RENATA CRISTINA FERREIRA BONOMO

TERMODINÂMICA, MODELAGEM E SIMULAÇÃO DO PROCESSO DE ADSORÇÃO E DESSORÇÃO DE BSA E β-LACTOGLOBULINA EM CROMATOGRAFIA DE INTERAÇÃO HIDROFÓBICA

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, para obtenção do título de *Doctor Scientiae*.

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Profª. Dr.ª Jane S. Reis Coimbra (Conselheiro)	Prof. Dr. Luis Henrique M. da Silva (Conselheiro)
Prof.ª Dr.ª Maria Helena C. de Andrade	Prof.ª Dr.ª Fabiana Queiroz Ferrua

Prof. Dr. Luis Antonio Minim

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RESUMO

BONOMO, Renata Cristina Ferreira, D.Sc., Universidade Federal de Viçosa, março de 2005. **Termodinâmica, modelagem e simulação do processo de adsorção e dessorção de BSA e β-lactoglobulina em cromatografia de interação hidrofóbica.** Orientador: Luis Antonio Minim. Conselheiros: Jane Sélia dos Reis Coimbra e Luis Henrique Mendes da Silva.

Neste trabalho foi estudado o processo de adsorção das proteínas albumina de soro bovino (BSA) e β-lactoglobulina (β-lg), provenientes do soro de queijo, em cromatografia de interação hidrofóbica variando-se as condições de temperatura e concentração de sal. Com os dados experimentais obtidos foi conduzida uma análise termodinâmica do processo. Foram realizadas, ainda, modificações no *software SimuCromWin*, desenvolvido por Saraiva (2003), que consistiram na inclusão da opção de utilização de outros modelos de isotermas além do modelo de Langmuir e o desenvolvimento de um programa que simula o processo de eluição em leito fixo. Avaliou-se, então, a influência dos modelos de isotermas na simulação do processo de adsorção assim como a otimização do

processo de adsorção e dessorção das referidas proteínas em cromatografia de interação hidrofóbica.

A partir dos resultados obtidos por meio do estudo da adsorção de BSA e β-lg em resina hidrofóbica (Streamline Phenil) foi possível observar que este processo é dependente da concentração de sal. Verificou-se, também, que o processo é espontâneo e entropicamente dirigido.

Com as alterações no *software SimuCRomWin*, foram realizados estudos que indicaram o modelo de isoterma de Langmuir o melhor para simulações em cromatografia preparativa. Além disso, foi realizada a otimização do processo de separação das proteínas albumina de soro bovino (BSA) e β-lactoglobulina (β-lg), utilizando o *software* SimuCromWin e a técnica de superfície de resposta, na qual verificou-se que em temperaturas e concentrações de sal mais altas, dentro da faixa estudada, a resolução entre os picos é maior.

ABSTRACT

BONOMO, Renata Cristina Ferreira, D.S., Universidade Federal de Viçosa, March, 2005. **Thermodynamic, modeling and simulation of adsorption and dessorption process of BSA e β-lactoglobulin at hydrophobic interaction chromatography.** Adviser: Luis Antonio Minim. Committe Members: Jane Sélia dos Reis Coimbra e Luis Henrique Mendes da Silva.

In this work was studied the adsorption process of whey proteins bovin serum albumin (BSA) and β-lactoglobulin (β-lg) at hydrophobic interaction chromatography (HIC) at four temperatures and salt concentrations. The thermodynamic analysis of the adsorptive process was made using the experimental data. Modifications were accomplished in the *software SimuCromWin*, developed by SARAIVA (2003), to promote the use of six types of isotherms and to include the elution stage of adsorption in fixed beds. Then, the influence of different isotherm models on the adsorption and dessorption process of BSA and β-lg and the optimization of these processes were studied. The results showed that the adsorption process of BSA and β-lg on Streamline Phenyl is dependent on salt and temperature for the two proteins. The adsorption

process is driven by entropy and is favorable for both proteins. Studies were made after the modifications on the *software SimuCromWin* and indicated the Langmuir isotherm was the best isotherm model to simulated adsorption process for both proteins. The obtained experimental data, *software SimuCromWin* and surface response analysis permitted to determine the best conditions for adsorption and dessorption processes. The values found to adsorption process were 0.9 M and 313.15 K and to dessorption process were 0.85 M and 313.15 K, for both proteins.

INTRODUÇÃO GERAL

O desenvolvimento de técnicas e metodologias para a separação e purificação de compostos de origem biológica, principalmente proteínas, tem sido essencial para os recentes avanços nas pesquisas de biotecnologia. O objetivo principal dos processos de purificação não é simplesmente a remoção de contaminantes indesejados, mas também a concentração do produto desejado e sua transferência para um meio onde seja estável e que mantenha suas características para a aplicação.

Dentre estas técnicas destaca-se a cromatografia como método de trabalho em escala preparativa, por seu elevado poder de purificação na obtenção de produtos em larga escala. Nos processos de purificação de proteínas, a cromatografia de interação hidrofóbica é uma das técnicas de cromatografia mais utilizada devido à rapidez de separação com baixa degradação do produto e pequena quantidade de solvente, conduzindo a um elevado grau de purificação.

No entanto, os processos cromatográficos apesar de freqüentemente utilizados na purificação de macromoléculas, ainda exibem baixa produtividade quando comparados com técnicas tradicionais usadas na indústria química e farmacêutica. Portanto, tendo em vista que a cromatografia líquida é um processo de custo relativamente elevado e, ao mesmo tempo, uma etapa de alta resolução e seletividade, um entendimento abrangente de sua dinâmica é essencial aos propósitos de projeto e operação de equipamentos. A modelagem é, portanto,

uma ferramenta muito útil para o desenvolvimento amplo do conhecimento das etapas cromatográficas, assim como a sua otimização.

Neste trabalho foi estudado o processo de adsorção das proteínas albumina de soro bovino (BSA) e β-lactoglobulina (β-lg), provenientes do soro de queijo, em cromatografia de interação hidrofóbica variando-se as condições de temperatura e concentração de sal. Os dados obtidos permitiram avaliar termodinamicamente o processo adsortivo. Foram realizadas, ainda, modificações no *software SimuCromWin*, desenvolvido por Saraiva (2003), que consistiram na inclusão da opção de utilização de outros modelos de isotermas além do modelo de Langmuir e o desenvolvimento de um programa que simula o processo de eluição em leito fixo. Avaliou-se, então, a influência dos modelos de isotermas na simulação do processo de adsorção assim como realizou-se a otimização do processo de adsorção e dessorção das referidas proteínas em cromatografia de interação hidrofóbica.

MODELAGEM E SIMULAÇÃO DE PROCESSOS CROMATOGRÁFICOS

RESUMO

O modelo de taxa geral é considerado o mais amplo modelo de representação da cromatografia líquida, uma vez que inclui vários mecanismos de transferência de massa e isotermas não-lineares. Portanto, este modelo é uma importante ferramenta para o estudo dos processos cromatográficos em colunas. Neste trabalho foram realizadas modificações no *software SimuCromWin*, que utiliza o modelo de taxa geral, a fim de promover a utilização de seis tipos de isotermas e incluir o processo de eluição em coluna de leito fixo. Verificou-se que o tempo gasto, nos os casos estudados, para a simulação do processo de eluição é, em geral, aproximadamente dez vezes maior que o tempo gasto para o processo de adsorção em leito fixo. Com as alterações realizadas no SimuCromWin, este, agora, auxilia na escolha do melhor modelo de isoterma e na otimização dos parâmetros para o processo de separação.

Palavras-chave: modelagem, simulação, cromatografia líquida

ABSTRACT

Among all kinds of models used for liquid chromatography, the general rate model is the wide one. It accounts for various mass transfer mechanisms and nonlinear isotherms. Therefore, this model is a very useful tool for the study of the chromatographic processes on columns. In this work, modifications were accomplished in the *software SimuCromWin*, which uses the general rate model, to promote the use of six types of isotherms and to include the elution stage of adsorption in fixed beds. The results shown that the spend time to simulate the elution stage is, in general, approximately ten times larger than the simulation time for the adsorption process. With

the modifications accomplished in the *SimuCromWin*, it become possible in the choice of the best isotherm model and in the optimization of the parameters for the separation process.

Keywords: mathematical modeling, simulation, liquid chromatography

1. INTRODUÇÃO

A Cromatografia é um poderoso método de separação que foi desenvolvido inicialmente para extração e purificação de misturas complexas de origem vegetal. Frações coletadas eram analisadas, identificadas e utilizadas em aplicações futuras. Ao longo do tempo, a cromatografia tornou-se um importante método preparativo, pois seu poder de separação atraiu a atenção de pesquisadores interessados na produção de produtos quimicamente puros em larga escala (GUIOGHON, 2002). Este poder de separação deve-se à facilidade de promover a retirada individual de vários componentes de uma mistura, sob condições experimentais, em que as duas fases de um sistema estão sempre próximas do equilíbrio, devido à rápida transferência de massa entre estas (BELLOT e CONDORET, 1993). Pode-se dizer que o poder de separação de uma coluna é uma função direta da taxa de transferência de massa, do coeficiente de dispersão axial e do equilíbrio de adsorção.

O grande sucesso das separações cromatográficas de macromoléculas é, contudo, devido a sua capacidade em atingir elevado grau de pureza a partir de misturas complexas com reduzidas concentrações de tais compostos (BOSCHETTI e COFFMAN, 1998).

