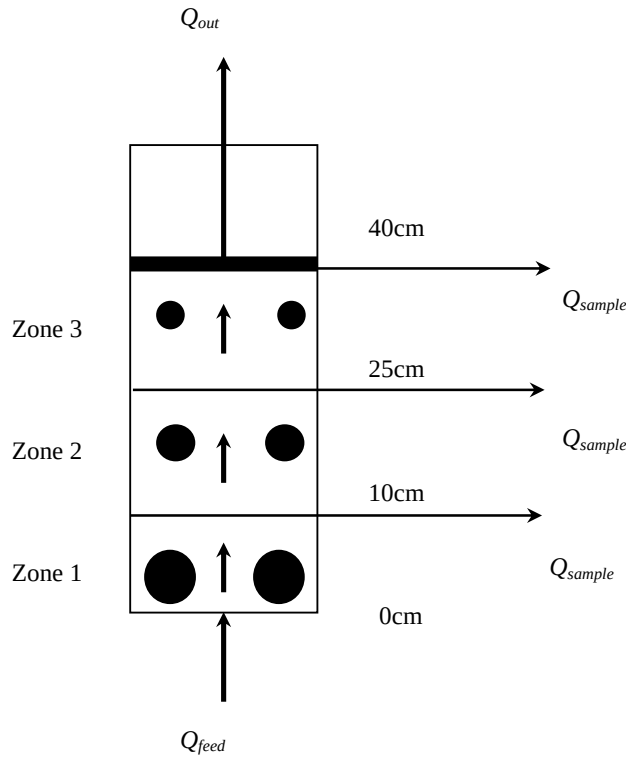


Fig. 5.1. Expanded bed with three zones and flow configuration in Bruce and Chase experimental system (2001). $Q_{feed} = 60.2 \text{ ml/min}$, $Q_{out} = 58.9 \text{ ml/min}$, $Q_{sample} = 1.3/3.0 \text{ ml/min}$.

The expanded bed is divided into three zones, as shown in Fig. 5.1. Zone 1: at the bottom of the column, where the adsorbent particle size is big, the bed voidage is small, and there exists significant liquid axial dispersion. Zone 2: at the middle of the column, where the adsorbent

particle size becomes smaller, the bed voidage increases, and the liquid axial dispersion become weaker than that at the bottom of the column. Zone 3: at the top of the column, where the adsorbent particle size is small, bed voidage is high, and the liquid axial dispersion is smallest. Fig. 5.1 also shows the flow configuration for in-bed monitoring breakthrough curve reported by Bruce and Chase (2001). We simulate these in-bed breakthrough curves using the 3-zone model described as follows.

5.2.2 Model development

The model equations are written for each zone (the bottom zone, the middle zone and the top zone of the column), and intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion and adsorbent axial dispersion all are taken into account. In the model equations, the adsorbent particle diameter, bed voidage and liquid axial dispersion coefficient can be assigned different values for different zones in order to represent the actual particle size, bed voidage and liquid dispersion axial variations measured in an expanded bed. The boundary conditions between the zones are concentration continuity and flux continuity. The liquid-phase concentration of protein in the adsorbent pores is assumed to be in equilibrium with the adsorbed-phase concentration of protein at any radial position in the adsorbent. The pore diffusion model is used to describe the protein diffusion in adsorbent. Radial concentration gradients in the column are neglected relative to axial concentration gradients, and so the hydrodynamic behavior can be described by the axial dispersion model.

Based on the mathematical model suggested by Wright and Glasser (2001) for a fluidized bed protein adsorption, the material balance equation for bulk liquid phase in each zone ($k = 1, 2, 3$) is

$$D_{Lk} \frac{\partial^2 C_k}{\partial Z^2} - \frac{u_k}{\varepsilon_{Bk}} \frac{\partial C_k}{\partial Z} - \frac{\partial C_k}{\partial t} - \frac{(1 - \varepsilon_{Bk})}{\varepsilon_{Bk}} \frac{3}{R_k} k_{fk} [C_k - (c_k)_{r=R_k}] = 0 \quad (5.1)$$

with the boundary conditions

$$D_{L1} \left(\frac{\partial C_1}{\partial Z} \right)_{Z=0} = \frac{u_1}{\varepsilon_{B1}} [(C_1)_{Z=0} - C_0] \quad (5.1a)$$

$$\left[u_1 C_1 + \varepsilon_{B1} D_{L1} \frac{\partial C_1}{\partial Z} \right]_{Z=L_1^-} = \left[u_2 C_2 + \varepsilon_{B2} D_{L2} \frac{\partial C_2}{\partial Z} \right]_{Z=L_1^+} \quad (5.1b)$$

$$(C_1)_{Z=L_1^-} = (C_2)_{Z=L_1^+} \quad (5.1c)$$

$$\left[u_2 C_2 + \varepsilon_{B2} D_{L2} \frac{\partial C_2}{\partial Z} \right]_{Z=(L_1+L_2)^-} = \left[u_3 C_3 + \varepsilon_{B3} D_{L3} \frac{\partial C_3}{\partial Z} \right]_{Z=(L_1+L_2)^+} \quad (5.1d)$$

$$(C_2)_{Z=(L_1+L_2)^-} = (C_3)_{Z=(L_1+L_2)^+} \quad (5.1e)$$

$$\left(\frac{\partial C_3}{\partial Z} \right)_{Z=L_1+L_2+L_3} = 0 \quad (5.1f)$$

and the initial condition

$$t = 0, C_k(Z, 0) = 0 \quad (5.1g)$$

where D_{Lk} is the liquid axial dispersion coefficient in zone k , C_k is the concentration in the fluid phase, u_k denotes superficial velocity, c_k is the concentration in the adsorbent pore, ε_{Bk} denotes the local bed voidage in zone k , hence $(1 - \varepsilon_{Bk})$ denotes the fractional volume occupied by the solid phase, Z is the axial distance from column entrance, R_k is the radius of the adsorbent in zone k , t is the time, k_{fk} is the film mass transfer coefficient in zone k , L_1 denotes the expanded height in zone 1, L_2 denotes the expanded height in zone 2, and L_3 denotes the expanded height in zone 3. Here, L_1^- and L_1^+ refer to the axial positions immediately before and after the section of the column at $Z = L_1$, respectively.

The mass balance for the adsorbent phase in each zone is described as

$$(1 - \varepsilon_{Bk}) \frac{\partial \bar{q}_k}{\partial t} = D_{Sk} \frac{\partial^2 \bar{q}_k}{\partial Z^2} + (1 - \varepsilon_{Bk}) \frac{3}{R_k} k_{fk} [C_k - (c_k)_{r=R_k}] \quad (5.2)$$

with the boundary conditions

$$\left(\frac{\partial \bar{q}_1}{\partial Z} \right)_{Z=0} = 0 \quad (5.2a)$$

$$D_{s1} \left(\frac{\partial \bar{q}_1}{\partial Z} \right)_{Z=L_1^-} = D_{s2} \left(\frac{\partial \bar{q}_2}{\partial Z} \right)_{Z=L_1^+} \quad (5.2b)$$

$$(\bar{q}_1)_{Z=L_1^-} = (\bar{q}_2)_{Z=L_1^+} \quad (5.2c)$$

$$D_{s2} \left(\frac{\partial \bar{q}_2}{\partial Z} \right)_{Z=(L_1+L_2)^-} = D_{s3} \left(\frac{\partial \bar{q}_3}{\partial Z} \right)_{Z=(L_1+L_2)^+} \quad (5.2d)$$

$$(\bar{q}_2)_{Z=(L_1+L_2)^-} = (\bar{q}_3)_{Z=(L_1+L_2)^+} \quad (5.2e)$$

$$\left(\frac{\partial \bar{q}_3}{\partial Z} \right)_{Z=L_1+L_2+L_3} = 0 \quad (5.2f)$$

and the initial condition

$$t = 0, \bar{q}_k(Z, 0) = 0 \quad (5.2g)$$

where the $\bar{q}_k$ is the average adsorbent phase concentration, and D_{sk} is the adsorbent particle axial dispersion coefficient in zone k in the expanded bed.

The pore diffusion equation in the adsorbent in each zone is described as

$$\varepsilon_p \frac{\partial c_k}{\partial t} + \frac{\partial q_k}{\partial t} = \varepsilon_p D_p \left(\frac{\partial^2 c_k}{\partial r^2} + \frac{2}{r} \frac{\partial c_k}{\partial r} \right) \quad (5.3)$$

with the boundary conditions

$$\varepsilon_p D_p \left(\frac{\partial c_k}{\partial r} \right)_{r=R_k} = \frac{R_k D_{sk}}{3(1 - \varepsilon_{Bk})} \frac{\partial^2 \bar{q}_k}{\partial Z^2} + k_{fk} [C_k - (c_k)_{r=R_k}] \quad (5.3a)$$

$$\left(\frac{\partial c_k}{\partial r} \right)_{r=0} = 0 \quad (5.3b)$$

and the initial condition

$$t = 0, c_k(r, 0) = 0, q_k(r, 0) = 0 \quad (5.3c)$$

where D_p is the intraparticle diffusion coefficient, ε_p is the adsorbent porosity, q_k is the adsorbed concentration in adsorbent, and r is the radial distance inside the adsorbent.

The relationship between q_k and c_k depends on the adsorption equilibrium of the selected experimental system, for example, in the Langmuir isotherm,

$$q_k = \frac{q_m c_k}{k_d + c_k} \quad (5.4)$$

where q_m is the adsorption capacity, and k_d is the dissociation constant.

5.2.3 Model parameters

The liquid axial dispersion coefficient, D_{Lk} , is usually measured experimentally by the residence time distribution method in advance, as described in Bruce and Chase (2001). In this chapter, D_{Lk} values were taken from Bruce and Chase (2001).

The adsorbent axial dispersion coefficient D_{Sk} is estimated by the correlation of Van Der Meer et al. (1984), using experimental values for superficial velocity, u_k , for each expansion zone.

$$D_{Sk} = 0.04u_k^{1.8} \text{ m}^2/\text{s} \quad (5.5)$$

The intraparticle diffusion coefficient, D_p , is usually determined by independently experimental measurement, such as by the methods of batch uptake curves, breakthrough curves et al. In this chapter, D_p value was taken from Bruce and Chase (2001).

The film mass transfer coefficient, k_{fk} is calculated for expanded-bed adsorption as a function of bed voidage using the correlation of Fan et al. (1960)

$$k_{fk} = \frac{D_m}{d_{pk}} \left[2 + 1.5(1 - \varepsilon_{Bk})^{1/2} \text{Re}_p^{1/2} \text{Sc}^{1/3} \right] \quad (5.6)$$

where Re_p is the modified Reynolds number ($= \rho d_{pk} u_k / \mu$), Sc is the Schmidt number ($= \mu / (\rho D_m)$), and D_m is the molecular diffusion coefficient.

The local bed voidages for each zone are experimental values, measured using the mean residence time's method (Bruce and Chase, 2001). In addition, the whole bed voidage, $\bar{\varepsilon}_B$, may be also estimated as a function of the whole expanded bed height (McCabe et al., 1985)

$$\bar{\varepsilon}_B = 1 - \left[(1 - \varepsilon_0) \frac{H_0}{H_{\text{exp}}} \right] \quad (5.7)$$

where ε_0 and H_0 are the settled bed voidage and the settled bed height.

The adsorbent particle diameter in each zone is average values, based on the measured data by withdrawing the particle samples from the different position of the column (Bruce and Chase, 2001).

5.2.4 Numerical method

The model equations are numerically solved by the orthogonal collocation method. In each zone, Eqs 5.1 and 5.2 are discretized at collocation points in the axial direction in the column, and Eq. 5.3 is discretized at collocation points in the particle radial direction, leading to a set of ordinary differential equations with initial values that are integrated in the time domain using Gear's stiff variable step integration routine. This yields concentration curves for the bottom, the middle and the top of the column. In each zone, 15 bed axial collocation points and 16 particle radial collocation points are used in order to obtain a stable numerical solution.

5.3. Results and discussion

5.3.1 Comparison between the experimental data and simulation results

Bruce and Chase (2001) measured the breakthrough curves in three positions of the expanded bed column, the bottom (10cm), the middle (25cm) and the top (40cm). The flow configuration for measuring in-bed breakthrough curves is shown in Fig. 5.1, where a Streamline 50 column was used with 21.2cm settled bed height and 40cm expanded bed height when the flow rate was up to 184cm/h; the adsorbent was Streamline SP and the feed concentration of protein (lysozyme) was 4.7kg/m³. Adsorbent particle diameters were also measured at 10, 25 and 40cm position by withdrawing the particle samples during the bed expansion. The zonal average particle size is calculated by averaging the experimental data at 10cm and 25cm for zone 2, at 25cm and 40cm for zone3, and 0cm and 10 cm for zone 1(the particle diameter at $Z = 0$ was taken as 300 μm due to the lack of experimental data, since the

manufacturer stated Streamline SP resin has particle size distribution from $100\ \mu\text{m}$ to $300\ \mu\text{m}$).

The liquid axial dispersion coefficient and the local bed voidage were measured by the residence time distribution method. These results are also shown in Table 5.1 for zone value (Bruce and Chase, 2001). The liquid axial dispersion coefficient in each zone is estimated from Bodenstein numbers ($Bo = 5.2$ for zone 1, $Bo = 21 - 5.2 = 15.8$ for zone 2, and

$Bo = 44 - 21 = 23$ for zone 3 where $Bo_k = \frac{u_k L_k}{\varepsilon_{Bk} D_{Lk}}$) presented by Bruce and Chase (2001).

The actual breakthrough curves at the bottom (10cm), middle (25cm) and top (40cm) of the column are shown in Fig. 5.2, marked with circle points. Bruce and Chase (2001) reported breakthrough curves as concentration versus liquid volume. In this chapter, we present the breakthrough curves as concentration as a function of time; the time is the liquid volume divided by flow rate.

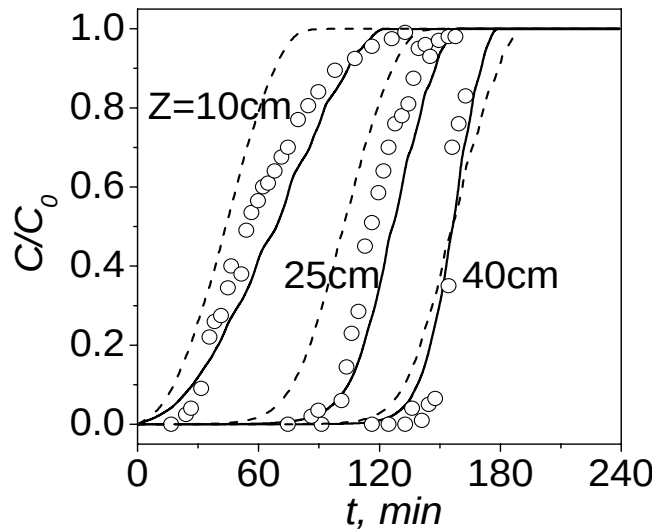


Fig. 5.2. Comparison among the in-bed experimental breakthrough curves and the simulation results. Circle points: experimental data(Bruce & Chase, 2001); solid lines: simulation results with 3-zone model; dashed lines: simulation results with uniform model.

Based on their experimental measurements, the local bed voidage indeed is very different for the bottom zone, the middle zone and the top zone of the column: 0.5 for zone 1, 0.69 for zone 2 to 0.86 for zone 3. Liquid axial dispersion is significantly higher in the bottom zone of the column (D_{L1} up to $19.6 \times 10^{-6} \text{ m}^2/\text{s}$) then decreases gradually to $D_{L2} = 7.0 \times 10^{-6} \text{ m}^2/\text{s}$ for

the middle zone and $D_{L3} = 3.8 \times 10^{-6} \text{ m}^2/\text{s}$ for the top zone of the column. The zonal average adsorbent particle diameter also varies axially, from $258 \mu\text{m}$ for the bottom zone, $194 \mu\text{m}$ for the middle zone to $156 \mu\text{m}$ for the top zone of the column.

Based on the operating conditions and model parameters measured by Bruce and Chase (2001), in-bed breakthrough curves are predicted by the 3-zone model. The zonal parameters used are summarized in Table 5.1. In Fig. 5.2, solid lines represent the simulation results calculated by 3-zone model. We found that the experimental data are well fitted by the simulation results of the 3-zone model at the bottom (10cm) at the middle (25cm) and the top (40cm) of the column. It should be mentioned that in the mathematical model, the isotherm parameter q_m is expressed in kg/m^3 (adsorbent particle volume).

In the past, uniform models, where the model parameters are average values all over the column (average particle diameter, average bed voidage and average liquid axial dispersion coefficient), were used to describe the adsorption kinetics in expanded beds due to their simplicity. In Fig. 5.2, dashed lines represent simulation results calculated by the uniform model. The simulation results are not in agreement with the experimental data, especially at the bottom and the middle of the column.

For example, the actual bed voidage at the bottom zone of the column (0.5) is smaller than the average bed voidage (0.706). So, at the 10cm position of the column, the actual breakthrough curve occurs later relative to the predicted results of the uniform model. A similar situation occurs at the 25cm position of the column. But at the top of the column, the effect of the bed voidage on the breakthrough curves become weaker because it must obey the mass balance over the whole column. However, in the 3-zone model, the zonal bed voidage values are taken into account, and the deviation of in-bed breakthrough curves are avoided at the bottom and the middle of the column.

According to the experimental data, it is found that at the bottom of the column (10cm position) in-bed breakthrough curve is broader due to serious mass transfer resistances and axial dispersion, and at the top of the column (40cm position), the breakthrough curve becomes steeper due to the favorable nature of the adsorption equilibrium and smaller mass transfer

resistance and axial dispersion. In the 3-zone model, higher liquid axial dispersion and intraparticle diffusion resistance are taken into account in the bottom zone by using bigger D_{L1} value and bigger particle diameter d_{p1} value, and, at the top of the column, smaller D_{L3} and d_{p3} values are used to fit the steeper breakthrough curves. However, in the uniform model, averaged particle size and averaged liquid axial dispersion coefficient are used. This means that the mass transfer resistances and axial dispersion are the same in the bottom and top of the column, which leads to almost the same broad breakthrough curves predicted for the bottom, the middle and the top of the column. Therefore, the simulation results are not in agreement with the experimental data, as shown in Fig. 5.2, and will fail to describe the adsorption behavior in the expanded bed.

Table 5.1. The model parameters used in the simulations based on the experimental data of Bruce and Chase (2001)

Parameters for 3-zone model				Parameters for uniform model		
Parameters	Zone 1 0~10cm	Zone 2 10~25cm	Zone 3 25~40cm	Zone 1 0~10cm	Zone 2 10~25cm	Zone 3 25~40cm
u_k , m/s	5.11×10^{-4}	5.07×10^{-4}	5.04×10^{-4}	5.11×10^{-4}	5.07×10^{-4}	5.04×10^{-4}
d_{pk} , μm	258	194	156	196	196	196
ε_{Bk}	0.5	0.69	0.86	0.706	0.706	0.706
D_{Lk}^a , m^2/s	19.6×10^{-6}	7.0×10^{-6}	3.8×10^{-6}	6.5×10^{-6}	6.5×10^{-6}	6.5×10^{-6}
D_{Sk}^b , m^2/s	4.8×10^{-8}	4.7×10^{-8}	4.6×10^{-8}	4.7×10^{-8}	4.7×10^{-8}	4.7×10^{-8}
k_{fk}^b , m/s	4.4×10^{-6}	4.8×10^{-6}	3.8×10^{-6}	4.3×10^{-6}	4.3×10^{-6}	4.3×10^{-6}
D_p , m^2/s	1.0×10^{-10}	1.0×10^{-10}	1.0×10^{-10}	1.0×10^{-10}	1.0×10^{-10}	1.0×10^{-10}
ε_p^c	0.35	0.35	0.35	0.35	0.35	0.35
q_m^a , kg/m ³ (particle)	178	178	178	178	178	178
k_d , kg/m ³	0.05	0.05	0.05	0.05	0.05	0.05
^a calculated from the experimental data of Bruce and Chase (2001). ^b calculated by the correlation of Van Der Meer et al. (1984) and Fan et al. (1960). ^c calculated from the experimental data of Tong et al., 2002 and Wright and Glasser, 2001.						

5.3.2 Parametric sensitivity analysis on the breakthrough curves

In modeling expanded bed adsorption, the effect of intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion and solid axial dispersion on the breakthrough

curves are considered. Because of the coupling of these effects, it is difficult to know which one is dominant. The typical model parameters, characterizing mass transfer resistances and axial dispersion, include the pore diffusivity, D_p , film mass transfer coefficient, k_{fk} , the liquid axial dispersion coefficient, D_{Lk} , and adsorbent axial dispersion coefficient, D_{Sk} . Here we study the individual contribution of each parameter on breakthrough behavior by parametric sensitivity analysis, where an individual effect is changed while the other parameters are fixed at above-mentioned experimental conditions.

5.3.2.1 Effect of intraparticle diffusion coefficient

In Bruce and Chase (2001) experimental system, the recommended value of the pore diffusivity is, $D_p = 1.0 \times 10^{-10} \text{ m}^2/\text{s}$, slightly smaller than the free diffusivity in infinite dilution, $1.12 \times 10^{-10} \text{ m}^2/\text{s}$. Based on the experimental measurement in batch adsorber; the effective pore diffusion coefficient of lysozyme in Streamline SP adsorbent is estimated as $\varepsilon_p D_p = 3.5 \times 10^{-11} \text{ m}^2/\text{s}$ (Tong et al., 2002; Wright and Glasser, 2001), so we can estimate $\varepsilon_p = 0.35$. Here we assumed constant adsorbent porosity ε_p , and changed the pore diffusion coefficient, D_p , increasing and decreasing it by a factor of 2, to see the effect on the breakthrough curves, as shown in Fig. 5.3. Significant changes on in-bed breakthrough curves are observed when changing D_p values, especially for the case by decreasing pore diffusivity from $D_p = 1.0 \times 10^{-10} \text{ m}^2/\text{s}$ to $D_p = 5.0 \times 10^{-11} \text{ m}^2/\text{s}$. Table 2 shows the effect of changes in D_p value on the breakthrough time at $C/C_0 = 0.05$, extracted from Fig. 5.3. Increasing D_p value by a factor of 2, which means decreasing the intraparticle diffusion resistance, the breakthrough time can be increased by 5.2% relatively to that calculated with the experimental D_p value; in contrast, decreasing D_p value by a factor of 2, the breakthrough time significantly decreases (13.5%) due to the limitation of the intraparticle diffusion resistance.

The model protein of the Bruce and Chase experiment (2001), lysozyme, is small protein

molecule (molecule weight, $M_w = 13,930$), thus the diffusion coefficient should be bigger relative to the other macromolecular proteins. Parametric sensitivity analysis shows that even for small protein molecules, the intraparticle diffusion resistance is also higher in expanded bed adsorption. When capturing macromolecular proteins (e.g. BSA, $M_w = 65,400$ with $D_m = 6.15 \times 10^{-11} \text{ m}^2/\text{s}$, molecule diffusivity in diluted aqueous solution; IgG, $M_w = 161,000$ with $D_m = 3.8 \times 10^{-11} \text{ m}^2/\text{s}$; and DNA, $M_w = 400,000$ with $D_m = 1.13 \times 10^{-12} \text{ m}^2/\text{s}$) in expanded beds, the intraparticle diffusion resistance should be more important, and significantly affect the breakthrough curves.

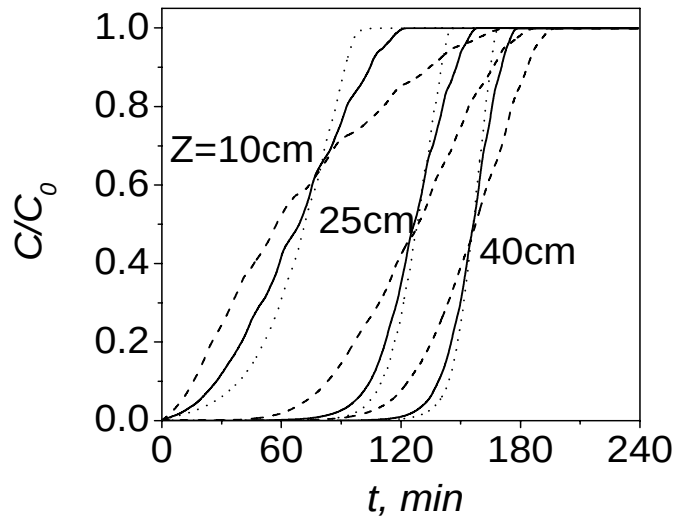


Fig. 5.3. Parametric sensitivity analysis of intraparticle diffusion coefficient to in-bed breakthrough curves in expanded bed. Solid lines: 3-zone model with $D_p = 1.0 \times 10^{-10} \text{ m}^2/\text{s}$; dashed lines: 3-zone model with $D_p = 5.0 \times 10^{-11} \text{ m}^2/\text{s}$; dot lines: 3-zone model with $D_p = 2.0 \times 10^{-10} \text{ m}^2/\text{s}$.

Moreover, in expanded beds, high liquid flow rate is used to expand the bed in order to allow the feed to pass through the column freely without clogging. Here we discuss a case with liquid flow rate as 184cm/h and expanded height about twice its settled height. Liquid flow rates up to 300-400cm/h or more are reported in the literature. For such high liquid flow rates, the effect of intraparticle diffusion resistance on the breakthrough curves becomes more significant.

5.3.2.2 Effect of film mass transfer coefficient

The film mass transfer coefficient k_{fk} is estimated by correlations, such as Eq. (5.6), in the above-mentioned experimental conditions, $k_{fk} = 3.8 \times 10^{-6}$ m/s to 4.8×10^{-6} m/s. In Fig. 5.4, the effect of film mass transfer coefficient on in-bed breakthrough curves is shown by changing k_{fk} by a factor of 2. The effect of increasing k_{fk} value, results in steeper breakthrough curves with a 4% increase in breakthrough time, while decreasing k_{fk} leads to broader breakthrough curves with a 7% decrease in breakthrough time, as shown in Table 5.2. Although the effect of the film mass transfer resistance on in-bed breakthrough curves is weaker than that of the intraparticle diffusion resistance, it is not negligible for protein adsorption in expanded beds.

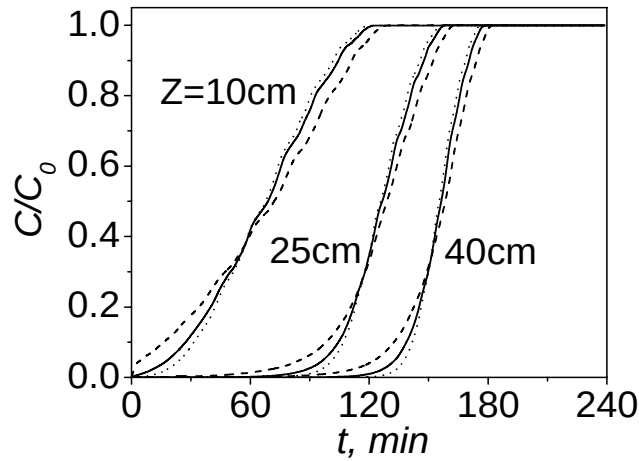


Fig. 5.4. Parametric sensitivity analysis of film mass transfer coefficient to in-bed breakthrough curves in expanded bed. Dot lines: 3-zone model with $k_{f1} = 8.8 \times 10^{-6}$ m/s, $k_{f2} = 9.6 \times 10^{-6}$ m/s, $k_{f3} = 7.6 \times 10^{-6}$ m/s; solid lines: 3-zone model with $k_{f1} = 4.4 \times 10^{-6}$ m/s, $k_{f2} = 4.8 \times 10^{-6}$ m/s, $k_{f3} = 3.8 \times 10^{-6}$ m/s; dashed lines: 3-zone model with $k_{f1} = 2.2 \times 10^{-6}$ m/s, $k_{f2} = 2.4 \times 10^{-6}$ m/s, $k_{f3} = 1.9 \times 10^{-6}$ m/s.

Table 5.2. Parametric sensitivity analysis to assess the impact of changes in D_p , k_{fk} , D_{Lk} , and D_{Sk} on the breakthrough time at $C/C_0 = 0.05$ in expanded beds (40cm position)

Model Parameters	state	Breakthrough time (min)
$D_p = 1.0 \times 10^{-10} \text{ m}^2/\text{s}$	Experimental conditions	132.3
$D_p = 5.0 \times 10^{-11} \text{ m}^2/\text{s}$	$D_p / 2$	114.4 (-13.5%)
$D_p = 2.0 \times 10^{-11} \text{ m}^2/\text{s}$	$2D_p$	139.2 (5.2%)
$k_{f1} = 4.4 \times 10^{-6} \text{ m/s}$, $k_{f2} = 4.8 \times 10^{-6} \text{ m/s}$, $k_{f3} = 3.8 \times 10^{-6} \text{ m/s}$	experimental conditions	132.3
$k_{f1} = 2.2 \times 10^{-6} \text{ m/s}$, $k_{f2} = 2.4 \times 10^{-6} \text{ m/s}$, $k_{f3} = 1.9 \times 10^{-6} \text{ m/s}$	$k_{fk} / 2$	123.1 (-7.0%)
$k_{f1} = 8.8 \times 10^{-6} \text{ m/s}$, $k_{f2} = 9.6 \times 10^{-6} \text{ m/s}$, $k_{f3} = 7.6 \times 10^{-6} \text{ m/s}$	$2k_{fk}$	137.6 (4.0%)
$D_{L1} = 19.6 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L2} = 7.0 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L3} = 3.8 \times 10^{-6} \text{ m}^2/\text{s}$	Experimental conditions	132.3
$D_{L1} = 9.8 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L2} = 3.5 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L3} = 1.9 \times 10^{-6} \text{ m}^2/\text{s}$	$D_{Lk} / 2$	134.2 (1.5%)
$D_{L1} = 39.2 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L2} = 14.0 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L3} = 7.6 \times 10^{-6} \text{ m}^2/\text{s}$	$2D_{Lk}$	129.8 (-1.9%)
$D_{L1} = 78.4 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L2} = 28.0 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L3} = 15.2 \times 10^{-6} \text{ m}^2/\text{s}$	$4D_{Lk}$	125.2 (-5.4%)
$D_{Sk} = 4.8 \times 10^{-8} \text{ m}^2/\text{s}$, $D_{S2} = 4.7 \times 10^{-8} \text{ m}^2/\text{s}$, $D_{S3} = 4.6 \times 10^{-8} \text{ m}^2/\text{s}$	Experimental conditions	132.3
$D_{Sk} = 1.2 \times 10^{-8} \text{ m}^2/\text{s}$, $D_{S2} = 1.2 \times 10^{-8} \text{ m}^2/\text{s}$, $D_{S3} = 1.2 \times 10^{-8} \text{ m}^2/\text{s}$	$D_{Sk} / 4$	133.1 (0.6%)
$D_{Sk} = 19.2 \times 10^{-8} \text{ m}^2/\text{s}$, $D_{S2} = 18.8 \times 10^{-8} \text{ m}^2/\text{s}$, $D_{S3} = 18.4 \times 10^{-8} \text{ m}^2/\text{s}$	$4D_{Sk}$	130.0 (-1.7%)
$D_{Sk} = 4.8 \times 10^{-7} \text{ m}^2/\text{s}$, $D_{S2} = 4.7 \times 10^{-7} \text{ m}^2/\text{s}$, $D_{S3} = 4.6 \times 10^{-7} \text{ m}^2/\text{s}$	$10D_{Sk}$	124.9 (-5.6%)

5.3.2.3 Effect of liquid axial dispersion

Although a specially designed liquid distributor is usually set at the bottom of the column to help obtain a stable expansion at the higher liquid flow rate, liquid axial dispersion in the expanded bed is still higher than in a fixed bed, especially at the bottom of the expanded bed. The effect of the liquid axial dispersion on in-bed breakthrough curves is shown in Fig. 5.5 by changing D_{Lk} values by a factor of 2. An apparent effect on in-bed breakthrough curves can

been observed at the bottom zone of the column, then the effect on in-bed breakthrough curves becomes smaller gradually from the middle to the top of the column. The corresponding breakthrough times, extracted from the Fig. 5.5, are shown in Table 5.2, and increasing D_{Lk} values by a factor of 2 leads to a 1.9% decrease in breakthrough time, while decreasing D_{Lk} values by a factor of 2 get a 1.5% increase in breakthrough times.

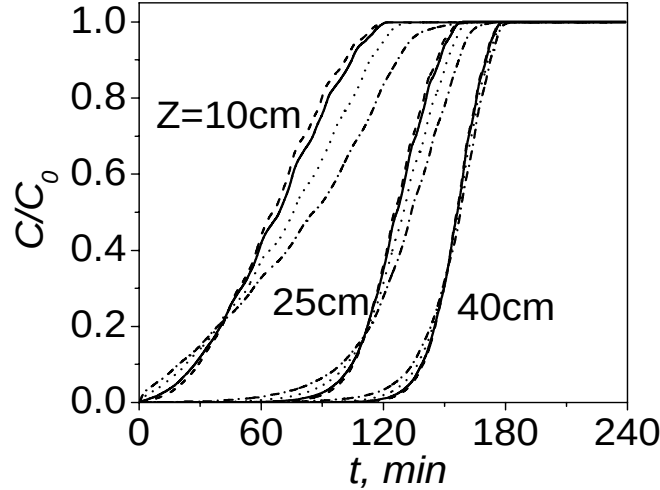


Fig. 5.5. Parametric sensitivity analysis of liquid axial dispersion coefficient to in-bed breakthrough curves in expanded bed. Dashed lines: 3-zone model with $D_{L1} = 9.8 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L2} = 3.5 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L3} = 1.9 \times 10^{-6} \text{ m}^2/\text{s}$; solid lines: 3-zone model with $D_{L1} = 19.6 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L2} = 7.0 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L3} = 3.8 \times 10^{-6} \text{ m}^2/\text{s}$; dot lines: 3-zone model with $D_{L1} = 39.2 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L2} = 14.0 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L3} = 7.6 \times 10^{-6} \text{ m}^2/\text{s}$; dashed dot lines: 3-zone model with $D_{L1} = 78.4 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L2} = 28.0 \times 10^{-6} \text{ m}^2/\text{s}$, $D_{L3} = 15.2 \times 10^{-6} \text{ m}^2/\text{s}$.

Under the experimental conditions of Bruce and Chase (2001), the effect of the liquid axial dispersion on the breakthrough curves is smaller than that of the protein intraparticle diffusion resistance. However, it should be emphasized that when D_{Lk} value is higher than $1.0 \times 10^{-5} \text{ m}^2/\text{s}$ due to unstable expansion, the effect of liquid axial dispersion on in-bed breakthrough curves will be significant, as shown in Fig. 5.5 (dashed dot lines), representing the simulation results with increasing D_{Lk} values by a factor of 4. According to literature data, liquid axial dispersion coefficient ranges from $1.0 \times 10^{-6} \text{ m}^2/\text{s}$ to $1.0 \times 10^{-5} \text{ m}^2/\text{s}$ in stable

expanded bed operation (Palsson et al., 2001; Thommes et al., 1995; Fenneteau et al., 2003), therefore, the adsorption behavior of expanded beds is comparable to that of fixed beds due to little effect of liquid axial dispersion coefficient on the adsorption kinetics.

5.3.2.4 Effect of adsorbent axial dispersion

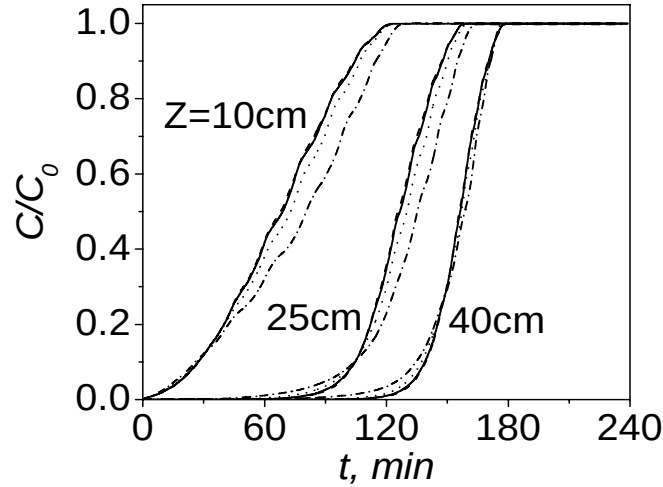


Fig. 5.6. Parametric sensitivity analysis of adsorbent axial dispersion coefficient to in-bed breakthrough curves in expanded bed. Dashed lines: 3-zone model with $D_{Sk} = 1.2 \times 10^{-8} \text{ m}^2/\text{s}$; solid lines: 3-zone model with $D_{Sk} = 4.7 \times 10^{-8} \text{ m}^2/\text{s}$; dot lines: 3-zone model with $D_{Sk} = 1.9 \times 10^{-7} \text{ m}^2/\text{s}$; and dashed dot lines: 3-zone model with $D_{Sk} = 4.7 \times 10^{-7} \text{ m}^2/\text{s}$.