Tendo em vista que a cromatografia líquida é um processo de custo relativamente elevado e, ao mesmo tempo, uma etapa de alta resolução e seletividade,

um entendimento abrangente de sua dinâmica é essencial aos propósitos de projeto e operação de equipamentos. A modelagem é, portanto, uma ferramenta muito útil para o desenvolvimento amplo do conhecimento das etapas cromatográficas, assim como a sua otimização (GUIOCHON, 2002).

A modelagem matemática da cromatografia líquida tem se constituído em uma importante área de estudo desde 1960, e vem possibilitando mais eficientemente o uso de colunas cromatográficas. Assim, valiosas informações são obtidas sobre o fenômeno de transferência de massa através do meio poroso e os parâmetros necessários para caracterizar a coluna cromatográfica, como a dispersão axial e difusividade na partícula (BELLOT e CONDORET, 1993).

Vários modelos matemáticos estão disponíveis para avaliar os perfis de banda e as curvas de ruptura obtidas nos processos cromatográficos. Os mais utilizados atualmente são o ideal, cinético agrupado, de equilíbrio-dispersivo e de taxa geral. Dentre estes, o de equilíbrio-dispersivo é o recomendado quando a resistência à transferência de massa é pequena e tem uma pequena influência nos perfis. Em casos mais complexos um dos outros modelos é utilizado, sendo o modelo de taxa geral o mais amplo (MIYABE e GUIOCHON, 2000, e KACZMARSKI et al, 2001).

O objetivo deste trabalho é aplicar o modelo de taxa geral na modelagem e simulação do processo de adsorção e eluição das proteínas de soro de queijo BSA e β-lactoglobulina. Para isto são apresentadas as modificações realizadas no programa SimuChromWin, as quais implementaram a utilização de vários tipos de isotermas e a opção de realização do processo de eluição.

2. MODELAGEM MATEMÁTICA

O uso de modelos matemáticos para descrever fenômenos existentes em engenharia objetiva a redução de custos e aumento da rapidez na obtenção dos resultados. Os modelos matemáticos podem ser classificados em teóricos ou empíricos, em dinâmicos ou estacionários e em parâmetros concentrados (agrupados) ou distribuídos (SPIEKER et al, 1998).

O emprego de métodos matemáticos para simulação e análise de processos é uma técnica de potencial reconhecido, principalmente depois do aparecimento de métodos matemáticos mais sofisticados e do desenvolvimento de computadores que permitem a solução de problemas em tempo relativamente curto.

Segundo GUIOCHON (2002), existem cinco modelos de grande importância que podem ser aplicados tanto na cromatografia linear como na cromatografia não linear. Esses modelos são:

a. Modelo ideal:

O modelo assume que a coluna tem uma eficiência infinita. Portanto, os perfis de banda surgem a partir das características de equilíbrio termodinâmico. Este modelo tem a vantagem de ser simples. Todos os outros modelos fogem da idealidade e usam diferentes aproximações que consideram a eficiência finita da coluna (GUIOCHON, 2002).

Este modelo negligencia completamente a influência das cinéticas de transferência de massa e da dispersão axial nos perfis de banda. A equação do balanço de massa é escrita como (GUIOCHON et al, 1994):

$$\frac{\partial C}{\partial t} + \varphi \frac{\partial C_p^s}{\partial t} + v \frac{\partial C}{\partial Z} = 0 \quad (1)$$

b. Modelo de equilíbrio dispersivo:

Este modelo considera que a transferência de massa através da coluna é infinitamente rápida, e uma magnitude finita da dispersão axial. Neste modelo a taxa finita da cinética de transferência de massa é considerada como outra contribuição para a dispersão axial. Deste modo, o modelo de equilíbrio dispersivo relaciona dispersão axial aparente e altura efetiva de pratos teóricos. Esta aproximação é válida quando a eficiência da coluna é alta, como na Cromatografia Líquida de Fase Reversa (CLFR) de pequenas moléculas de polaridade moderada. Devido ao fato deste modelo considerar a influência para uma coluna de eficiência finita como uma pequena correção, não deve ser aplicado quando esta eficiência é baixa (GUIOCHON, 2002 e KACZMARSKI et al, 2001).

c. Modelo cinético agrupado:

Em contraste com o modelo de equilíbrio dispersivo, o qual baseia-se nas considerações de que o equilíbrio termodinâmico é alcançado entre as fases estacionárias e que a influência da dispersão axial e das várias contribuições de origem cinética para o alargamento da banda podem ser avaliadas usando um coeficiente de dispersão aparente, de magnitude apropriada, o modelo cinético agrupado baseia-se no uso de uma equação cinética. A equação de balanço de massa é escrita como:

$$\frac{\partial C}{\partial t} + \varphi \frac{\partial C_p^s}{\partial t} + v \frac{\partial C}{\partial Z} = D_p \frac{\partial^2 C}{\partial Z^2} \quad (2)$$

No modelo cinético agrupado, as contribuições de todos os mecanismos envolvidos no alargamento de banda, devido a cinéticas relativamente lentas, são agrupados num único coeficiente de taxa.

Vários modelos cinéticos podem ser deduzidos dependendo da principal causa da demora no alcance do equilíbrio na coluna. Se a cinética do mecanismo de retenção for mais lenta que as outras etapas do processo cromatográfico, o modelo utilizado é o reação-dispersivo. Se a etapa mais lenta no processo cromatográfico é a cinética de transferência de massa, o modelo é o de transporte dispersivo (GUIOCHON, 2002).

e. Modelo de taxa geral:

O modelo de taxa geral considera simultaneamente todas as possíveis contribuições à cinética de transferência de massa, que são a dispersão axial, a resistência à transferência de massa no filme extrapartícula, a difusão intrapartícula e a taxa de adsorção-dessorção (GUIOCHON et al, 1994). Uma breve descrição das características do modelo de taxa geral utilizado na construção do programa para simulação de processos cromatográficos é apresentada a seguir.

Segundo KACZMARSKI et al (2001), são feitas as seguintes considerações para o modelo de taxa geral:

1. o processo cromatográfico é isotérmico;
2. a velocidade da fase móvel é constante;
3. o leito é empacotado com partículas porosas, esféricas e de tamanho uniforme;
4. o gradiente de concentração na direção radial do leito é desprezível;
5. existe equilíbrio local, para cada componente, entre a superfície do poro (monocamada) e a fase fluida estagnada dentro dos macroporos;
6. os coeficientes de dispersão são constantes.

Com base nas considerações acima, pode-se escrever duas equações de balanço de massa para cada componente, uma da fase móvel percolando o leito de partículas (Equação 3) e outra para o interior das partículas (Equação 4).

$$-D_{bi}\frac{\partial^2 C_{bi}}{\partial Z^2}+v\frac{\partial C_{bi}}{\partial Z}+\frac{\partial C_{bi}}{\partial t}+\frac{3k_i\left(1-\varepsilon_b\right)}{\varepsilon_b R_p}\left(C_{bi}-C_{pi,R=R_P}\right)=0 \tag{3}$$

$$\left(1-\varepsilon_p\right)\frac{\partial C_{pi}^s}{\partial t}+\varepsilon_p\frac{\partial C_{pi}}{\partial t}-\varepsilon_p D_{pi}\left[\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2\frac{\partial C_{pi}}{\partial R}\right)\right]=0 \tag{4}$$

Com as seguintes condições iniciais e de contorno

$$\left(1-\varepsilon_p\right)\frac{\partial C_{pi}^s}{\partial t}+\varepsilon_p\frac{\partial C_{pi}}{\partial t}-\varepsilon_p D_{pi}\left[\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2\frac{\partial C_{pi}}{\partial R}\right)\right]=0 \tag{5, 6}$$

$$Z=0,\quad \frac{\partial C_{bi}}{\partial Z}=\frac{v}{D_{bi}}\left(C_{bi}-C_{fi}(t)\right)\qquad Z=L,\quad \frac{\partial C_{bi}}{\partial Z}=0 \tag{7, 8}$$

$$R=0,\quad \frac{\partial C_{pi}}{\partial R}=0\qquad R=R_p,\quad \frac{\partial C_{pi}}{\partial R}=\frac{k_i}{\varepsilon_p D_{pi}}\left(C_{bi}-C_{pi,R=R_p}\right) \tag{9, 10}$$

Na Equação (4), C_{pi}^s é a concentração do componente i na fase sólida do adsorvente por volume de adsorvente, excluindo os poros. Portanto, C_{pi}^s é descrito por um modelo de isoterma.

A forma adimensional do sistema de equações acima é:

$$-\frac{1}{Pe_{Li}}\frac{\partial^2 c_{bi}}{\partial z^2}+\frac{\partial c_{bi}}{\partial z}+\frac{\partial c_{bi}}{\partial \tau}+\xi_i\left(c_{bi}-c_{pi,r=1}\right)=0 \tag{11}$$

$$-\frac{1}{Pe_{Li}}\frac{\partial^2 c_{bi}}{\partial z^2}+\frac{\partial c_{bi}}{\partial z}+\frac{\partial c_{bi}}{\partial \tau}+\xi_i\left(c_{bi}-c_{pi,r=1}\right)=0 \tag{12}$$

Condição inicial

$$\tau = 0, \quad c_{bi} = c_{bi}(0, z), \qquad c_{pi} = c_{pi}(0, r, z) \tag{13, 14}$$

Condição de contorno

$$z = 0, \quad \frac{\partial c_{bi}}{\partial z} = Pe_{Li}\left(c_{bi} - C_{fi}(\tau)/C_{0i}\right) \tag{15}$$

Para adsorção frontal $C_{fi}(\tau)/C_{0i} = 1$

Para eluição

$$C_{fi}(\tau)/C_{0i} = \begin{cases} 1 & 0 \le \tau \le \tau_{imp} \\ 0 & senão \end{cases}$$

$$z = 1, \qquad \frac{\partial c_{bi}}{\partial z} = 0 \tag{16}$$

$$r = 0 \qquad \frac{\partial c_{pi}}{\partial z} = 0 \qquad\qquad r = 1, \qquad \frac{\partial c_{pi}}{\partial r} = Bi_i(c_{bi} - c_{pi,r=1}) \tag{17, 18}$$

em que:; $c_{pi} = \frac{C_{pi}}{C_{0i}}$; $c_{pi}^{s} = \frac{C_{pi}^{s}}{C_{0i}}$; $r = \frac{R}{R_p}$; $z = \frac{Z}{L}$; $\tau = \frac{vt}{L}$; $Pe_{Li} = \frac{vL}{D_{bi}}$; $Bi_i = \frac{k_i R_p}{\varepsilon_p D_{pi}}$;

$\eta_i = \frac{\varepsilon_p D_{pi} L}{R_p^2 v}$; $\xi_i = \frac{3Bi_i\eta_i(1-\varepsilon_b)}{\varepsilon_b}$.

GU et al (1990) utilizaram o modelo de taxa geral multicomponente com a isoterma de Langmuir multicomponente para estudar o efeito dos parâmetros da isoterma de um deslocador na eficiência da cromatografia de dessorção. Em 1991, GU et al utilizaram o modelo de taxa geral com uma nova isoterma, extensão da isoterma de Langmuir, desenvolvida para adsorção multicomponente com capacidades de saturação diferentes. Estes autores verificaram que as simulações baseadas no modelo de taxa geral usando a nova isoterma demonstraram satisfatoriamente o fenômeno eluição (GU et al, 1991).

KACZMARSKI et al (2001) reavaliaram resultados experimentais de um estudo prévio das cinéticas de transferência de massa de albumina de soro bovino (BSA) em cromatografia de troca iônica, sob condições não-lineares, utilizando o modelo de taxa geral. Os resultados obtidos com este modelo foram comparados com aqueles obtidos usando o modelo de transporte-dispersivo e o modelo de difusão no poro para os mesmos dados experimentais. Os autores observaram que a utilização de um modelo mais simples (transporte-dispersivo) pode conduzir a interpretações errôneas dos dados experimentais e dos fundamentos dos processos envolvidos.

SARAIVA (2003) desenvolveu um programa computacional (*SimuCromWin*) que simula processos cromatográficos. O processo de adsorção em leito fixo foi estudado com base no modelo de taxa geral utilizando a isoterma de Langmuir. Por meio de simulação das curvas de ruptura para diferentes valores de vazão da fase móvel e de concentração dos solutos (α-lactoalbumina e β-lactoalbumina) na alimentação foi demonstrada a capacidade do modelo de predizer de forma adequada o processo de adsorção em leito fixo destas proteínas.

3. PROGRAMA COMPUTACIONAL

Ao programa computacional desenvolvido por SARAIVA (2003) foram implementadas modificações, as quais permitem o uso de outros modelos de isotermas de equilíbrio além do modelo de Langmuir e a simulação do processo de eluição, que pode ser uma ferramenta útil na otimização do processo de separação de biocompostos.

3.1. Solução Numérica

As equações adimensionais (11 e 12) do modelo de taxa geral foram discretizadas utilizando a técnica de diferenças finitas (na coluna) e o método de colocação ortogonal (na partícula).

3.2. Estimação dos parâmetros numéricos

O valor do coeficiente de transferência de massa é calculado de acordo com a equação utilizada por TRUEI et al. (1992):

$$Sh = \frac{k_i 2R_p}{D_{pi}} = 2 + 1{,}45\,\mathrm{Re}^{1/2}\,Sc^{1/3} \tag{19}$$

Onde o número de Schmidt, Sc, é dado por

$$Sc = \frac{\mu}{\rho D_{pi}} \tag{20}$$

A difusividade molecular, D_{pi}, foi deduzida a partir da correlação citada por KACZMARSKI et al. (2001):

$$D_{pi} = 8{,}31x10^{-8}\,\frac{T}{\mu M_i^{1/3}} \tag{21}$$

O coeficiente de dispersão axial pode ser determinado a partir de experimentos ou ser estimado usando correlações empíricas, como a apresentada por CHUNG e WEN (1968), que correlaciona o número de Peclet (Pe) como:

$$Pe_L = \frac{vL}{D_b} = \frac{L}{2R_p\varepsilon_b}\left(0{,}2 + 0.011\,\mathrm{Re}^{0{,}48}\right) \tag{22}$$

Com o número de Reynolds (Re)

$$\mathrm{Re} = \frac{2R_p\varepsilon_b v\rho}{\mu} \tag{23}$$

3.3. Implementação de novos modelos de isotermas

O processo cromatográfico é analisado com base no equilíbrio entre as fases envolvidas por meio de isotermas de adsorção e nos balanços de massa. No estudo de

equilíbrio, a isoterma de adsorção representa o equilíbrio sólido-líquido de um soluto adsorvido, em dada massa de fase estacionária, em contato com uma solução que contém o soluto (GUIOCHON et al, 1994). Este estudo é de fundamental importância para a modelagem precisa de um processo cromatográfico, pois um dos principais termos dos modelos matemáticos é os dados que descrevem o equilíbrio na adsorção de cada um dos componentes presentes (JACOBSON e FRENZ, 1990). Embora, o modelo de isoterma mais empregado para representar o equilíbrio sólido-líquido seja o de Langmuir (GUIOCHON et al, 1994), outros modelos podem ser eficazes e às vezes até melhores para descrever esses dados. Além do modelo de Langmuir outros modelos também utilizados são o de Toth, de Bi-Langmuir, de Jovanovic, de Freundlich e o modelo de Langmuir Exponencialmente Modificado (principalmente para cromatografia de interação hidrofóbica), dentre outros.

a. Modelo de isoterma de Langmuir

Este modelo foi proposta por Langmuir, em 1916, para adsorção num sistema gás-sólido. Langmuir assumiu um calor de adsorção constante e um número finito de sítios de adsorção. Com estas considerações, o máximo de adsorção corresponde à formação de uma monocamada saturada de moléculas de soluto na superfície adsorventes (LANGMUIR, 1916, e JACOBSON et al, 1984). Este modelo é escrito da seguinte forma:

$$Q = q_s \frac{bC}{1 + bC} \tag{24}$$

Neste modelo, q_s é a capacidade de saturação da monocamada e b é a constante de equilíbrio de adsorção.

b. Modelo de isoterma de Bi-Lagmuir

O modelo de isoterma de Bi-Langmuir foi proposto por GRAHAM (1953) para avaliar o comportamento de adsorção em superfícies não homogêneas. A superfície é

considerada como tendo dois tipos diferentes de domínio químico, os quais se comportam independentemente (JACOBSON et al., 1991). Portanto, esta isoterma de equilíbrio é o resultado da adição de duas isotermas de Langmuir.

$$Q = q_{s,1}\frac{b_1 C}{1 + b_1 C} + q_{s,2}\frac{b_2 C}{1 + b_2 C} \tag{25}$$

Neste modelo, existem duas capacidades de saturação, $q_{s,1}$ e $q_{s,2}$. A capacidade total de saturação é a soma dessas duas capacidades e b_1 e b_2 são as constantes de equilíbrio (GRITTI et al, 2003).

c. Modelo de isoterma de Toth

Originalmente derivado do estudo de equilíbrio gás-sólido, o modelo de isoterma de Toth (TOTH, 1971) possue três parâmetros. Como o modelo da isoterma de Langmuir, este pode ser extendido para o caso de equilíbrio sólido-líquido. Esta isoterma é utilizada para avaliar dados de equilíbrio experimentais que são obtidos em adsorventes não homogêneos.

$$Q = q_s \frac{bC}{\left[1 + (bC)^n\right]^{1/n}} \tag{26}$$

Nesta equação, q_s e b tem o mesmo significado que na isoterma de Langmuir e n é o parâmetro de heterogeneidade ($0 < n < 1$). Quando n = 1, a isoterma de Toth torna-se idêntica a isoterma de Langmuir. Os parâmetros b e n permitem o ajuste independente da inclinação inicial e da curvatura da isoterma (GUIOCHON et al, 1994 e GRITTI et al, 2003).

d. Modelo de isoterma de Jovanovic

Este modelo foi deduzido para a adsorção numa superfície sólida homogênea, considerando o fenômeno não-localizado, sem interações laterais e cobertura da superfície com uma monocamada de soluto (QUIÑONES e GUIOCHON, 1996a). O

modelo para adsorção de um componente pode ser escrito como (HUANG e HORVÁTH, 1987; QUIÑONES e GUIOCHON, 1996a e 1996b):

$$Q = q_s[1 - \exp(-b \cdot C)] \tag{27}$$

em que q_s é a concentração superficial na saturação e b é a constante de ligação apropriada.