Due to the fluidized nature of the expanded bed, there is adsorbent axial dispersion in the column. In this chapter, the correlation of Van Der Meer et al. (1984) was used to estimate the adsorbent axial dispersion coefficient D_{Sk} . D_{Sk} is increased and decreased by a factor of 4 to analyze the effect on in-bed breakthrough curves. The effect of the adsorbent axial dispersion on the breakthrough curves is weak, as shown in Fig. 5.6, the corresponding breakthrough time is increased by 0.6% if decreasing D_{Sk} value by a factor of 4, and is decreased by 1.7% if increasing D_{Sk} value by a factor of 4, only by increasing D_{Sk} value up to $4.7 \times 10^{-7} \text{ m}^2/\text{s}$, a significant effect on the breakthrough curves is observed, and the corresponding breakthrough time is decreased by 2%. Under the experimental conditions described here, the effect of the adsorbent axial dispersion coefficient on the breakthrough curves is negligible.

5.3.3 Effect of adsorbent particle size on the breakthrough curves

Compared with protein intraparticle diffusion resistance, the effects of film mass transfer resistance, liquid axial dispersion and adsorbent particle axial dispersion on the breakthrough curves are small in the stable expanded bed operation. The particle size should have a significant effect on the breakthrough curves due to particle diameter characterizing the diffusion path in the adsorbent particle (small particle diameters having a shorter diffusion path length, leading to less diffusion resistance than larger particle).

The specially designed adsorbents used in expanded beds, usually have a wide size distribution; for example, Streamline SP adsorbent size range is $100\ \mu\text{m}$ to $300\ \mu\text{m}$. In Bruce and Chase (2001) experimental measurements, the sample particle diameters are $217\ \mu\text{m}$ (std. dev. $45\ \mu\text{m}$) at the 10cm position of the column, $171\ \mu\text{m}$ (std. dev. $34\ \mu\text{m}$) at the 25cm position of the column, and $140\ \mu\text{m}$ (std. dev. $20\ \mu\text{m}$) at the 40cm position of the column. Here, we used the lower limits of experimental values ($217\text{-}45$ at $Z = 10\text{cm}$, $171\text{-}34$ at $Z = 25\text{cm}$ and $140\text{-}20$ at $Z = 40\text{cm}$) to calculate the average zonal values ($d_{p1} = 236\ \mu\text{m}$, $d_{p2} = 155\ \mu\text{m}$, and $d_{p3} = 129\ \mu\text{m}$) and analyze the effect of the particle size on the in-bed breakthrough curves. The simulation results are shown in Fig. 5.7, solid lines. Comparing with the initial simulation results (shown in Fig. 5.2, solid lines), in-bed breakthrough curves become steeper when decreasing the particle size.

Decreasing the adsorbent's diffusion path can effectively reduce the intraparticle diffusion resistance and increase the column efficiency for capturing the macromolecule protein by expanded bed adsorption at high liquid velocities. This work supports observations that use of pellicular and inert core adsorbents can improve the separation performance of proteins in expanded beds (Jahanshahi et al., 2002; Palsson et al., 2000; Li et al., 2003). These pellicular or inert core adsorbents that have been increased in density by the incorporation of a heavier, inert core, are not only being adequate for stable expansion at high flow rates, but also are expected to show improved efficiency by reducing the diffusion path in the adsorbent.

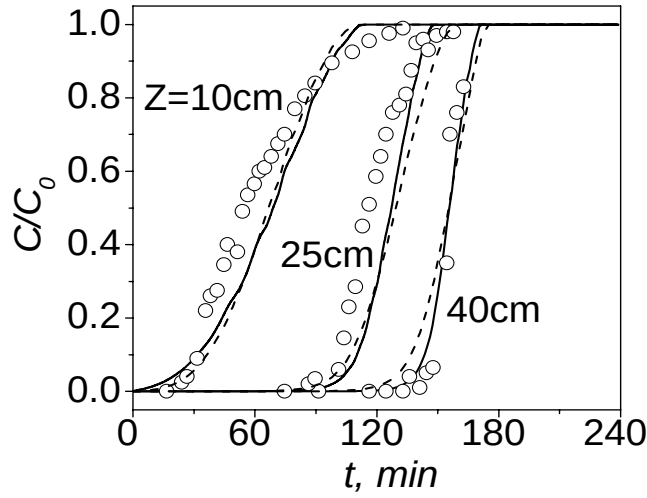


Fig. 5.7. Effect of adsorbent particle size on in-bed breakthrough curves in expanded bed and comparison between the experimental data and the simulation results predicted by the modified uniform model. Circle points: experimental data (Bruce and Chase, 2001); solid lines: 3-zone model with $d_{p1} = 236\mu\text{m}$, $d_{p2} = 155\mu\text{m}$, and $d_{p3} = 129\mu\text{m}$; dashed lines: modified uniform model with Eq. (5.8) for particle size distribution and Eq. (5.9) for bed voidage.

5.3.4 Modified uniform model by taking into account the adsorbent particle size and bed voidage axial distributions

In uniform models, all model parameters are averaged values over the whole column; therefore, the accuracy of the simulation results does not acceptably describe adsorption kinetics in expanded beds, as shown in Fig. 5.2. Here, the uniform model is revised by taking into account the adsorbent particle size and bed voidage axial distribution, but the liquid axial dispersion coefficient is assumed to be uniform due to its small effect on the breakthrough curves at the stable expansion. The experimental data for the adsorbent particle diameter and local bed voidage at different positions (Bruce and Chase, 2001) are described by the following linear equations; the fits of these relationships are shown in Fig. 5.8.

$$\varepsilon_B = 0.4444 + 0.5213 \frac{Z}{H_{\text{exp}}} \quad (5.8)$$

$$d_p = 240.17 - 102.67 \frac{Z}{H_{\text{exp}}} \quad (\mu\text{m}) \quad (5.9)$$

The simulation results for in-bed breakthrough curves, calculated by the modified uniform model with Eq. (5.8) for bed voidage axial variation and Eq. (5.9) for the particle diameter

axial distribution, are shown in Fig. 5.7 (dashed lines). The accuracy of the modified uniform model is improved compared to that of the uniform model (presented in Fig. 5.2, dashed lines). But the fitting with the experimental breakthrough curves is not good for the bottom zone and at the top zone of the column. Recently, Yun et al. (2004) found the adsorbent particle diameter and local bed voidage varied almost linearly with the bed height only in the middle region of the expanded bed; the nonlinear changes are observed near the bottom and the top of the column, which may be the reason why deviations are found between the experimental breakthrough curves and those predicted using the modified uniform model at the bottom position and at the top position of the column.

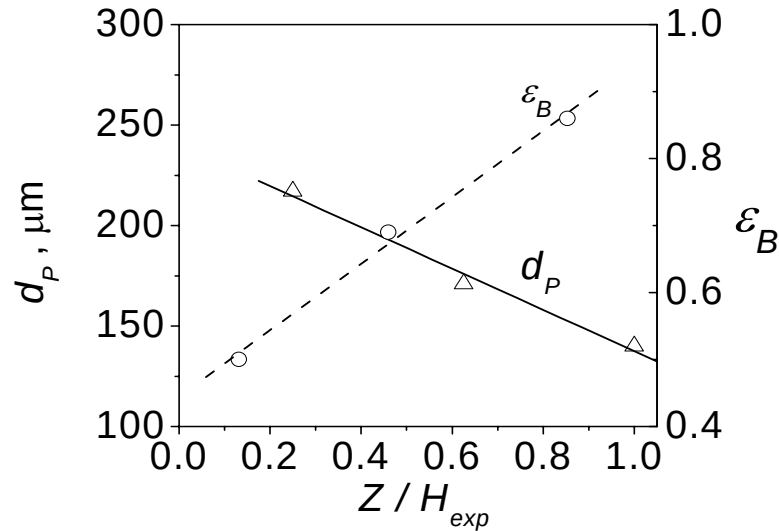


Fig. 5.8. Streamline SP adsorbent particle size axial distribution and bed voidage axial variation in an expanded bed. Points: experimental data (Bruce and Chase, 2001), straight lines: linear fitting with Eq. (5.8) for ε_B and Eq. (5.9) for d_p .

5.4. Conclusions

In expanded beds, the adsorbent particle size, bed voidage, and liquid axial dispersion coefficient are very different at the bottom zone, the middle zone and the top zone of the column, and significantly affects on the adsorption behavior. The 3-zone model, in which the zonal values for these parameters are used, can predict simultaneously in-bed breakthrough curves at the bottom, the middle and the top of the column. The simulation results by this 3-zone model are closely fit with the experimental data from literature. By contrast, the

conventional uniform model, in which all the model parameters are estimated by averaged values over the whole column, does not satisfactorily predict in-bed breakthrough curves. When the uniform model is modified by taking into account the bed voidage and the adsorbent particle size axial distribution, the accuracy of the modified uniform model is improved.

With the stable bed expansion, such as at experimental conditions reported in literature (Bruce and Chase, 2001), although there is higher liquid axial dispersion than that in fixed bed and there is adsorbent axial dispersion due to the fluidization in the expanded bed, the parametric sensitivity analysis shows that the liquid axial dispersion and the adsorbent axial dispersion both have a small effect on the breakthrough curves. By contrast, the protein intraparticle diffusion coefficient has a significant effect on the breakthrough curves. It is found that even for small proteins (i.e. lysozyme), the intraparticle diffusion resistance is also high in expanded bed adsorption. When capturing macromolecular protein at high flow rates in expanded beds, the intraparticle diffusion resistance is expected to be significant. This work supports observations that of use of pellicular and inert core adsorbents can improve the separation performance of proteins in expanded beds due to significant decrease of the intraparticle diffusion resistance by shortening the adsorbent's diffusion path.

It should be emphasized when liquid axial dispersion coefficient is larger than $1.0 \times 10^{-5} \text{ m}^2/\text{s}$ due to unstable bed expansion, the effect of liquid axial dispersion on the breakthrough curves will be significant.

Notation

c_k	concentration in particles in zone k (kg/m^3)
C_k	concentration in fluid in zone k (kg/m^3)
C_0	inlet concentration in fluid (kg/m^3)
d_{pk}	diameter of adsorbent in zone k (m)
D_{Lk}	liquid axial dispersion coefficient in zone k (m^2/s)
D_m	molecular diffusion coefficient (m^2/s)

D_p	intraparticle diffusion coefficient (m^2/s)
D_{Sk}	adsorbent axial dispersion coefficient in zone k (m^2/s)
H_0	settled bed height (m)
H_{exp}	expanded bed height ($= L_1 + L_2 + L_3$) (m)
k_d	dissociation constant defined by Equation (4) (m^3/kg)
k_{fk}	film mass transfer coefficient in zone k (m/s)
L_k	zone bed height in the column (m)
q_k	adsorbed concentration of lysozyme in zone k ($\text{kg}/\text{m}^3(\text{particle})$)
$\bar{q}_k$	average adsorbent phase concentration in zone k (kg/m^3)
q_m	maximum adsorbed concentration of lysozyme, defined by Eq.(5.4) $\text{kg}/\text{m}^3(\text{particle})$
Q_{feed}	feed flowrate (m^3/s)
Q_{sample}	sample flowrate (m^3/s)
Q_{out}	effluent flowrate (m^3/s)
r	radial distance from center of particle (m)
R_k	radius of adsorbent in zone k (m)
Re_p	particle Reynolds number ($= \rho d_{pk} u_k / \mu$)
S_C	Schmidt number ($= \mu / \rho D_m$)
t	time (s)
u_k	superficial linear velocity in zone k (m/s)
Z	axial distance from column entrance (m)

Greek Letters

ε_0	settled bed voidage (m^3/m^3)
ε_{Bk}	bed voidage of expanded bed in zone k (m^3/m^3)
ε_p	adsorbent porosity (m^3/m^3)
μ	liquid viscosity (Pa·s)
ρ	liquid density (kg/m^3)

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6. Experimental and Modeling Study of Protein Adsorption in Expanded Bed ^{*}

Streamline DEAE is the first generation adsorbent developed for expanded bed adsorption (low-density base matrix with wide particle size distribution and ligand sensitive to ionic strength and salt concentration), and Streamline direct CST I is the second generation adsorbent (high-density base matrix with narrow particle size distribution and ligand not sensitive to ionic strength and salt concentration). In this chapter, experiments were carried out for bovine serum albumin (BSA) protein adsorption in expanded beds, where a Streamline 50 column was packed either with Streamline DEAE or with Streamline direct CST I. The hydrodynamics, BSA dynamic binding capacity, and BSA recovery in the whole expanded bed adsorption process were compared for both adsorbents.

A mathematical model, in which intraparticle diffusion, film mass transfer, liquid axial dispersion, solid axial dispersion, particle size axial distribution, and bed voidage axial variation were taken into account, was developed to predict the breakthrough curves in expanded bed adsorption. BSA breakthrough curves in expanded bed adsorption were measured for both Streamline DEAE and Streamline CST I, and compared with the predictions from this mathematical model. The effects of intraparticle diffusion, film mass transfer, liquid and solid axial dispersion, particle size axial distribution, and bed voidage axial variation on the breakthrough curves were evaluated for expanded bed adsorption with both adsorbents.

^{*}This chapter is based on the paper by Li, P., Xiu, G. H. and Rodrigues, A. E., “Experimental and modeling study of protein adsorption in expanded bed”, *AIChE Journal*, 51, 2965-2977, 2005.

6.1. Introduction

Expanded bed adsorption is a single operation in which desired proteins are purified from particulate-containing feedstocks without the need for separate clarification, concentration, and initial purification. This technology has been widely applied to capture proteins directly from crude feedstocks, such as *E. coli* homogenate, yeast, fermentation, mammalian cell culture, milk, animal tissue extracts, and other unclarified feedstocks, and various applications have been reported from lab-scale to pilot-plant and large-scale production (Chase, 1994; Hjorth, 1997; Thommes, 1996; Ujam et al., 2003; Clemmitt and Chase, 2002; Smith et al., 2002; Bai and Glatz, 2003; Anspach et al., 1999; Jahanshahi et al., 2002).

With specially designed adsorbents and columns, the adsorption behavior in expanded beds is comparable to that in fixed beds (Chase, 1994). Streamline DEAE and Streamline SP are the typical first generation adsorbents, developed for expanded bed adsorption. The modified Sepharose matrices allow capture of biomolecules directly from unclarified feedstocks; the adsorbents have high binding capacities and product yields, attributed to stable expanded beds, and long life as the results of high chemical and mechanical stability. Many theoretical and experimental researches were reported in literature with respect to the hydrodynamics and protein adsorption kinetics in expanded beds packed with the first generation adsorbents (Change and Chase, 1996; Bruce and Chase, 2001, 2002; Karau et al., 1997; Wright and glasser, 2001; Willoughby et al., 2000a, b; Tong et al., 2003). Recently, Streamline direct CST I, a second-generation adsorbent recently developed for use in expanded bed adsorption, has two special features compared with the first generation adsorbents: a high-density base matrix and a salt-tolerant ligand. The high density matrix means minimizing dilution arising from biomass or viscosity and reducing dilution buffer consumption; and the legend's lack of sensitivity to ionic strength means there is no need for dilution of feedstock because of high ionic strength. However, hydrodynamics and protein adsorption kinetics in expanded beds packed with the second-generation adsorbent have not been investigated in detail.

In this chapter, experiments were carried out for protein (bovine serum albumin (BSA))

adsorption in expanded beds, where a Streamline 50 column was packed with 300ml Streamline DEAE and with 300 ml Streamline direct CST I, respectively. The experimental results are compared for both adsorbents to give a comprehensive evaluation of the hydrodynamics, BSA dynamic binding capacity, and BSA recovery in the whole expanded bed adsorption process.

The hydrodynamics and adsorption kinetics in expanded beds are more complex than that in fixed beds. The liquid axial dispersion in expanded beds is more significant than that in fixed beds; because of the fluidized nature of the expanded bed, adsorbent particle axial dispersion occurs. Moreover, there are variations of particle size axial distribution and bed voidage axial variation in expanded beds for the specially designed adsorbents with wide particle size distribution (Bruce and Chase, 2001; Willoughby et al., 2000a, b; Yun et al., 2004). Models available for fixed beds may be not adequate to describe the hydrodynamic and adsorption behavior in expanded beds.

Wright and Glasser (2001) developed a mathematical model to predict the breakthrough curve for protein adsorption in a fluidized bed, where intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion, and adsorbent particle axial dispersion were taken into account. Later, Tong et al. (2002) and Chen et al. (2003) used this model to predict the breakthrough curves in the expanded bed adsorption. When capturing proteins in an expanded bed with a high flow velocity, the slow diffusion rate of proteins results in high intraparticle diffusion resistance, significantly affecting the breakthrough curve. It is argued that, in this case, the particle size, characterizing the diffusion path in the adsorbent particles, should have a substantial effect on the breakthrough curves (Karau et al., 1997). Therefore, simulation results should be improved when the particle size axial distribution and bed voidage axial variations are taken into account in the model. Tong et al. (2003) modified the mathematical model by taking into account the particle size axial distribution in expanded beds. Following their experimental research using in-bed monitoring in expanded beds, Bruce and Chase (2002) predicted the in-bed breakthrough curves in expanded bed by using zonally measured parameters. Li et al. (2004) developed a 3-zone model to predict in-bed breakthrough curves and confirmed the

effect of the particle size axial distribution and bed voidage axial variation on the breakthrough curves in expanded beds. Recently, Kaczmaarski and Bellot (2004) also made the theoretical investigation about the effects of the axial and local particle size distribution and bed voidage axial variation on the breakthrough curves in expanded beds.

Up to now, theoretical and experimental researches have focused on protein adsorption onto Streamline DEAE or Streamline SP in expanded beds. Streamline DEAE and Streamline SP are first-generation adsorbents; the adsorbent matrix has low density. And large particle diameter with wide particle size distribution, 100-300 μm . The matrix of the second generation adsorbent, Streamline direct CST I, has high density and small particle diameter with narrow particle size distribution (80-165 μm). In this study, BSA breakthrough curves in expanded bed adsorption are measured for both Streamline direct CST I and Streamline DEAE, and a mathematical model is developed to predict the breakthrough curves and to compare with the experimental results; the effects of intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion, solid axial dispersions, adsorbent particle size axial distribution, and bed voidage axial variation on the breakthrough curves will be evaluated for expanded beds packed with Streamline DEAE and with Streamline direct CST I, respectively.

Although the adsorbents and the columns are designed specially for expanded beds to maintain stable bed expansion, the liquid axial dispersion is more significant than that in fixed beds. Sometimes, some inadequate operation----such as, the column not being in a vertical position, trapped air in the bottom distribution system, clogging of the bottom distribution system and pump pulse---would make the liquid axial dispersion more significant. Therefore, it is necessary to exactly measure and predict the liquid axial dispersion in expanded beds to allow stable bed expansion during protein adsorption.

Usually, the liquid axial dispersion coefficient in expanded beds is measured from residence time distribution (RTD) by using the moment method (Palsson et al., 2001; Thommes et al., 1995; Fenneteau et al., 2003; Bruce and Chase, 2001). Based on the experimental data of RTD curves, the mean residence time, and the variance of distribution can be calculated. Then, by letting the first absolute moment of the dispersion model equal

the mean residence time, and the second central moment of the dispersion model equal the variance of distribution, the liquid axial dispersion coefficient can be obtained easily. However, the validity of the dispersion model cannot be judged by this moment analytical method because the fitting degree of the calculated response curves to that measured experimentally cannot be evaluated directly. Sometimes, it will cause a significant deviation for the estimation of the liquid axial dispersion coefficient with the baseline drift and the baseline fluctuation of experimental RTD curves during measurements. Fernandez-Lahore et al. (1999) fitted the experimental RTD curves with the theoretical model in Laplace domain using the expression originally developed by Villiermaux and van Swaij (1969). In this paper, an analytical solution for Dirac input mode, in which liquid axial dispersion, tracer intraparticle diffusion, and film mass transfer all are taken into account, is used to fit experimental RTD curves to better estimate the liquid axial dispersion coefficient.

6.2. Experimental

6.2.1 Equipment, adsorbents and model protein

A pilot-scale Streamline 50 column (Amersham Pharmacia Biotech, Uppsala Sweden) was used in all expanded bed experiments. Masterflex® peristaltic pumps (Cole-Parmer Instrument Co., Vernon Hills, IL) were used for buffer and feed application and to raise and lower the hydraulic adaptor of Streamline 50 column. A JASCO 7800 UV detector (Tokyo, Japan) equipped with a flowcell was used to monitor online BSA effluent concentration from the expanded bed and tracer (acetone) effluent concentration in RTD experiments, and the absorbance signal was logged using the data-acquisition software in a personal computer.

Streamline DEAE and Streamline direct CST I were purchased from Amersham Pharmacia Biotech. Streamline DEAE is a weak anion exchanger with $-O-CH_2CH_2-N^+(C_2H_5)_2H$ functional group, with the following characteristics: its matrix consists of macroporous crosslinked 6% agarose constraining crystalline quartz core materials, with a particle density of 1200kg/m^3 , a particle size distribution of 100-300 μm ,

and a mean particle size of 200 μm . Streamline direct CST I is an ion exchanger with multimodal functional group, with the following characteristics: its matrix consists of macroporous crosslinked 4% agarose constraining stainless steel core materials, with a particle density of 1800kg/m^3 , a particle size distribution of 80-165 μm , and a mean particle size of 135 μm .

The model protein, bovine serum albumin (BSA; product number A3059, further purified fraction V, ~99%), was purchased from Sigma (St. Louis, MO). The molecular weight of BSA is about 65400 g mole^{-1} , diffusion coefficient at infinite dilution in water is about $6.15 \times 10^{-11}\text{ m}^2/\text{s}$, radius of gyration is $29.8\text{\AA}$, and isoelectric point is about 4.7.

A sample solution of BSA was prepared with the appropriate buffer; in the case of Streamline DEAE the buffer is 20 mM phosphate buffer (pH=7.5), a mixture of $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, and in the case of Streamline direct CST I the buffer is 50mM acetate buffer(pH=5), a mixture of acetate sodium and acetic acid. Distilled water was used in all experiments.

6.2.2 Batch adsorption experiments

Before performing adsorption isotherm experiments, the adsorbents must be saturated by phosphate buffer or acetate buffer. Adsorbents, in the amount of 0.5mL by particle volume, are equilibrated with 30 mL of different concentrations of BSA solution about 8 h at $25\text{ }^{\circ}\text{C}$ on a shaking incubator (about 30 rpm); then BSA concentration in supernatant liquid is measured by UV 7800 detector at 280 nm (using a 2-mL quartz cuvette). The adsorption capacity is calculated by mass balance.

For the kinetic experiment 2 mL of adsorbent was mixed with 100 mL of BSA solution in a flask. The adsorption was carried out in the shaking incubator at $25\text{ }^{\circ}\text{C}$ at 150 rpm. Every few minutes, about 2 ml of the liquid phase was aspirated using a suction tube to determine protein concentration, and the sample was immediately returned to the vessel. By this procedure, the time course of BSA concentration in batch adsorber was determined to estimate the effective pore diffusivity of BSA in Streamline DEAE and Streamline direct

CST I.

6.2.3 Residence time distribution experiments

A Streamline 50 column is packed either with 300mL Streamline DEAE or with 300mL Streamline direct CST I to maintain a stable expansion. For Streamline direct CST I, the settled bed height is 15.6 cm, and the settled bed voidage is 0.39; For Streamline DEAE, the settled bed height is 16.5 cm, and the settled bed voidage is 0.4. The liquid axial dispersion coefficient in expanded beds is measured by the RTD method. Acetone inert tracer is used in all RTD experiments. About 50%v/v concentration of acetone (1.5 mL buffer aqueous solution) is used for the dirac input mode, and input position at the bottom of the column.

Before carrying out the RTD measurement, the bed is expanded about 1 h by 20 mM phosphate buffer (pH=7.5) for Streamline DEAE, and by 50 mM acetate buffer (pH=5) for Streamline direct CST I. A dirac input of acetone sample is applied to the column at the bottom of the bed; the effluent liquid sample passes through the flowcell online, where the acetone concentration is monitored by UV detector at 280-nm wavelength; and the ABS (absorbance) signal of acetone is logged by the data-acquisition software in a personal computer.

6.2.4 Experimental procedures for the whole expanded bed adsorption process

Expansion/equilibration stage: First, the equilibration buffer is pumped through the column with upward flow to the expected expansion degree. Second, the expanded bed is allowed to stabilize at this degree of expansion for about 30-40 min. Then, the liquid axial dispersion in expanded bed is checked by the RTD method; the liquid axial dispersion coefficient should be as small as possible by avoiding inadequate operation. The adaptor will be positioned about 0.5 cm above the height to which the bed expands, to reduce the dead volume in expanded beds.

Adsorption stage: When the expanded bed is stable and equilibrated with the appropriate buffer, the process switches to feedstock application. Because of protein

adsorption on adsorbents, the expanded bed height gradually drops, especially in the expanded bed of low-density Streamline DEAE; therefore, the liquid flow velocity will be increased gradually during the adsorption process to maintain the constant degree of bed expansion. The average liquid velocity was calculated from the ratio of total feed volume supplied to column to the operation time in the loading process. The effluent stream from the top of the column will pass through the flowcell where BSA effluent concentration is monitored online by UV 7800 detector, and the ABS (absorbance) signal of BSA is logged by the data acquisition software in a personal computer. The BSA concentration in the feed was 2kg/m^3 , showing a linear relation between the UV detector signal (ABS) and BSA effluent concentration during the adsorption stage.

Washing stage: When the measurement of the breakthrough curve was completed, the process switches to the wash buffer to wash out the excess protein, others loosely bound materials and particulates from the column in expanded mode until the effluent absorbance reached a relative stable value. In the expanded bed of Streamline direct CST I, because of the highly favorable adsorption of BSA, the effluent absorbance approaches the baseline, meaning almost irreversible adsorption.

Elution stage: After washing, the pump is turn off and the bed is allowed to settle. When the adsorbent has settled, the adaptor is moved down towards the surface until the edge of the adaptor net touches the bed. Elution buffer is then pumped through the settled bed with downward flow to elute BSA protein. Because the BSA concentration in the effluent is very high, the effluent samples are collected by small sample tubes, and the BSA concentration in these samples is then monitored by UV 7800 detector.

6.3. Mathematical model for the protein adsorption in expanded beds

Mathematical model

The mathematical model is developed based on the work of Wright and Glasser (2001), where intraparticle diffusion, film mass transfer, liquid axial dispersion and solid axial dispersion were taken into account. In addition, the particle size axial distribution and bed voidage axial variation in expanded beds are also included in the model.

The material balance equation for liquid bulk phase in an expanded-bed is

$$D_L \frac{\partial^2 (\varepsilon_B(Z)C)}{\partial Z^2} - u \frac{\partial C}{\partial Z} - \varepsilon_B(Z) \frac{\partial C}{\partial t} - \frac{3k_f(Z)(1-\varepsilon_B(Z))}{R(Z)} [C - (c)_{r=R(Z)}] = 0 \quad (6.1)$$

where D_L is the axial dispersion coefficient; C is the concentration in the fluid phase; u denotes superficial velocity; c is the concentration in the adsorbent pore; Z is the axial distance from column entrance; $\varepsilon_B(Z)$ denotes bed voidage at the axial distance Z of the column, and thus $[1-\varepsilon_B(Z)]$ denotes the fractional volume taken up by the solid phase; r is the radial coordinate in the adsorbent particle; $R(Z)$ is the radius of the adsorbent at the axial distance Z of the column; t is the time; and $k_f(Z)$ is the film mass transfer coefficient at the axial distance Z of the column.

Boundary conditions

$$D_L \left(\frac{\partial C}{\partial Z} \right)_{Z=0} = \frac{u}{\varepsilon_B(0)} [(C)_{Z=0} - C_0] \quad (6.1a)$$

$$\left(\frac{\partial C}{\partial Z} \right)_{Z=H} = 0 \quad (6.1b)$$

Initial condition

$$t = 0, \quad C(Z,0) = 0 \quad (6.1c)$$

The mass balance for adsorbent bulk phases in the expanded bed column is described as

$$[1-\varepsilon_B(Z)] \frac{\partial \bar{q}}{\partial t} = D_{ax,S} \frac{\partial^2 \bar{q}}{\partial Z^2} + [1-\varepsilon_B(Z)] \frac{3}{R(Z)} k_f(Z) [C - (c)_{r=R(Z)}] \quad (6.2)$$

where the $\bar{q}$ is the average adsorbent phase concentration and $D_{ax,S}$ is the solid axial dispersion coefficient in the expanded bed.

Boundary conditions

$$\left(\frac{\partial \bar{q}}{\partial Z} \right)_{Z=0} = 0 \quad (6.2a)$$

$$\left(\frac{\partial \bar{q}}{\partial Z} \right)_{Z=H} = 0 \quad (6.2b)$$

Initial condition

$$t = 0, \quad \bar{q}(Z, 0) = 0 \quad (6.2c)$$

The pore diffusion equation in the adsorbent is described as

$$\varepsilon_p \frac{\partial c}{\partial t} + \frac{\partial q}{\partial t} = D_e \left(\frac{\partial^2 c}{\partial r^2} + \frac{2}{r} \frac{\partial c}{\partial r} \right) \quad (6.3)$$

where q is the adsorbed amount concentration in adsorbent, ε_p is the particle porosity, and D_e is the adsorbate effective pore diffusivity.

Boundary conditions

$$D_e \left(\frac{\partial c}{\partial r} \right)_{r=R(Z)} = \frac{R(Z) D_{ax,S}}{3[1 - \varepsilon_B(Z)]} \frac{\partial^2 \bar{q}}{\partial Z^2} + k_f(Z) [C - (c)_{r=R(Z)}] \quad (6.3a)$$

$$\left(\frac{\partial c}{\partial r} \right)_{r=0} = 0 \quad (6.3b)$$

Initial condition

$$t = 0, \quad c(r) = 0, \quad q(r) = 0 \quad (6.3c)$$

In Eq. 6.3, the relationship between q and c depends on the adsorption equilibrium of the selected experimental system. Based on the experimental measurements for BSA protein adsorption on Streamline DEAE and on Streamline direct CST I, the Langmuir isotherm is assigned as

$$q = \frac{q_m c}{k_d + c} \quad (6.4)$$

where q_m is the adsorption capacity and k_d is the dissociation constant, both of which are determined by experiments.

The correlations recommended to estimate the particle size axial distribution and bed voidage axial variation in expanded beds, packed with the first-generation adsorbents, are

$$R(Z) = \frac{\bar{d}_p}{2} \left(1.20 - 0.51 \frac{Z}{H} \right) \quad (6.5)$$

$$\varepsilon_B(Z) = \bar{\varepsilon}_B \left(0.629 + 0.738 \frac{Z}{H} \right) \quad (6.6)$$

where the averaged adsorbent particle diameter $\bar{d}_p$ is an averaged value over the wider particle size distribution, and the bed averaged voidage $\bar{\varepsilon}_B$ is estimated as a function of the whole expanded bed height (McCabe et al., 1985).

$$\bar{\varepsilon}_B = 1 - \left[(1 - \varepsilon_0) \frac{H_0}{H} \right] \quad (6.7)$$

where ε_0 and H_0 are the settled bed voidage and the settled bed height, respectively.

The correlations given by Eq. 6.5 and Eq. 6.6 are based on the experimental data of Bruce and Chase (2001), where a Streamline 50 column was packed with Streamline SP to measure both the particle size axial distribution and bed voidage axial variation in expanded bed. Because that system is similar to our experimental setup, that is a Streamline 50 column packed with Streamline DEAE (the same Streamline matrix), we used in the modeling the hydrodynamic results obtained by Bruce and Chase. Kaczmariski and Bellot(2004) recommended two different equations for the particle size axial distribution and bed voidage axial variation; simulated breakthrough curves using correlations from both groups are similar.

Model parameters

The effective pore diffusivity D_e in adsorbents is determined by independent batch experiments.

The liquid axial dispersion coefficient D_L is measured experimentally during the expansion stage by the residence time distribution method.

The adsorbent axial dispersion coefficient $D_{ax,S}$ is estimated by the correlation of Van Der Meer et al.(1984), using experimental values for superficial velocity u , as follows:

$$D_{ax,S} = 0.04u^{1.8} \text{ m}^2/\text{s} \quad (6.8)$$

The Wilson-Geankopli equation (1966), applicable to low Reynolds number (Eq. 6.9), is used to estimate the film mass transfer coefficient, $k_f(Z)$ in an expanded bed:

$$Sh = \frac{1.09}{\varepsilon_B(Z)} Re^{1/3} Sc^{1/3} \quad (0.0015 < Re < 55) \quad (6.9)$$

where Re is Reynolds number $[= 2R(Z)\rho u / \mu]$, Sc is Schmidt number $[= \mu / (\rho D_m)]$, Sh is Sherwood number $[= 2k_f(Z)R(Z) / D_m]$, and D_m is molecular diffusion coefficient.

Numerical method

The model equations are numerically solved by the orthogonal collocation method. Equations. 6.1 and 6.2 are discretized at collocation points in the axial direction in the column, and Eq. 6.3 is discretized at collocation points in the particle radial direction, leading to a set of ordinary differential equations with initial values that are integrated in the time domain using Gear's stiff variable step integration routine. To obtain a stable numerical solution for highly favorable adsorption isotherm, 21 bed axial collocation points and 21 particle radial collocation points are used.

6.4. Results and discussion

6.4.1 Adsorption isotherm and BSA effective pore diffusivity

Based on the independent batch adsorption equilibrium experiments, as shown in Fig.6.1, circle points represent the experimental data at room temperature (25 °C). BSA protein adsorption isotherm on both Streamline DEAE and Streamline direct CST I can be approximately described by the Langmuir equation as follows.

BSA adsorption on Streamline DEAE

$$q = \frac{92.59c}{0.065 + c} \quad (6.10a)$$

BSA adsorption on Streamline direct CST I

$$q = \frac{82.15c}{0.0109 + c} \quad (6.10b)$$

According to the Langmuir equation, the separation factor f can be defined as

$$f = \frac{1}{1 + c_0 / k_d} \quad (6.11)$$

which characterizes the adsorption conditions. For a BSA concentration of 2 kg/m^3 , which will be used in the adsorption kinetics experiments in batch adsorber and in expanded bed adsorption later, the separation factors are 0.032 for BSA adsorption on Streamline DEAE and 0.0055 for BSA adsorption on Streamline direct CST I, respectively, indicating highly favorable adsorption for BSA on Streamline adsorbents, especially on Streamline direct CST I almost taking place irreversible adsorption.

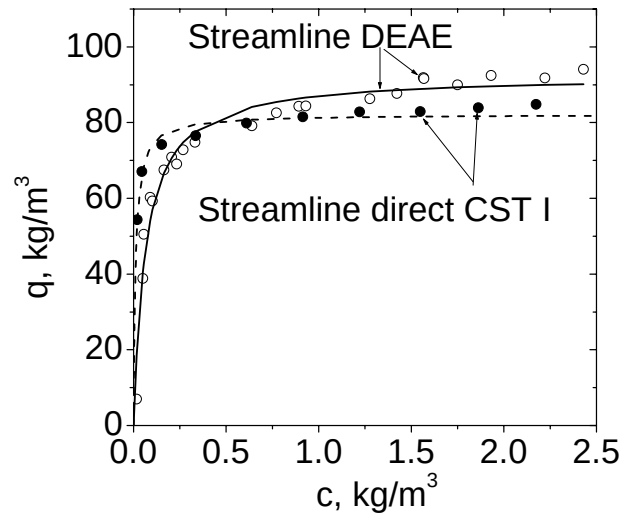


Fig. 6.1. BSA adsorption isotherms on Streamline DEAE and on Streamline direct CST I at the room temperature ($\sim 25^\circ \text{C}$). Circle points: experimental data; lines: the calculating results by Langmuir equations, Eq. 6.10a and 6.10b.