e. Modelo de Isoterma de Langmuir Exponencialmente Modificado

A isoterma de Langmuir promove uma descrição insatisfatória da adsorção de proteínas em adsorventes hidrofóbicos devido a duas razões: a) a ligação de muitas proteínas em adsorventes hidrofóbicos é baseada em interações multivalentes (JENNISSEN, 1978), b) a adsorção de proteínas em meio hidrofóbico é altamente influenciada pela concentração de sal, mas o modelo de Langmuir não pode expressar este comportamento e os parâmetros do modelo são funções implícitas da concentração de sal (MELANDER et al, 1984, OSCARSSON and KÅRSNÄS, 1998 e CHEN and SUN et al, 2003). Para contornar este problema, um modelo da isoterma de Langmuir exponencialmente modificado foi proposto para inserir a contribuição da concentração de sal nas isotermas de adsorção de proteínas (ANTIA e HORVATH, 1989):

$$Q = \frac{\lambda b \exp(-kC_s)C}{1 + b\exp(-kC_s)C} \tag{28}$$

em que λ, b and k são parâmetros da equação, C_s é a concentração de sal na fase líquida e C a concentração de proteína na fase líquida.

f. Modelo de isoterma de Freundlich

O modelo de isoterma empírica, proposto por Boedeker em 1885, que é a base da isoterma de Freundlich, descreve a adsorção de componentes polares em adsorventes polares ou de compostos fortemente polares em solventes cuja polaridade é baixa ou média (GUIOCHON et al, 1994):

$$Q = aC^{1/n} \tag{29}$$

No programa SimuCromWin, como dito anteriormente, só era possível a utilização do modelo de isoterma de Langmuir para simular o processo de adsorção em leito fixo (ALF). Para inserir a opção de se utilizar outros tipos de isotermas, foi construída uma sub rotina denominada Hisoterma, a qual contém os seis modelos de isotermas descritos acima. Na tela inicial de ALF o usuário deve escolher o número de componentes e o tipo de isoterma para cada um deles (Figura 1). Na tela de simulação, o usuário deve fornecer os valores dos parâmetros da isoterma escolhida para cada um dos componentes, antes de iniciar o processo de simulação (Figura 2).

As Figuras 3 e 4 mostram as curvas de rupturas utilizando-se os modelos de Langmuir, Toth e Jovanovic, como exemplo, e o balanço de massa total do processo realizado, respectivamente. Depois de encerrada a etapa de ALF o usuário tem a opção de realizar do processo de eluição naquele momento ou salvar os dados para simular o processo de eluição em outra ocasião.

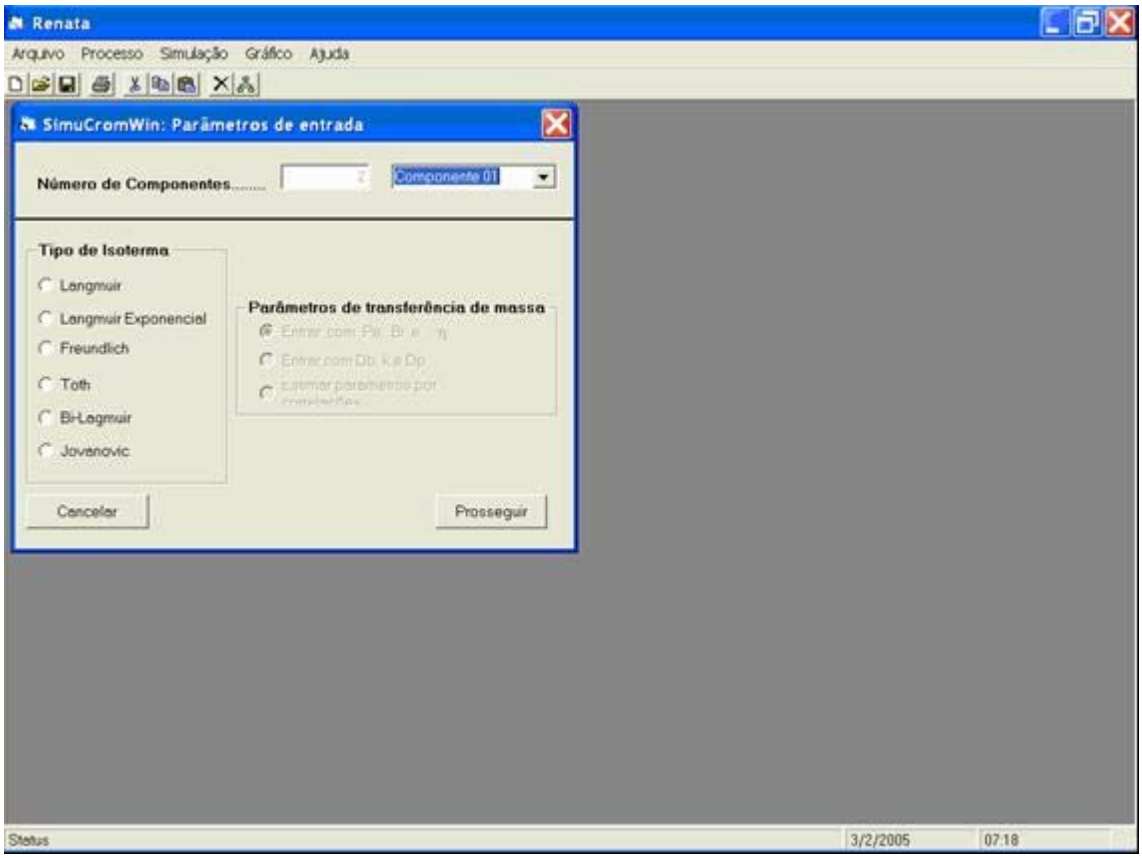

Figura 1. Tela inicial do processo ALF do *software SimuCromWin* modificado para utilização de seis tipos de isotermas.

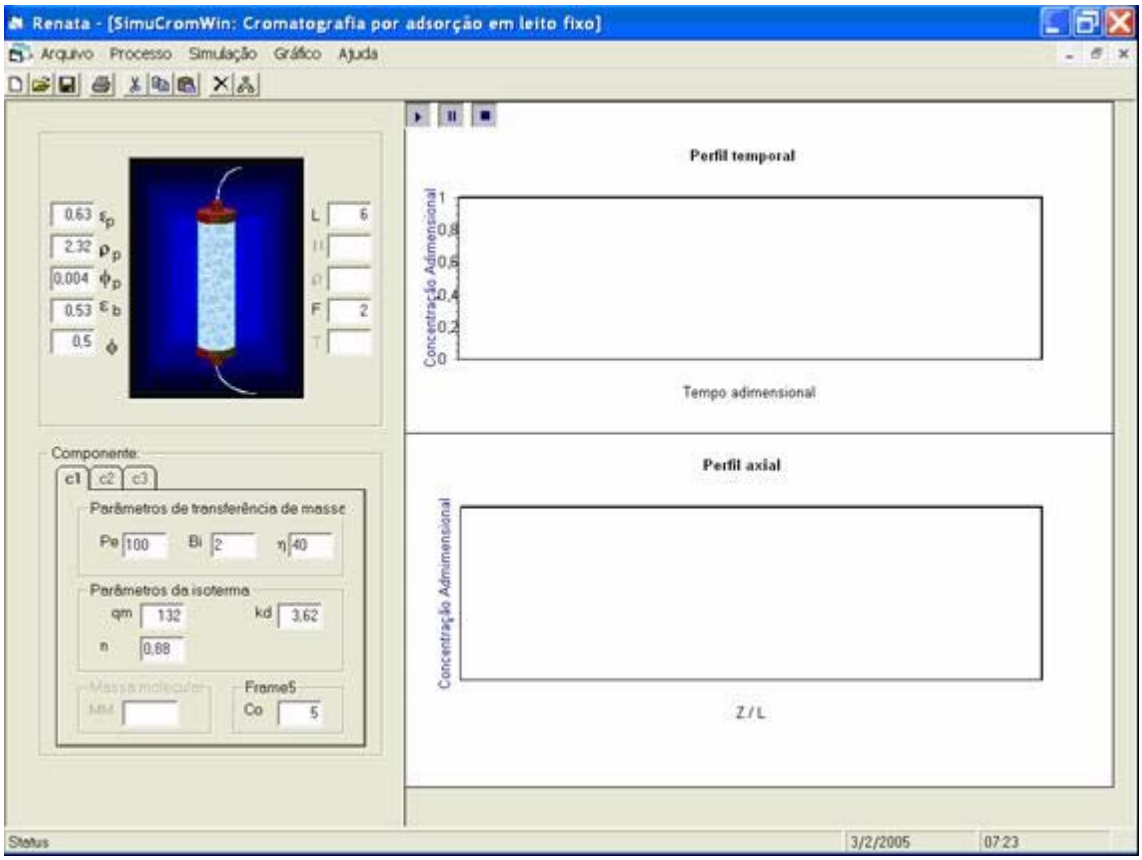

Figura 2. Tela de simulação da ALF do *software SimuCromWin* modificado.

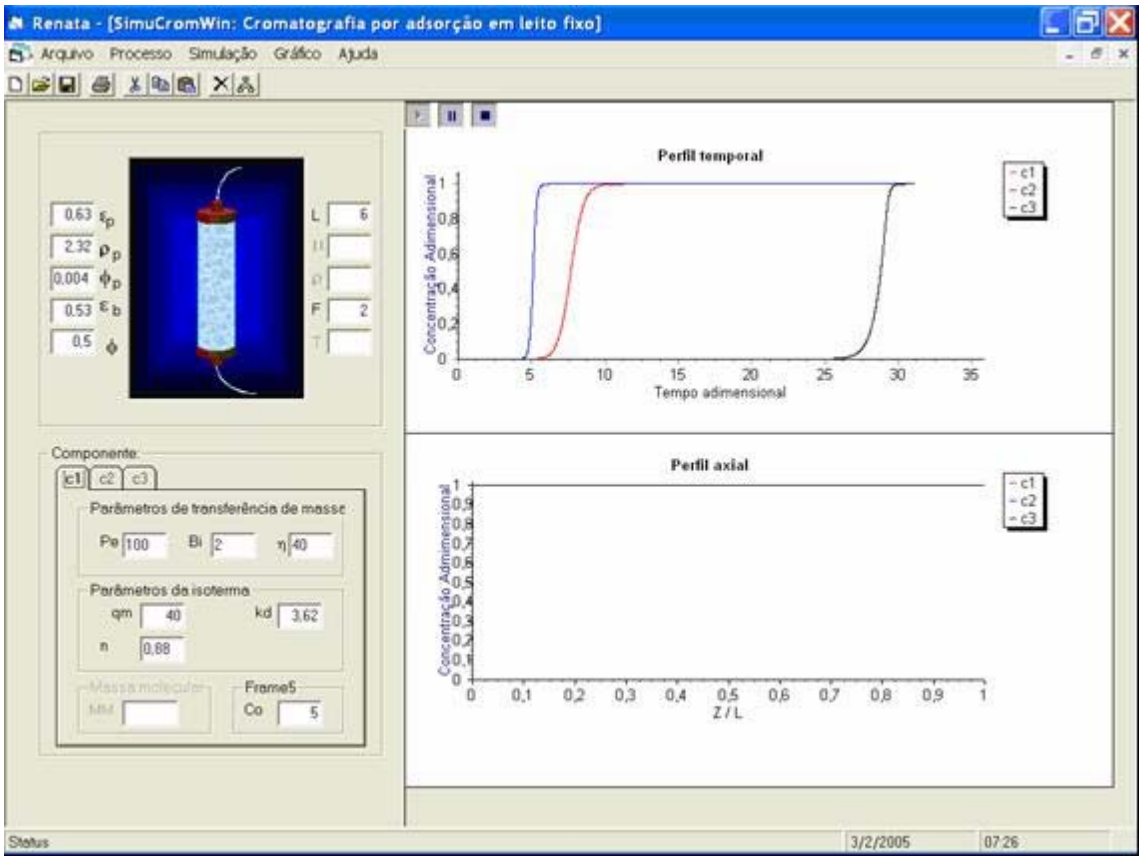

Figura 3. Curvas de ruptura simuladas utilizando os modelos de isoterma de Langmuir, Toth e Jovanovic, respectivamente.

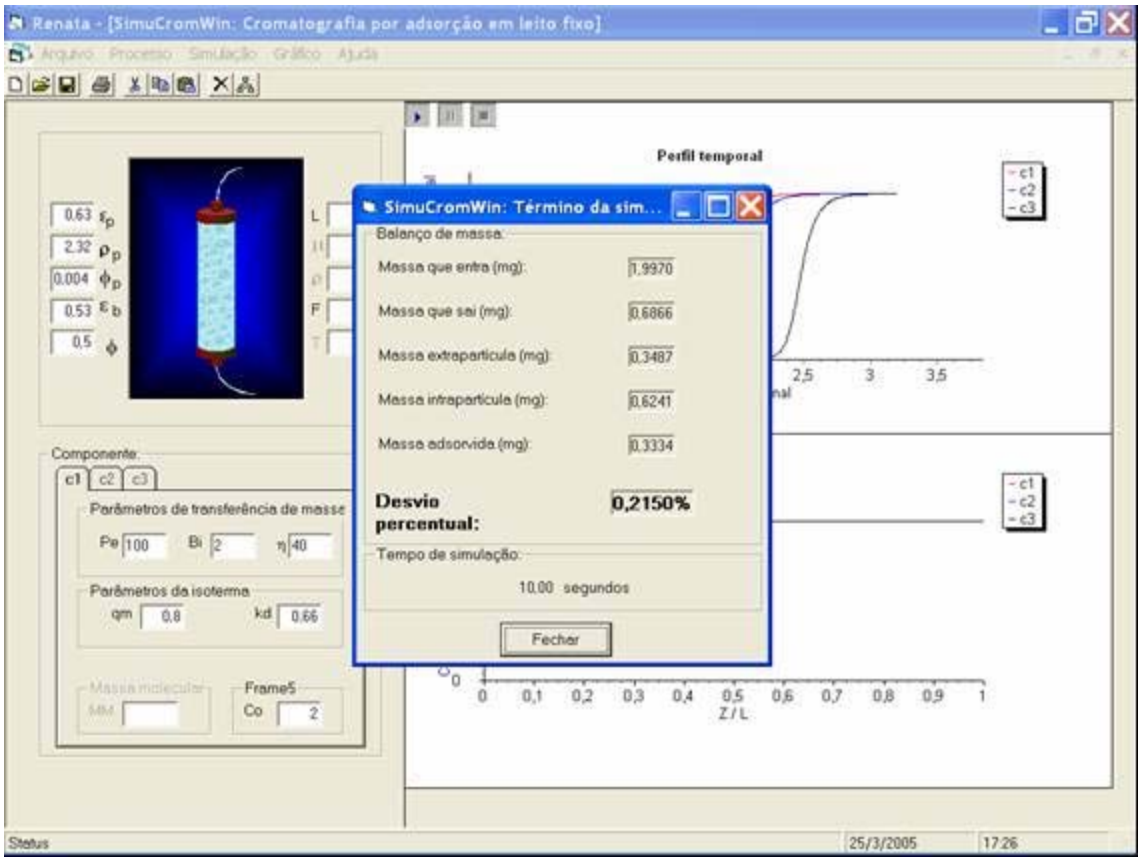

Figura 4. Balanço de massa total do processo ALF utilizando os modelos de isoterma de Langmuir, Toth e Jovanovic , respectivamente..

3.4. Programa para Simulação do Processo de eluição

A etapa de dessorção (eluição) tem recebido considerável atenção, nos processos de recuperação de biocompostos a partir de uma solução diluída, depois de terem sido adsorvidas numa coluna (GU et al, 1990). Sendo assim, a modelagem e simulação do estágio de eluição foi realizada para que haja um bom conhecimento dos fatores que influenciam esta etapa, tendo em vista uma satisfatória otimização do estágio de dessorção.

Estudos como o ARVES e LIAPIS (1987) apresentaram um modelo matemático descrevendo a dinâmica do estágio de eluição da adsorção bioespecífica em leitos fixos. O modelo considera a eluição específica e não-específica do adsorvato e também a

resistência à transferência de massa no filme de líquido na superfície do adsorvente, assim como a taxa de reação entre o adsorvato e o adsorvente. A curva de equilíbrio, obtida entre o adsorvente e o adsorvato, foi representada pelo modelo de Langmuir.

ARNOLD et al. (1985) apresentaram um modelo para a eluição (dessorção) em colunas e em sistemas em batelada, em que a taxa de dessorção foi considerada um parâmetro agrupado em todos os sistemas, exceto naqueles em que a taxa de dessorção foi muito menor que as taxas de difusão no fluido, no interior da partícula, e no filme de líquido, ao redor das partículas adsorventes.

Neste trabalho, foi utilizado o modelo de taxa geral citado anteriormente com as condições iniciais e de contorno referentes a etapa de eluição. Foi considerado que a coluna passou por um processo de lavagem, portanto, $c_b = 0$ em toda a coluna, no início desta etapa.

No programa, depois de terminada a simulação do processo ALF, o usuário tem a opção de iniciar a simulação do processo de eluição ou de salvar os dados e iniciá-la em outro momento. Os parâmetros das isotermas podem ser modificados, assim como os parâmetros de transferência de massa, a concentração do soluto na alimentação, a velocidade da alimentação e as propriedades reológicas e físico-químicas. Ao final do processo, se esse for para dois ou mais componentes, é exibido na tela do programa o valor da resolução entre os picos dos componentes, que é diretamente proporcional ao grau de separação dos compostos, calculado pela seguinte equação (FISCHER, 1974):

$$R_s = 2\frac{(V_{R2} - V_{R1})}{(W_{H1} + W_{H2})} \tag{30}$$

em que: V_{R1} é o volume de retenção do pico 1, V_{R2} é o volume de retenção do pico 2, W_{H1} é a largura do pico 1 e W_{H2} é a largura do pico 2.

A partir dos resultados obtidos na simulação deste processo, as condições de operação podem ser otimizadas, reduzindo desta forma custo e tempo de experimentos.

A Figura 5, apresenta a interface gráfica do programa para a simulação do processo de eluição de dois compomentes usando a isoterma de Langmuir, com mesmo valor de q_m e valores de k_d diferentes. A Figura 6 apresenta a interface gráfica com os valores de resolução calculados para a simulação executada acima (R_1 é a resolução entre os picos 1 e 2, R_2 é a resolução entre os picos 1 e 3 e R_3 é a resolução entre os picos 2 e 3).