Batch adsorption kinetic experiments are carried out to estimate BSA effective pore diffusivity in Streamline DEAE and in Streamline direct CST I; Fig.6.2 shows typical experimental data of the bulk concentration profiles in batch adsorber (with 100 mL of 2 kg/m^3 BSA aqueous solution, and 2 mL adsorbent). Then, the experimental data of the bulk concentration profile are fitted with the simulation results of the pore diffusion model to

estimate BSA effective pore diffusion coefficient in Streamline DEAE as $3.2 \times 10^{-11} \text{ m}^2/\text{s}$ and in Streamline direct CST I as $1.7 \times 10^{-11} \text{ m}^2/\text{s}$. It should be emphasized that in the pore

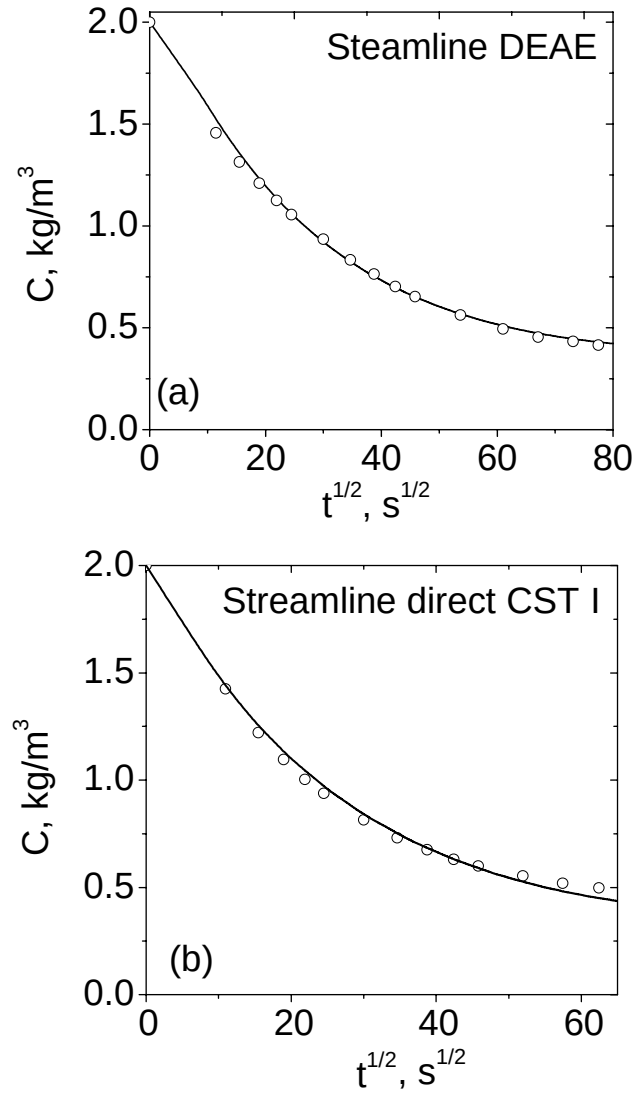


Fig.6.2. Estimation of BSA effective pore diffusivity in Streamline DEAE adsorbents and in Streamline CST I adsorbents in batch adsorber. Circle points: experimental data; solid lines: simulation results of the pore diffusion model with $D_e = 3.2 \times 10^{-11} \text{ m}^2/\text{s}$ and $k_f = 8.6 \times 10^{-6} \text{ m/s}$ for Streamline DEAE (a) and with $D_e = 1.7 \times 10^{-11} \text{ m}^2/\text{s}$, and $k_f = \infty$ for Streamline direct CST I(b).

diffusion model, the particle size is assigned an average particle diameter over the particle size distribution ($\bar{d}_p = 200 \mu\text{m}$ for Streamline DEAE ; and $\bar{d}_p = 135 \mu\text{m}$ for Streamline

direct CST I), and the particle porosity is assigned 0.55, accounting to the published literature (Boyer and Hsu, 1992). The film mass transfer resistance is not negligible for BSA adsorption on Streamline DEAE; in contrast, the film mass transfer can be neglected for BSA adsorption on Streamline direct CST I in the batch experimental system. A method to estimate both film mass transfer coefficient and effective pore diffusivity from a single bulk concentration-time curve in a batch adsorber has been given elsewhere (Li et al., 2003a).

6.4.2 Bed expansion and liquid axial dispersion coefficient in expanded bed

The expansion degree of expanded beds packed either with Streamline DEAE or with Streamline direct CST I is measured at various superficial flow velocities, as shown in Fig.6.3, where a Streamline 50 column is packed either with 300mL Streamline DEAE or with 300mL Streamline direct CST I the settled bed height is 15.6 cm for Streamline direct CST I, and 16.5 cm for Streamline DEAE. It is apparent that at the same expansion degree, the superficial liquid flow velocity for the new Streamline direct CST I is higher than that for the old Streamline DEAE, given that the particle density of the “new” adsorbent ($\sim 1800 \text{ kg/m}^3$) is greater than that of the “old” adsorbent ($\sim 1200 \text{ kg/m}^3$). When the expansion degree is equal to 2, the superficial flow velocity for Streamline DEAE is only 228 cm/h, but for Streamline CST I, it allows a higher velocity of the feedstock (up to 553 cm/h) to pass through its expanded bed.

The residence time distribution method with a dirac tracer (acetone) input mode is used to estimate the liquid axial dispersion coefficient in expanded beds packed with Streamline DEAE and packed with Streamline direct CST I, respectively; the experimental data of RTD curves are shown in Fig.6.4, marked as circle points.

Based on the experimental data of RTD curves, the mean residence time ($\bar{t}_m$) and the variance of distribution (σ^2) are calculated as

$$\bar{t}_m = \frac{\int_0^\infty t C dt}{\int_0^\infty C dt} = \frac{(\sum t_i C_i) \Delta t}{(\sum C_i) \Delta t} \quad (6.12)$$

$$\sigma^2 = \frac{\int_0^\infty t^2 C dt}{\int_0^\infty C dt} - \bar{t}_m^2 = \frac{(\sum t_i^2 C_i) \Delta t}{(\sum C_i) \Delta t} - \bar{t}_m^2 \quad (6.13)$$

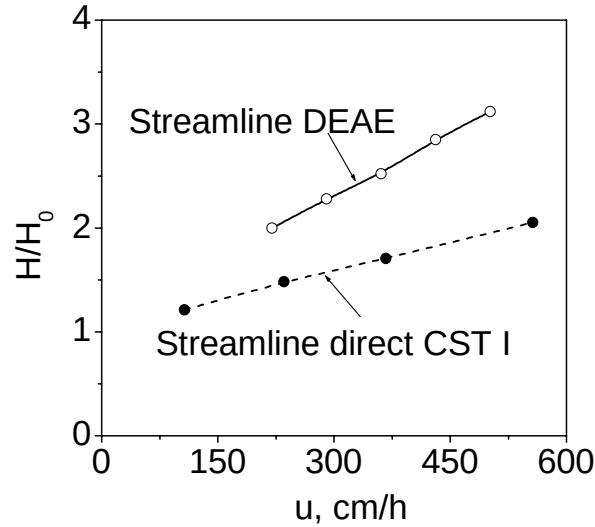


Fig.6.3. Relationship between bed expansion degree and superficial liquid flow velocity in expanded beds packed with Streamline DEAE and with Streamline CST I.

If we then let the first absolute moment μ_1 of the dispersion model equal the mean residence time ($\bar{t}_m$) and the second central moment (μ'_2) of the dispersion model equal the variance of distribution (σ^2) the liquid axial dispersion coefficient can be easily obtained. Some common calculation formula, are summarized as follows:

$$\frac{\sigma^2}{\bar{t}_m^2} = \frac{2\bar{\varepsilon}_B D_L}{uH} \quad (\text{Aris, 1959}) \quad (6.14)$$

$$\frac{\sigma^2}{\bar{t}_m^2} = \frac{2(\bar{\varepsilon}_B D_L / uH) + 3(\bar{\varepsilon}_B D_L / uH)^2}{1 + 2(\bar{\varepsilon}_B D_L / uH) + (\bar{\varepsilon}_B D_L / uH)^2} \quad (\text{Bruce and Chase, 2001}) \quad (6.15)$$

$$\frac{\sigma^2}{\bar{t}_m^2} = 2 \frac{\bar{\varepsilon}_B D_L}{uH} - 2 \left(\frac{\bar{\varepsilon}_B D_L}{uH} \right)^2 \left(1 - e^{-uH / (\bar{\varepsilon}_B D_L)} \right) \quad (\text{Levenspiel, 1972}) \quad (6.16)$$

When considering the tracer intraparticle diffusion resistance ,film mass transfer resistance and liquid axial dispersion, one has

$$\frac{\sigma^2}{\bar{t}_m^2} = \frac{2u\bar{R}^2\bar{\varepsilon}_p^2}{15H} \frac{(1-\bar{\varepsilon}_B)}{(\bar{\varepsilon}_B + \bar{\varepsilon}_p(1-\bar{\varepsilon}_B))^2} \left(\frac{1}{\bar{\varepsilon}_p D_p} + \frac{5}{k_f \bar{R}} \right) + \frac{2\bar{\varepsilon}_B D_L}{uH} \quad (6.17)$$

In Eq. 6.17, estimations of acetone intraparticle diffusivity and particle porosity for acetone are described in the Appendix.

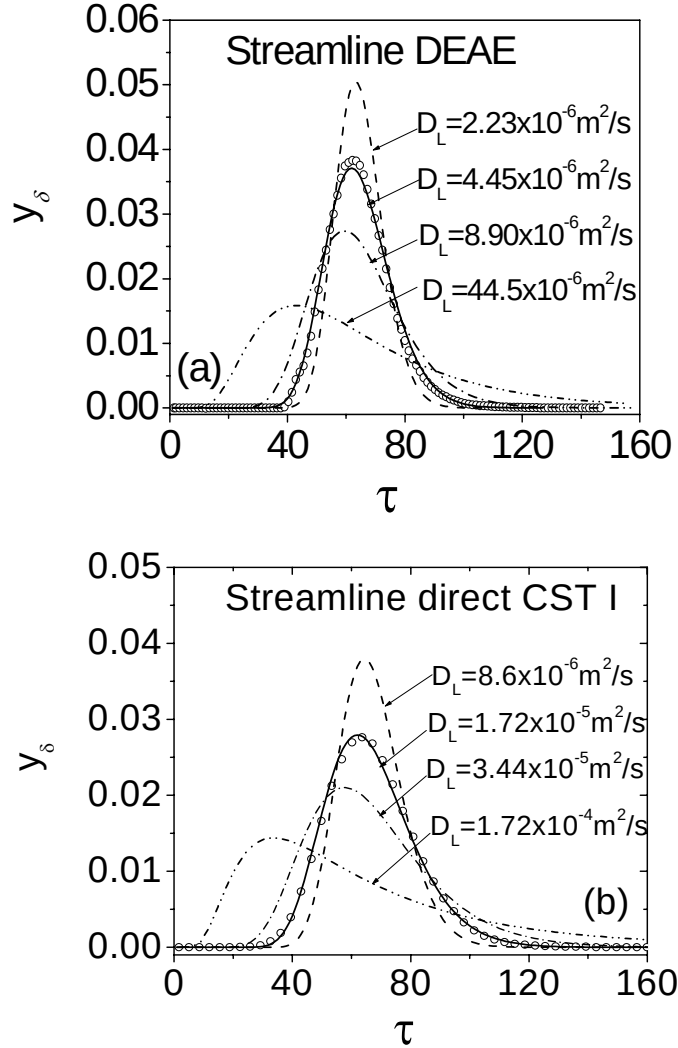


Fig.6.4. Experimental data of RTD curves are fitted by the analytical solution with the dirac input of acetone tracer at expanded beds packed with Streamline DEAE and packed with Streamline direct CST I. (a) settled bed height 16.5 cm, expanded degree as 2, superficial liquid flow velocity 228 cm/h, $\tau = D_p t / \bar{R}^2 = 0.128t$; (b) settled bed height as 15.6 cm, expansion degree as 2, superficial liquid flow velocity 553 cm/h, $\tau = D_p t / \bar{R}^2 = 0.281t$

The results calculated by different formula are similar. Because of the small size of the tracer molecule, acetone, the effect of the tracer intraparticle diffusion is negligible. For

Streamline DEAE, when the bed expands to about twice the settled bed height with 228 cm/h superficial flow velocity, the liquid axial dispersion coefficient is 4.45×10^{-6} m/s; for Streamline direct CST I, when the bed expands to about twice the settled bed height with 553 cm/h superficial flow velocity, the liquid axial dispersion coefficient increases to 17.2×10^{-6} m/s as a result of the liquid flow velocity increase. In the calculation, the mean residence time and variance of the residence time distribution of the sampling system and the precolumn tubing were subtracted from the measured mean residence time and variance of the overall residence time distribution to yield the corrected mean residence time and variance of the RTD of the expanded bed column. As an example, the mean residence time of extra-column volume and variance are $\bar{t}_c = 38\text{s}$ and $\sigma_c^2 = 352\text{s}^2$ at 228cm/h flow rate and $\bar{t}_c = 18\text{s}$ and $\sigma_c^2 = 126\text{s}^2$ for 553cm/h, respectively.

If there is a the baseline drift or a baseline fluctuation for experimental RTD curves, the liquid axial dispersion coefficient estimated by the previous calculation method may deviate from the real value of liquid axial dispersion coefficients. Therefore, in Fig.6.4, the experimental data of RTD curves are fitted by the analytical solutions with the dirac input of acetone tracer to confirm the calculation accuracy, and at the same time, the parametric sensitivity is analyzed. The analytical solution is given in the Appendix for reference, in which the tracer intraparticle diffusion resistance, film mass transfer resistance and liquid axial dispersion coefficient are all taken into account. In Fig.6.4, the mean residence time has been corrected, and the variance associated with extra-column volumes was negligible compared to the variance associated with the expanded bed.

6.4.3 BSA protein breakthrough behavior in expanded beds packed with Streamline direct CST I and with Streamline DEAE

BSA breakthrough behavior in expanded beds packed with Streamline direct CST I

When a given volume (300 mL) of Streamline direct CST I is packed into a Streamline 50 column, the settled bed height is 15.6 cm. BSA aqueous solution with concentration 2

kg/m^3 prepared with 50 mM acetate buffer (pH=5) is introduced to the column in upward flow with 181 ml/min flow velocity. The experimental data of the BSA breakthrough curve are shown in Fig.6.5, marked as circle points.

The uniform model, where the model parameters are average values all over the column (average particle diameter and average bed voidage), is used to predict the breakthrough curve, as shown in Fig.6.5 (solid line). Because of the heavier adsorbent, Streamline direct CST I, with narrower particle size distribution (80-165 μm), the effects of the particle size axial distribution and the bed voidage axial variation on the breakthrough curves are smaller, so the uniform model predicts the breakthrough curve in expanded beds reasonably well.

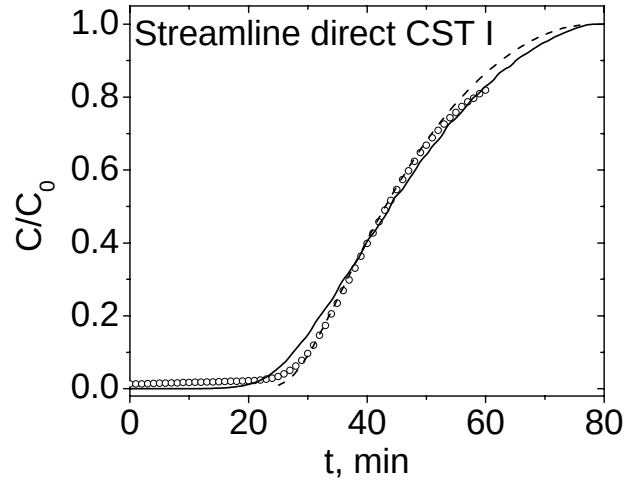


Fig.6.5. Experimental and predicted BSA breakthrough curve in expanded bed packed with Streamline direct CST I. Circle points: experimental data; solid line: uniform model; dashed line: the analytical solution (Eq. 6.18) with irreversible adsorption ($q_m = 82.15 \text{ kg/m}^3$). The experimental conditions and model parameters are summarized in Table 6.1.

The model parameters D_e , k_f , D_L and $D_{ax,S}$ characterize the effects of intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion and solid axial dispersion on the breakthrough curves during expanded bed adsorption. Fig.6.6 demonstrates the individual contribution of each model parameter (D_e , k_f , D_L or $D_{ax,S}$) on the breakthrough curve during expanded bed adsorption. First, by controlling film mass

transfer resistance, liquid and solid axial dispersion must be as small as possible to neglect their effects on the breakthrough curves (k_f increased 10 times, D_L and $D_{ax,S}$ decreased 10 times); the effect of the model parameter D_e on the breakthrough curve is demonstrated in Fig.6.6, represented by a dashed line. It is apparent that the contribution of BSA effective pore diffusivity to the breakthrough curves is dominant (dashed line). Then, in turn, the effects of film mass transfer resistance, liquid axial dispersion and solid axial dispersion are considered in the model; the simulation results are demonstrated in Fig.6.6, respectively. The dashed-double-dotted line represents the simulation result when both the intraparticle diffusion resistance and the film mass transfer resistance are taken into account in the model. By comparing with the simulation result (dashed line, considering only D_e), the effect of the film mass transfer coefficient is not negligible for such highly favorable protein adsorption isotherm, because the breakthrough time is significantly shortened as the result of the effect of k_f . The dashed dotted line represents the simulation results where intraparticle diffusion resistance, film mass transfer resistance and liquid axial dispersion are taken into account in the model, and the solid line represents the simulation results when intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion and solid axial dispersion all are taken into account in the model. It is apparent that the effects of the liquid axial dispersion coefficient and the solid axial dispersion coefficient are smaller even at high liquid flow velocity (up to 553 cm/h) if the bed expansion is stable.

Based on the simulation results, as shown in Fig.6.6, the effect of the liquid axial dispersion and solid axial dispersion on the breakthrough curves are smaller in a stable expanded bed packed with Streamline direct CST I. BSA protein adsorption on Streamline direct CST I is highly favorable, leading to almost irreversible adsorption (Fig.6.1); therefore, instead of the numerical solution, a simple analytical solution derived at the irreversible adsorption isotherm with $q_m = 82.15 \text{ kg/m}^3$ (Eq.6.18), which takes into account both the intraparticle diffusion and film mass transfer, may be used approximately to predict the breakthrough curves in the expanded bed (as shown in Fig.6.5, dashed line).

The analytical solution is indeed close to the experimental data in the expanded bed adsorption. Moreover, the analytical solution (dashed line in Fig.6.5) is similar to the simulated breakthrough curve from a model where only the intraparticle diffusion and film mass transfer are significant (dashed-double-dotted line in Fig.6.6). This analytical solution was reported by Weber and Chakravorti (1974) based on the assumption of constant pattern, as follows.

$$(\tau_1 - 1)N_{pore} = \frac{15}{\sqrt{3}} \tan^{-1} \left[\frac{2\eta + 1}{\sqrt{3}} \right] - \frac{15}{2} \left[\ln(1 + \eta + \eta^2) - \frac{1}{3} \right] + \frac{5}{Bi} [\ln(1 - \eta^3) + 1] - \frac{5\pi}{2\sqrt{3}} \quad (6.18)$$

where the dimensionless parameters are defined as

$$\tau_1 = \frac{\left(\frac{ut}{H} - \bar{\varepsilon}_B \right)}{\Lambda}, \quad N_{pore} = \frac{15(1 - \bar{\varepsilon}_B)D_e H}{u\bar{R}^2}, \quad \eta = \left(1 - \frac{C}{C_0} \right)^{1/3}, \quad \Lambda = \frac{(1 - \bar{\varepsilon}_B)q_m}{C_0}, \quad Bi = \frac{k_f \bar{R}}{D_e}$$

It should be emphasized that this formula requires that $\tau_1 N_{pore} > 2.5 + 1/Bi$.

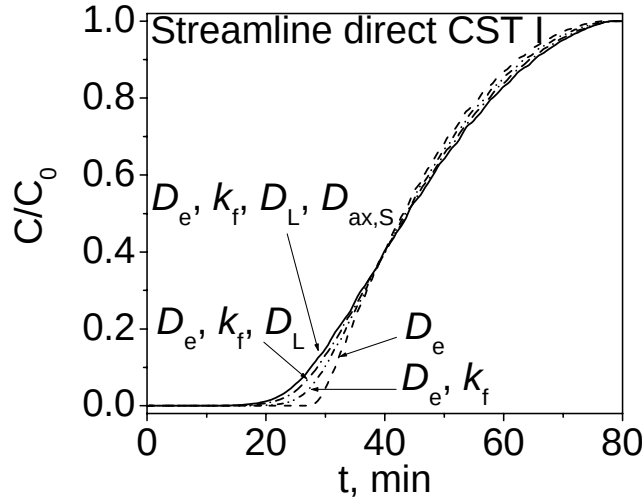


Fig.6.6. Contribution of each model parameter (D_e , k_f , D_L or $D_{ax,S}$) to the breakthrough curve in expanded bed packed with Streamline direct CST I. Dashed line: uniform model with $D_e = 1.7 \times 10^{-11} \text{ m}^2/\text{s}$ (neglect k_f , D_L and $D_{ax,S}$ effects); dashed-double-dotted line: uniform model with $D_e = 1.7 \times 10^{-11} \text{ m}^2/\text{s}$ and $k_f = 10.6 \times 10^{-6} \text{ m/s}$ (neglect D_L and $D_{ax,S}$ effects); dashed dot line: uniform model with $D_e = 1.7 \times 10^{-11} \text{ m}^2/\text{s}$, $k_f = 10.6 \times 10^{-6} \text{ m/s}$, and $D_L = 17.2 \times 10^{-6} \text{ m}^2/\text{s}$ (neglect $D_{ax,S}$ effect); solid line: uniform model with considering D_e , k_f , D_L and $D_{ax,S}$ effects ($D_e = 1.7 \times 10^{-11} \text{ m}^2/\text{s}$, $k_f = 10.6 \times 10^{-6} \text{ m/s}$, $D_L = 17.2 \times 10^{-6} \text{ m}^2/\text{s}$, and $D_{ax,S} = 3.45 \times 10^{-7} \text{ m}^2/\text{s}$). The other calculation conditions are the same as in Fig.6.5.

Table 6.1 Experimental conditions and model parameters used for the simulation of the breakthrough curves in expanded beds	
Expanded bed of Streamline direct CST I	Expanded bed of Streamline DEAE
$H_0 = 15.6 \text{ cm}$ $\bar{\varepsilon}_{B0} = 0.39$	$H_0 = 16.5 \text{ cm}$ $\bar{\varepsilon}_{B0} = 0.4$
$\bar{d}_p = 135 \text{ }\mu\text{m}$ $\varepsilon_p = 0.55$	$\bar{d}_p = 200 \text{ }\mu\text{m}$ $\varepsilon_p = 0.55$
$C_0 = 2 \text{ kg/m}^3$	$C_0 = 2 \text{ kg/m}^3$
$u = 15.37 \times 10^{-4} \text{ m/s}^*$	$u = 7.10 \times 10^{-4} \text{ m/s}^*$
$H = 32.0 \text{ cm}$ $H / H_0 = 2.05$ $\bar{\varepsilon}_B = 0.7025$	$H = 33.5 \text{ cm}$ $H / H_0 = 2.03$ $\bar{\varepsilon}_B = 0.7045$
$q_m = 82.15 \text{ kg/m}^3$ $k_d = 0.0109 \text{ kg/m}^3$	$q_m = 92.59 \text{ kg/m}^3$ $k_d = 0.065 \text{ kg/m}^3$
$D_e = 1.7 \times 10^{-11} \text{ m}^2/\text{s}$	$D_e = 3.2 \times 10^{-11} \text{ m}^2/\text{s}$
$D_L = 17.2 \times 10^{-6} \text{ m}^2/\text{s}$	$D_L = 4.45 \times 10^{-6} \text{ m}^2/\text{s}$
$k_f = 10.6 \times 10^{-6} \text{ m/s}$	$\bar{k}_f = 6.6 \times 10^{-6} \text{ m/s}$
$D_{ax,S} = 3.45 \times 10^{-7} \text{ m}^2/\text{s}$	$D_{ax,S} = 8.78 \times 10^{-8} \text{ m}^2/\text{s}$
* u is the average velocity during the adsorption stage, with the interval minimum - maximum liquid velocity of $6.68 \times 10^{-4} \sim 7.52 \times 10^{-4} \text{ m/s}$ for Streamline DEAE, and of $15.20 \times 10^{-4} \sim 15.54 \times 10^{-4} \text{ m/s}$ for Streamline direct CST I.	

The effects of particle size axial distribution, bed voidage axial variation, liquid axial dispersion and solid axial dispersion on the breakthrough curve are smaller than those of intraparticle diffusivity and film mass transfer coefficient for BSA adsorption in expanded bed packed with streamline direct CST I; therefore, the uniform model and the analytical solution give a reasonable fit of experimental breakthrough curves. However, in the uniform model, the effects of the particle size axial distribution and bed voidage axial variation on the breakthrough curve are neglected, which results in predicted breakthrough curves slightly broader than the experimental data (greater amount of larger-size adsorbent at the bottom of the column and low amount of smaller size adsorbent at the top of the column, will make the real breakthrough curve steeper). In the analytical solution, the effects of the particle size axial distribution and the bed voidage axial variation on the breakthrough curve (making the breakthrough curves slightly steeper), and the effects of

the liquid axial dispersion and the solid axial dispersion on the breakthrough curve (making the breakthrough curves slightly broader) are all neglected; it happens that the sharpening effect almost equals the broadening effect for our experimental conditions, leading to an analytical solution closer to experimental data.

BSA breakthrough behavior in expanded beds packed with Streamline DEAE.

When Streamline DEAE (300 mL) is packed into a Streamline 50 column, the settled bed height is 16.5 cm. A 2 kg/m^3 BSA aqueous solution, prepared with 20 mM phosphate buffer (pH=7.5) is introduced in upward flow at the column bottom with 83.6 ml/min flow velocity. The bed is expanded about twice settled bed height. The experimental data of the BSA breakthrough curve are shown in Fig.6.7, marked as circle points. First, the uniform model is used to predict the breakthrough curve, and the simulation result (dashed line, in Fig.6.7) is compared with the experimental data. The simulation result does not fit the experimental data very well.

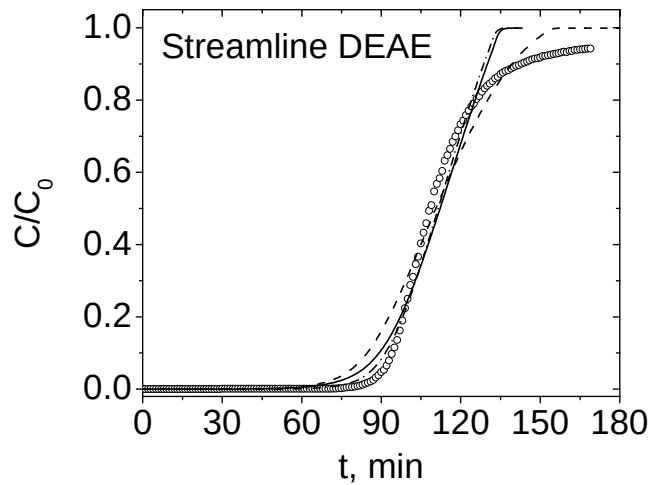


Fig.6.7. Experimental and predicted BSA breakthrough curve in expanded bed packed with Streamline DEAE. Circle points: experimental data; dashed line: uniform model; solid line: modified uniform model; dashed dotted line: modified uniform model with double k_f value. The experimental conditions and model parameters summarized in Table 6.1.

Based on the experimental results, at the initial breakthrough stage, the real adsorption behavior in expanded beds is better than the predicted results by the uniform model; however, there is a tailing behavior of the breakthrough curves when effluent concentration

approaches the feed concentration. In previously published papers, the tailing behavior was explained by the presence of the dimer in BSA samples, or microporous diffusion in the macroporous adsorbent, or protein steric hindrance on active sites of the surface of the adsorbent. Until now, the explanation is still unclear for the tailing behavior of the breakthrough curves that often occur for medium size and large size protein adsorption. Because the Streamline adsorbents are macroporous, at the initial adsorption stage, the macroporous diffusion should be considered; that is, the breakthrough curves may be predicted by the macroporous diffusion model for the initial breakthrough stage.

For the protein adsorption in a stable expanded bed, the slow diffusion rate of macromolecular protein in adsorbent significantly affects the breakthrough behavior in expanded bed. Therefore, the particle size should have an effect on the breakthrough curves as the result of particle diameter characterizing the diffusion path in the adsorbent particle (small particle diameters having a shorter diffusion path length, leading to lower diffusion resistance than in larger particle). Compared with Streamline direct CST I, Streamline DEAE has wider particle size distribution (100-300 μm); when the bed expands, the smaller and lighter particles move to positions at the top of the expanded bed, the larger and heavier particles to the bottom, and more adsorbents will be present at the bottom of the column. At the top zone of the column there is a small amount of adsorbent, and thus the real adsorption behavior in expanded beds is better than the predicted result by the uniform mode. The simulation result by the modified uniform model with tacking into account the particle size axial distribution (Eq. 6.5) and bed voidage axial variation (Eq. 6.6) is shown in Fig.6.7, denoted by the solid line. Compared with the uniform model, the simulation results with the modified uniform model better describe the initial breakthrough. For such a highly favorable adsorption isotherm, BSA protein diffuses by a shrinking core mode in the Streamline DEAE, so the more significant effect of the particle size axial distribution and bed voidage axial variation on the breakthrough curves will be observed at the end of the breakthrough curves, not at the initial breakthrough stage. In our previous work (Li et al., 2003a) for the small size protein (lysozyme) adsorption on Streamline SP in expanded beds, where the tailing behavior of the breakthrough curves was not observed, a

significant improvement in the simulation results by the modified uniform model can be observed.

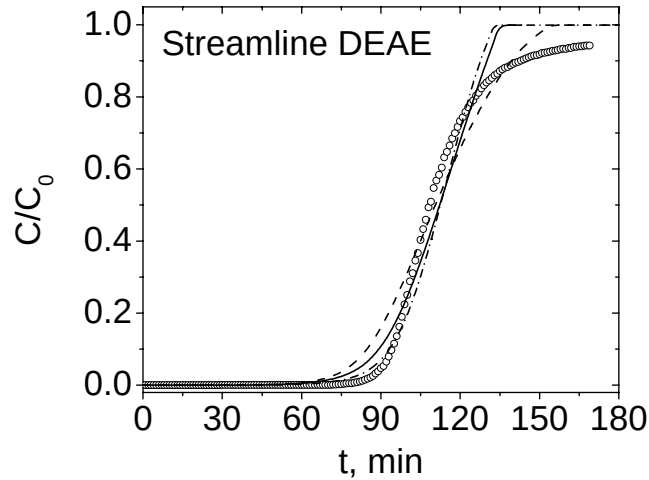


Fig.6.8. Effect of the particle size axial distribution and bed voidage axial distribution on the breakthrough curves in expanded beds packed with Streamline DEAE. Circle points: experimental data; dashed line: uniform model; dash dotted line: modified uniform model with considering particle size axial distribution; solid line: modified uniform model with considering both particle size axial distribution and bed voidage axial distribution. The experimental conditions and model parameters are the same as in Fig.6.7.

In Fig.6.8, we give the detailed comparisons among three simulation results; the dashed line represents the uniform model, the dashed-dotted line represents the modified uniform model by taking into account the particle size distribution, and the solid lines represents the modified uniform model by tacking into account both the particle size distribution and bed voidage axial variation. From Fig.6.8, by comparing the simulation results of the uniform model (dashed line) and modified uniform model with particle size axial distribution (dashed-dotted line), the effect of the particle size distribution on the breakthrough curve is significant; however, by comparing the simulation results of the modified uniform model with particle size axial distribution (dashed-dotted line) and the modified uniform model with both particle size axial distribution and bed voidage axial variation(solid line), the effect of the bed voidage axial variation on the breakthrough curve is small. It should be emphasized that there is an effect of bed voidage axial variation on the breakthrough curve when adsorbents have larger particle diameter with wider particle size distribution. Kaczmariski and Bellot (2004) claimed that the bed voidage axial variation

had no effect on breakthrough curves only for the case of smaller size adsorbents ($\bar{d}_p = 50\mu m$ or $\bar{d}_p = 150\mu m$) with relatively narrow particle size distribution.

It is observed that the real bed adsorption behavior at the initial breakthrough stage is still better than the predicted results even if we modify the uniform model by taking into account the particle size axial dispersion and bed voidage axial variation. Based on the theoretical analysis, as shown in Fig.6.6, the film mass transfer coefficient also has an important effect on the breakthrough time for such a highly favorable adsorption isotherm. It is very important to correctly estimate k_f in the simulation. From the published papers, the film mass transfer coefficient k_f is estimated by the correlations for fixed beds or their revised formula. In expanded beds, the adsorbent particles are suspended in the liquid phase and fluctuate slightly up and down, which will favor the film mass transfer; that is, the real k_f value should be larger than the estimated value of k_f by the previous correlations. In Fig.6.7, simulation results with double k_f value ($k_f \cong 13.2 \times 10^{-6}$ m/s) will more nearly reflect the real breakthrough time.

6.4.4 Comprehensive evaluations on the whole expanded-bed protein adsorption process with Streamline DEAE and with Streamline CST I

Experiments are carried out for the whole expanded-bed BSA protein adsorption process with Streamline direct CST I (Fig.6.9) and with Streamline DEAE (Fig.6.10). A Streamline 50 column is packed either with Streamline direct CST I or Streamline DEAE at the same amount of the adsorbents (300 ml). With the same degree of expansion (twice settled bed height), 2 kg/m³ BSA aqueous solution is applied to the expanded beds, and BSA protein is adsorbed by adsorbents; after adsorption stage, the bed is washed and BSA protein recovery processes at the elution stage. The detailed operation procedures have been previously described.

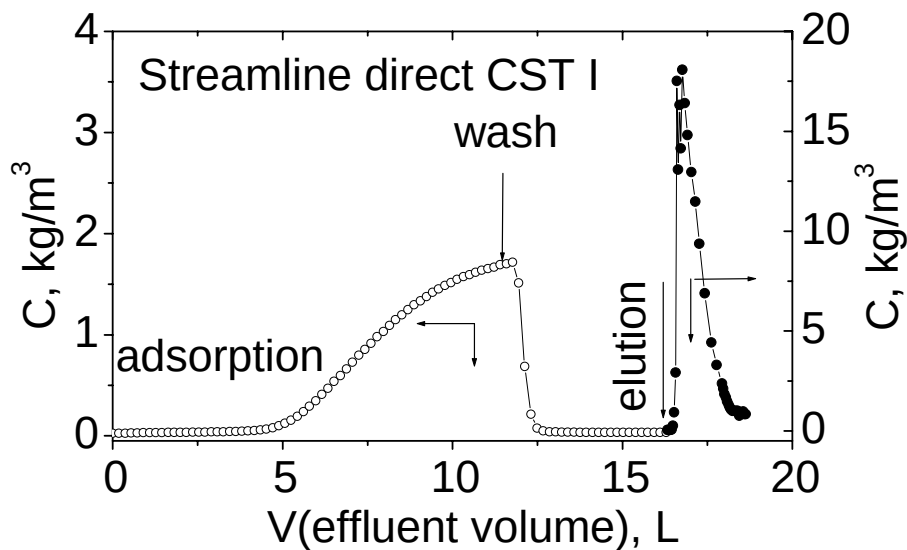


Fig.6.9. Effluent curves of BSA protein during adsorption, washing, and elution stages in expanded bed packed with Streamline direct CST I. At the adsorption stage, 2 kg/m³ BSA aqueous solution, prepared with 50 mM acetate buffer, pH=5, is applied from the bottom of the expanded bed at 181 ml/min flow velocity; at the washing stage, 50 mM acetate buffer, pH=5, is applied from the bottom of the expanded bed; and at the elution stage, 50mM acetate buffer with 1 M NaCl, pH=7, is applied from the top of the settled bed at 39 ml/min.

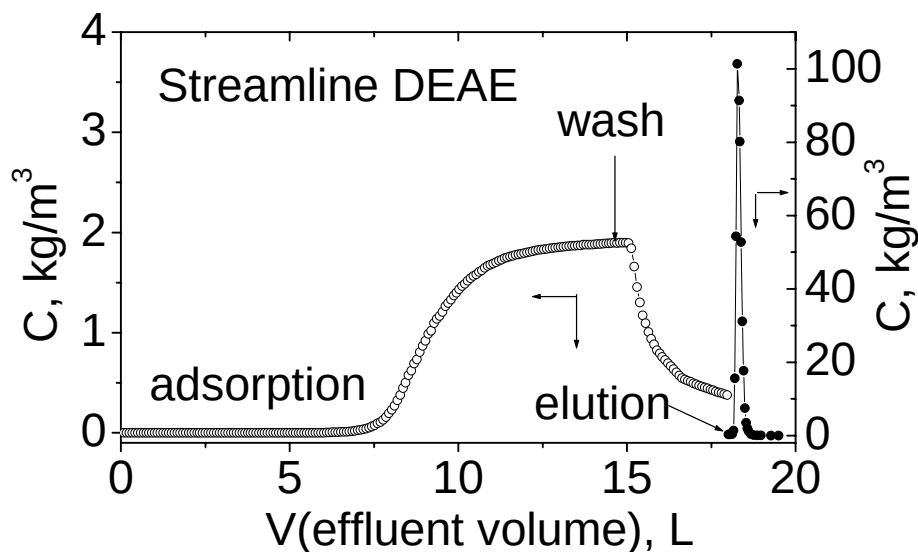


Fig.6.10. Effluent curves of BSA protein during adsorption, washing, and elution stages in expanded bed packed with Streamline DEAE. At adsorption stage, 2 kg/m³ BSA aqueous solution, prepared with 20 mM phosphate buffer, pH=7.5, is applied from the bottom of the expanded bed at 83.6 ml/min flow rate; at washing stage, 20 mM phosphate buffer, pH=7.5, is applied from the bottom of the expanded bed; and at the elution stage, 20 mM phosphate buffer with 0.5 M NaCl, pH=7.5, is applied from the top of the settled bed at 37 ml/min.

Based on the experimental results shown in Fig.6.9 and 6.10, the comprehensive evaluations on the hydrodynamics, BSA dynamic adsorption capacity, and BSA recovery in the expanded bed adsorption process with Streamline DEAE and with Streamline direct CST I are summarized as follows.

- 1) For the same degree of expansion and the same expanded bed height, the high-density Streamline direct CST I allows a higher feed flow velocity (553 cm/h) to pass through the expanded bed; in contrast, a low feed flow velocity (259 cm/h) is allowed to pass through the expanded bed packed with low density Streamline DEAE. Because of high flow velocity, the liquid axial dispersion coefficient is correspondingly increased in expanded beds of Streamline direct CST I. Based on our experimental and theoretical research on the BSA breakthrough behavior in an expanded bed of Streamline direct CST I, the effect of the liquid axial dispersion on the breakthrough curve is also small, even at such a high flow velocity if the bed expansion is stable.
- 2) At 5% BSA breakthrough point during expanded bed adsorption, BSA dynamic binding capacity on Streamline direct CST I is 34 mg (BSA)/ml of settled bed volume and BSA dynamic binding capacity on Streamline DEAE is 50 mg (BSA)/ml of settled bed volume. However, BSA binding capacity on Streamline direct CST I is not sensitive to ionic strength in feedstock, as shown in Fig.6.11b, which means there is no need for dilution of feedstock arising from high ionic strength. In contrast, BSA binding capacity on Streamline DEAE is very sensitive to the ionic strength in feedstock, as shown in Fig.6.11a; when the ionic strength in feedstock is increases to 50mM, the BSA adsorption capacity decreases by half. BSA dynamic binding capacity, $Q_{5\%}$, is calculated from BSA breakthrough curve at expanded bed adsorption stage; the formula is defined as

$$Q_{5\%} = \frac{\int_0^{V_{5\%}} (C_0 - C) dV}{V_A} \quad (6.19)$$

where V is the effluent liquid volume from expanded beds, $V_{5\%}$ is the effluent liquid volume at 5% BSA breakthrough point, and V_A is the settled bed volume of adsorbents.

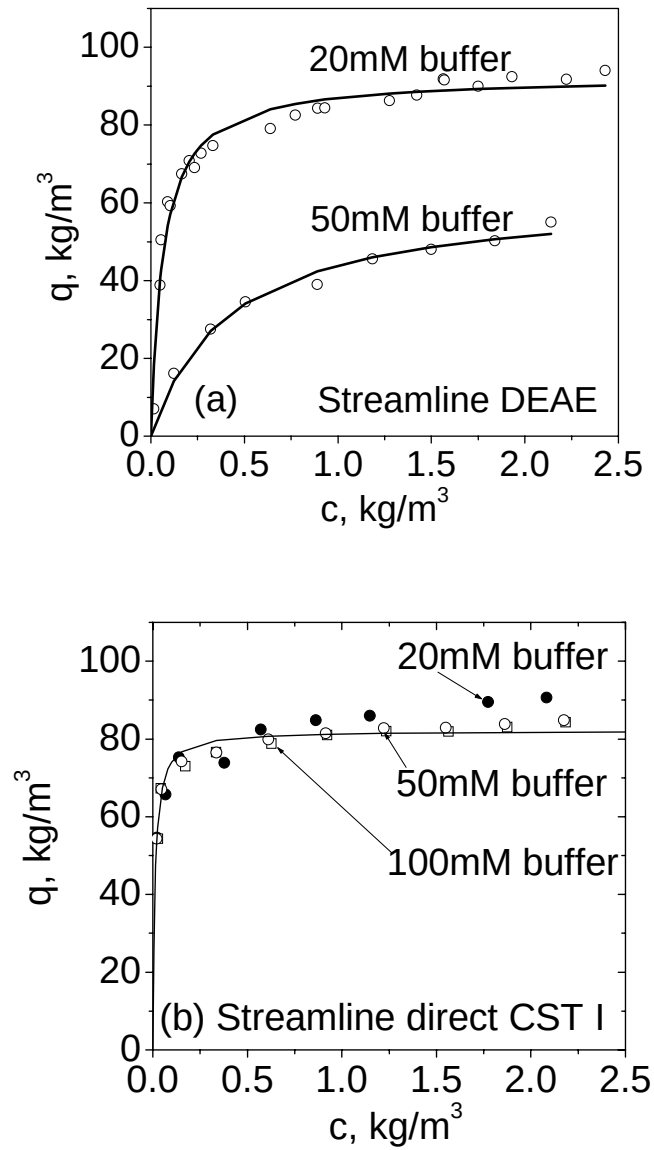


Fig. 6.11. Effect of ionic strength of buffer on BSA adsorption isotherms on Streamline DEAE (Fig. 6.11a, phosphate buffer, pH=7.5) and on Streamline direct CST I (Fig. 6.11b, acetate buffer, pH=5). Circle points: experimental data.

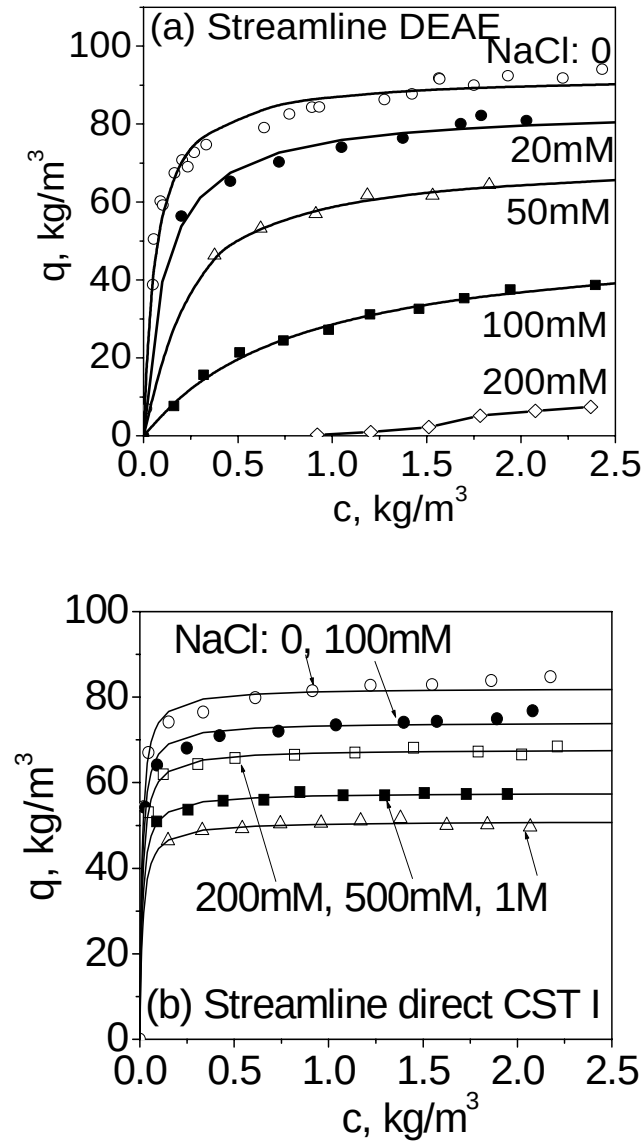


Fig. 6.12. Effect of salt concentration in buffer on BSA adsorption isotherms on Streamline DEAE (Fig. 6.12a, 20mM phosphate buffer, pH=7.5) and on Streamline direct CST I (Fig. 6.12b, 50mM acetate buffer, pH=5). Circle points: experimental data.

- 3) At the washing stage, it is found that BSA effluent concentration quickly drops and approaches the baseline for the expanded bed of Streamline direct CST I, which means almost irreversible adsorption for BSA binding to Streamline CST I. BSA adsorption on Streamline DEAE is reversible; when washing, some BSA can be desorbed from Streamline DEAE so that the effluent concentration approaches a relatively stable value.

4) The ligand of Streamline DEAE is very sensitive to salt concentration in buffer, as shown in Fig.6.12a, so BSA adsorbed on Streamline DEAE can be eluted easily by increasing the salt concentration to 0.5 M in 20 mM phosphate buffer, pH=7.5. BSA recovery in the whole expanded bed adsorption process can reach 91%. From the BSA effluent concentration curve during the elution stage, it is observed that BSA can be eluted almost completely from Streamline DEAE; however, due to desorption during the washing stage, BSA recovery is not up to 100%; a small amount of the elution buffer is consumed, as shown in Fig.6.10. Streamline direct CST I has a multimodal ligand that is not sensitive to the salt concentration, as shown in Fig.6.12b. Therefore, it is very difficult to elute BSA from Streamline CST I in the column only by increasing the salt concentration in 50 mM acetate buffer, pH=5. Therefore, to accomplish elution of adsorbed BSA proteins, both salt concentration and pH value in acetate buffer are increased. Here, when the elution buffer is 50 mM acetate buffer with 1 M NaCl at pH=7, BSA recovery attains 87%. From BSA effluent concentration curve during the elution stage, it can be noted that BSA cannot be eluted completely, and the amount of the elution buffer consumed is also larger, as shown in Fig.6.9.

6.5 Conclusions

With the specially designed adsorbents (Streamline DEAE and Streamline direct CST I), a stable expanded bed can be formed. At the same expansion degree, the high-density Streamline direct CST I allows a higher feed flow velocity to pass through the expanded bed; in contrast, a lower feed flow velocity is allowed to pass through the expanded bed of lower-density Streamline DEAE. With the high feed flow velocity, the liquid axial dispersion is more significant in the expanded bed of Streamline direct CST I than that in the expanded bed of Streamline DEAE.

In spite of the existence of intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion and solid axial dispersion during expanded bed adsorption, the contribution of BSA effective pore diffusivity to the breakthrough curves is dominant. The film mass transfer coefficient has a significant effect on initial

breakthrough time for the highly favorable protein adsorption isotherm; liquid axial dispersion and solid axial dispersion have a lesser effect on the breakthrough curves, even at high liquid flow velocity (up to 553 cm/h for Streamline direct CST I), if the bed expansion is stable.

Because of the narrow particle size distribution of Streamline direct CST I, the effects of the particle size axial dispersion and the bed voidage axial variation on the breakthrough behavior in the expanded bed are small, and thus the uniform model can be used to predict the breakthrough curves with acceptable accuracy. In contrast, because of the wide particle size distribution of Streamline DEAE, the effects of the particle size axial distribution and bed voidage axial variation on the breakthrough curves in expanded bed should be taken into account in the model.

Based on the experimental results, at 5% BSA breakthrough point during expanded bed adsorption, the BSA dynamic binding capacity on Streamline DEAE is 50 mg (BSA)/ml of settled bed volume, larger than that on Streamline direct CST I (34 mg (BSA)/ml of settled bed volume). However, the BSA binding capacity on Streamline CST I is not sensitive to ionic strength in feedstock, which means no need for dilution of feedstock even at high ionic strength. In contrast, the BSA binding capacity on Streamline DEAE is very sensitive to the ionic strength in feedstock; when the ionic strength in feedstock is increased to 50 mM, BSA adsorption capacity decreases by half.

The ligand of Streamline DEAE is very sensitive to salt concentration in buffer, so BSA adsorbed on Streamline DEAE can be easily eluted by increasing the salt concentration to 0.5 M in 20 mM phosphate buffer, pH=7.5. BSA recovery in the whole expanded bed adsorption process reaches 91%, and a small amount of the elution buffer is consumed. Streamline direct CST I has a multimodal ligand that is not sensitive to the salt concentration. Both salt concentration and pH value should be increased in elution buffer; for example, 50 mM acetate buffer with 1 M NaCl, pH=7, is used as elution buffer in this experiment, and BSA recovery in the whole expanded bed adsorption process is up to 87%, and the amount of the elution buffer is greater than that consumed for Streamline DEAE. In addition, from the BSA effluent concentration curve during the elution stage, it is observed

that BSA cannot be eluted completely from Streamline CST I.

Notation

c	concentration in particle pore, kg/m^3
C	concentration in fluid, kg/m^3
C_0	inlet concentration in fluid, kg/m^3
$\bar{d}_p$	average diameter of adsorbent, m
D_L	liquid axial dispersion coefficient, m^2/s
D_m	molecular diffusion coefficient, m^2/s
D_p	intraparticle pore diffusivity, m^2/s
D_e	intraparticle effective pore diffusivity, m^2/s
$D_{ax,S}$	solid axial dispersion coefficient, m^2/s
H_0	settled bed height, m
H	expanded bed height, m
k_d	dissociation constant defined by Eq. 6.4, m^3/kg
k_f	film mass transfer coefficient, m/s
n	amount of sample injected, kg
q	adsorbed concentration in adsorbent, kg/m^3 particle
$\bar{q}$	averaged adsorbent phase concentration, kg/m^3
q_m	maximum adsorbed concentration, defined by Eq. 6.4, kg/m^3 particle
r	radial distance from center of particle, m
R	radius of adsorbent, m
Re	Reynolds number
S_C	Schmidt number

Sh	Sherwood number
t	time, s
u	superficial liquid flow velocity, m/s
V_c	column volume, m ³
Z	axial distance from column entrance, m

Greek Letters

ε_0	settled bed voidage, m ³ /m ³
ε_B	bed voidage of expanded bed, m ³ /m ³
ε_p	adsorbent porosity, m ³ /m ³
μ	liquid viscosity, Pa s
ρ	liquid density, kg/m ³

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Appendix:**Analytical solution for the residence time distribution (RTD) curve**

In the mathematical model, the liquid axial dispersion, intraparticle diffusion and film mass transfer resistance all are taken into account. The material balance equation for fluid phase is

$$D_L \frac{\partial^2 C}{\partial Z^2} - \frac{u}{\bar{\varepsilon}_B} \frac{\partial C}{\partial Z} - \frac{\partial C}{\partial t} - \frac{(1 - \bar{\varepsilon}_B)}{\bar{\varepsilon}_B} \frac{3}{R} \varepsilon_p D_p \left(\frac{\partial C}{\partial r} \right)_{r=\bar{R}} = 0 \quad (A1)$$

The material balance equation in pore particle is

$$\varepsilon_p \frac{\partial C}{\partial t} = \varepsilon_p D_p \left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \frac{\partial C}{\partial r} \right) \quad (0 \leq r \leq \bar{R}) \quad (A2)$$

The initial and boundary conditions for Eqs A1 and A2 are

$$C(Z, 0) = 0 \quad (A3)$$

$$c(r, Z, 0) = 0 \quad (A4)$$

$$C(0, t) = C^0 t_c \delta(t) \quad \text{with Dirac input mode} \quad (A5)$$

$$C(\infty, t) \text{ is limited} \quad (A6)$$

$$\left(\frac{\partial C}{\partial r} \right)_{r=0} = 0 \quad (A7)$$

$$\varepsilon_p D_p \left(\frac{\partial C}{\partial r} \right)_{r=\bar{R}} = k_f [C - (c)_{r=\bar{R}}] \quad (A8)$$

Based on the published works (Rasmuson, 1981; Xiu et al., 1997; Li et al., 2003b), the analytical solution for RTD curve at Dirac input mode, is

$$y_\delta(\tau) = \frac{1}{\pi} \int_0^\infty \exp \left(\frac{Pe \zeta}{2} - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} + a}{2}} \right) \cos \left(\beta \tau - \zeta \sqrt{\frac{\sqrt{a^2 + b^2} - a}{2}} \right) d\beta \quad (A9)$$

where

$$a = Pe \left[\frac{Pe}{4} + 3\theta v \frac{Bi^2 I_1 + Bi(I_1^2 + I_2^2)}{(Bi + I_1)^2 + I_2^2} \right] \quad (A10)$$

$$b = \theta Pe \left[\beta + 3\nu \frac{Bi^2 I_2}{(Bi + I_1)^2 + I_2^2} \right] \quad (A11)$$

$$I_1 = \sqrt{\frac{\beta}{2}} \left(\frac{\sinh \sqrt{2\beta} + \sin \sqrt{2\beta}}{\cosh \sqrt{2\beta} - \cos \sqrt{2\beta}} \right) - 1 \quad (A12)$$

$$I_2 = \sqrt{\frac{\beta}{2}} \left(\frac{\sinh \sqrt{2\beta} - \sin \sqrt{2\beta}}{\cosh \sqrt{2\beta} - \cos \sqrt{2\beta}} \right) \quad (A13)$$

The dimensionless variables are

$$y(\delta) = \frac{C}{C^0 \tau_c}, \quad \zeta = \frac{Z}{H}, \quad \nu = \left(\frac{1 - \bar{\varepsilon}_B}{\bar{\varepsilon}_B} \right) \varepsilon_p, \quad Bi = \frac{k_f \bar{R}}{\varepsilon_p D_p}, \quad \theta = \frac{\bar{\varepsilon}_B H D_p}{u \bar{R}^2}, \quad Pe = \frac{uH}{\bar{\varepsilon}_B D_L}, \quad \text{and}$$

$$\tau = \frac{D_p t}{\bar{R}^2}$$

where the reference concentration is the ratio between the amount injected and the fluid volume in the column, $C^0 = n / \bar{\varepsilon}_B V_c$ and $\tau_c = D_p t_c / \bar{R}^2$ ($t_c = \bar{\varepsilon}_B H / u$ is the space time).

The tracer (acetone) intraparticle pore diffusivity is estimated approximately by Wilke and Chang (1955) formula as $D_p = 1.28 \times 10^{-9} \text{ m}^2/\text{s}$; the film mass transfer coefficient k_f is estimated by the correlation of Eq. 6.9, and the particle porosity for acetone is estimated by first moment μ_1 , equal to the mean resistance time of the experimental RTD curve, as suggested by Boyer and Hsu (1992)

7. Expanded Bed Adsorption/Desorption of Proteins with Streamline Direct CST I Adsorbent*

Streamline Direct CST I is a new type of ion exchanger with multi-modal functional groups, specially designed for an expanded bed adsorption (EBA) process, which can capture directly the proteins from the high ionic strength feedstocks with a high binding capacity. In this chapter, an experimental study is carried out for two-component proteins (BSA and myoglobin) competitive adsorption and desorption in an expanded bed packed with Streamline Direct CST I. Based on the measurements of the single- and two-component BSA/myoglobin adsorption isotherm on Streamline Direct CST I, the binding and elution conditions for the whole EBA process are selected; and then frontal analysis for a longer timescale and column displacement experiments in a fixed bed (XK16/20 column) are carried out to evaluate the two-component proteins (BSA and myoglobin) competitive adsorption and displacement on Streamline Direct CST I. Finally, the feasibility of capturing both BSA and myoglobin by an expanded bed packed with streamline Direct CST I is addressed in a Streamline 50 column packed with 300mL Streamline Direct CST I.

* This chapter is based on the paper by Li, P., Xiu, GH., Mata, VG, Grande, CA and Rodrigues, AE., "Expanded bed adsorption/desorption of proteins with Streamline Direct CST I, Biotechnology and Bioengineering, 2006, in press.

7.1. Introduction

The technology of an expanded bed adsorption (EBA) has been widely applied to capture proteins directly from crude unclarified feedstocks, such as, *E. coli* homogenate, yeast, fermentation, mammalian cell culture, milk, animal tissue extracts (Anspach et al., 1999; Bai and Glatz, 2003; Chase, 1994; Clemmitt and Chase, 2002; Chen et al., 2003; Hjorth, 1997; Smith et al., 2002; Thommes, 1996; Ujam et al., 2003). The objectives of EBA process are both the removal of the bulk impurities, such as cells, cell debris, particulate matter and contaminants, and the concentration and stabilization of the target protein molecules from the source materials. Therefore, an expanded bed is designed in a way that the suspended adsorbent particles capture target protein molecules while cells, cell debris, particulate matter and contaminants pass through the column unhindered. After loading and washing, the bound proteins can be eluted with buffer and be concentrated in a small amount of elution solution, apart from the bulk impurities and contaminants in source materials.

The EBA process will be more effective for those adsorbents that have both high-density base matrix and salt-tolerant ligand. The high-density matrix means minimizing dilution arising from biomass or viscosity in feedstocks and reducing dilution buffer consumption; the lack of sensitivity of the ligand to ionic strength and salt concentration means there is no need for dilution of feedstocks. Recently, Streamline Direct CST I, a second-generation adsorbent, was developed for the EBA process. Its base matrix is based on macroporous cross-linked 4% agarose, modified by the inclusion of inert stainless steel core materials to provide the particle density up to 1800kg/m^3 ; the ligand is a new type of multi-modal functional groups, which can bind proteins with a high capacity even in high ionic strength and salt concentration feedstocks. In a previous work (Li et al., 2005), experiments were carried out for bovine serum albumin (BSA) adsorption in expanded beds, where a Streamline 50 column was packed either with Streamline Direct CST I or with Streamline DEAE (a first-generation adsorbent, developed for the EBA process). The hydrodynamics, BSA dynamic binding capacity, and BSA recovery in the

whole EBA process were compared for both adsorbents.

In practical applications, various proteins in source materials prepared from an upstream process or prepared from biological raw materials can be present. When the target protein is captured from these crude feedstocks by an EBA process, the other proteins also competitively bind to adsorbents with the target protein. Therefore, it will be more significant to research multi-component proteins competitive adsorption in an EBA process. Many authors have investigated single-component protein ion exchange rates in a fixed bed and in an EBA process (Alan and Carta, 2000; Bruce and Chase, 2001; Chen et al., 2003, Dziennik et al., 2005; Kaczmarek et al., 2001; Li et al., 2004; Skidmore et al., 1990; Wright and Glasser, 2001), and some authors also have considered the case of multi-component proteins competitive adsorption in a fixed bed (Skidmore and Chase, 1990; Weinmrrner and Etzel, 1994; Martin et al., 2005; Lewus and Carta, 1999; Hubbuch et al., 2003), but few studies have been reported about protein competitive adsorption and desorption in an EBA process.

Furthermore, small molecules such as small peptides interact with the ligand on adsorbent by single point attachment. Their migration velocity depends directly on the binding constant of a single bond. With the multi-component competitive adsorption, the strongly adsorbed component may easily displace the weakly adsorbed component, which leads to roll up in breakthrough curves. However, large molecules, such as proteins and nucleic acids interact with the ligand on adsorbent by multi-point attachment. Their migration velocity depends on the sum of several bonds. Therefore, proteins competitive adsorption on adsorbent is more complex than small molecules. In the published works, Skidmore and Chase(1990) investigated the simultaneous adsorption of lysozyme and BSA in a fixed bed packed with the cation exchanger SP-Sepharose-FF; Weinbrenner and Etzel (2004) investigated the simultaneous adsorption of BSA and lactalbumin on cation exchange membranes; Martin et al. (2005) and Lewus and Carta (1999) investigated the adsorption of lysozyme/cytochrome C mixtures on the cation exchanger SP-Sepharose-FF and S HyperD-M, respectively; and Hubbuch et al. (2003) measured the breakthrough curves of an IgG/BSA solution in the fixed bed packed with the cation exchanger

SP-Sepharose-FF. These authors reached the same conclusion with regard to the competitive nature of protein binding also leading to a roll up of concentration of the less strongly adsorbed protein in some binding conditions.

However, the above motioned adsorbents, SP-Sepharose-FF and S HyperD-M (specially designed for the conventional chromatography), together with Streamline DEAE and Streamline SP (specially designed for an expanded bed), are classical ion-exchangers, in which binding proteins are primarily based on interactions between charged amino acids on the protein surface and oppositely charged immobilized ligands. Protein retention on an ionic surface of adsorbent can be simply explained by the *pI*-value (isoelectric point) of a protein. But in practical applications, it is found these ion exchangers have a lower binding capacity to proteins in high ionic strength and salt concentration feedstocks. Streamline direct CST I is a new type of cation exchanger with multi-modal functional groups, which not only takes advantage of electrostatic interaction, but also takes advantage of hydrogen bond interaction and hydrophobic interaction to tightly bind proteins. In other words, the new type of multi-modal ligand on adsorbent is able to interact with proteins through various intermolecular forces to get a high binding capacity in high ionic strength and salt concentration feedstocks (Johansson et al., 2003a, 2003b).

In this study, we will investigate two-component protein competitive adsorption and desorption in an expanded bed packed with Streamline Direct CST I. A target protein is still selected as BSA, a medium molecular weight protein, which also has a relatively low price and is easily available in market; the other proteins are represented as myoglobin, a relative low molecular weight protein. Moreover, the concentrations of BSA and myoglobin in the sample mixture can be detected easily by spectrophotometer. The investigation contents involve:

1. single- and two-component BSA/myoglobin adsorption isotherm on Streamline Direct CST I
2. frontal analysis and column displacement measurements for BSA and myoglobin competitive adsorption system
3. BSA and myoglobin competitive adsorption and desorption in an expanded bed packed

with Streamline Direct CST I.

7.2. Materials and methods

7.2.1 Equipment, Streamline Direct CST I, and model proteins

A pilot-scale Streamline 50 column (Amersham Pharmacia Biotech, Uppsala, Sweden) was used in an expanded bed experiments. Masterflex peristaltic pumps (Cole-Parmer Instrument Co., Vernon Hills, IL) were used for buffer and feed applications and raise and lower the hydraulic adapter of Streamline 50 column. A XK16/20 column (Amersham Pharmacia Biotech) was used for the frontal analysis and the column displacement experiments. A Jasco 7800 UV detector (Tokyo, Japan) equipped with flowcell was used to monitor online proteins concentrations, and the absorbance signal was logged using the data-acquisition software in a personal computer.

Streamline Direct CST I was purchased from Amersham Pharmacia Biotech, and some properties given by the manufacturer are listed in Table 7.1. The model proteins, BSA and myoglobin were purchased from Sigma (St. Louis, MO). Some properties of BSA and myoglobin are listed in Table 7.2. A sample solution of BSA and myoglobin was prepared with acetate buffer. Acetate buffer is a mixture of acetate sodium and acetic acid. The sample solution of myoglobin should be filtered before used due to impurities. Distilled water was used in all experiments.

BSA has light yellow color, and has a stronger absorbance (ABS) at 280-nm wavelength; myoglobin is deep brown color, there exist both a stronger absorbance of ultraviolet at 280-nm wavelength and a stronger absorbance of visible light at 405-nm wavelength. So the independent concentration for each protein in a mixture of BSA and myoglobin can be measured by spectrophotometry. Myoglobin concentration is measured at 405nm, and a total ABS value of two proteins is measured at 280nm. By subtracting the ABS value of a given myoglobin concentration at 280nm, BSA concentration in the sample is calculated. It should be noted that this method based on the addition of ABS values of proteins, requires a linear relationship between concentration and ABS value during

monitoring by a spectrophotometer.

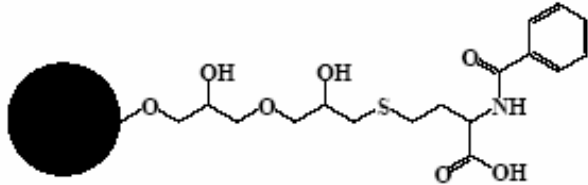
Table 7.1 Some properties of Streamline Direct CST I adsorbent ^a	
Type of exchanger	High salt-tolerant
Functional group ^b	Multimodal function
Matrix structure	Macroporous cross-linked 4% agarose containing stainless steel materials
Particle form	Spherical, 80-165 μm
Mean particle size	135 μm
Mean particle density	1.8g/mL
Degree of expansion(H/H ₀) at 800cm/h	3
^a Streamline Direct CST I has been changed by the manufactures to Streamline Direct HST I . ^b The structure formula of the multimodal functional groups is $\text{—O—CH}_2\text{—CH(OH)—CH}_2\text{—O—CH}_2\text{—CH(OH)—CH}_2\text{—S—CH}_2\text{—CH}_2\text{—CH(COOH)—NH—CO—C}_6\text{H}_5$	
	

Table 7.2 Some properties of BSA and myoglobin	
BSA	myoglobin
Product number A3059	Product number M0630
Purity:99%	Purity: 96%
Color: light yellow	Color: deep brown
Molecular weight: 65,400 g/mol	Molecular weight: 16,890 g/mol
Isoelectric point: 4.7.	Isoelectric point: 7.4
Diffusivity in water: $6.15 \times 10^{-11} \text{ m}^2/\text{s}$,	Diffusivity in water: $11.3 \times 10^{-11} \text{ m}^2/\text{s}$,
Radius of gyration: 29.8Å	Radius of gyration: 16.0 Å

7.2.2 Batch adsorption equilibrium isotherm experiments

Before carrying adsorption equilibrium isotherm experiments, the adsorbents need to be saturated with the appropriate buffer. Adsorbents (0.25 mL or 0.5mL) (particle volume) are equilibrated with 30 mL or 40mL of solutions with different proteins concentration for

about 20 h at 25 °C in a shaking incubator with about 30 rpm; then proteins concentration in supernatant liquid are measured by Jasco7800 UV detector (using a 2 mL quartz cuvette). The adsorption capacity is calculated by mass balance.

7.2.3 Column displacement experiments

XK16/20 column is used for the displacement adsorption measurements between BSA and myoglobin competitive adsorption system. 10mL Streamline Direct CST I is packed to XK16/20 column, where the inside diameter of the column is 16mm, the packed height is 50mm. The bed voidage is measured as 0.35; a given mass of drained adsorbent is packed in the XK16/20 column and the packed height is measured. Since the density of the drained adsorbent measured by pycnometry is 1800kg/m³, the bed voidage is calculated. The adsorbents in the column are reused after regeneration with the solution of 1M NaOH and 1M NaCl. Streamline Direct CST I packed in the column is equilibrated with the binding buffer (50mM acetate buffer(pH 5)) in advance. One protein (BSA or myoglobin) is loaded to the column in downward flow until Streamline Direct CST I adsorbents almost are saturated, and then the column is washed with the binding buffer to remove the unbound protein in the bed voidage and in the pores of the adsorbents. After loading and washing, the other protein (myoglobin or BSA) is fed to the column to displace the bound protein on Streamline Direct CST I. The effluent stream from the bottom of the column will pass through the flowcell where the ABS value of single-component protein or total protein effluent concentration is monitored online by UV at 280nm, and the ABS signal is logged by the data acquisition software in a personal computer. At the same time, the effluent samples are collected by small sample tubes for two component protein adsorption system. Then BSA and myoglobin concentration in these samples are measured off-line by spectrophotometer at 280nm and 405nm, respectively.

7.2.4 Frontal analysis in a fixed bed

XK16/20 column is used for the frontal analysis of BSA and myoglobin competitive adsorption. 15mL Streamline Direct CST I is packed to XK16/20 column, where the inside

diameter of the column is 16mm, the packed height is 75mm, and the bed voidage is 0.35. The adsorbents in the column are reused after regeneration with the solution of 1M NaOH and 1M NaCl. The binding buffer (50mM acetate buffer (pH 5)) is pumped through the column in downward flow to equilibrate Streamline Direct CST I adsorbents, and then the process switches to feedstock application. The proteins concentration in the effluent stream from the bottom of the column was measured as indicated in the previous section.

7.2.5 Experimental procedures for the whole expanded bed adsorption process

A pilot-scale Streamline 50 column is used for the experiments of the EBA process. 300mL Streamline Direct CST I adsorbent is packed in a Streamline 50 column and the settled bed height is 15.3cm.

Expansion/equilibration stage: First, the equilibration buffer (50mM acetate buffer (pH 5)) is pumped through the column with upward flow to an expected expansion degree. Second, the expanded bed is allowed to stabilize at this degree of expansion for about 30-40 min. Then, the liquid axial dispersion in the expanded bed is checked by the Residence Time Distribution (RTD) method; the liquid axial dispersion coefficient should be as small as possible by avoiding inadequate operation. The adaptor will be positioned about 0.5 cm above the height to which the bed expands, to reduce the dead volume in expanded beds.

Adsorption stage: When an expanded bed is stable and equilibrated with the binding buffer (50mM acetate buffer (pH 5)), the process switches to feedstock application. Because of proteins adsorption on adsorbents, the expanded bed height gradually drops. Therefore, the liquid flow velocity will be increased gradually during the adsorption process to maintain a constant degree of bed expansion. The average liquid velocity was calculated from the ratio of total feed volume supplied to the column to the operation time in the loading process. The effluent stream from the top of the column will pass through the flowcell, where the ABS value of total protein effluent concentration is monitored online by spectrophotometry at 280nm, and the ABS signal is logged by the data acquisition software in a personal computer. At the same time, the effluent samples are collected by small

sample tubes. BSA and myoglobin concentration in these samples are measured off-line by spectrophotometer at 280nm and 405nm, respectively

Wash stage: When the measurements of the breakthrough curves were completed, the process switches to the wash buffer (50mM acetate buffer (pH 5)) to wash out the unbound proteins, others loosely bound materials and particulates from the column in expanded mode until the effluent absorbance reaches a relative stable value.

Elution stage: After washing, the pump is turned off and the bed is allowed to settle. When the adsorbent has settled, the adaptor is moved down toward the surface until the edge of the adapter net touches the bed. Elution buffer is then pumped through the settled bed with a downward flow to elute BSA and myoglobin. The effluent samples are collected by small sample tubes, and the concentrations of BSA and myoglobin in these samples are monitored off-line by spectrophotometer, respectively.

7.3. Results and discussion

7.3.1 Single- and two-component BSA /myoglobin adsorption isotherm

In this research, BSA is assigned as a target protein in source materials for proteins preparation. Figures 7.1a-c demonstrate the effects of ionic strength of buffer, salt concentration and pH value in feedstock on BSA adsorption isotherm on Streamline Direct CST I, respectively. From Figure 7.1a, it is apparent that the ligand indeed lacks sensitivity to ionic strength, and the adsorbent has a high binding capacity to BSA even at 100mM acetate buffer. It is sensitive to salt concentration, as shown in Figure 7.1b. However, even at high salt concentration, such as 1M NaCl, the ligand still has a higher binding capacity to BSA, as opposed to classical ion exchangers(such as Streamline SP and Streamline DEAE, specially designed for the expanded bed). Of particular interest is the effect of pH value in feedstock on BSA adsorption isotherm, as shown in Figure 7.1c. The isoelectric point of BSA is about 4.7. When pH is in the range 5-7, BSA has negative charges, so the adsorption of BSA on Streamline Direct CST I takes advantage of the hydrogen bond interaction between BSA and ligand instead of the charge-charge interaction. The

multi-modal ligand is so designed that the hydrogen bond interactions between ligand and proteins are greater in acidic conditions. So the binding capacity of BSA increases as the pH value is reduced from 7 to 5. When pH 4 in the buffer, BSA has positive charges, the adsorption of BSA on Streamline Direct CST I takes advantage of the charge-charge interaction and the hydrogen bond interaction. But the maximum binding capacity to BSA occurs near the isoelectric point of BSA (pH 5) instead of that at pH 4, which means there exists the evident contribution of the hydrophobic interaction between BSA and ligand at pH 5. As stated by the manufacturer, Streamline direct CST I has a ligand with the multi-modal function groups, and this new type of ligand is able to interact with protein through various intermolecular forces to tightly bind proteins. When the ionic strength or salt concentration in feedstock is increased, although the electrostatic interaction becomes significantly weak, but there exist still the hydrogen bond interaction and the hydrophobic interaction between protein and ligand that lead to a higher binding capacity to protein even at a high ionic and salt concentration. Based on the experimental results, the optimum binding condition, with 50mM acetate buffer (pH 5), would be recommended for BSA adsorption in an expanded bed packed with streamline Direct CST I.

The multi-modal ligand on Streamline Direct CST I is not more sensitive to the salt concentration. So it is difficult to elute bound BSA from Streamline Direct CST I only by increasing the salt concentration in the binding condition (50mM acetate buffer (pH 5)). However, when the pH value in the buffer is increased to 7, the binding interactions between BSA and the ligand become significantly weak. Furthermore, when some salt is added to the buffer with pH 7, such as 0.5M salt concentration in the buffer, Streamline Direct CST I also can lose the binding capacity to BSA, as shown in Figure 7.2. Therefore, the elution condition for bound BSA in an EBA process discussed later, should be recommended as the buffer with 0.5M or 1M salt ($\text{pH} \geq 7$), which is very moderate for the recovery of the bound BSA on Streamline Direct CST I.