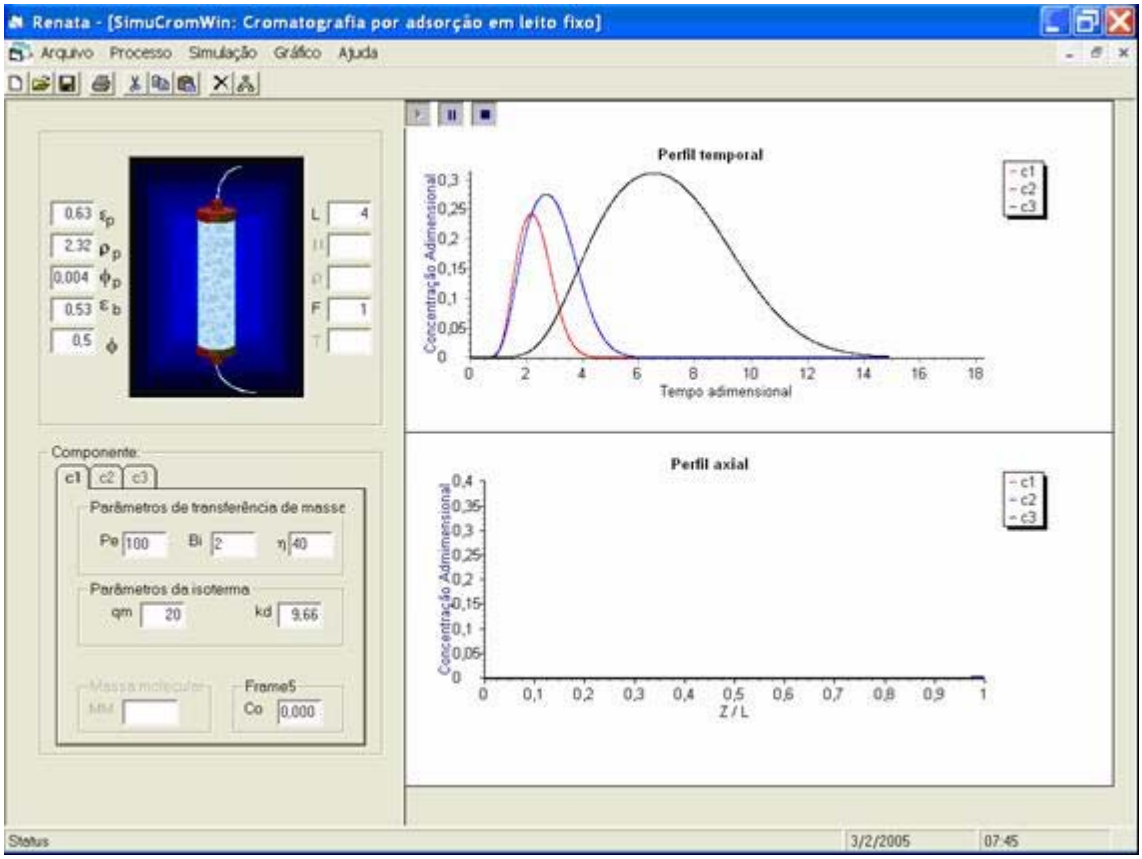

Figura 5. Interface gráfica do programa para o processo de eluição.

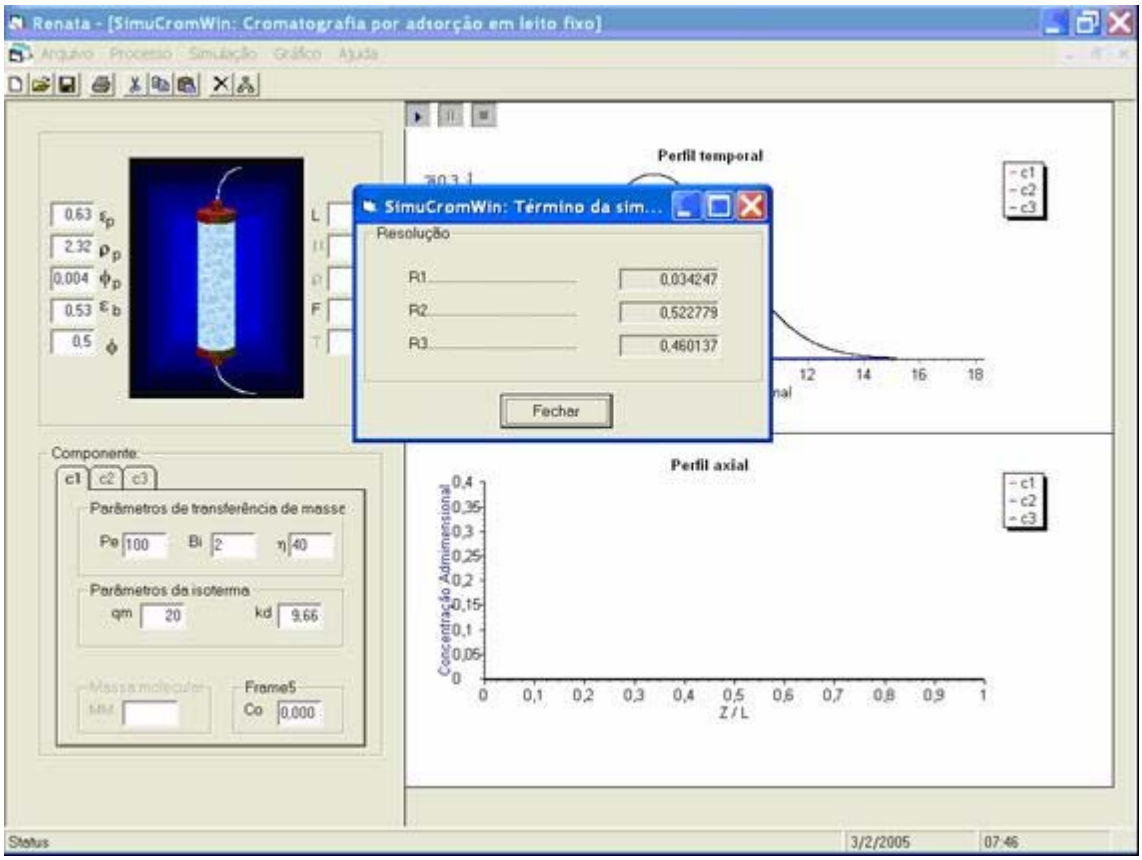

Figura 6. Interface gráfica do programa para a exibição dos valores de resolução.

4. CONCLUSÕES

O modelo de taxa geral mostrou-se apropriado tanto para o estágio de adsorção como para o estágio de eluição. O *software SimuCromWin* que já era uma ferramenta rápida e eficiente para a simulação de processos cromatográficos, tornou-se mais completo após a inclusão da opção da escolha de outros modelos de isotermas, além do modelo de Langmuir, e da opção de simulação do processo de eluição. Com estas modificações, podem ser realizados estudos para a seleção do melhor modelo de isoterma para simulações em cromatografia preparativa além da otimização dos parâmetros do processo de separação.

SÍMBOLOS USADOS

SÍMBOLO	*DESCRIÇÃO*
b	**Constante de equilíbrio de adsorção da isoterma de Langmuir, constante de ligação apropriada da isoterma de Jovanovic, parâmetro da isoterma de Langmuir exponencialmente Modificada.**
Bi	**Número de Biot de transferência de massa**
C_{bi}	**Concentração do componente i na fase móvel**
C_{fi}	**Concentração do componente i na alimentação**
C_{oi}	**Concentração usada para adimensionalização, max $\{c_{fi}(t)\}$**
C_{pi}	**Concentração do componente i na fase líquida da fase estacionária**
C^{s}_{pi}	**Concentração do componente i na fase sólida da fase estacionária**
C_s	**Concentração de sal da fase móvel**
c_{bi}	**Concentração adimensional do componente i na fase móvel**
c_{pi}	**Concentração adimensional do componente i na fase líquida da fase estacionária**
c^{s}_{pi}	**Concentração adimensional do componente i na fase sólida da fase estacionária**
D_{bi}	**Coeficiente de dispersão axial do componente i**
D_{pi}	**Coeficiente de difusão do componente i**
k	**Parâmetro do modelo de isoterma de Langmuir Exponencialmente Modificado.**
L	**Comprimento do leito de partículas**

n	**Parâmetro de heterogeneidade da isoterma de Toth, parâmetro do modelo de isoterma de Freundlich.**
Pe_{Li}	**Número de Peclet para o componente i**
Q	**Quantidade de proteína adsorvida por volume de adsorvente**
q_s	**Capacidade de saturação**
r	**Coordenada radial para a partícula**
Re	**Número de Reynolds**
R_p	**Raio da partícula**
r	**Coordenada radial adimensional para a partícula**
Sc	**Número de Schmidt**
Sh	**Número de Sherwood**
t	**Tempo**
v	**Velocidade intersticial da fase móvel**
Z	**Coordenada axial**
z	**Coordenada axial adimensional**

LETRAS GREGAS	**DESCRIÇÃO**
ε_b	**Fração de volume vazio do leito**
ρ	**Densidade da fáse móvel**
λ	**Parâmetro da isoterma de Langmuir Exponencialmente Modificado**
μ	**Viscosidade da fase móvel**
ε_p	**Porosidade da partícula**
η_i	**Número adimensional para o componente i**

ξ_i	**Número adimensional para o componente i**
τ	**Tempo adimensional**
τ_{imp}	**Tempo de duração adimensional para um pulso retangular da amostra**

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STUDY OF HYDROPHOBIC INTERACTION ADSORPTION OF WHEY PROTEINS: EFFECT OF TEMPERATURE AND SALT CONCENTRATION AND THERMODYNAMIC ANALYSIS

ABSTRACT

The adsorptive behavior of bovine serum albumin (BSA) and β-lactoglobulin (β-lg) on hydrophobic adsorbent was studied at four temperatures and salt concentrations. The Langmuir model was fitted by experimental equilibrium data showing that increasing temperature and salt concentrations increased the capacity factor of both proteins. The thermodynamic analysis of the adsorptive process using the non-linear van't Hoff equation showed that the adsorption process of the two proteins is spontaneous and entropically driven.