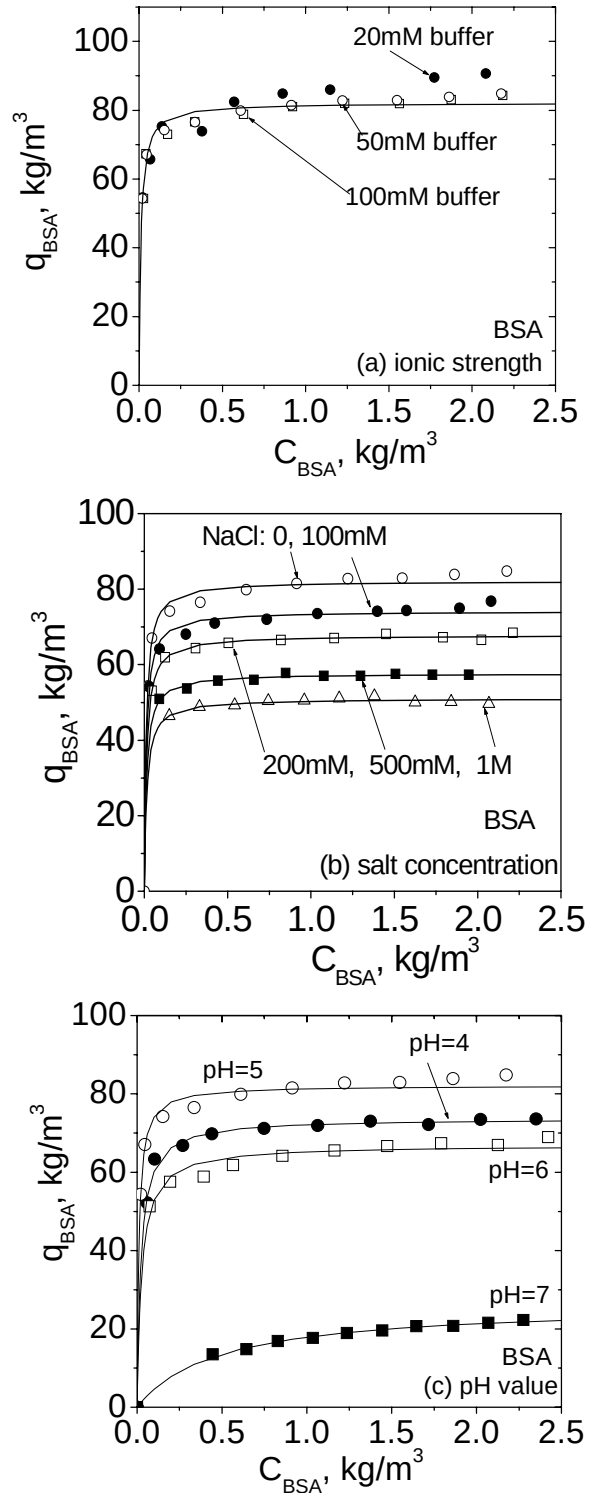


Fig. 7.1 Effect of ionic strength, salt concentration and pH value in feedstocks on BSA adsorption isotherm on Streamline Direct CST I at room temperature ($\sim 25^\circ\text{C}$). (a): different ionic strengths in acetate buffer (pH 5); (b): different salt concentration in 50mM acetate buffer (pH 5); (c): different pH value in 50mM acetate buffer.

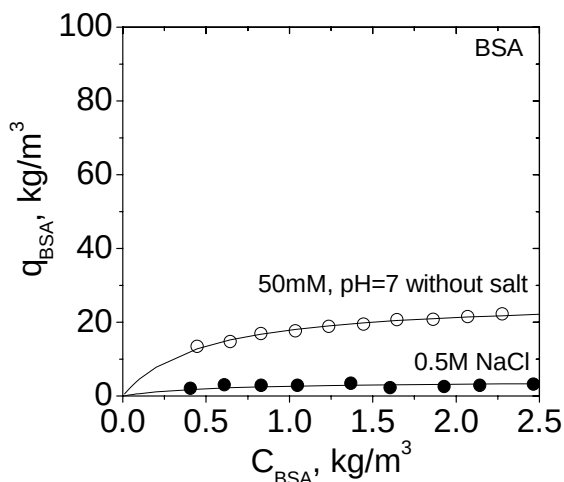


Fig. 7.2 Effect of salt concentration on BSA adsorption isotherm on Streamline Direct CST I at 50mM acetate buffer (PH 7) at room temperature ($\sim 25^{\circ}\text{C}$).

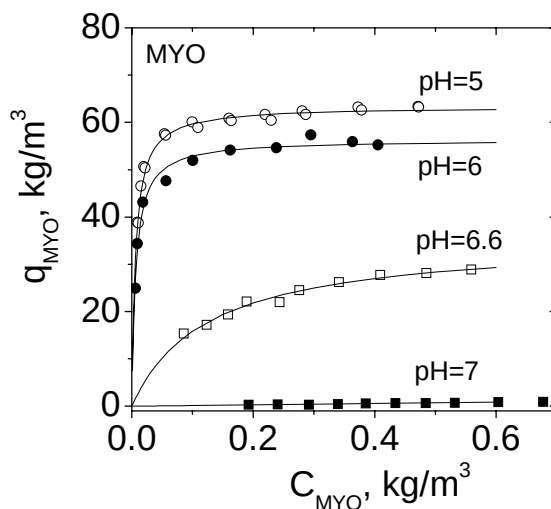


Fig. 7.3 Myoglobin adsorption isotherm on streamline Direct CST I in 50mM acetate buffer with different pH value at room temperature ($\sim 25^{\circ}\text{C}$).

In source materials for proteins preparation, the other proteins, are here represented as myoglobin; its adsorption capacity on streamline direct CST I in the optimum binding condition for BSA (50mM acetate buffer (pH 5)), is shown in Figure 7.3. The multi-modal ligand also can bind myoglobin with a high capacity. BSA maximum adsorbed capacity is about $82.15\text{kg}/\text{m}^3$ and myoglobin maximum adsorbed capacity is up to $63.35\text{kg}/\text{m}^3$.

Therefore, when capturing target protein BSA from feedstock at the binding condition with 50mM acetate buffer(pH 5), myoglobin will compete for adsorption with BSA on Streamline Direct CST I. Moreover, in contrast to BSA adsorption behavior, myoglobin retention on an ionic surface of adsorbent can be simply explained by the *pI*-value of myoglobin. When increasing pH value up to 7, that is, approaching to the isoelectric point of myoglobin (pI 7.4), the myoglobin binding capacity decreases significantly as a result of the decrease of the net charges of myoglobin.

BSA and myoglobin competitive adsorption isotherm on streamline direct CST I is measured by static batch experiments at the binding condition with 50mM acetate buffer (pH 5). In the serial batch experiments, the initial sample solutions are prepared with a mixture of 1kg/m^3 BSA and different amounts of myoglobin. Experimental results are shown in Figure 7.4. Without myoglobin in initial BSA solution, Streamline Direct CST I has a high adsorption capacity to BSA up to 82.4kg/m^3 (per particle volume). When there exists myoglobin in initial BSA solution, BSA binding capacity decreases significantly as a result of myoglobin competitive adsorption with BSA on Streamline Direct CST I.

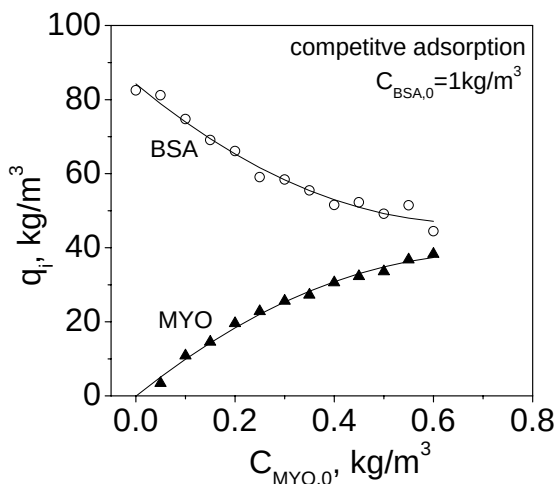


Fig. 7.4 BSA and myoglobin competitive adsorption isotherm on Streamline Direct CST I measured in static batch experiments at 50mM acetate buffer (pH 5) at room temperature ($\sim 25^\circ\text{C}$).

7.3.2 Frontal analysis and column displacement measurements

Column displacement experiments are carried out for the evaluation of the displacement adsorption between BSA and myoglobin on Streamline Direct CST I at the binding condition (50mM acetate buffer (pH 5)), where a XK16/20 column is packed with 10mL of Streamline Direct CST I.

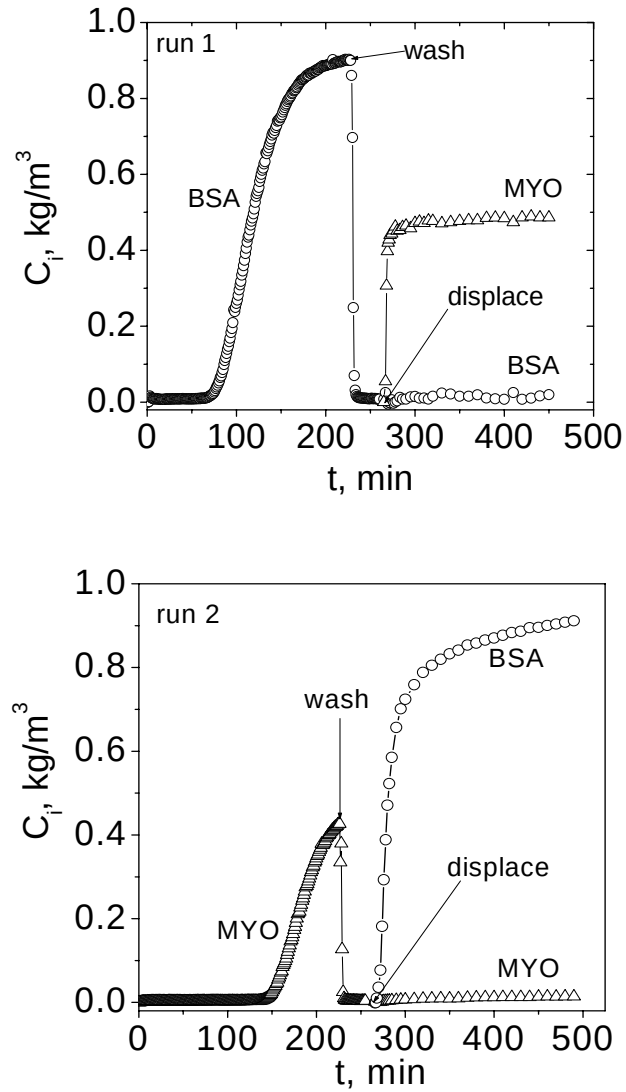


Fig. 7.5 Displacement adsorption between BSA and myoglobin in a fixed bed packed with Streamline Direct CST I. (a) in run 1, with 4.4mL/min flow rate at loading and washing stages and 5.1mL/min at displacing stage; (b) in run 2, with 4.1mL/min flow rate at loading, washing and displacing stages.

For the case of run 1 in Figure 7.5a, BSA with 1kg/m^3 concentration is loaded to the column until Streamline Direct CST I adsorbents almost are saturated, and then the column is washed with the binding buffer (50mM acetate buffer (pH 5)) to remove the unbound BSA in the bed voidage and in the pore of the adsorbents. After loading and washing, myoglobin with 0.5kg/m^3 concentration is applied to the column to displace the bound BSA on Streamline Direct CST I. Based on the experimental results shown in Figure 7.5a (run1), it seems that myoglobin can not displace the bound BSA at the binding condition with 50mM acetate buffer(pH 5). In turn, for the case of run 2 in Figure 7.5b, myoglobin with 0.5kg/m^3 concentration is loaded to the column until Streamline Direct CST I adsorbents are almost saturated, and then the column is washed with the binding buffer (50mM acetate buffer (pH 5)) to remove the unbound myoglobin in the bed voidage and in the pore of the adsorbents. After loading and washing, BSA with 1.0kg/m^3 concentration is applied to the column to displace the bound myoglobin. Based on the experimental results shown in Figure 7.5b(run 2), BSA slightly displace the bound myoglobin on Streamline Direct CST I, but the displacement rate is extremely slow as result of myoglobin multi-point binding tightly by ligand on Streamline Direct CST I at the binding condition with 50mM acetate buffer (pH=5). The very weak displacement adsorption between BSA and myoglobin also suggests that the two proteins are adsorbed on different sites at the binding condition (50mM acetate buffer with pH 5).

Frontal analysis is carried out in a fixed bed to measure the breakthrough curves of single-component BSA and two-component BSA/myoglobin for a longer timescale, where a XK16/20 column is packed with 15mL of Streamline Direct CST I.

Figure 7.6a shows the breakthrough curve of single-component BSA with 1kg/m^3 feed concentration. Without myoglobin competitive adsorption, the dynamic binding capacity of BSA is 33.8 mg (BSA)/mL of fixed bed volume at 5% breakthrough point in the column. BSA dynamic binding capacity, $Q_{i,5\%}$, is calculated from BSA breakthrough curve measured. The formula is defined as

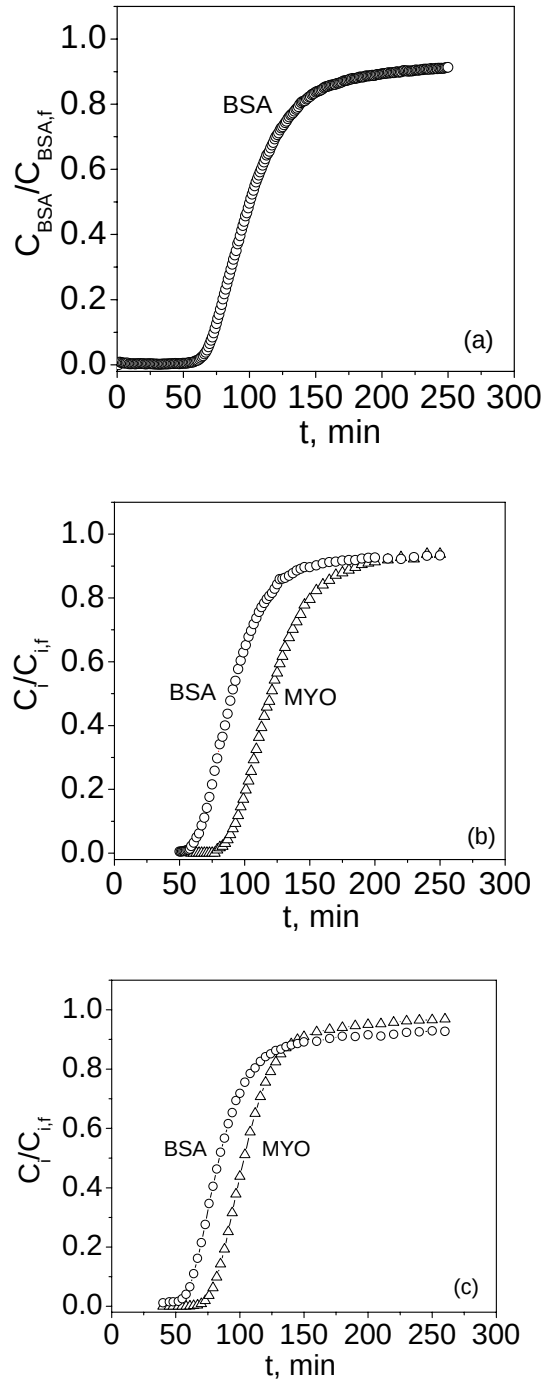


Fig. 7.6 Breakthrough curves in a fixed bed (XK16/20 column) packed with Streamline Direct CST I for single- and two-component BSA/myoglobin adsorption system. (a) single-component, BSA, with $C_{BSA,f} = 1 \text{ kg/m}^3$ at 7.4 mL/min flow rate; (b) two-component, BSA and myoglobin, $C_{BSA,f} = 1 \text{ kg/m}^3$ and $C_{MYO,f} = 0.1 \text{ kg/m}^3$ at 7.5 mL/min flow rate; (c) two-component, BSA and myoglobin, with $C_{BSA,f} = 1 \text{ kg/m}^3$ and $C_{MYO,f} = 0.2 \text{ kg/m}^3$ at 7.1 mL/min flow rate.

$$Q_{i,5\%} = \frac{\int_0^{V_{5\%}} (C_{i,f} - C_i) dV}{V_A} \quad (1)$$

where V is the effluent liquid volume from the fixed bed, $V_{5\%}$ is the effluent liquid volume at 5% BSA breakthrough point, and V_A is the packed volume of adsorbent. With myoglobin in feedstock, such as with 0.1kg/m^3 myoglobin (in Figure 7.6b) and with 0.2kg/m^3 myoglobin (in Figure 7.6c), the dynamic binding capacity of BSA (still with 1kg/m^3 feed concentration) decreases to 31.5 mg (BSA)/mL of fixed bed volume (calculated from Figure 7.6b) and decreases to 27.9 mg (BSA)/mL of fixed bed volume (calculated from Figure 7.6c), respectively, as a result of myoglobin competitive adsorption with BSA on Streamline Direct CST I. By the way, in the feedstock, the difference of mass concentrations between BSA and myoglobin assigned are bigger for the convenient monitoring by UV detector, but the molar concentrations close with each other for the case of feedstock with 0.2kg/m^3 concentration of myoglobin and 1kg/m^3 concentration of BSA.

Although we intentionally measured the breakthrough curves for a longer timescale, there was no roll-up in breakthrough curves for BSA and myoglobin competitive adsorption in the fixed bed packed with Streamline Direct CST I, as shown in Figure 7.6b and Figure 7.6c. The roll up of the concentration curve usually is caused by the displacement adsorption between the stronger adsorption component and the weaker adsorption component. Therefore, the fact that there is no roll up suggests that the two proteins are adsorbed on different sites at the binding condition (50mM acetate buffer with pH 5).

At the binding condition (50mM acetate buffer (pH 5)), the competitive adsorption between BSA and myoglobin is more significant in a fixed bed packed with Streamline Direct CST I. The adsorption isotherms of both proteins are high favorable, and almost irreversible because of the ligand tightly binding either BSA or myoglobin with various intermolecular forces; the displacement adsorption between two proteins is very weak as result of the two proteins adsorbed on different sites. Therefore, BSA and myoglobin will take competitively the adsorption sites on Streamline Direct CST I packed in the column.

However, the lower molecular myoglobin has a fast transport rate (smaller film mass transfer resistance and smaller intraparticle diffusion resistance) than that larger molecular BSA has, myoglobin will take the adsorption sites in adsorbent more easily; and myoglobin also can bind to some adsorption sites inaccessible to larger molecular BSA. So it will be found that BSA breaks the bed always earlier than that of myoglobin, as shown in Figure 7.6b and 7.6c.

There exists the tailing behavior of the breakthrough curves for both proteins when effluent concentration approaches the feed concentration. For the lower molecular protein, myoglobin, the tailing behavior is not significant, in contrast, for the larger molecular protein, BSA, the tailing behavior is very significant. In previous published papers, the tailing behavior was explained by the presence of the impurities in protein samples, or microporous diffusion in the macroporous adsorbent, or protein steric hindrance on active sites of the surface of the adsorbent. Up to now, it is still unclear the explanation for the tailing behavior of the breakthrough curves often occurring for medium size and big size proteins adsorption.

Here, based on the experimental data of the breakthrough curves, the binding capacities for both BSA and myoglobin on Streamline Direct CST I packed in a fixed bed are calculated as 88.8kg(BSA)/m³ of particle volume in Figure 7.6a, 80.2kg (BSA)/m³ of particle volume and 10.3kg(myoglobin)/m³ of particle volume in Figure 7.6b, and 73.3.kg/m³ (BSA)/m³ of particle volume and 16.6kg(myoglobin)/m³ of particle volume in Figure 7.6c, respectively. Comparing these results with the experimental data in Figure 7.4 measured by static batch experiments, myoglobin binding capacities in the fixed bed almost are close to that measured in static batch experiments. In contrast, BSA binding capacities in the fixed bed are more than those measured in static batch experiments. This deviation is caused by the tailing behavior of BSA breakthrough curve, which is explained as interactions with itself and the consequent formation of dimers in the packed bed (Skidmore et al., 1990).

7.3.3 BSA and myoglobin competitive adsorption and desorption in an expanded bed

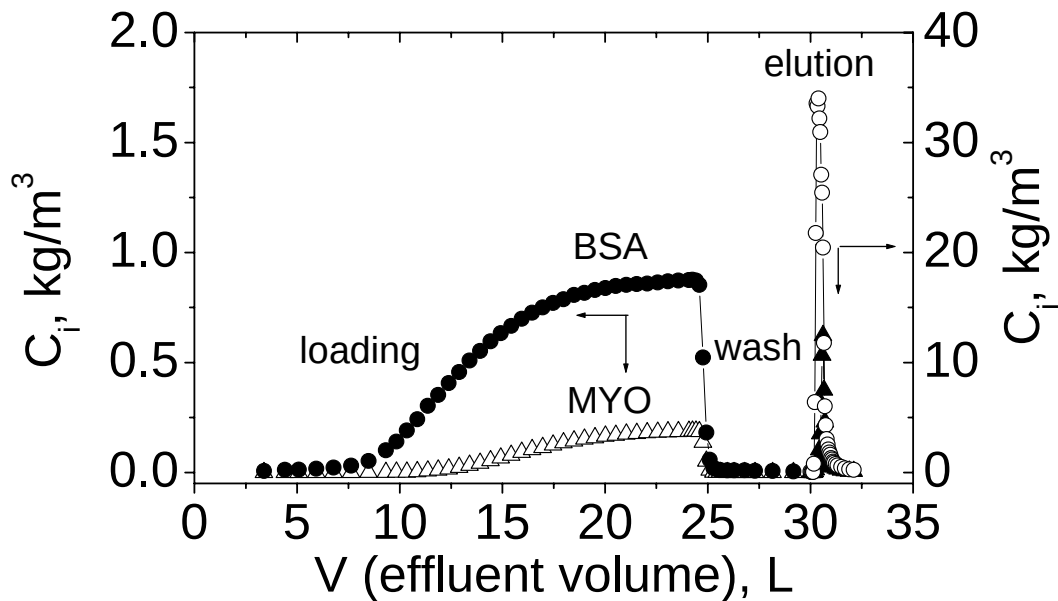


Fig. 7.7 Effluent curves of BSA and myoglobin during adsorption, wash and elution stages in expanded bed packed with Streamline direct CST I. At the adsorption stage, the feedstock with 1 kg/m^3 BSA and 0.2 kg/m^3 myoglobin, prepared with 50 mM acetate buffer (pH 5), is applied from the bottom of the expanded bed at 169 mL/min flow velocity; at the wash stage, 50 mM acetate buffer with pH 5, is applied from the bottom of the expanded bed at about 169 mL/min flow velocity; and at the elution stage, 50 mM phosphate buffer with 1 M NaCl, (pH 7) is applied from the top of the settled bed at 44 mL/min.

An experiment is carried out to capture both BSA and myoglobin from feedstock by an EBA process, where a Streamline 50 column is packed with 300 mL of Streamline Direct CST I. The feedstock with a mixture of 1 kg/m^3 BSA and 0.2 kg/m^3 myoglobin is applied to the expanded bed at 517 cm/h flow rate. The expansion degree is about twice settled bed height (30.3 cm/15.3 cm). BSA and myoglobin are adsorbed by suspended Streamline Direct CST adsorbents in an expanded bed. After the adsorption stage, the bed is washed and the bound BSA and myoglobin are desorbed at the elution stage. The detailed operation procedures have been previously described. The experimental results are shown in Figure 7.7, where circle points marked for BSA and triangle points marked for myoglobin. Before

the application of the feedstock to an expanded bed, the liquid axial dispersion coefficient in an expanded bed is measured by tracer technique using Residence Time Distribution (RTD) method. About 50%v/v concentration of acetone (1.5 mL buffer aqueous solution) is injected as dirac input, at the bottom of the column. The liquid axial coefficient is measured as $9.6 \times 10^{-6} m^2 / s$.

At the binding condition with 50mM acetate buffer (pH 5), Streamline Direct CST I can efficiently capture both BSA and myoglobin from the feedstock in the expanded bed. At the breakthrough point of 5% BSA feed concentration, BSA dynamic binding capacity is 28.5 mg (BSA)/mL of settled bed volume, and myoglobin dynamic binding capacity is 5.65 mg (myoglobin)/mL of settled bed volume. The dynamic binding capacities of both BSA and myoglobin in this EBA process are close to the experimental results measured in a fixed bed adsorption process with 212cm/h feed flow rate (Figure 7.6c), where BSA dynamic binding capacity is 27.9 mg (BSA)/mL of fixed bed volume, and myoglobin dynamic binding capacity is 5.58 mg (myoglobin)/mL of fixed bed volume. Therefore, the two-component protein competitive adsorption behaviors in a stable expanded bed are comparable to that in a fixed bed.

At the washing stage, 50mM acetate buffer (pH 5) is used to wash the column. It is found that the effluent concentrations both of BSA and myoglobin quickly drop and approach the baseline in an expanded bed of Streamline direct CST I, which means almost irreversible adsorption of both BSA and myoglobin on Streamline Direct CST I.

When the elution buffer, 50mM phosphate buffer with 1M NaCl (pH 7), is used in the elution stage, BSA recovery in the whole EBA process can reach 95% and myoglobin recovery reaches 88%, the consumption amount of the elution buffer is very small, as shown in Figure 7.7. After the elution stage, the solution of a mixture of 1M NaOH and 1MNaCl is used to regenerate the column, a small concentration peak is found, that means proteins are not eluted completely during the elution stage. There are some strong adsorption sites on Streamline Direct CST I, which bind proteins so tightly that the bounded proteins can not be eluted easily at the moderate elution condition. Because of the

lower molecular myoglobin having a fast transport rate, these strong adsorption sites maybe bind more myoglobin than BSA, which results that the recovery of myoglobin is lower than that of BSA.

7.4 Conclusions

Streamline Direct CST I can capture BSA from high ionic strength and salt concentration feedstocks with a high binding capacity, as opposed to classical ion exchangers, such as Streamline SP and Streamline DEAE. At the optimum binding condition of BSA, 50mM acetate buffer (pH 5), Streamline Direct CST I also can capture myoglobin with a high binding capacity.

The competitive adsorption between BSA and myoglobin on Streamline Direct CST I is more significant, but the displacement adsorption between the two proteins becomes very weak. Furthermore, there are no a roll up in breakthrough curves caused by the displacement adsorption between BSA and myoglobin that suggests the two proteins are adsorbed on different sites at the binding condition, 50mM acetate buffer(pH 5).

A Streamline 50 column packed with 300mL Streamline Direct CST I was used to capture both BSA and myoglobin from the feedstock of 1kg/m³ BSA and 0.2kg/m³ myoglobin at 517cm/h feed flow rate. At the binding condition with 50mM acetate buffer (pH 5), BSA dynamic binding capacity is 28.5 mg(BSA)/mL of settled bed volume, and myoglobin dynamic binding capacity is 5.65 mg (myoglobin)/mL of settled bed volume in the EBA process. Then, 50mM phosphate buffer with 1M NaCl (pH 7) is used to elute both bound BSA and bound myoglobin, the recovery of BSA and myoglobin are 95% and 88% respectively, and a little elution solution is consumed.

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8. Proteins separation and purification by salt gradient ion-exchange simulated moving bed

The process performance of the separation and purification of proteins by ion-exchange simulated moving bed (SMB) can be improved when a lower salt concentration is formed in section III and IV to increase the adsorption of proteins and a higher salt concentration is formed in section I and II to improve the desorption of the bound proteins, called salt gradient ion-exchange SMB. In this chapter, a real gradient SMB model is used to analyze the performance of salt gradient ion-exchange SMB for linear and nonlinear ion-exchange equilibrium isotherm of proteins. Some strategies will be discussed for the selection of salt gradient and the selection of flowrate in each section of salt gradient ion-exchange SMB for two cases, (a) binary separation of proteins with complete recovery from extract stream and raffinate stream; and (b) protein purification from a stream with some impurities. The gradient SMB configurations, open loop, closed loop and closed loop with a holding vessel, are compared, and when a gradient SMB is run with closed loop, a holding vessel with a given volume is added to the system to mix the desorbent with the recycled liquid stream from section IV during a switch time interval, in order to reduce the fluctuation of salt or solvent strength in the column. Moreover, we will present a comparison of the two strategies of modeling, equivalent gradient TMB model and real gradient SMB model, for the prediction of internal concentration profiles in gradient SMB with open loop and closed loop.

8.1 Introduction

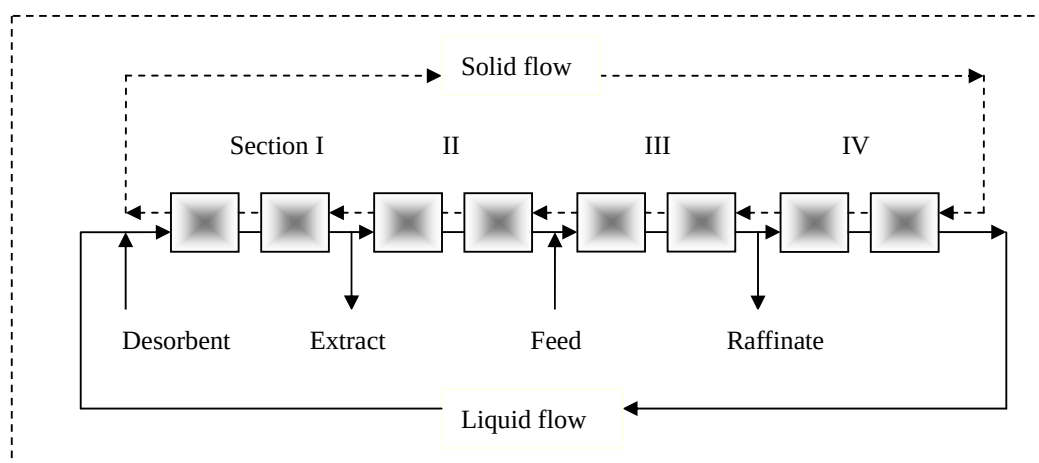
Simulated moving bed (SMB) chromatography is a continuous process, which for preparative purposes can replace the discontinuous regime of elution chromatography. Furthermore, the countercurrent contact between fluid and solid phase used in SMB chromatography maximizes the mass transfer driving force, leading to a significant reduction in mobile and stationary phase consumption when compared with elution chromatography. With the established theory and technique (Broughton and Gerhold, 1961; Ruthven and Ching, 1989; Ma and Wang, 1997; Mazzotti et al, 1997; Rodrigues and Pais, 2004), the resolution of very similar products on preparative scale (ktons/year) can be performed efficiently using SMB chromatography. Successful examples are the separation of para-xylene from a mixture of C8 isomers, the separation of glucose and fructose, and the resolution of enantiomers on chiral stationary phases (Broughton, 1984; Ruthven and Ching, 1989; Pais et al., 1997; Juza et al., 2000; Azevedo and Rodrigues, 2001). The new challenge for the SMB technology is its application to the separation and purification of biomolecules; examples of products that are considered for SMB separation and purification are therapeutical proteins, antibodies, nucleosides and plasmid DNA et al. (Gottschlich and Kasche, 1997; Imamoglu, 2002; Houwing et al., 2002; Xie et al., 2002; Paredes et al., 2005a; Geisser et al., 2005; Andersson and Mattiasson, 2006).

In downstream processing, the frequently used chromatographic methods for separating and purifying proteins take advantage of physical properties that vary from one protein to the other, including size, charge, hydrophobicity, and specially binding capacity. The separation method based on the difference of protein size or shape is called size exclusion (gel filtration) chromatography (SEC). Ion exchange chromatography (IEC) takes advantage of the charge-charge interaction between protein and ligand, whilst hydrophobic interaction chromatography (HIC) or reversed-phase chromatography (RPC) takes advantage of the hydrophobic interaction between protein and ligand, and affinity chromatography (AC) is based on the specific binding between protein and ligand.

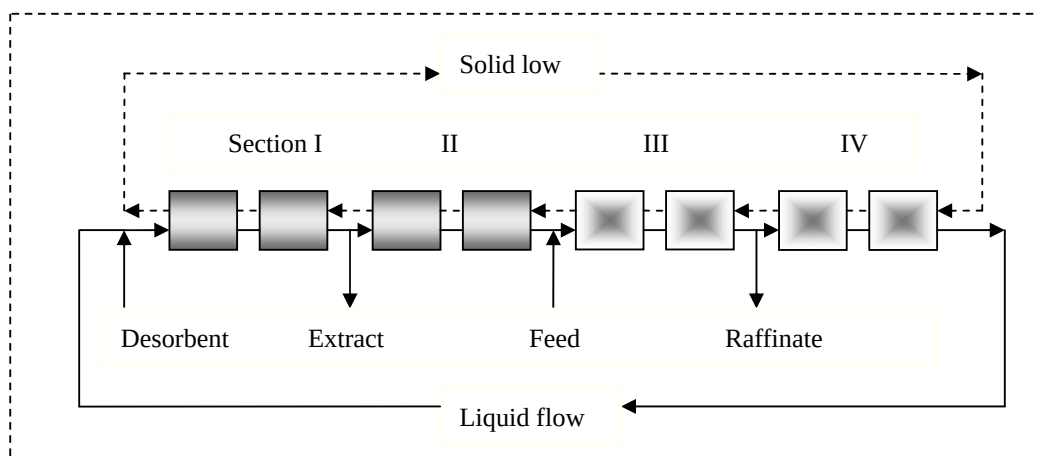
Research on the separation and purification of proteins by SMB technology started with size exclusion –simulated moving bed (SE-SMB) chromatography, for its design

simplicity in terms of the liquid and solid flow rate ratios (linear distribution coefficients for all proteins on porous stationary phases). Experimental examples are the purification of plasmid DNA by SE-SMB packed with Sepharose FF particles (Galatea et al., 2005a, 2005b), the separation of bovine serum albumin (BSA) and myoglobin by SE-SMB packed with Sepharose Big Beads (Houwing et al., 2003) and the insulin purification by SE-SMB packed with Sephadex G50 gel (Xie et al., 2002). Because of the absence of ligand in these particles, the distribution coefficients of proteins only depend on the accessible porosity in the particles. A large protein has a smaller distribution coefficient, as weakly retained component, and will elute in the raffinate stream in the SE-SMB (a four-zone SMB); in contrast, a small protein has a bigger distribution coefficient, as strongly retained component, will elute from the extract stream in the SE-SMB. It is easy to obtain a high purity protein (large molecular weight) from the raffinate stream, but in the extract stream, it is very difficult to obtain the high purity protein (low molecular weight) as a result of the limitation of the slow mass transfer resistance of larger molecular protein (steric hindrance), as shown in the experimental results reported by Houwing et al (2003).

Due to the limitation of SE-SMB technology for the separation and purification of the proteins, research was extended to the application of ion exchange-simulated moving bed (IE-SMB), reversed phase-simulated moving bed (RP-SMB) or affinity-simulated moving bed (A-SMB) (Jensen et al, 2000; Houwing et al, 2002; Abel et al, 2004). By carefully selecting the buffer, pH value, solvent strength and ligand of adsorbent to let the larger molecular protein elute from the extract stream, and the smaller protein elute from the raffinate stream, the two proteins can be separated efficiently by SMB chromatography. When the binding capacities of proteins on adsorbent are close to each other, an isocratic SMB mode may be used to separate and purify the proteins, where the adsorbents have the same affinity capacity to proteins in all sections in SMB chromatography, as shown in Figure 8.1a. However, usually the binding capacities of proteins are so different in IEC, HIC or RPC, and AC that we can not separate them by the isocratic mode with a reasonable retention time. In conventional elution chromatography, a gradient mode should be used for the separation of proteins. It is most commonly applied in reversed phase and ion exchange chromatography, by changing the concentration of the organic solvent and salt in



(a) isocratic SMB with closed loop



(b) gradient SMB with closed loop

Figure 8.1. Schematic diagram of a four-zone isocratic/gradient SMB with closed loop

 : higher solvent strength;
  : lower solvent strength.

a step-wise gradient or with a linear gradient, respectively. For SMB chromatography, only a step-wise gradient can be formed by introducing a solvent mixture with a lower strength at the feed inlet port compared to the solvent mixture introduced at the desorbent port; then the adsorbents have a lower binding capacity to proteins in section I and section II to improve the desorption and have a stronger binding capacity in section III, and IV to increase adsorption in SMB chromatography, as shown in Figure 8.1b. Some authors state

that the solvent consumption by gradient mode can be decreased significantly when compared with isocratic SMB chromatography (Jensen et al, 2000; Antos et al, 2001, 2002; Abel et al., 2002, 2004; Houwing et al, 2002; Ziomek et al, 2005). Moreover, when a given feed is applied to gradient SMB chromatography, the protein obtained from the extract stream can be enriched if protein has a medium or high solubility in the solution with the stronger solvent strength, while the raffinate protein is not diluted at all (Jensen et al., 2000).

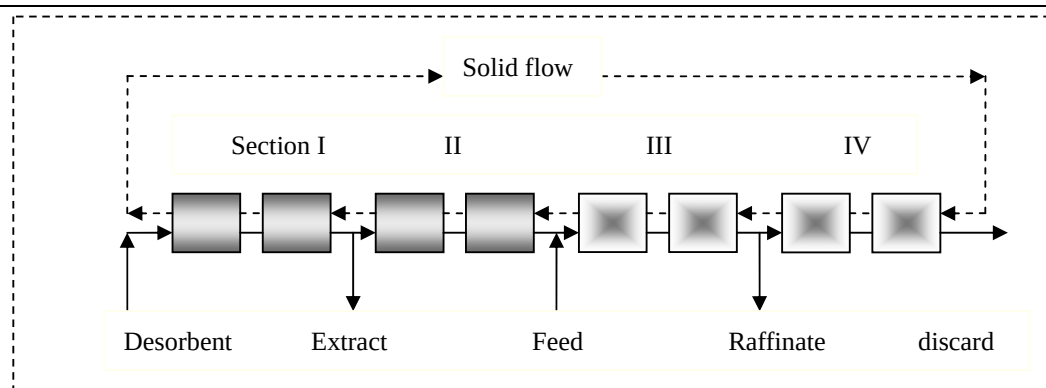
Experimental research for the separation of proteins by salt gradient ion exchange SMB chromatography (Houwing et al, 2002) and for the separation of antibodies by solvent gradient reversed phase SMB chromatography (Jensen, 2003) allowed a qualitative analysis of process feasibility. Moreover, theoretical analysis for gradient SMB chromatography was reported by some authors (Jensen et al, 2000; Antos et al, 2001; Houwing et al 2002; Abel et al., 2004; Ziomek et al, 2005), confirming the potential application of gradient SMB chromatography in bioseparations. Up to now, the research is just underway, because of the expensive experiments for practical proteins separation and purification by gradient SMB chromatography; also authors used an equivalent gradient TMB (true moving bed) model to simplify the simulation, and only linear adsorption was dealt with. Therefore, a detailed mathematical simulation, using a real gradient SMB model instead of an equivalent gradient TMB model, is more significant to understand the behavior of gradient SMB chromatography.

In this chapter, we will begin our research with simulation, using a real gradient SMB model, to study the separation performance of proteins by salt gradient ion exchange SMB chromatography. Ion exchange is probably the most frequently used chromatographic technique for the separation and purification of proteins. The reasons for the success of ion exchange are its widespread applicability, its high resolving power, its high capacity, and the simplicity and controllability of the method. In addition, instead of the common Langmuir competitive adsorption isotherm, in our simulation, the steric mass action (SMA) model (Brooks and Cramer, 1992) is used to describe the proteins competitive adsorption isotherm on ion exchange resin.

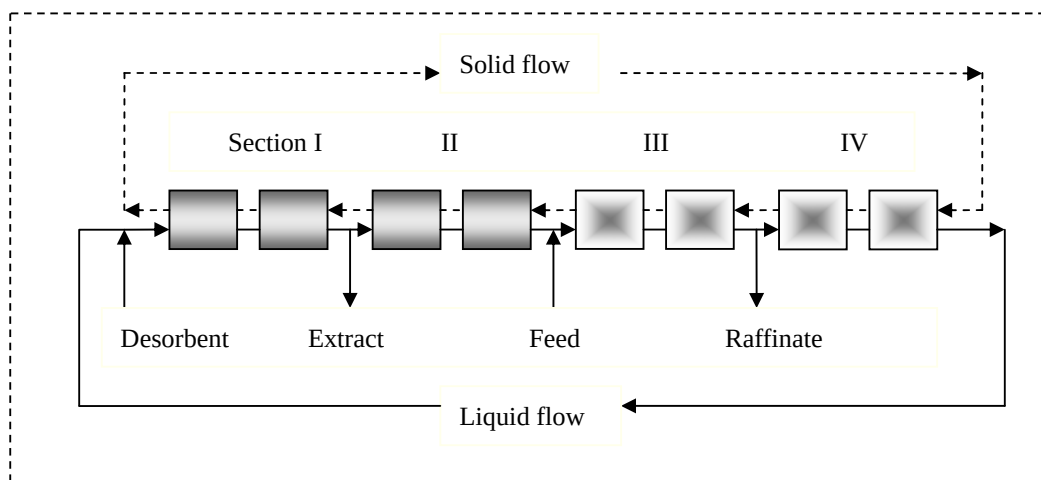
Furthermore, in the separation and purification of proteins by SMB chromatography,

the open loop configuration was used by many authors in order to avoid the accumulation of contaminants in the columns, as shown in Figure 8.2a, where the liquid stream from section IV is discarded, instead of being recycled to the desorbent stream for further reduction of desorbent consumption. It is well known that one of the biggest advantages of SMB chromatography compared with fixed bed chromatography is the reduced desorbent consumption. This can be achieved in the closed loop configuration by recycling the liquid stream from section IV to the desorbent inlet of section I, as shown in Figure 8.2b, which is very important for RP-SMB chromatography because of the large amount of organic solvent being consumed. However, in gradient SMB chromatography, the recycling of liquid stream is more complicated. The solvent strength in the eluent is different in section I and IV, and the effluent composition from section IV varies in a dynamic manner during a switch time interval. This complicates the direct recycling of the eluent (Jensen, 2003). In Figure 8.2c, a holding vessel with a given volume is added to the system to mix the desorbent with the recycled liquid stream from section IV during a switch time interval, in order to reduce the fluctuation of the solvent strength in the columns.

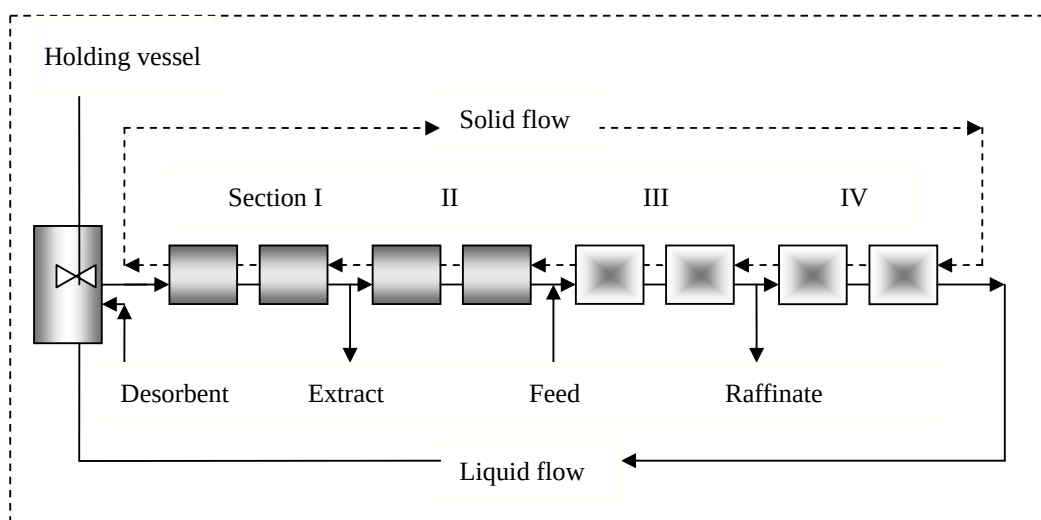
Therefore, in this chapter, the simulation will be carried out for gradient SMB chromatography with open loop (Figure 8.2a), closed loop (Figure 8.2b), and closed loop and a holding vessel (Figure 8.2c).



(a) gradient SMB with open loop



(b) gradient SMB with closed loop



(c) gradient SMB with closed loop and a holding vessel

Figure 8.2. Operation modes for gradient SMB.

 : higher solvent strength;  : lower solvent strength.

8.2. Gradient SMB strategies of modeling

8.2.1 Formation of salt gradient in ion-exchange SMB chromatography

A four-zone SMB chromatography is used, as shown in Figure 8.1, and is constituted by a set of identical fixed-bed columns, which are connected in series. Each column is packed with anion or cation exchangers. Here, Q-Sepharose FF anion exchanger is used to separate BSA and myoglobin, as done by Houwing et al.(2002). Some properties of Q-Sepharose FF resin are shown in Table 8.1. It is well known that the binding capacity of the classical ion exchanger to proteins is very sensitive to salt concentration in the feedstock; the higher the salt concentration is, the lower the binding capacity to the proteins is. Therefore, a step-wise gradient is formed by introducing a lower salt concentration at the feed inlet port compared to a higher salt concentration introduced at the desorbent port; then the ion exchanger has a lower binding capacity to proteins in section I and II to improve the desorption and has a stronger binding capacity in section III, and IV to increase adsorption in ion –exchange SMB chromatography.

Table 8.1 Some properties of Q-Sepharose-FF anion exchanger	
Matrix structure	6% highly cross-linked agarose
Mean particle size	90 μm (range 45-165 μm)
Charged group Quaternary ammonium (Q)	$-\text{CH}_2-\text{N}^+(\text{CH}_3)_3$
Type of medium	Strong anion
Total ionic capacity	0.18-0.25mmol Cl^-/mL medium
Pore diameter	About 350Å
Polymer string diameter	About 56Å
Dynamic binding capacity	120 mg HSA/mL medium (50% breakthrough)
Pressure/flow spec	400-700cm/h (100kPa)

8.2.2 Model equations for the real gradient SMB model

Model equations for the gradient SMB model result from the mass balances over a volume element of the bed and inside the particle. Axial dispersion flow for the bulk fluid phase is included and the linear driving force (LDF) approximation is used to describe the

intraparticle mass-transfer rate.

Mass balance over a volume element of the bed k for proteins and salt:

$$\frac{\partial C_{ik}}{\partial t} = D_{Lk} \frac{\partial^2 C_{ik}}{\partial Z^2} - \frac{u_k}{\varepsilon_B} \frac{\partial C_{ik}}{\partial Z} - \frac{(1-\varepsilon_B)}{\varepsilon_B} k_{pik} [q_{ik}^* - q_{ik}] \quad (8.1)$$

Mass balance in the particle for proteins and salt described by linear driving force (LDF) model:

$$\frac{\partial q_{ik}}{\partial t} = k_{pik} (q_{ik}^* - q_{ik}) \quad (8.2)$$

where C is the concentration in the fluid phase; q is the average adsorbed phase concentration in adsorbent; q^* is the average adsorbed phase concentration in equilibrium with fluid phase concentration; Z is the axial distance from the column entrance; t is the time; ε_B is the bed voidage in column; u is the superficial velocity; D_L is the axial dispersion coefficient; k_p is the mass transfer coefficient, k refers to the column number, total N columns in gradient SMB; and i refers to proteins and salt.

Initial conditions:

$$t = 0: C_{ik} = q_{ik} = 0 \text{ for proteins} \quad (8.3a)$$

Before feed is applied to the column, a salt gradient has been formed in the columns of SMB as

$$C_{Sk} = C_S^F, \quad q_{Sk} = q_{Sk}^*(C_S^F) \text{ in section III and IV} \quad (8.3b)$$

$$C_{Sk} = C_S^D, \quad q_{Sk} = q_{Sk}^*(C_S^D) \text{ in section I and II} \quad (8.3c)$$

Boundary conditions in each column for proteins and salt

$$D_{Lk} \varepsilon_B \frac{\partial C_{ik}}{\partial Z} \Big|_{Z=0} = u_k [C_{ik}|_{Z=0} - C_{ik,0}] \quad (8.4a)$$

$$\frac{\partial C_{ik}}{\partial Z} \Big|_{Z=L_k} = 0 \quad (8.4b)$$

Mass balances at nodes:

at desorbent node

$$C_{i1,0} = C_i^D \quad \text{open loop} \quad (8.5a)$$

$$C_{i1,0} = \frac{Q_D C_i^D + Q_{rec} C_{iN} \big|_{Z=L_N}}{Q_I} \quad \text{closed loop} \quad (8.5b)$$

$$V_S \frac{dC_{i1,0}}{dt} + Q_I C_{i1,0} = Q_D C_i^D + Q_{rec} C_{iN} \big|_{Z=L_N} \quad \text{closed loop with a holding vessel} \quad (8.5c)$$

The holding vessel has a constant volume V_S with initial condition of $C_{i1,0} = C_i^D$ at $t = 0$

at extract node

$$C_{ik+1,0} = C_{ik} \big|_{Z=L_k} \quad (8.5d)$$

at feed node

$$C_{ik+1,0} = \frac{Q_F C_i^F + Q_{II} C_{ik} \big|_{Z=L_k}}{Q_{III}} \quad (8.5e)$$

at raffinate node

$$C_{ik+1,0} = C_{ik} \big|_{Z=L_k} \quad (8.5f)$$

At the nodes between other columns

$$C_{ik+1,0} = C_{ik} \big|_{Z=L_k} \quad (8.5g)$$

Global balances:

$$Q_I = Q_D \quad \text{open loop} \quad (8.6a)$$

$$Q_I = Q_D + Q_{rec} \quad \text{closed loop} \quad (8.6b)$$

$$Q_{II} = Q_I - Q_E \quad (8.6c)$$

$$Q_{III} = Q_{II} + Q_F \quad (8.6d)$$

$$Q_{IV} = Q_{III} - Q_R \quad (8.6e)$$

where Q_I , Q_{II} , Q_{III} , Q_{IV} are flowrate in Section I, II, III, and IV, respectively; Q_D , Q_E ,

Q_F , Q_R and Q_{rec} are desorbent flowrate, extract flowrate, feed flowrate, raffinate

flowrate, and recycle flowrate, respectively; C_i^D and C_i^F are concentration in desorbent

and in feed, respectively.

As a result of the switch of inlet and outlet lines, each column plays different functions during a whole cycle, depending on its location (section). As a consequence, we shall notice that the boundary conditions for each column change after the end of each switch time interval. When the cyclic steady state is reached, the internal concentration profiles vary during a given cycle, but they are identical at the same time for two successive cycles.

8.2.3 Proteins and salt adsorption equilibrium isotherm on ion exchanger

In the above mentioned real gradient SMB model, the mass balance in the particles is described simply by LDF model, so the protein adsorption amount on ion exchange resin includes two terms, one is the protein ion-exchange amount, and the other is the distribution amount in the accessible porosity (size exclusion). When the salt concentration is high, the protein ion-exchange amount becomes very weak, the distribution amount in the accessible porosity will be very important.

Protein ion-exchange equilibrium at various salt concentrations can be described by the steric mass action (SMA) model, which was developed by Brook and Cramer (1992). In the SMA model, ion exchange is regarded as a reaction of a characteristic number of charges of a protein with many salt ions bound to the ion-exchanger, and the protein ion-exchange equilibrium isotherm can be expressed as

$$C = \frac{q^{IE} C_s^z}{K [q_0 - (z + \sigma) q^{IE}]^z} \quad \text{for single component protein} \quad (8.7)$$

and

$$C_i = \frac{q_i^{IE} C_s^{z_i}}{K_i [q_0 - \sum (z_i + \sigma_i) q_i^{IE}]^{z_i}} \quad \text{for multicomponent proteins} \quad (8.8)$$

where, the parameters z , K and σ , should be measured by independent experiments, such as using isocratic/gradient elution chromatography, recommended by Book and Cramer (1992) and Pedersen et al.(2003). The characteristic charge z means the number of sites that the protein interacts with on the resin surface; K (binding constant) means the binding constant for the stoichiometric binding reaction between the protein and the salt counterions; σ (steric factor) means the number of sites on the resin surface that are

shielded by the protein and prevented from exchange. In the equations, q_0 (ionic or bed capacity) means the total number of binding sites available on the resin surface, usually provided by manufacturer, it should be measured as done by Whitley et al. (1989).

For dilute protein solution or at high salt concentration, the protein ion-exchange amount, q^{IE} , is very small, that is $q_0 \gg (\sigma + z)q^{IE}$, then Equation (8.7) becomes a linear ion exchange equilibrium isotherm, such as:

$$q^{IE} = \frac{Kq_0^z}{C_s^z} C \quad (8.9)$$

The linear ion-exchange equilibria for BSA and myoglobin on Q Sepharose FF resin (in 10mM Tris buffer, pH 8) are given by Houwing et al. (2002) as

$$q_{BSA}^{IE} = 0.00161C_s^{-5.61}C_{BSA} \quad (8.10a)$$

$$q_{MYO}^{IE} = 0.0761C_s^{-1.31}C_{MYO} \quad (8.10b)$$

BSA ion exchange amount predicted by Equation (8.10a) is more close to our experimental results in the linear region, as shown in Figure 8.13a. The predicted value by Equation (8.10b) for myoglobin ion exchange amount is higher than our experimental results. Houwing et al (2002) also pointed out it as a referenced formula to go on the analysis. Here, we still use it in the simulation as a reference case and then compare with our experimental data.

With the accessible porosity of BSA and myoglobin in Q-Sepharose FF is 0.49 and 0.64, respectively, measured by Houwing et al (2002), then the adsorption equilibrium isotherm of BSA and myoglobin on Q-Sepharose FF are expressed as

$$q_{BSA}^* = (0.49 + 0.00161C_s^{-5.61})C_{BSA} \quad (8.11a)$$

$$q_{MYO}^* = (0.64 + 0.076C_s^{-1.31})C_{MYO} \quad (8.11b)$$

Where the salt concentration C_s unit is M.

The nonlinear ion-exchange equilibrium isotherms of BSA and myoglobin on Q-Sepharous FF resin over a wide proteins concentration range were measured in our laboratory, and the detailed results are shown later in results and discussion.

The salt adsorption equilibrium on Q-Sepharose FF resin is expressed as

$$q_s = 0.22 \left(-0.5 + 0.5 \sqrt{1 + 4 \times 1.09 \times C_s^2 / 0.22^2} \right) = 0.11 \left(-1 + \sqrt{1 + 90.083 C_s^2} \right) \quad (8.12)$$

which was given by Houwing et al. (2003), and salt concentration C_s unit is as M.

8.2.4 Model parameters and numerical method

The diffusivities of BSA and myoglobin in water are $D_{BSA,0} = 6.15 \times 10^{-11} m^2 / s$, and $D_{MYO,0} = 11.3 \times 10^{-11} m^2 / s$, respectively. The effective pore diffusivities of BSA and myoglobin in Q-Sepharose FF resin are estimated as $D_{pe,BSA} = 1.5 \times 10^{-11} m^2 / s$, and $D_{pe,MYO} = 3.6 \times 10^{-11} m^2 / s$, here $D_{pei} = \varepsilon_{pi} D_{0i} / \tau$ and tortuosity factor τ assigned as 2.

The mass transfer coefficient k_{pik} in Equation (8.2) is calculated by

$$k_{pik} = \frac{15 D_{pei}}{(0.5 d_p)^2} \left/ \frac{dq_{ik}}{dC_{ik}} \right. \quad (8.13)$$

The mass transfer coefficient k_{pSk} for salt is bigger, about $2.0 s^{-1}$. When $k_{pSk} \geq 0.5 s^{-1}$, the effect of k_{pSk} on the simulation results is negligible, so we set $k_{pSk} = 0.5 s^{-1}$ in order to get quickly the stable numerical solution.

Liquid axial dispersion coefficient D_{Lk} is estimated by (Chung and Wen (1968))

$$D_{Lk} = \frac{d_p u_k}{0.20 + 0.011 (d_p u_k \rho / \mu)^{0.48}} \quad (8.14)$$

here $\rho = 1000 kg / m^3$ and $\mu = 0.89 cP$ approximately taken from water.

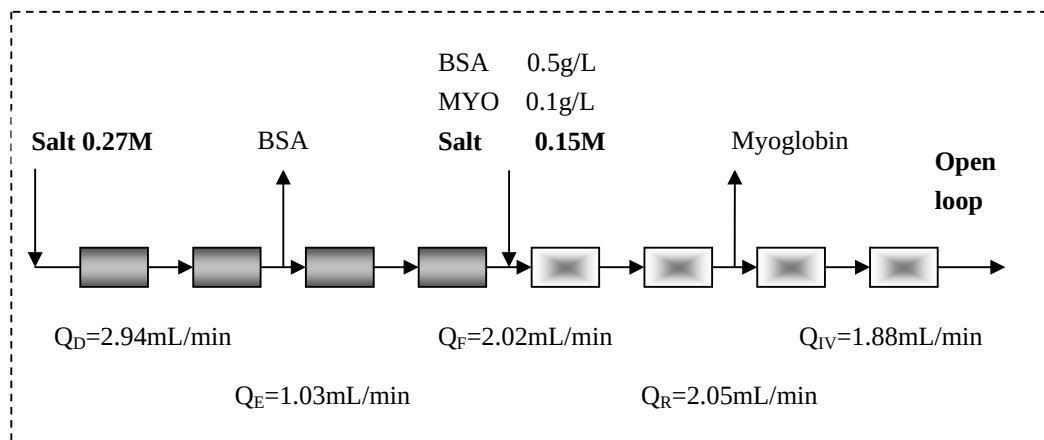
The orthogonal collocation method or the finite difference method may be used to discretize Equation (8.1) in axial direction in each column, leading to a set of ordinary differential equations with initial values; at these discretized points in each column Equation (8.2) is also represented as a set of ordinary differential equations with initial values. Then, all ordinary differential equations will be solved using Gear's stiff variable step integration routine. If the discretized points are assigned 32 in each column, for 8 column and three components (BSA, myoglobin and salt), a total 1536 ordinary differential

equations will be obtained. Using Gear's stiff variable step integration routine to solve these ordinary differential equations, it takes about 3 hours to calculate 20 cycles (about 160 switch times) for the linear case (using a finite difference method). It should be noticed that the initial state in each column varies with switch if the columns are fixed during simulation.

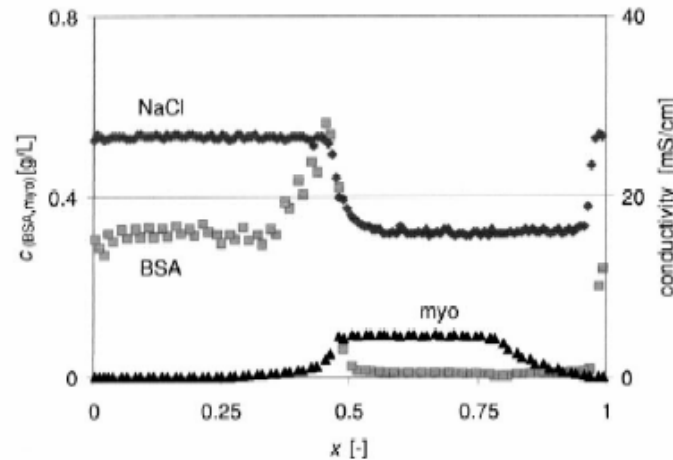
8.3 Results and discussion

8.3.1 Proteins separation by salt gradient ion-exchange SMB with linear ion-exchange equilibrium isotherm

Houwing et al. (2002) reported the experimental results for the separation of BSA and myoglobin by salt gradient ion-exchange SMB chromatography, where the experimental conditions and SMB configuration are shown in Figure 8.3a-b. The salt gradient is formed in ion-exchange SMB chromatography by input feed with a lower salt concentration (0.15M NaCl) and desorbent with a higher salt concentration (0.27M NaCl). When the feed with a mixture of BSA and myoglobin is applied to salt gradient ion-exchange SMB, as a weakly adsorbed component, myoglobin is eluted from raffinate stream, and large molecular BSA is eluted from extract stream as a strongly adsorbed component. Their experimental results confirmed qualitatively that BSA and myoglobin can be separated efficiently by salt gradient ion-exchange SMB chromatography.



(a) Experimental conditions and configuration in salt gradient ion-exchange SMB with open loop.



(b) experimental results of Houwing et al. (2002)—profiles at half switch time in salt gradient ion-exchange SMB with open loop

Figure 8.3. Experimental results and experimental conditions and configuration in salt gradient ion-exchange SMB with open loop. Switch time 4.182min, column packed with Q-Sepharose FF resin, and column diameter 10mm, packed length 90.4mm, bed voidage 0.39

Figure 8.4, 8.5 and 8.6 show a series of the transient concentration profiles formed in salt gradient ion-exchange SMB chromatography, which are calculated by the real gradient SMB model based on the experimental conditions and configuration shown in Figure 8.3, to demonstrate the process of the salt gradient formation and the efficient separation process of BSA and myoglobin by salt gradient ion-exchange SMB chromatography. It is not clear about proteins concentrations in feed from the work reported by Houwing et al. (2002); here we estimate the feed with 0.5g/L BSA and 0.1g/L myoglobin in the simulation.

Figure 8.4 shows the cases in the first full cycle (switch 8 times), where the transient concentration profiles before next switch are shown successively from the initial state in salt gradient ion-exchange SMB. Before the feed is applied to the column, the salt gradient in ion-exchange SMB has been formed by inputting the relative salt aqueous solutions to the columns, as shown in Figure 8.4 for the case with $t = 0$. Then the feed with a mixture of BSA (0.5g/L), myoglobin (0.1g/L) and salt (0.15M NaCl) are applied to the column in section III, and desorbent with 0.27M NaCl is simultaneously applied to the column in section I to desorb the bound proteins. With the switch of inlet and outlet lines, the strongly adsorbed component BSA moves gradually downward to the extract port, and the weakly

adsorbed component myoglobin moves gradually upward to the raffinate port, as shown in Figure 8.4. The salt gradient will redistribute dynamically according to the salt concentrations in feed and desorbent and switch frequency.

Going on above 10 cycles (about switch 80 times), a cycle steady state can be reached, as shown in Figure 8.5, where concentration profiles shown at half switch time are almost identical for two successive cycles. Salt gradient is formed easily in ion-exchange SMB and reaches quickly the cyclic steady state. The internal concentration profile for the weakly adsorbed component myoglobin quickly reached the cyclic steady state also. However, it takes above 10 cycles to reach the cyclic steady state for BSA concentration profile.

After cyclic steady state is reached, the concentration profiles at the end of a switch time interval are the same as at the beginning of this interval, but they are advanced one column. These profiles will be reproduced in the same way column after column. A typical evolution of the internal concentration profiles during a switch time interval at the cyclic steady state (20 cycles), is shown in Figure 8.6.

When comparing the simulation results with the experimental data reported by Houwing et al. (2002), it was found that the internal concentration profiles of salt and myoglobin at half switch time calculated by the real gradient SMB model agree reasonably with the experimental results, but a deviation is found for the internal concentration profile of BSA, where the simulation results are higher than the experimental data. From the view of global BSA mass balance, it seems that the cyclic steady state was not reached for BSA if BSA concentration in feed was 0.5g/L in this experiment. As shown in Figure 8.5, it takes above 10 cycles to reach the cyclic steady state for BSA concentration profile.

Moreover, the poor regeneration of adsorbents is found in section I. Since the binding capacity of BSA is very sensitive to the salt concentration, a simple and easy method to increase the salt concentration in desorbent can improve the regeneration of adsorbents in section I. Figure 8.7 shows the simulation results by increasing salt concentration to 0.3M NaCl in desorbent, and the salt concentration in the feed is adjusted correspondingly as 0.13M NaCl in order to keep similar separation in Section III and IV as before (Figure 8.6). As shown in Figure 8.7, the adsorbents in section I indeed can be regenerated very well,

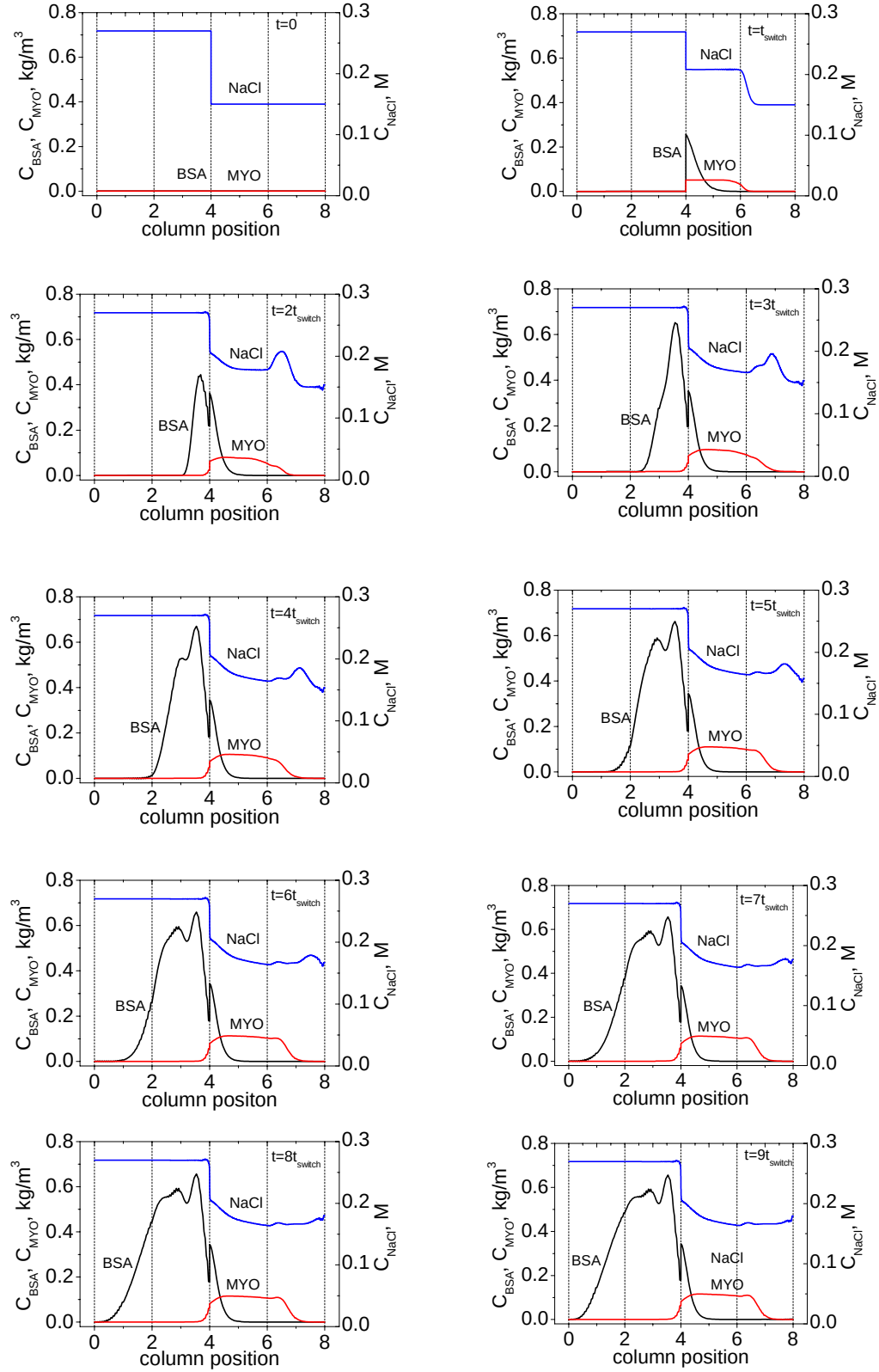


Figure 8.4. Transient concentration profiles before next switch in salt gradient ion exchange SMB with open loop in the first full cycle, calculation conditions shown in Figure 8.3.

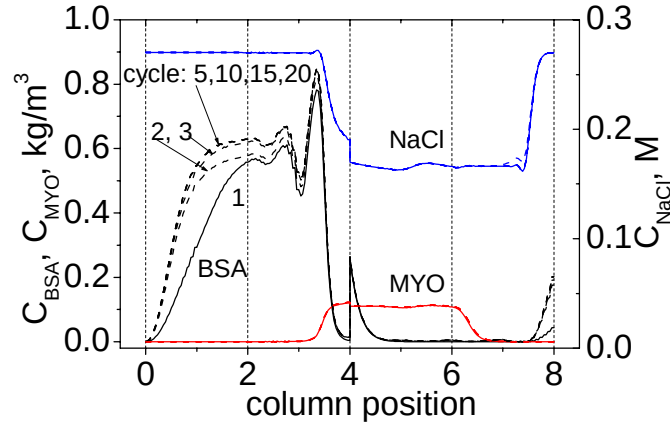


Figure 8.5. Concentration profiles at half switch time with different cycles in salt gradient ion-exchange SMB with open loop, calculation conditions shown in Figure 8.3.

and there is almost no loss of BSA in the effluent stream from section IV, BSA averaged concentration at extract stream can be increased to 1g/L. Enrichment of BSA in extract stream up to 2 means BSA is concentrated relative to the feed flow during separation. Enrichment is defined as

$$E_i = \frac{C_i^E}{C_i^F} \quad \text{in extract stream} \quad (8.15a)$$

$$E_i = \frac{C_i^R}{C_i^F} \quad \text{in raffinate stream} \quad (8.15b)$$

In raffinate stream, myoglobin averaged concentration is about 0.1g/L, not diluted relative to the feed flow. Enrichment of protein concentration in the extract stream is a significant improvement in gradient SMB when compared with isocratic SMB. However, the local protein concentrations are high in section II in salt gradient SMB, which would cause precipitation if the protein has a lower solubility in the solution with medium or high salt concentration.

With the increase of salt concentration to 0.3M in desorbent, the local concentration of BSA in section II becomes very high; the simulation with the linear ion-exchange equilibrium isotherm would result in deviation from the actual process. Based on our experimental results shown later, with 0.3M NaCl in buffer, over a wide BSA concentration range (0-3g/L), ion-exchange equilibrium isotherm between BSA and Q-Sepharose FF resin is still linear. But in section II and III of gradient SMB, there exist lower salt

concentration zone for adsorption and desorption of high concentration BSA, where the linear ion-exchange equilibrium isotherm is not valid. Therefore, nonlinear ion-exchange equilibrium isotherm should be considered. Later, we will extend the simulation to the case of nonlinear ion-exchange equilibrium isotherm.

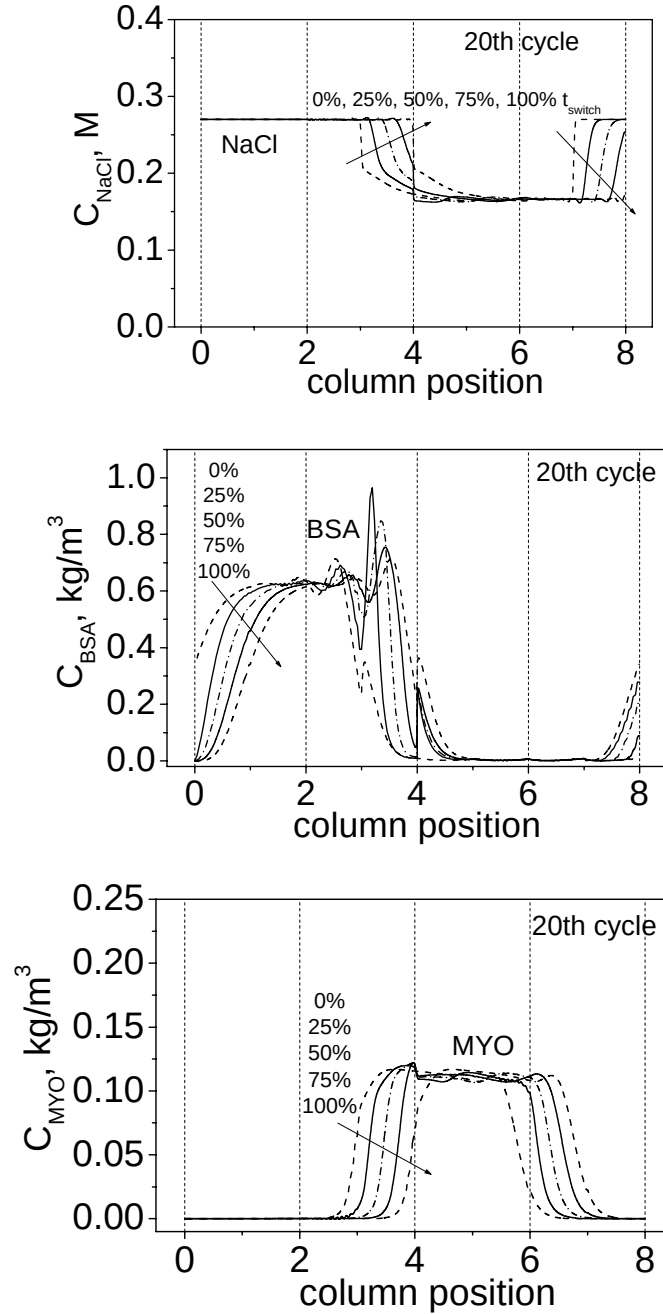


Figure 8.6 Cyclic steady state internal concentration profiles during a switch time interval in salt gradient ion-exchange SMB with open loop, calculation conditions shown in Figure 8.3.