Keywords: adsorption, hydrophobic interaction, thermodynamic parameters, proteins.

1. INTRODUCTION

Hydrophobic interaction chromatography (HIC) is a very popular methodology used in the purification of biomolecules (DIAS-CABRAL et al, 2003). This technology is based on the hydrophobic interactions between hydrophobic ligands and non-polar regions on the surface of biomolecules (HJERTEN, 1973 and LIN et al, 2001). It is a powerful adsorptive separation technique due to the fast separations achieved with little product degradation, low solvent requirements and very good purification levels (QUEIROZ et al, 2001).

The mechanism of hydrophobic interactions between solutes has been studied because of its importance in protein precipitation by salting-out (CHEN and SUN, 2003). It is well known that the type of salt and salt concentration greatly influences the hydrophobic interactions between proteins, with hydrophobic media and HIC processes being often carried out by gradient elution with decreasing salt concentrations (RUAAN et al, 1998 and OSCARSSON and KARSNAS, 1998).

Temperature is another factor affecting HIC. Increasing temperature enhances protein retention and decreasing temperature generally promotes protein elution (HJERTEN et al, 1974). CHEN et al (1997) showed that the exposed hydrophobic regions of the protein increased with temperature, resulting in the binding mechanism changing from adsorption to partition in some cases. To study the interaction between proteins and hydrophobic solid surfaces, researchers have traditionally developed thermodynamic analyses based on the van't Hoff dependencies (BOYSEN et al, 1999). Generally, the classical linear van't Hoff equation has been used to calculate the thermodynamic parameters in experiments performed in a narrow temperature range. Since heat capacity, enthalpy changes and entropy changes are expected to be invariable, the enthalpy and entropy of the interaction can be obtained by linear plotting from the logarithm of the equilibrium constant with inversed temperature (CHEN et al, 2003). When the heat capacity changes with temperature, the non-classical van't Hoff equations are used to obtain a proper analysis. Enthalpy and entropy changes at different temperatures can be obtained, being important to estimate a significant sub process in the adsorption procedure (CHEN et al, 2003, and VAILAYA and HORVATH, 1998).

In this article we describe the adsorption behavior of bovine serum albumin (BSA) and β-lactoglobulin (β-lg) on a Streamline Phenyl® resin, a hydrophobic interaction

support, at different salt concentrations and temperatures. We have also analyzed the thermodynamic parameters of HIC data from linear and non-linear van't Hoff equations.

2. THEORY

2.1 Determination of single-component isotherms by frontal analysis

The most convenient and fast methods for our purpose are the frontal analysis (FA), elution by characteristic point (ECP) and pulse methods (GUIOCHON et al, 1994). Among the methods used for determination of single-component isotherm, the frontal analysis is the most accurate (JACOBSON et al, 1984, GUIOCHON et al, 1994 and GUIOCHON, 2002). The adsorbed amount Q_{i+1} is given by:

$$Q_{i+1} = Q_i + \frac{(C_{i+1} - C_i)(V_{F,i+1} - V_0)}{V_a} \quad (1)$$

where Q_i and Q_{i+1} are the amounts of compound adsorbed by volume of adsorbent after the i^{th} and the $(i+1)^{th}$ step, when in equilibrium with the concentrations C_i and C_{i+1}, respectively, $V_{F,i+1}$, is the retention volume of the inflection point of the (i+1) th breakthrough curve, V_0 is the column void volume, and V_a is the volume of the adsorbent in the column.

2.1.1 The Linear isotherm

This isotherm, which relates to the stationary phase concentration, Q, with the fluid concentration, C, is written as:

$$Q_{i+1} = Q_i + \frac{(C_{i+1} - C_i)(V_{F,i+1} - V_0)}{V_a} \quad (2)$$

where a is the slope of the isotherm (Henry's adsorption constant), k' is the retention factor (k'= $(t_R-t_0)/t_0$, t_R, retention time, t_0, dead time), φ is the phase ratio, φ = (1-ε)/ε, and ε is the total porosity of the column (JACOBSON et al, 1984 and GUIOCHON et al, 1994).

2.1.2. The Langmuir isotherm

Langmuir proposed this model for adsorption in a gas-solid system in 1916. He assumed a constant adsorption heat and finite number of surface adsorption sites. With these assumptions, maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface (LANGMUIR, 1916 and JACOBSON et al, 1984), written as:

$$Q = q_s \frac{bC}{1 + bC} \tag{3}$$

In this model, q_s is the monolayer saturation capacity of the adsorbent and b is the equilibrium constant of adsorption.

2.2. Calculation of the thermodynamic parameters:

For a better understanding of the influences of temperature and hydrophobicity of the column and composition of the mobile phase in the selectivity of HIC, it is essential to quantify the mechanisms that establish equilibrium characteristics, such as capacity and selectivity (DIAS-CABRAL et al, 2003). Thus, GENG et al (1990) proposed the stoichiometric displacement retention model for the HIC of proteins. This model assumes that no matter how different the interactions between adsorbent and solute or solvent molecules are, or how heterogeneous the distribution of these active sites is, a rational mechanism for adsorption in a liquid-solid system based on the stoichiometric displacement for solute adsorption can be used (DIAS-CABRAL et al, 2003 and GENG et al, 1990). When applied to linear chromatography, the model is reduced to:

$$Q = q_s \frac{bC}{1 + bC} \tag{3}$$

where,

$$I = K(L_d)^{n'}\Phi \tag{4}$$

and Z, when salt concentration, ligand and temperature are fixed, is a characteristic constant related to protein conformation. The intercept of this equation, lnI, contains a number of constants related to the affinity of a protein to the HIC column. K is the equilibrium constant, L_d corresponds to the hydrated ligand in salt solution, n' is the number of ligand interactions with protein molecule and φ, the column phase ratio.

WU et al (1986) used a plot ln k' versus the concentration of water (%B, volume fraction) to characterize protein adsorption in HIC. They demonstrated that the slope of the plot $[\partial(\ln k')/\partial(\ln \%B)]$ is a sensitive measure of protein conformation, which is related to the contact area of the adsorbed protein on the surface. The Z value then can be obtained taking the derivative $\partial(\ln k')/\partial(\ln \%B)$. Thus, there is no fundamental difference between the Z values obtained by GENG et al (1990) and WU et al (1986).

All of the models discussed above are applicable only to linear chromatography. For the overloaded region, the commonly used approach is to characterize the behavior with isotherm measurements and calculation of the thermodynamic parameters. The thermodynamic treatment of adsorption effectively began with the Gibbs equation, which provides a convenient definition of the interfacial region (JACOBSON at al, 1984). Besides free energy, enthalpy and entropy are other important parameters for the study of the process of adsorption.

The linear and the non-linear van't Hoff equations are used for the calculation of these parameters. The former equation shows the dependence of k' (capacity ratio) on temperature, and is defined as:

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \Phi \tag{6}$$

where ΔH and ΔS are the enthalpy change and entropy change, respectively, associated with the transfer of the solute from the mobile to the stationary phase, R is the gas constant and Φ is a system constant depending on the phase ratio in the column. This equation assumes that either the phase ratio does not change significantly with temperature and ΔH includes contributions due to changes in φ (GRUSHKA et al, 1982). The value of ΔH is calculated from the slope of the plot of ln k' versus 1/T. According to JACOBSON et al (1984) the constant k' is related to the equilibrium constant for the sorption process in the domain of Henry's law, K, by

$$k' = K\varphi \tag{7}$$

where φ represents the phase ratio and K = *a*. The parameter *a* is the Langmuir isotherm slope at low solute concentration ($a = q_s b$) (Jacobson et al., 1984).