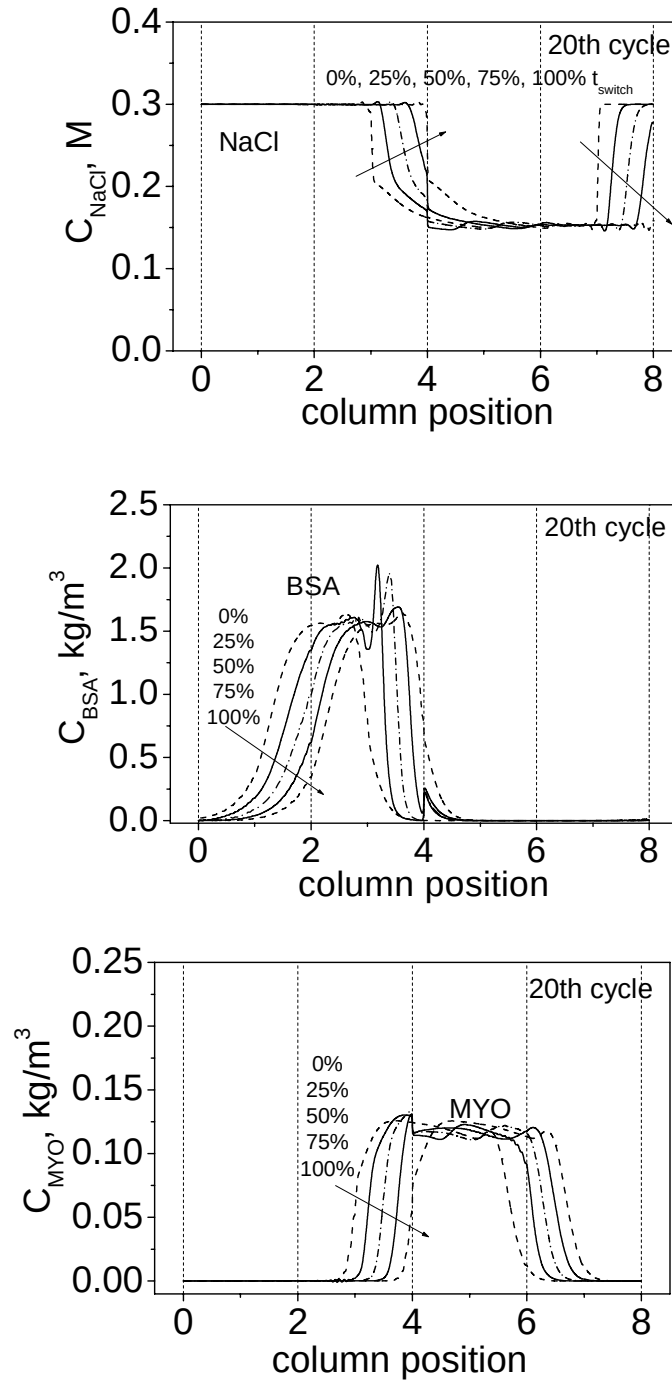


Figure 8.7 Cyclic steady state internal concentration profiles during a switch time interval in salt gradient ion-exchange SMB with open loop, $C_S^D = 0.3M$ and $C_S^F = 0.13M$ and other calculation conditions shown in Figure 8.3.

8.3.2. Comparison of gradient SMB configurations: open loop, closed loop and closed loop with a holding vessel.

Three configurations for gradient SMB chromatography and the detailed calculation conditions are shown in Figure 8.8, for gradient SMB with open loop (Figure 8.8a), gradient SMB with closed loop (Figure 8.8b) and gradient SMB with closed loop and a holding vessel (Figure 8.8c). In the case of open loop, desorbent with 0.3M NaCl is applied to the columns at 2.94mL/min; for closed loop, desorbent flow rate can be decreased to 1.06mL/min due to the recycle of liquid from section IV, but the salt concentration should be increased to 0.34M NaCl in desorbent to keep the similar salt gradient in columns of SMB. The simulation results at cycle steady state (20 cycles) are shown in Figure 8.9 (open loop), Figure 8.10 (closed loop) and Figure 8.11 (closed loop and a holding vessel $V_S=10\text{mL}$), respectively. Here, BSA concentration in feed is assigned as 0.3g/L, instead of 0.5g/L used before.

In open loop, as shown in Figure 8.9, in the position of column 4 of section II and column 8 of section IV, salt concentrations dynamically vary during a switch time interval. The direct recycling of liquid stream from section IV to section I in closed loop, will cause the dynamic change of salt concentration in section I also, as shown in Figure 8.10, where the salt concentration decreases gradually and makes the regeneration of adsorbents in section I inefficient. In Figure 8.8c, a holding vessel with 10mL volume (about 1.5 column volume) is used to mix the desorbent with the recycled liquid stream from section IV before applied to column 1 in section I. As shown in Figure 8.11, the dynamic change of salt concentration in section I during a switch time interval indeed becomes very weak, which favors the adsorbent efficient regeneration in section I.

Because the similar salt gradients are formed in open loop, closed loop and closed loop with a holding vessel, at the given calculation conditions (Figure 8.8), BSA and myoglobin can be separated with almost identical efficiency by these three kinds of configurations of gradient SMB.

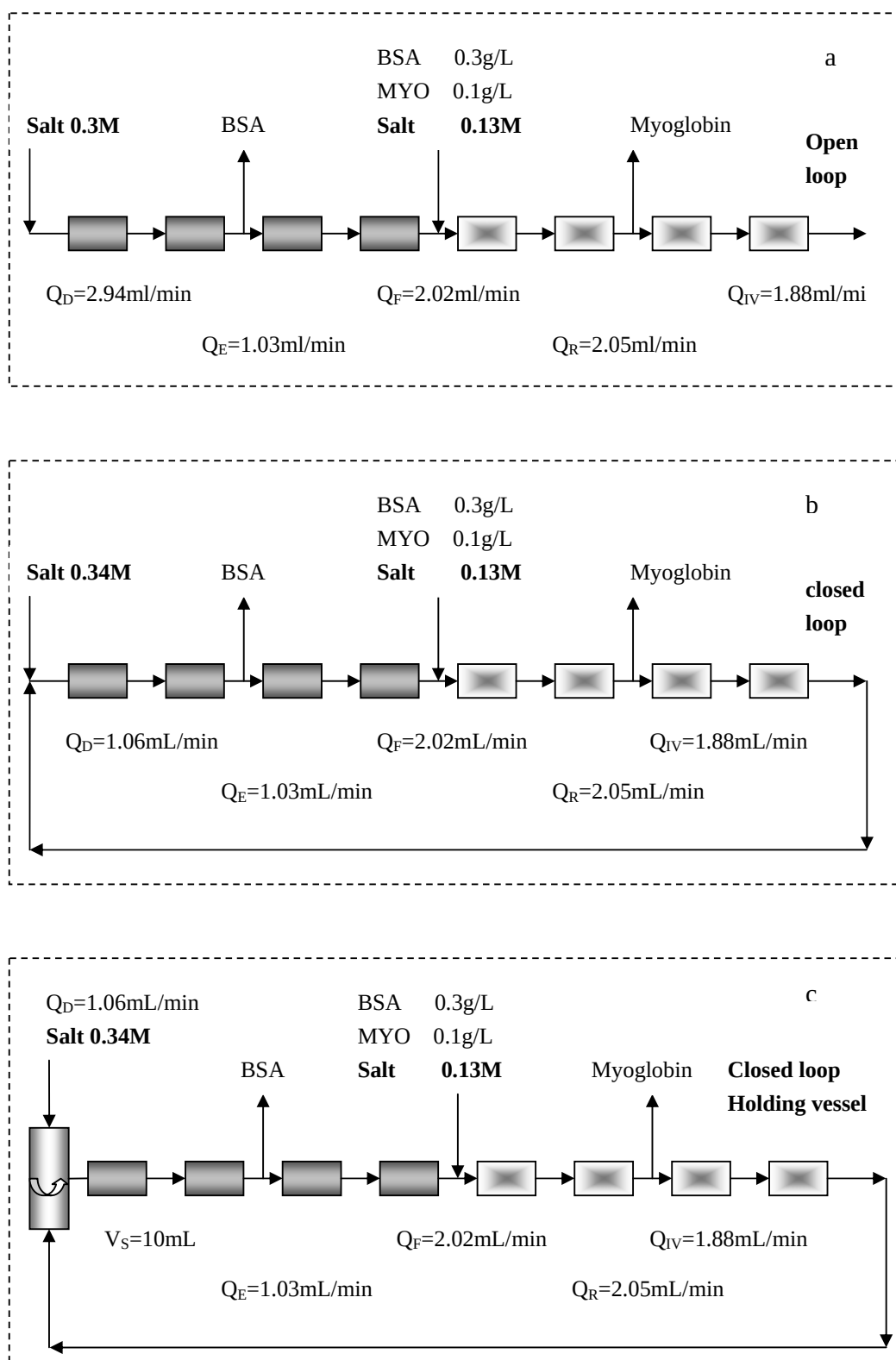


Figure 8.8 Simulation conditions and configurations of salt gradient ion-exchange SMB. Switch time 4.182min, column packed with Q-Sepharose FF resin, and column diameter 10mm, packed length 90.4mm, bed voidage as 0.39

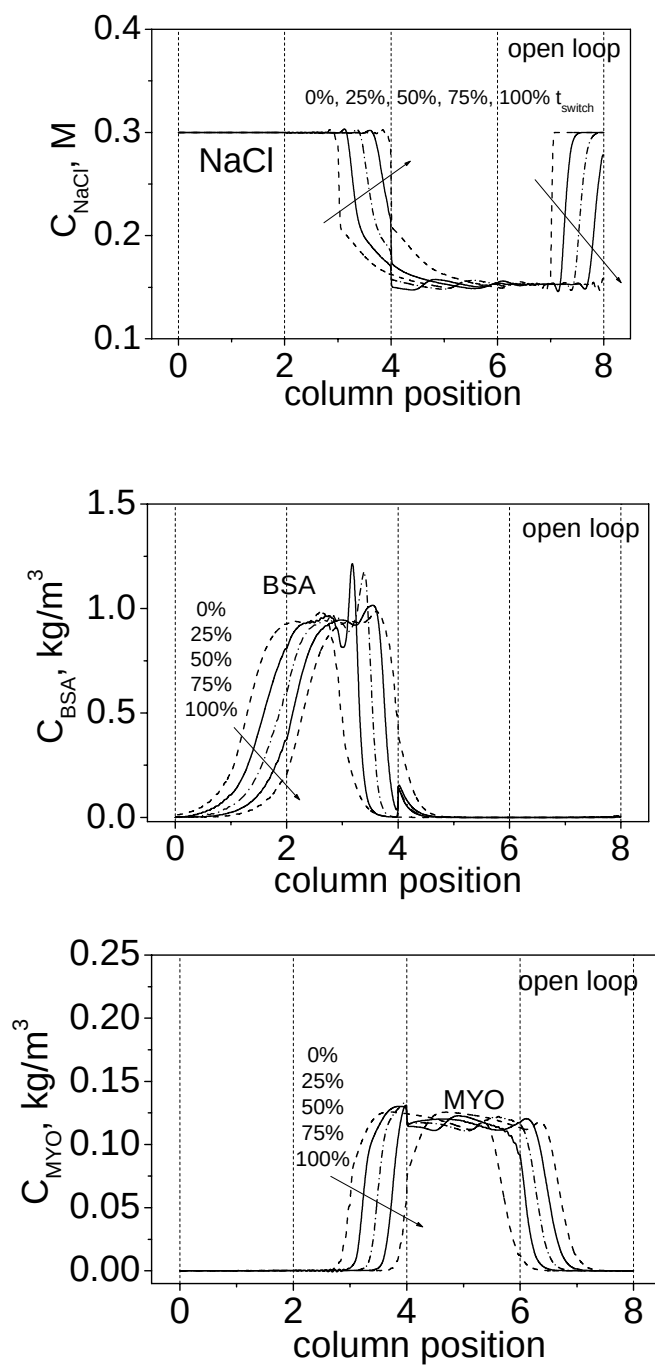


Figure 8.9 Internal concentration profiles at cyclic steady state (20 cycles) in salt gradient ion-exchange SMB with open loop, calculation conditions shown in Figure 8.8a.

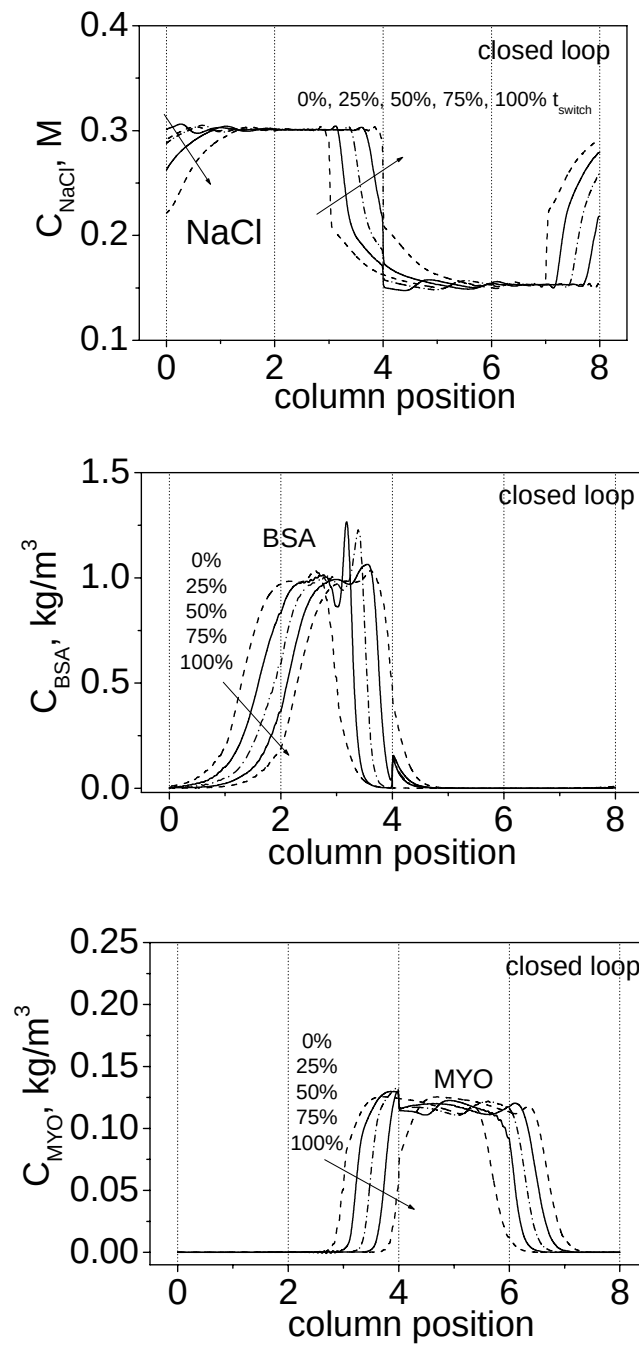


Figure 8.10 Internal concentration profiles at cyclic steady state (20 cycles) in salt gradient ion-exchange SMB with closed loop, calculation conditions shown in Figure 8.8b.

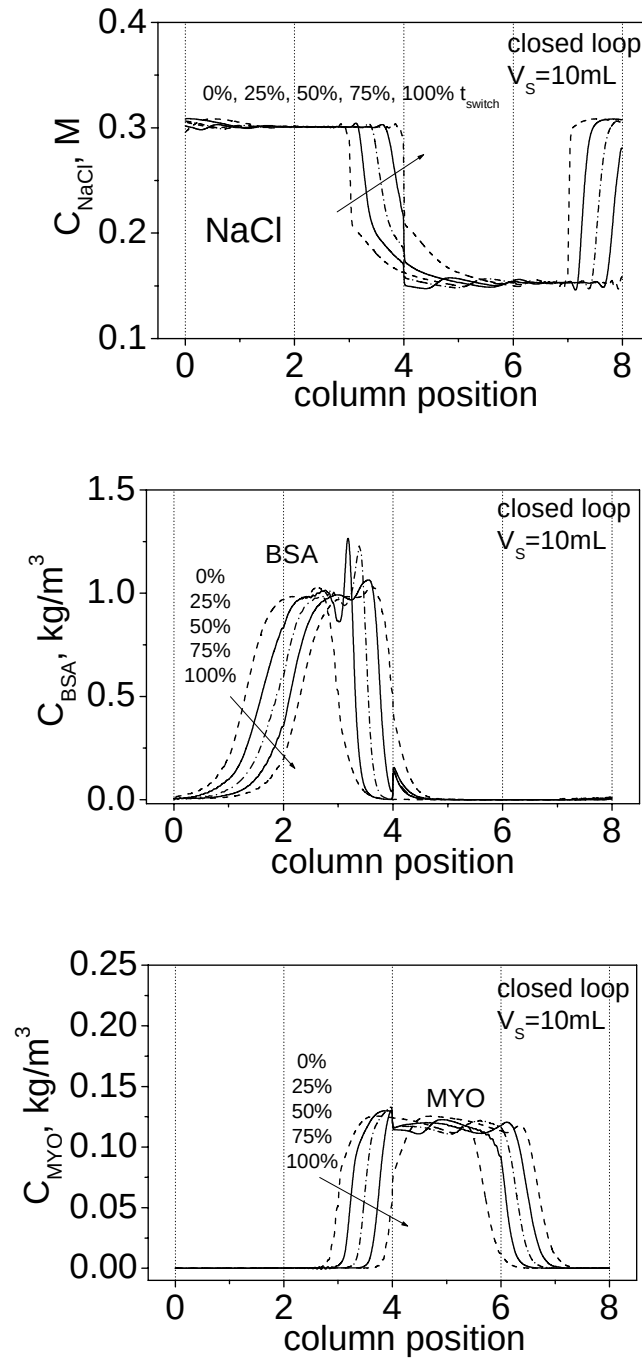


Figure 8.11 Internal concentration profiles at cyclic steady state (20 cycles) in salt gradient ion-exchange SMB with closed loop and a holding vessel ($V_s=10\text{mL}$), calculation conditions shown in Figure 8.8c.

8.3.3 Comparison of the two strategies of modeling, gradient SMB/TMB model

Two models are frequently used to simulate an SMB unit: one is the true moving bed (TMB) model, which assumes equivalence with an ideal unit where the solid and the liquid phases move counter-currently; the other is the real SMB model, where the dynamics associated with the periodic shift of the inlet and outlet lines is taken into account. It is known that the prediction of the isocratic SMB operation can be carried out through the equivalent isocratic TMB approach when the SMB unit is constituted by, at least, two columns per section (a total of eight columns). Here, we will present the comparison of the two strategies of modeling, equivalent gradient TMB model and real gradient SMB model, for the prediction of internal concentration profiles in gradient SMB chromatography.

Figure 8.12 demonstrates the internal concentration profiles at steady state in salt gradient ion-exchange SMB with open loop and closed loop, respectively, predicted by equivalent gradient TMB model. The mathematical model equations for equivalent gradient TMB model are given in Appendix. Calculated conditions are identical to those in Figure 8.9 for the corresponding open loop predicted with real gradient SMB model, and to those in Figure 8.10 for the corresponding closed loop predicted with real gradient SMB model, respectively. The salt gradient formed in ion exchange SMB chromatography, that is in section I and II with higher salt concentration and in section III and IV with lower salt concentration, are identical when predicted by real gradient SMB model and by gradient TMB model. However, the actual dynamical changes of salt concentrations in the positions of column 4 in section II and column 8 in section IV, and column 1 in section I (special for closed loop) can not be predicted by gradient TMB model; also the corresponding significant change in BSA concentration can not be represented by gradient TMB model. It seems that the deviation between the predictions by real gradient SMB model and gradient TMB model becomes small for the weakly retained myoglobin, as a result of the small effect of salt concentration on myoglobin ion exchange amount. From the view of global mass balance of BSA, myoglobin and salt in gradient ion exchange SMB chromatography, when two proteins can be separated completely, the protein concentrations in extract stream and raffinate stream predicted by two models should be identical, so as primary design of gradient SMB chromatography, the simple gradient TMB model is applicable, because it

can save computer time for the selection and optimization of flow rates in each section.

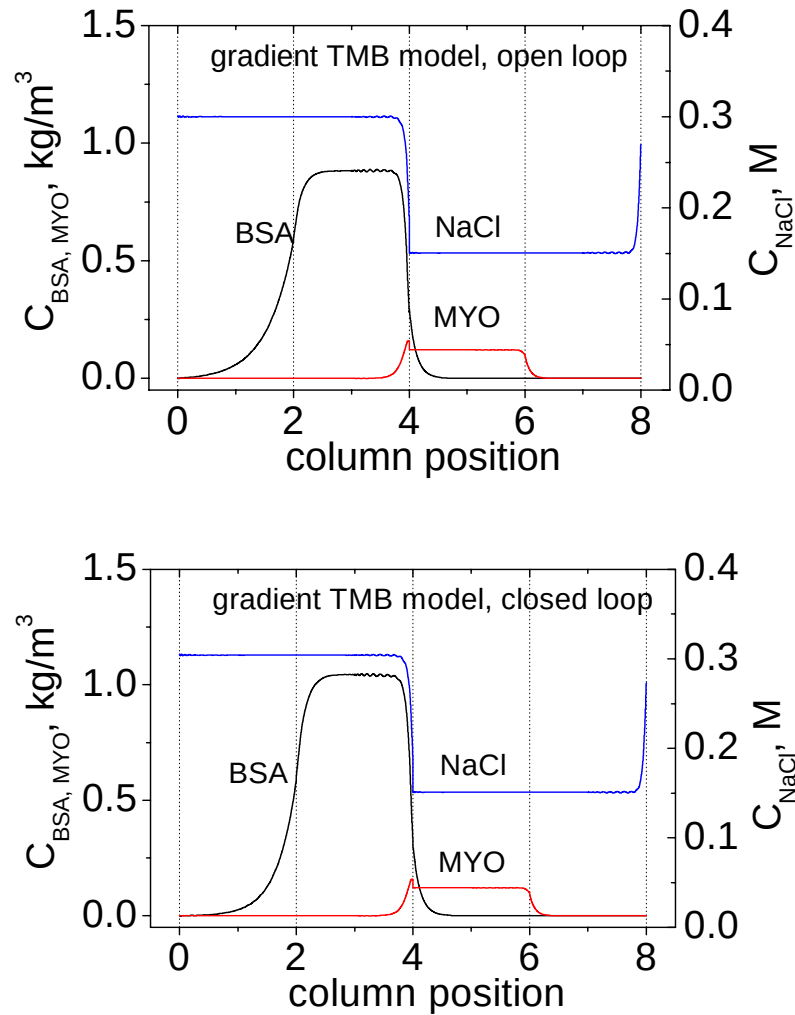


Figure 8.12. Internal concentration profiles at the steady state in salt gradient ion-exchange SMB with open loop and closed loop, respectively, predicted by gradient TMB model. Calculated conditions are identical with Figure 8.9 (open loop, predicted by gradient SMB model) and Figure 8.10 (closed loop, predicted by gradient SMB model).

8.3.4 Salt gradient ion-exchange SMB with nonlinear ion exchange equilibrium isotherm of proteins

8.3.4.1 Nonlinear ion exchange equilibrium isotherms of BSA and myoglobin on Q-Sepharose FF anion exchanger

Ion-exchange equilibrium isotherms of BSA and myoglobin on Q-Sepharose FF resin were studied experimentally in our laboratory over a wide proteins concentration range, as shown in Figure 8.13, where the equilibrium data are measured in a static batch adsorber. Before performing the protein ion exchange equilibrium experiments, the resins must be saturated by the corresponding buffer. Resins, in the amount of 0.5~2.5mL of particle volume, are equilibrated with 30mL of different concentrations of BSA or myoglobin solution for about 20 hours at 25°C on a shaking incubator (about 30rpm); then BSA or myoglobin concentration in supernatant liquid is measured by UV 7800 detector at 280nm (using a 2-mL quartz cuvette). The ion exchange capacity is calculated by mass balance. Here, the corresponding buffer is 10 mM Tris buffer (pH 8) with various NaCl concentrations, BSA product number as A3059 and myoglobin product number as M0630, both are from Sigma-Aldrich Company.

BSA has a high ion exchange capacity on Q-Sepharose FF resin at pH 8 Tris buffer (10mM), with a high favorable nonlinear equilibrium isotherm, as a result of isoelectric point (pI 4.7) of BSA far from buffer pH value (pH 8). The ion exchange equilibrium isotherm of BSA on Q-Sepharose FF resin with the dependence on NaCl concentrations can be represented by the SMA model as

$$C_{BSA} = \frac{q_{BSA}^{IE} C_S^{z_{BSA}}}{K_{BSA} [q_0 - (z_{BSA} + \sigma_{BSA}) q_{BSA}^{IE} / M_{BSA}]^{z_{BSA}}} = \frac{q_{BSA}^{IE} C_S^{6.03}}{10.83 [0.210 - (6.03 + 75) q_{BSA}^{IE} / M_{BSA}]^{6.03}} \quad (8.16)$$

where q_{BSA}^{IE} , C_{BSA} units are in kg/m³, C_S unit is in M, and M_{BSA} is BSA molecular weight, 65400, $q_0 = 0.21M$ for Q-Sepharose FF resin at pH 8 buffer measured by Whitley et al. (1989). The model parameters, $z_{BSA} = 6.03$ and $K_{BSA} = 10.83$, are

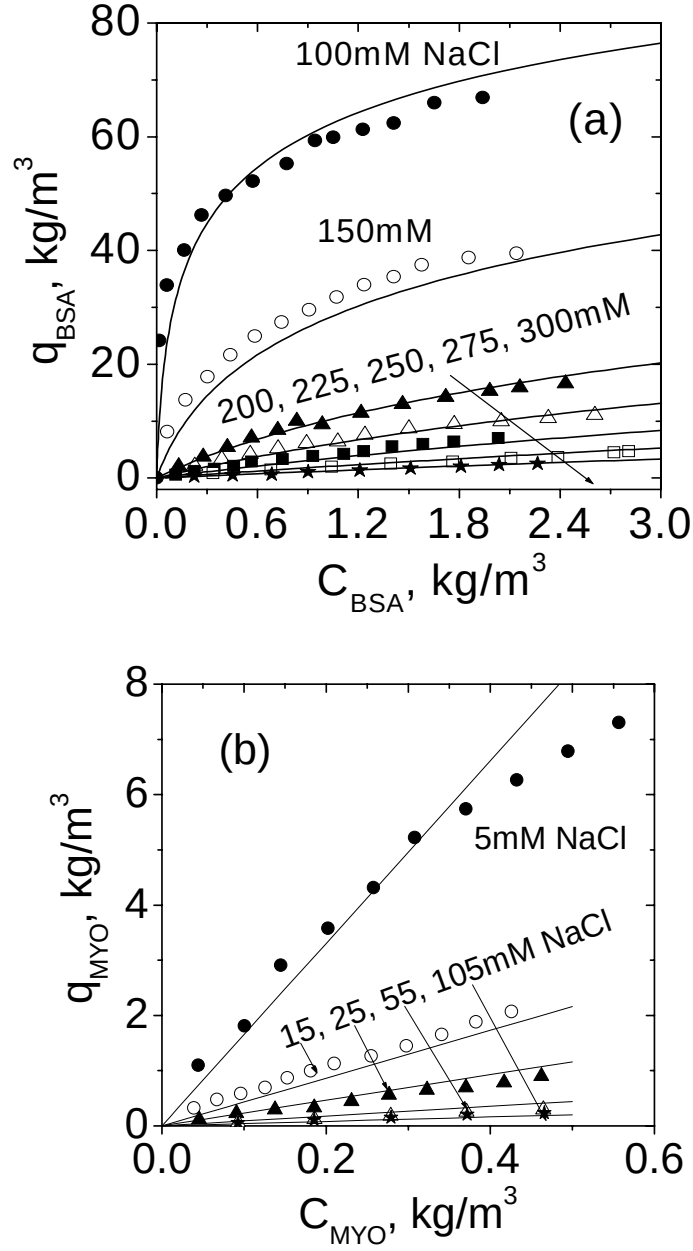


Figure 8.13. Ion exchange equilibrium isotherms of BSA and myoglobin on Q-Sepharose FF resin at room temperature ($\sim 25^{\circ}\text{C}$), in 10mM Tris buffer (pH 8). Points: experimental data; lines: calculated by Equation (8.16) for BSA, by Equation (8.17) for myoglobin.

estimated by the plot of $\log(q_{BSA}^{IE}/C_{BSA})$ versus $\log(C_S)$ with the slope of z_{BSA} , and the intercept of $\log(K_{BSA}q_0^{z_{BSA}})$ under the linear condition, as shown in Figure 8.14a, where experimental data are taken from Figure 8.13a for the cases with dilute BSA concentration

at high salt concentrations. The other model parameter, $\sigma_{BSA} = 75$, is measured by fitting the non-linear ion-exchange equilibrium data with Equation (8.16) at various salt concentrations, and then an average value over the range of salt concentration is assigned to σ_{BSA} . The predicted values by Equation (8.16) are close to the experimental results at various salt concentrations, as shown in Figure 8.13a.

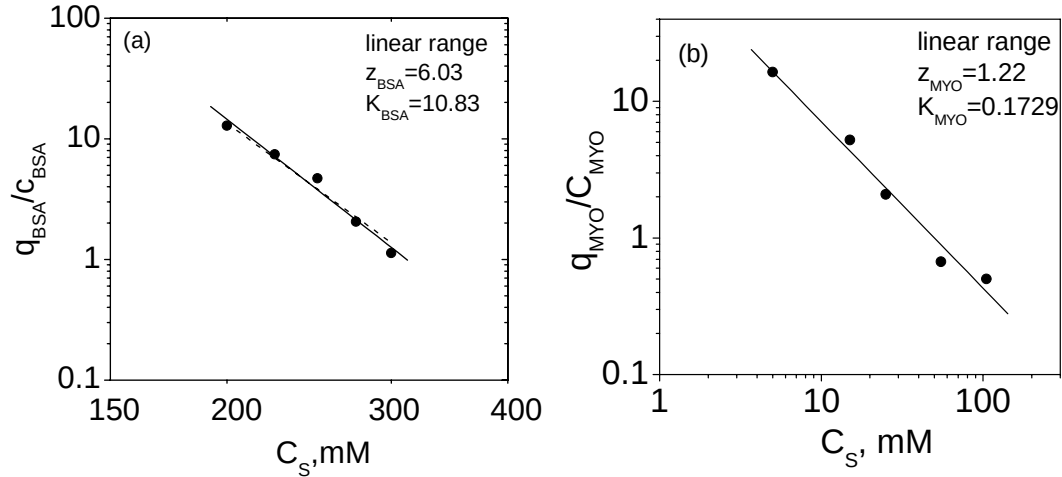


Figure 8.14. Plots of $\log(q/C)$ versus $\log(C_s)$ in the linear ion exchange equilibrium isotherm. Points: experimental data taken from Figure 8.13; solid lines: the linear regression lines; dashed line: calculated by Equation (8.10a).

Since pH value (pH 8) in 10mM Tris buffer approaches the isoelectric point of myoglobin (pI 7.4), the ion exchange amount of myoglobin on Q-Sepharose FF resin is very small even at lower salt concentrations. Over a wide myoglobin concentration range, linear ion exchange equilibrium isotherms can be found at various NaCl concentrations. Based on the experimental results shown in Figure 8.13b, the linear ion-exchange equilibrium isotherm of myoglobin on Q-Sepharous FF resin is expressed as

$$q_{MYO}^{IE} = \frac{K_{MYO} q_0^{z_{MYO}}}{C_s^{z_{MYO}}} C_{MYO} = 0.02576 C_s^{-1.22} C_{MYO} \quad (8.17)$$

where q_{MYO}^{IE} , C_{MYO} units are in kg/m^3 , C_s unit is in M, and $q_0 = 0.21M$. The model parameters, $z_{MYO} = 1.22$ and $K_{MYO} = 0.1729$, are estimated from the plot of $\log(q_{MYO}^{IE}/C_{MYO})$ versus $\log(C_s)$ under linear conditions, as shown in Figure 8.14b. The

predicted values by Equation (8.17) are close to the experimental results at various salt concentrations, as shown in Figure 8.13b.

Furthermore, breakthrough curves of BSA and myoglobin are measured in a fixed bed, as shown in Figure 8.15 a-c, where XK16/20 column is packed with Q-Sepharous FF anion exchangers, packed height as 100mm, column diameter as 16mm, bed voidage as 0.35 (estimated by a given mass of drained adsorbents to pack the column, drained adsorbents with 1050kg/m^3 density).

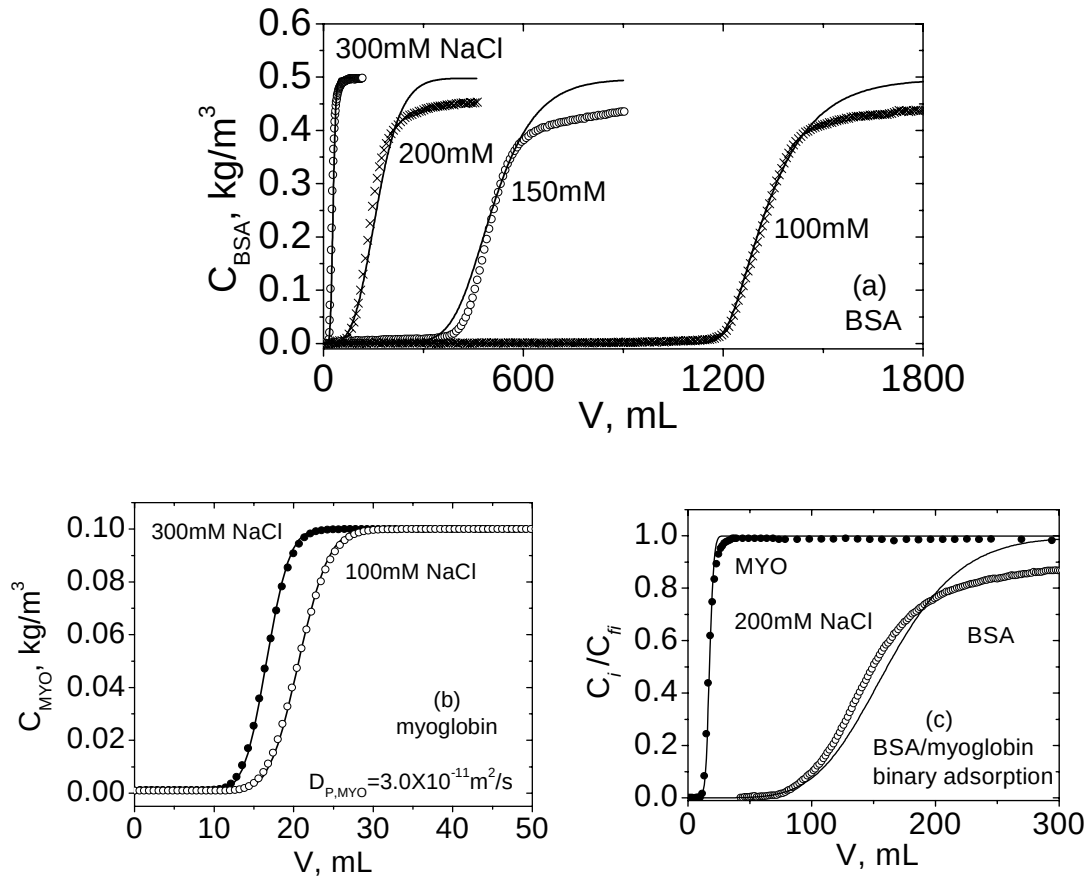


Figure 8.15. BSA and myoglobin breakthrough curves at various NaCl concentrations.

Circle points: experimental data; lines: simulation results with LDF model.

(a) single component breakthrough for BSA.

BSA: $Q = 7.2\text{mL/min}$ (100mM NaCl), $Q = 7.0\text{mL/min}$ (150mM NaCl)

$Q = 7.0\text{mL/min}$ (200mM NaCl), $Q = 3.5\text{mL/min}$ (300mM NaCl)

(b) single component breakthrough for myoglobin

MYO: $Q = 4.0\text{mL/min}$ (100mM NaCl) and $Q = 4.75\text{mL/min}$ (300mM NaCl)

(c) binary breakthrough curves BSA/MYO

$Q = 4.9\text{mL/min}$ (200mM NaCl), $C_{f,BSA}/C_{f,MYO} = 0.5\text{kg/m}^3/0.1\text{kg/m}^3$

The experimental data of breakthrough curves are compared with the simulation results, as shown in Figure 8.15, to confirm the accuracy of ion exchange equilibrium isotherm expressions (Equation (8.16) for BSA and Equation (8.17) for myoglobin). And the mass coefficients k_{p_i} of BSA and myoglobin are evaluated by fitting the experimental data of breakthrough curves with LDF model. For myoglobin with linear adsorption isotherm, $k_{p_{MYO}}$ can be calculated with Equation (8.13) with $D_{p_{e,MYO}} = 3.0 \times 10^{-11} \text{ m}^2 / \text{s}$; for BSA at 300mM and 200mM NaCl buffer, $k_{p_{BSA}}$ can be calculated with Equation (8.13) with $D_{p_{e,BSA}} = 1.5 \times 10^{-11} \text{ m}^2 / \text{s}$ also, and $k_{p_{BSA}} = 0.00278 \text{ s}^{-1}$ at 150mM NaCl and $k_{p_{BSA}} = 0.00139 \text{ s}^{-1}$ at 100mM NaCl. Moreover, as commonly observed by many authors, there exists a severe tailing behavior of BSA breakthrough curve as the effluent approaches the feed concentration. In previously published articles (Graham et al., 1990; Hunter and Carta, 2001; Leitao et al, 2002), the tailing behavior was explained by the presence of dimer in BSA sample, or microporous diffusion in the macroporous adsorbent, or protein aggregation into dimer or trimer on the adsorbed surface of adsorbents. Up to now, the explanation is still unclear to the tailing behavior occurred for larger size protein adsorption, further mechanic analysis is still necessary.

8.3.4.2 Proteins separation and purification by salt gradient ion-exchange SMB with nonlinear ion exchange equilibrium isotherm

Separation factor is defined as

$$S_{1,2} = \frac{q_1/C_1}{q_2/C_2} \quad (8.18)$$

In ion-exchange chromatography, the separation factor of proteins depends evidently on salt concentration. Table 8.2 lists the separation factors of BSA to myoglobin by Q-Sepharose FF anion exchanger at various salt concentration, that are estimated approximately based on the linear adsorption isotherm for BSA and myoglobin.

$$q_{BSA}^* = K_{BSA} C_{BSA} = (0.49 + 8.8636 \times 10^{-4} C_S^{-6.03}) C_{BSA} \quad (8.19)$$

$$q_{MYO}^* = K_{MYO} C_{MYO} = (0.64 + 0.02576 C_S^{-1.22}) C_{MYO} \quad (8.20)$$

Table 8.2 Separation factor of BSA to myoglobin by Q-Sepharose FF anion exchanger under linear adsorption equilibrium isotherm				
Salt concentration in 10mM Tris buffer (pH 8)	K_{BSA}	K_{MYO}	$S_{BSA,MYO}$	Comments
200mM NaCl	15.024	0.824	18.244	BSA: the more retained Myoglobin: the less retained
250mM NaCl	4.275	0.780	5.482	BSA: the more retained Myoglobin: the less retained
300mM NaCl	1.751	0.752	2.328	BSA: the more retained Myoglobin: the less retained
350mM NaCl	0.988	0.733	1.348	BSA: the more retained Myoglobin: the less retained
400mM NaCl	0.712	0.719	0.991	azeotrope
500mM NaCl	0.548	0.700	0.783	Myoglobin: the more retained BSA: the less retained Similar to size exclusion SMB

With a lower NaCl concentration in Tris buffer (pH 8), the separation factor of BSA to myoglobin is large; for example, with 200mM NaCl, $S_{BSA,MYO}$ up to 18.244, BSA and myoglobin can be separated easily by ion-exchange chromatography. With the increase of

salt concentration, the separation factor becomes small. When salt concentration is up to 400mM NaCl, the separation factor approaches to unity, that means BSA and myoglobin can not be separated by ion exchange chromatography. This phenomena is called azeotrope (Houwing et al, 2002), like azeotropic distillation. Further increasing salt concentration, it will be found a reversal of separation that myoglobin becomes more retained component and BSA becomes less retained component, and the separation behavior of BSA and myoglobin in ion-exchange chromatography is more close to that in size exclusion chromatography.

Therefore, the selection of salt gradient in ion exchange SMB chromatography is very important. In section III and IV of ion-exchange SMB packed with Q-Sepharose FF resins, a lower salt concentration will favor the separation of BSA and myoglobin as a result of the high separation factor. During the design, we assign salt concentration less than 0.16M in section III and IV, with the big separation factor, the separation of BSA and myoglobin becomes very easy, and a high feed throughput capacity can be carried out. In section I and II of ion-exchange SMB chromatography, a high salt concentration is assigned to desorb the bound BSA and myoglobin on Q-Sepharose FF resin in order to reduce the desorbent consumption. But the salt concentration should be less than 350mM NaCl, as a result of the significant decrease of separation factor in section II.

The selection of the flowrates in each section to obtain the desired separation can be done based on the equilibrium theory (Ruthven and Ching, 1989), triangle theory (Mazzotti, et al., 1997), standing wave (Ma and Wang, 1997), and separation volume (Rodrigues and Pais, 2004). Anyway, some constraints have to be met if one wants to recover the less adsorbed component in the raffinate stream and the more retained component in the extract stream. These constraints are expressed in terms of the net fluxes of components in each section, that is in section I, the more retained component must move upward, in section II and III, the less adsorbed component must move upward, while the net flux of the more retained component must be downward, and in section IV, the net flux of the less adsorbed component has to be downward. In addition, in salt gradient SMB operation, the additional constraints in terms of the net fluxes of salt in each section also must be taken into account in design. In order to form a stable salt gradient in ion exchange SMB, such as in

section I and II with high salt concentration by using desorbent with high salt concentration and section III and IV with lower salt concentration by using feed with lower salt concentration, the upward movement for the net salt flux in each section must be considered. Table 8.3 summarizes the constraints conditions to the net flux for the separation of BSA and myoglobin in salt gradient ion-exchange SMB.

Table 8.3 Some constraints to the net fluxes for BSA and myoglobin separation in salt gradient ion-exchange SMB			
	Salt	BSA	myoglobin
Section I	$\frac{Q_I^{TMB}}{Q_S} \frac{C_{SI}}{q_{SI}} > 1$	$\frac{Q_I^{TMB}}{Q_S} \frac{C_{BSAI}}{q_{BSAI}} > 1$	
Section II	$\frac{Q_{II}^{TMB}}{Q_S} \frac{C_{SII}}{q_{SII}} > 1$	$\frac{Q_{II}^{TMB}}{Q_S} \frac{C_{BSAII}}{q_{BSAII}} < 1$	$\frac{Q_{II}^{TMB}}{Q_S} \frac{C_{MYOII}}{q_{MYOII}} > 1$
Section III	$\frac{Q_{III}^{TMB}}{Q_S} \frac{C_{SIII}}{q_{SIII}} > 1$	$\frac{Q_{III}^{TMB}}{Q_S} \frac{C_{BSAIII}}{q_{BSAIII}} < 1$	$\frac{Q_{III}^{TMB}}{Q_S} \frac{C_{MYOIII}}{q_{MYOIII}} > 1$
Section IV	$\frac{Q_{IV}^{TMB}}{Q_S} \frac{C_{SIV}}{q_{SIV}} > 1$		$\frac{Q_{IV}^{TMB}}{Q_S} \frac{C_{MYOIV}}{q_{MYOIV}} < 1$

The definition of the net flowrate ratio in each section is

$$m_j = \frac{\text{net fluid flow rate}}{\text{adsorbed phase flow rate}} = \frac{Q_j^{TMB}}{Q_S} = \frac{Q_j^{SMB} - Q_S \varepsilon_B / (1 - \varepsilon_B)}{Q_S}$$

where, $Q_S = (1 - \varepsilon_B) V_C / t_{\text{switch}}$, V_C means the column volume, t_{switch} means the switch time interval.

Based on the constraint conditions shown in Table 8.3, the range of the net flowrate ratio of BSA, myoglobin, and salt in each section can be estimated for the separation and purification of BSA and myoglobin by salt gradient ion exchange SMB chromatography. Here we deal with two kinds of salt gradient, one is about 0.30M NaCl formed in section I and II and about 0.08M NaCl formed in section III and IV, applied to BSA and myoglobin separation with the complete recovery of myoglobin in raffinate stream (called *Case 1*), and the other is about 0.30M NaCl formed in section I and II and about 0.15M NaCl formed in

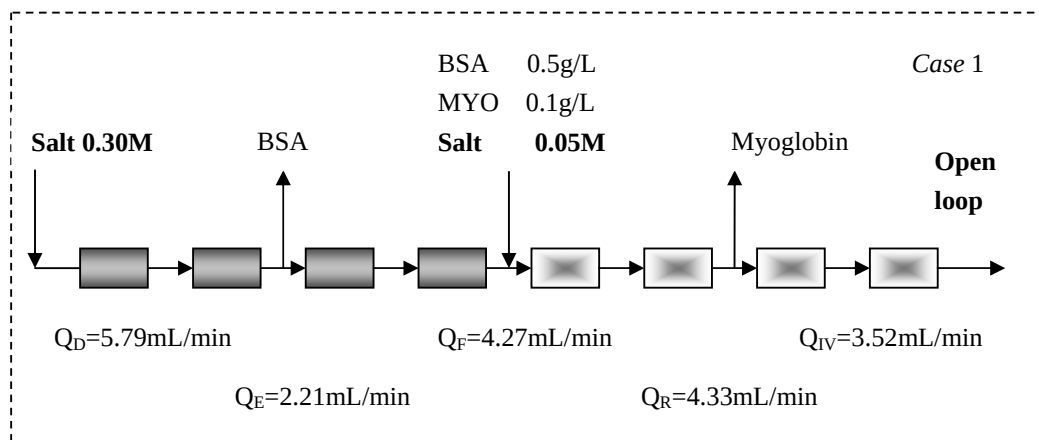
section III and IV, applied to BSA purification from myoglobin impurity without the recovery of myoglobin (called *Case 2*), special operation with open loop. During this estimation, we further assume BSA being linear adsorption equilibrium isotherm in section I and II due to high salt concentration and in section III, $q_{BSAIII} / C_{BSAIII} \approx \Delta q_{BSAIII} / \Delta C_{BSAIII}$; $q_s / C_s \approx \partial q_s / \partial C_s = 9.909 C_s / \sqrt{1 + 90.083 C_s^2}$ for salt in each section. The estimated range of the net flowrate ratio in each section for *Case 1* and *Case 2* are shown in Table 8.4, respectively. It should be emphasized that the effects of both protein mass transfer resistances and axial dispersion are neglected during the estimation, so the further optimization of flow rates in each section to obtain the desired purity of proteins in raffinate and extract streams is still necessary (Minceva and Rodrigues, 2005).

Table 8.4 Flowrate ratio range for the separation and purification of BSA and Myoglobin in salt gradient ion-exchange SMB with open loop					
Case 1 BSA and myoglobin separation with complete recovery of myoglobin in raffinate					
	Salt	BSA	myoglobin	Flow rate ratio range	In Figure 8.17
Section I $C_s^I \approx 0.30M$	$m_I > 0.981$	$m_I > 1.751$		$m_I > 1.751$	$m_I = 2.12$
Section II $C_s^{II} \approx 0.30M$	$m_{II} > 0.981$	$m_{II} < 1.751$	$m_{II} > 0.725$	$0.981 < m_{II} < 1.751$	$m_{II} = 1.11$
Section III $C_s^{III} \approx 0.08M$	$m_{III} > 0.631$	$m_{III} < 100$ (0.5g/L in feed)	$m_{III} > 1.201$	$1.201 < m_{III} < 100$	$m_{III} = 3.07$
Section IV $C_s^{IV} \approx 0.08M$	$m_{IV} > 0.981$ $C_s^{IV} \approx 0.30M$ for NaCl		$m_{IV} < 1.201$	$0.981 < m_{IV} < 1.201$	$m_{IV} = 1.08$
Case 2 BSA purification from myoglobin impurity without recovery of myoglobin					
	Salt	BSA	myoglobin	Flow rate ratio range	In Figure 8.18
Section I $C_s^I \approx 0.30M$	$m_I > 0.981$	$m_I > 1.751$		$m_I > 1.751$	$m_I = 2.12$
Section II $C_s^{II} \approx 0.30M$	$m_{II} > 0.981$	$m_{II} < 1.751$	$m_{II} > 0.725$	$0.981 < m_{II} < 1.751$	$m_{II} = 1.21$
Section III $C_s^{III} \approx 0.15M$	$m_{III} > 0.854$	$m_{III} < 35$ (0.5g/L in feed)	$m_{III} > 0.901$	$0.901 < m_{III} < 35$	$m_{III} = 3.16$
Section IV $C_s^{IV} \approx 0.15M$	$m_{IV} > 0.981$ $C_s^{IV} \approx 0.30M$ for NaCl		$m_{IV} < 0.901$	$m_{IV} > 0.981$ myoglobin upward	$m_{IV} = 1.18$

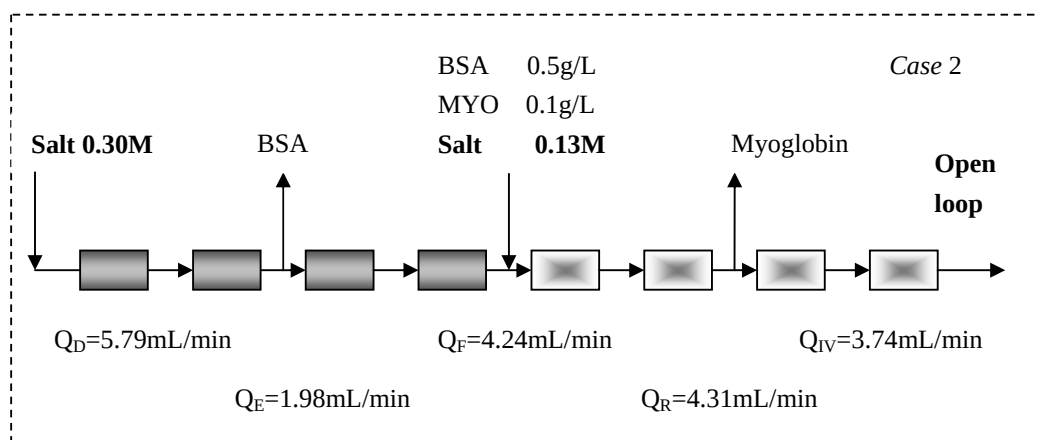
According to the range of the net flowrate ratio in each section, the actual net flowrate ratios are selected for *Case 1* and *Case 2* process, as shown in Table 8.4. Then with a given configuration of salt gradient ion-exchange SMB (two columns in each section, and column diameter as 16mm and packed height as 100mm, bed voidage as 0.35) and switch time (here, a longer switch time, 6min, used for macromolecular proteins separation), the actual flow rates of desorbent, extract, feed and raffinate can be calculated, as shown in Figure 8.16. The salt gradient is formed for *Case 1* by using desorbent with 0.3M NaCl and feed with 0.05M NaCl, for *Case 2* by using desorbent with 0.3M NaCl and feed with 0.13M NaCl, respectively. With the given operating conditions and configuration of salt gradient ion-exchange SMB with open loop, the simulations for *Case 1* and *Case 2* are carried out by the real gradient SMB model, and the simulation results are shown in Figure 8.17 (*Case 1*) and Figure 8.18 (*Case 2*), respectively, where nonlinear ion exchange equilibrium for BSA (Equation (8.16)) and Equation (8.17) for myoglobin ion exchange isotherm are used in simulation.

Simulation results in Figure 8.17 and Figure 8.18 demonstrate that BSA and myoglobin can also be separated very well by salt gradient ion-exchange SMB for nonlinear ion exchange equilibrium isotherm of proteins. From extract stream the high purity and enriched BSA can be obtained and myoglobin can be also recovered from raffinate stream with a high purity. For *Case 1*, BSA and myoglobin separation with myoglobin complete recovery in raffinate stream, a relative lower salt concentration is formed in section III and IV with feed of 0.05M NaCl, in order to increase the myoglobin ion exchange amount with Q-Sepharose FF resin and make the downward movement of myoglobin in section IV to raffinate port. However, with such low salt concentration in section III and IV, the other contaminants probably adsorb to the anion exchangers, so it is better to increase pH value in buffer to improve the myoglobin ion exchange amount, such as pH value in buffer increased to 8.5 or 9, in order that a relative high salt concentration can be used in section III and IV to avoid the other contaminants adsorption on Q-Sepharose FF resin. For *Case 2*, BSA purification from myoglobin impurity without the recovery of myoglobin from raffinate stream, a relative high salt concentration is formed in section III and IV by input feed with 0.13M NaCl, in order to decrease myoglobin and other

contaminants's ion exchange amount with Q-Sepharose FF resin; also pH value in buffer may be adjusted near the isoelectric point of impurity protein to decrease impurity protein ion exchange amount. Without the recovery of myoglobin from raffinate stream, the constraint to the net flux in section IV only depends on the net salt flux with an upward movement for open loop.



Case 1 BSA and myoglobin separation with complete recovery of myoglobin in raffinate



Case 2 BSA purification from myoglobin impurity without recovery of myoglobin

Figure 8.16. Operating conditions and configuration in salt gradient ion-exchange SMB with open loop. Switch time **6min**, column packed with Q-Sepharose FF resin, and column diameter **16mm**, packed length **100mm**, bed voidage **0.35**

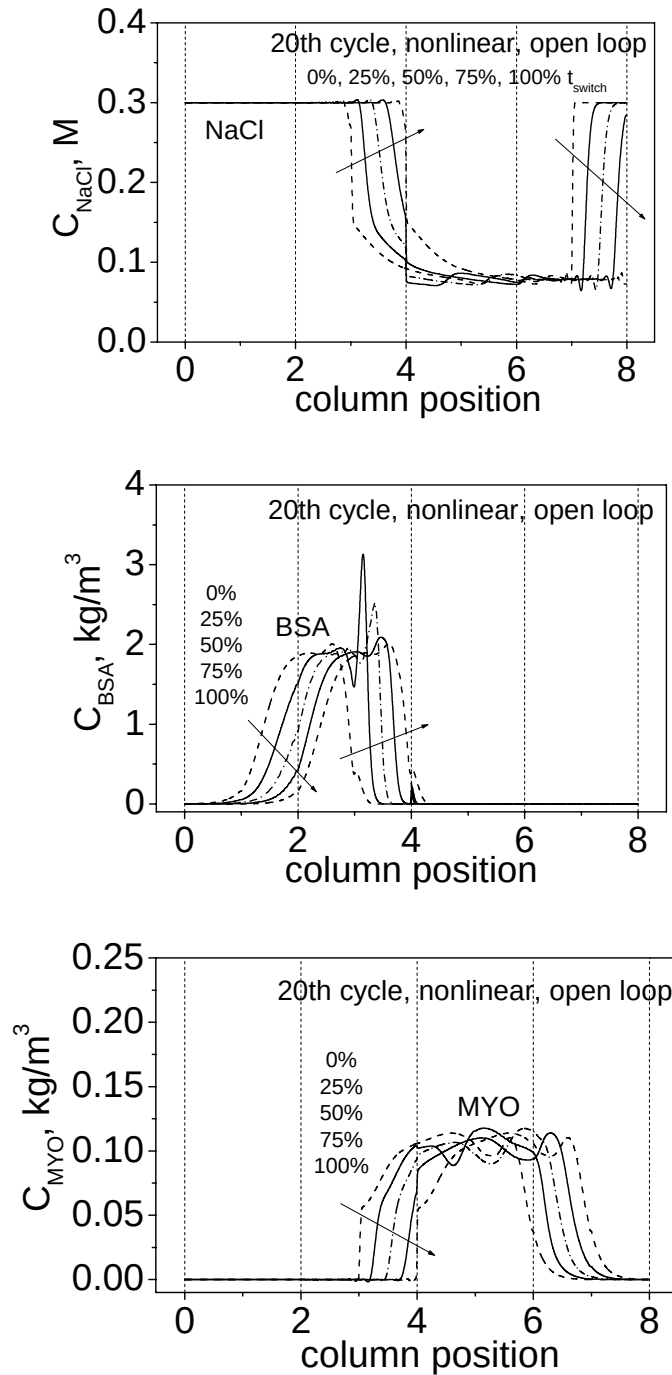


Figure 8.17. BSA and myoglobin separation with complete recovery of myoglobin in raffinate stream (Case 1). Cyclic steady state internal concentration profiles during a switch time interval in salt gradient ion-exchange SMB with open loop for nonlinear equilibrium isotherm. Calculation conditions shown in Figure 8.16.

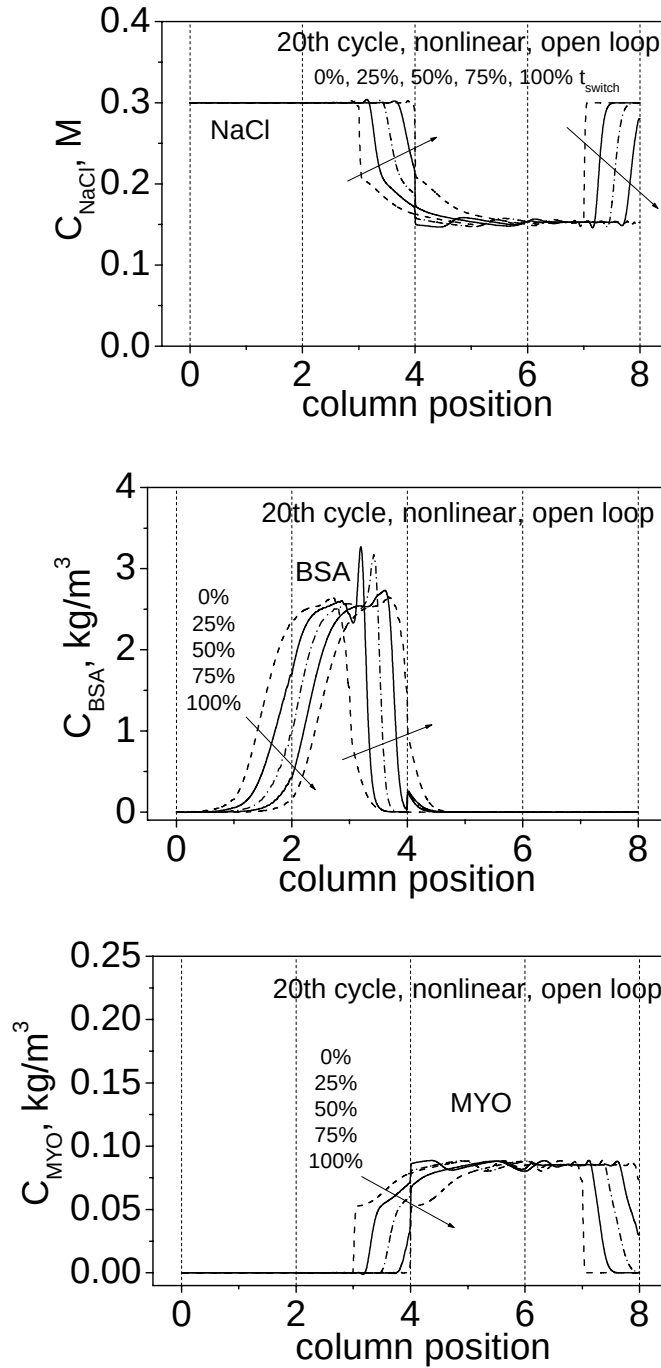


Figure 8.18 BSA purification from myoglobin impurity without the recovery of myoglobin (Case 2). Cyclic steady state internal concentration profiles during a switch time interval in salt gradient ion-exchange SMB with open loop for nonlinear equilibrium isotherm. Calculation conditions shown in Figure 8.16.

Conclusion

Based on theoretical analysis, it is demonstrated that the separation and purification of proteins can be performed effectively by salt gradient ion-exchange SMB chromatography. Although the experimental work done by Houwing and co-workers (2002) confirmed qualitatively the potential application of proteins separation by salt gradient ion exchange SMB, further experimental validation is still necessary.

The selection of salt gradient is a key issue and is also flexible in the design of proteins separation and purification by salt gradient ion-exchange SMB chromatography. In section I and II of ion exchange SMB, a high salt concentration is assigned to desorb the bound proteins on ion exchangers in order to reduce the desorbent consumption, but one should avoid using too high salt concentration, as a result of the significant decrease of separation factor in section II, and sometimes salt concentration may be limited by the proteins solubility; in section III and IV, a lower salt concentration is assigned to increase the adsorption of proteins, but for the case of protein purification from some impurities, the salt concentration should be raised a little to decrease the adsorption of impurities and contaminant on ion exchangers. Moreover, when the gradient SMB is run in closed loop to further reduce solvent consumption, it is better that a holding vessel with a given volume is added to the system to mix the desorbent with the recycled liquid stream from section IV in order to reduce solvent strength fluctuation in section I in the columns.

Compared to isocratic SMB, the selection of flowrates in each section to desired separation level becomes more complex in salt gradient ion exchange SMB; the additional constraints in terms of the net fluxes of salt in each section in order to keep a stable salt gradient formation, must also be taken into account in design of gradient SMB.

Although it is known that the prediction of the isocratic SMB operation can be carried out through the equivalent isocratic TMB approach when the SMB unit is constituted by, at least, two columns per section (a total of eight columns), some deviation is found between predictions from a real gradient SMB model and gradient TMB model. When the simple gradient TMB model is used to optimize flow rate in each section for the desired separation and purification of proteins by salt gradient ion exchange SMB chromatography, results must be used with caution.

Notation

C	=concentration in the fluid, kg/m ³
$D_{i,0}$	=diffusivities of proteins and salt in water, m ² /s.
D_L	=axial dispersion coefficient, m ² /s
d_p	=particle diameter, m
D_{pe}	=effective pore diffusivity in adsorbent, m ² /s.
E_i	=enrichment, defined by equation (8.15)
K	=binding constant in SMA model
k_p	=mass transfer coefficient, s ⁻¹ .
L_C	=column packed length, m
m	= net flowrate ratio, defined by equation (8.21)
q	=average adsorbed concentration in adsorbent, kg/m ³ particle
q^*	=average adsorbed concentration in equilibrium with fluid concentration, kg/m ³
q_0	=total ionic capacity of ion exchanger, mmol Cl ⁻¹ /mL particle for anion exchanger
Q	=volumetric liquid flowrate, m ³ /s
Q_S	=solid volumetric flowrate, m ³ /s, $Q_S = (1 - \varepsilon_B) V_C / t_{switch}$
$S_{1,2}$	=separation factor, defined by equation (8.18)
t	=time, s
t_{switch}	=switch time, s
u	=superficial liquid flow velocity, m/s
u_S	=solid flow velocity, m/s, $u_S = L_C / t_{switch}$
V_C	= the column packed volume, m ³
Z	= axial distance from the column entrance, m

V_s	=volume of the holding vessel, m ³
z	=characteristic charge in SMA model
τ	=tortuosity factor in pore of adsorbent
σ	=steric factor in SMA model
ε_B	=bed voidage in column, m ³ /m ³

Subscripts and superscripts

I, II, III, IV	=section I, section II, section III and section IV in SMB
D, E, F, R, rec	= desorbent, extract, feed, raffinate, and recycle
IE	=ion exchange
N	=total columns in SMB
SMB	=simulation moving bed
TMB	=true moving bed
i	=components, BSA, myoglobin and NaCl
j	=section in SMB
k	=column number
S	=salt, NaCl
0	=inlet

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Appendix: Model equations for gradient TMB model

In gradient TMB model, the solid phase is assumed to move in plug flow in the opposite direction of the fluid phase, solid flow velocity as $u_s = L_C / t_{switch}$, while the inlet and outlet lines remain fixed. As a consequence, each column plays the same function, depending on its location (section).

Mass balance in a volume element of the bed in section j :

$$\frac{\partial C_{ij}}{\partial t} = D_{Lk} \frac{\partial^2 C_{ij}}{\partial Z^2} - \frac{u_j^{TMB}}{\varepsilon_B} \frac{\partial C_{ij}}{\partial Z} - \frac{(1 - \varepsilon_B)}{\varepsilon_B} k_{p_{ij}} [q_{ij}^* - q_{ij}] \quad (A1)$$

Mass balance in the particle

$$\frac{\partial q_{ij}}{\partial t} = u_s \frac{\partial q_{ij}}{\partial Z} + k_{p_{ij}} (q_{ij}^* - q_{ij}) \quad (A2)$$

Initial conditions:

$$t = 0: C_{ij} = q_{ij} = 0 \text{ for proteins} \quad (A3a)$$

before feed application to the column, a salt gradient has been formed in the columns as

$$C_{sj} = C_s^F, \quad q_{sj} = q_{sj}^*(C_s^F) \text{ in section III and IV} \quad (A3b)$$

$$C_{sj} = C_s^D, \quad q_{sj} = q_{sj}^*(C_s^D) \text{ in section I and II} \quad (A3c)$$

Boundary conditions in each section j for proteins and salt

$$D_{Lj} \varepsilon_B \frac{\partial C_{ij}}{\partial Z} \Big|_{Z=0} = u_j^{TMB} [C_{ij}|_{Z=0} - C_{ij,0}] \quad (A4a)$$

$$\frac{\partial C_{ij}}{\partial Z} \Big|_{Z=L_j} = 0 \quad (A4b)$$

$$\frac{\partial q_{ij}}{\partial Z} \Big|_{Z=0} = \frac{\partial q_{ij-1}}{\partial Z} \Big|_{Z=L_{j-1}} \quad (A4c)$$

$$q_{ij}|_{Z=0} = q_{ij-1}|_{Z=L_{j-1}} \quad (A4d)$$

Mass balances at nodes:

at desorbent node

$$C_{ii,0} = C_i^D \quad \text{open loop} \quad (A5a)$$

$$C_{iI,0} = \frac{Q_D C_i^D + Q_{IV}^{TMB} C_{iIV} \big|_{Z=L_{IV}}}{Q_I^{TMB}} \quad \text{closed loop} \quad (\text{A5b})$$

at extract node:

$$C_{iII,0} = C_{iI} \big|_{Z=L_I} \quad (\text{A5c})$$

at feed node:

$$C_{iIII,0} = \frac{Q_F C_i^F + Q_{II}^{TMB} C_{iII} \big|_{Z=L_{II}}}{Q_I^{TMB}} \quad (\text{A5d})$$

at raffinate node

$$C_{iIV,0} = C_{iIII} \big|_{Z=L_{III}} \quad (\text{A5e})$$

Global balances:

$$Q_I^{TMB} = Q_D - [\varepsilon_B / (1 - \varepsilon_B)] Q_S \quad \text{open loop} \quad (\text{A6a})$$

$$Q_I^{TMB} = Q_D + Q_{IV}^{TMB} \quad \text{closed loop} \quad (\text{A6b})$$

$$Q_{II}^{TMB} = Q_I^{TMB} - Q_E \quad (\text{A6c})$$

$$Q_{III}^{TMB} = Q_{II}^{TMB} + Q_F \quad (\text{A6d})$$

$$Q_{IV}^{TMB} = Q_{III}^{TMB} - Q_R \quad (\text{A6e})$$

The equivalent between gradient TMB model and gradient SMB model can be made in terms of flowrates, $Q_j^{TMB} = Q_j^{SMB} - [\varepsilon_B / (1 - \varepsilon_B)] Q_S$, with $Q_S = (1 - \varepsilon_B) V_C / t_{switch}$. Here L_C is column length, V_C is column volume, and t_{switch} is the switch time interval in gradient SMB operation.

9. Conclusions and suggestions for future work

9.1 Conclusions

Inert core adsorbent:

Biomacromolecule protein diffusion resistance in adsorbents can be reduced significantly when the design of adsorbent matrix is improved by including a single inert core material in the macroporous crosslinked resin, which is called inert core adsorbent, pellicular adsorbent or poroshell adsorbent. The decreased intraparticle diffusion resistance by inert core adsorbents is quantitatively estimated by the parameter $1/\Theta$ (Eq. 3.53), derived in this thesis, and the analytical expression of linear resolution, Eq. 3.82, confirms that good resolution of two components can be obtained by chromatographic column packed with inert core adsorbent rather than with conventional adsorbent if the rate of the protein intraparticle diffusion is very slow.

Furthermore, the analytical expression, Eq. 4.66, for Height Equivalent to a Theoretical Plate (*HETP*), derived under the linear adsorption kinetics, can be used to evaluate the independent contribution of liquid axial dispersion, film mass transfer resistance, intraparticle diffusion resistance and restricted adsorption-desorption rate on *HETP* in the chromatography column packed with inert core adsorbents. And, a new method to estimate both film mass transfer coefficient and effective pore diffusivity from a single bulk concentration-time curve in batch adsorber is also given in this thesis.

Expanded bed:

Streamline Direct CST I can capture proteins from high ionic strength and salt concentration feedstocks with a high binding capacity, as opposed to classical ion

exchangers (such as Streamline SP and Streamline DEAE). When a Streamline 50 column packed with 300mL Streamline Direct CST I was used to capture proteins from the feedstock of 1kg/m^3 BSA and 0.2kg/m^3 myoglobin at 517cm/h feed flow rate, BSA dynamic binding capacity is 28.5 mg(BSA)/mL of settled bed volume, and myoglobin dynamic binding capacity is 5.65 mg (myoglobin)/mL of settled bed volume at the binding condition of 50mM acetate buffer (pH 5) (measured at 5% breakthrough points). Then, 50mM phosphate buffer with 1M NaCl (pH 7) is used to elute both bound BSA and bound myoglobin, the recoveries of BSA and myoglobin are 95% and 88%, respectively, and a little elution solution is consumed.

The competitive adsorption between BSA and myoglobin on Streamline Direct CST I is more significant, but the displacement adsorption between the two proteins becomes very weak. Furthermore, there are no a roll up in breakthrough curves caused by the displacement adsorption between BSA and myoglobin that suggests the two proteins are adsorbed on different sites at the binding condition of 50mM acetate buffer (pH 5).

In spite of the existence of protein intraparticle diffusion resistance, film mass transfer resistance, liquid axial dispersion and solid axial dispersion during expanded bed adsorption, the contribution of protein effective pore diffusivity to the breakthrough curves is dominant. The film mass transfer coefficient has a significant effect on initial breakthrough time for the highly favorable protein adsorption isotherm; liquid axial dispersion and solid axial dispersion have a lesser effect on the breakthrough curves, even at high liquid flow velocity (up to 553 cm/h for Streamline direct CST I), if the bed expansion is stable.

Because of the narrow particle size distribution of Streamline direct CST I, the effects of the particle size axial dispersion and the bed voidage axial variation on the breakthrough behavior in the expanded bed are small, and thus the uniform model can be used to predict the breakthrough curves with acceptable accuracy. In contrast, because of the wide particle size distribution of Streamline DEAE and Streamline SP, the effects of the particle size axial distribution and bed voidage axial variation on the breakthrough curves in expanded bed should be taken into account in the model.

Salt gradient ion-exchange SMB:

The process performance of the separation and purification of proteins by ion-exchange SMB can be improved when a lower salt concentration is formed in section III and IV to increase the adsorption of proteins and a higher salt concentration is formed in section I and II to improve the desorption of the bound proteins, called salt gradient ion-exchange SMB.

The selection of salt gradient is a key issue and also is flexible in the design of proteins separation and purification by salt gradient ion-exchange SMB chromatography. In section I and II of ion exchange SMB, a high salt concentration will favor the desorption of the bound proteins and reduce the desorbent consumption, but too high salt concentration should be avoided, as a result of the significant decrease of separation factor in section II; in section III and IV of ion exchange SMB, a lower salt concentration will favor the adsorption of proteins, but for the case of protein purification from a stream with some impurities, the salt concentration should be raised a little to decrease the adsorption of impurities and contaminant on ion exchangers. Moreover, when the gradient SMB is run in closed loop to reduce further desorbent consumption, it is better that a holding vessel with a given volume is added to the system to mix the desorbent with the recycled liquid stream from section IV in order to reduce salt or solvent strength fluctuation in the columns of section I.

Compared to isocratic SMB, the selection of flow rates in each section to desired separation level becomes more complex in salt gradient ion exchange SMB; the additional constraints in terms of the net fluxes of salt in each section also must be taken into account in design, in order to keep a stable salt gradient formation in ion-exchange SMB.

Although it is known that the prediction of isocratic SMB operation can be carried out through the equivalent isocratic TMB approach when the SMB unit is constituted by, at least, two columns per section (a total of eight columns), it is found some deviation between the real gradient SMB model and the equivalent gradient TMB model for the prediction of internal concentration profiles of proteins and salt. It should be paid attention when we use the simple gradient TMB model to optimize flow rate in each section for the

desired separation and purification of proteins by salt gradient ion-exchange SMB chromatography.

9.2 Suggestions for future work

Gradient SMB:

On theoretical analysis carried out in this thesis, it is demonstrated that the separation and purification of proteins can be performed effectively by salt gradient ion-exchange SMB. Although the experimental work done by Houwing and co-workers (2002) confirmed qualitatively the potential application of proteins separation by salt gradient ion exchange SMB, further experimental validation is still necessary.

Expanded bed:

The EBA process will be more effective for those adsorbents that have both high-density base matrix and salt-tolerant ligand. Recently, multi-modal functional groups are immobilized on Streamline direct CST I adsorbents, which not only takes advantage of electrostatic interaction, but also takes advantage of hydrogen bond interaction and hydrophobic interaction to tightly bind proteins, in order to get a high binding capacity in high ionic strength and salt concentration feedstocks. Moreover, the inert core adsorbents not only increase the particle density to form stable expansion at high feed flow rate in the expanded bed, but also reduce the protein diffusion resistance inside adsorbent due to shortening of the diffusion path. More work is needed when multi-modal ligands are immobilized on inert core adsorbents to effectively capture proteins in expanded bed adsorption process.

Tailing behavior of breakthrough curves of proteins

There exists the tailing behavior of the breakthrough curves when protein effluent concentration approaches the feed concentration. For the lower molecular protein, such as myoglobin, the tailing behavior is not significant; in contrast, for the larger molecular protein, such as BSA, the tailing behavior is very significant. In previous published papers,

the tailing behavior was explained by the presence of the impurities in protein samples, or microporous diffusion in the macroporous adsorbent, or protein steric hindrance on active sites of the surface of the adsorbent. Up to now, it is still unclear the explanation for the tailing behavior of the breakthrough curves often occurring for medium size and big size proteins adsorption. Our experimental data of the breakthrough curves agree to the explanations that the tailing behavior of protein breakthrough curves is caused by protein interactions with itself and the consequent formation of dimers in the packed bed. The further mechanical analysis is more important.

Practical applications of expanded bed chromatography and gradient SMB technology

Instead of using model solution of proteins with high purity, in the future, our research team will pay more attention to capture and purify the target proteins from real proteins sources by expanded bed chromatography and gradient SMB technology.