The parameters calculated from this equation are averaged for the entire range of T. For the calculation of these parameters at a temperature studied, the non-linear van't Hoff (ESQUIBEL-KING et al, 1999) equation is used. This equation, proposed by HORVÁTH and VAILAYA (1996), has a refinement that corrects the variation of ΔC_P^0 with temperature, resulting in the following equation:

$$\ln k' = a_1 + \frac{a_2}{T} + \frac{a_3}{T^2} + ... + \ln \Phi \quad (8)$$

Deriving the Equation (8) and (6) in function of 1/T, we have

$$\left(\frac{\partial \ln k'}{\partial (1/T)} \right) = a_2 + 2\frac{a_3}{T} + ... = -\frac{\Delta H}{R} \quad (9)$$

According to LEVINE (1995) and BOYSEN et al. (1999) the ΔG^0 can be calculated by

$$\Delta G^0 = -RT \ln K \quad (10)$$

According to GERSTNER et al (1999) for adsorption process, K is given by

$$K = \frac{k'}{\varphi} \quad (11)$$

Thus, the change in entropy is calculated from the Gibbs-Helmholtz relationship, given by

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (12)$$

3. EXPERIMENTAL

3.1 Materials

BSA and β-lg were purchased from Sigma (St. Louis, MO, USA). BSA is a globular ellipsoid, with a molecular mass of 69000 Da, and isoelectric point (pI) of 4.7. The β-lg has a molecular mass of 32000 Da, when in dimmer form, and isoelectric point of 5.2 (CAYOT and LORIENT, 1997). The HIC support was Streamline Phenyl®, packed in a column HR 5/5, purchased from Amershan Pharmacia Biotech (Uppsala, Sweden). Sodium phosphate (monobasic), sodium phosphate (dibasic) and sodium sulfate were of analytical grade.

3.2 Apparatus

Frontal chromatography was carried out using an Äkta Purifier System (MOD 10X) from Amershan Pharmacia Biotech (Uppsala, Sweden) with a UV detector fixed at 280 nm at a flow rate of 2.0 mL/min. The equipment was controlled using the software Unicorn v.1.0 (Amersham Biosciences). The system temperature was controlled by immersion of the column in a thermostatic bath (Quimis, precision of ± 0.1 K).

3.3 Procedures

3.3.1 Analysis

A column HR 5/5 packed with Streamline Phenyl® was initially equilibrated with 50 column volumes (CV) of the carrier buffer (20 mM phosphate, pH 7,0) containing various concentrations of sodium sulfate (50, 300, 600 and 900 mM) at different temperatures (283.15 K, 293.15 K, 303.15 K and 313.15 K). A 4x4 factorial design was applied to develop the experiments. Single component solutions at concentrations of 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 mg mL^{-1}, for each protein, were perfused through the column, until the complete formation of the breakthrough curve. At the end of each experiment, the column was regenerated with 30 CV of a buffer (20 mM phosphate, pH 7.0).

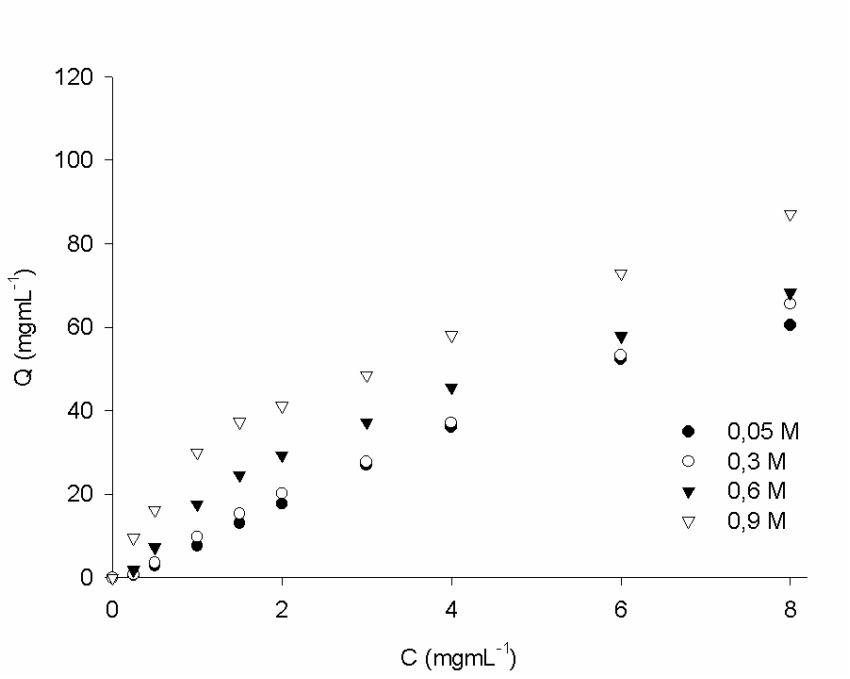
4. RESULTS AND DISCUSSION

4.1 Effect of salt concentration in the adsorptive equilibrium

Adsorption experiments were carried out with four concentrations of sodium sulfate at the following temperatures: 283.15 K, 293.15 K, 303.15 K and 313.15 K. The plots of Q (amount of compound adsorbed by the column packing, mg protein/mL adsorbent), versus C (protein concentration in the liquid phase, mg protein/mL) are presented in Figure 1, for BSA, and in Figure 2, for β-lg. As shown in the Figures, the

amounts of the proteins bound on the adsorbents increase with the concentration of sodium sulfate for both proteins. These results are consistent with the findings by CHEN et al (2003) and ARAKAWA (1986) on the interaction of protein with hydrophobic octyl-Sepharose and polysaccharide columns, respectively, in buffer solution with ammonium sulfate. Many reasons were proposed to explain the stronger hydrophobic interaction at higher salt concentrations in hydrophobic interaction systems. According to LIN et al (2001), the bound water prevents protein molecules from binding to the hydrophobic ligands on an adsorbent surface. However, in the presence of a salt, the protein will be dehydrated due to the hydration effect of salt molecules surrounding the protein. Thus, the hydrophobic zones of the protein will be gradually naked with increasing salt concentration, strengthening the hydrophobic interactions between protein and adsorbent surface (CHEN and SUN, 2003).

(A)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8
C (mgmL-1)
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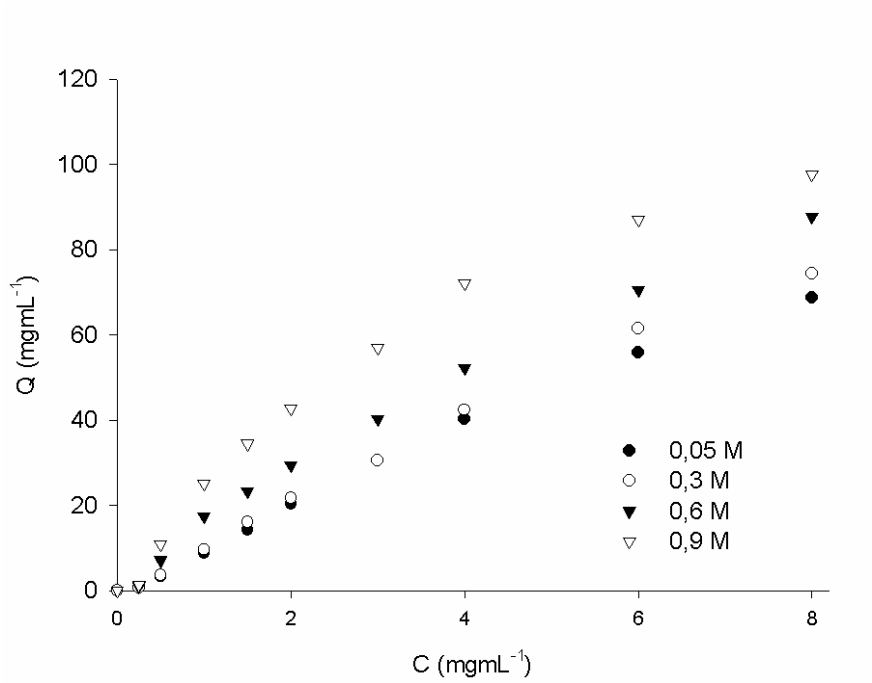
0,3 M
0,6 M
0,9 M

(B)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8
C (mgmL-1)
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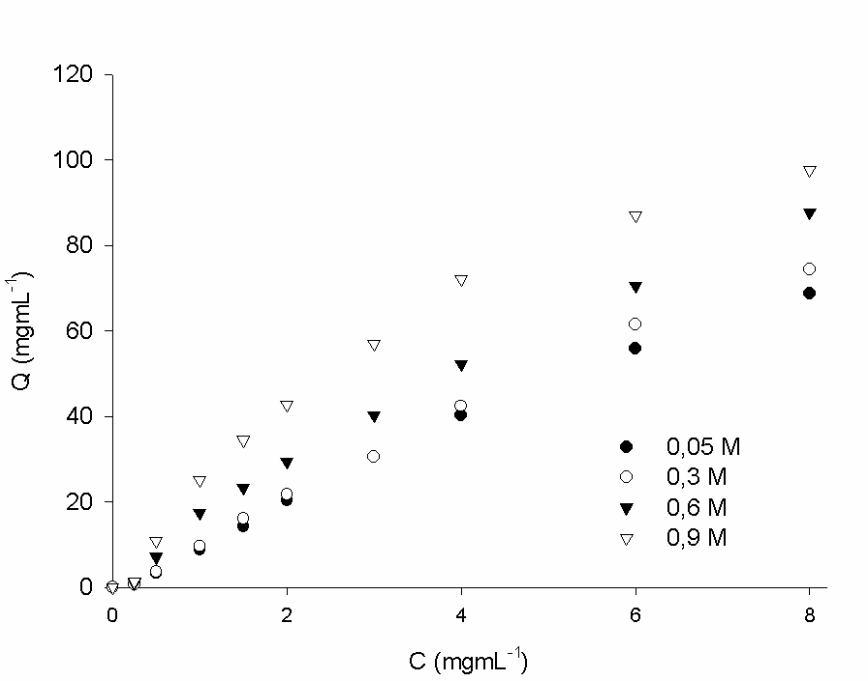
0,6 M
0,9 M

(C)



(D)

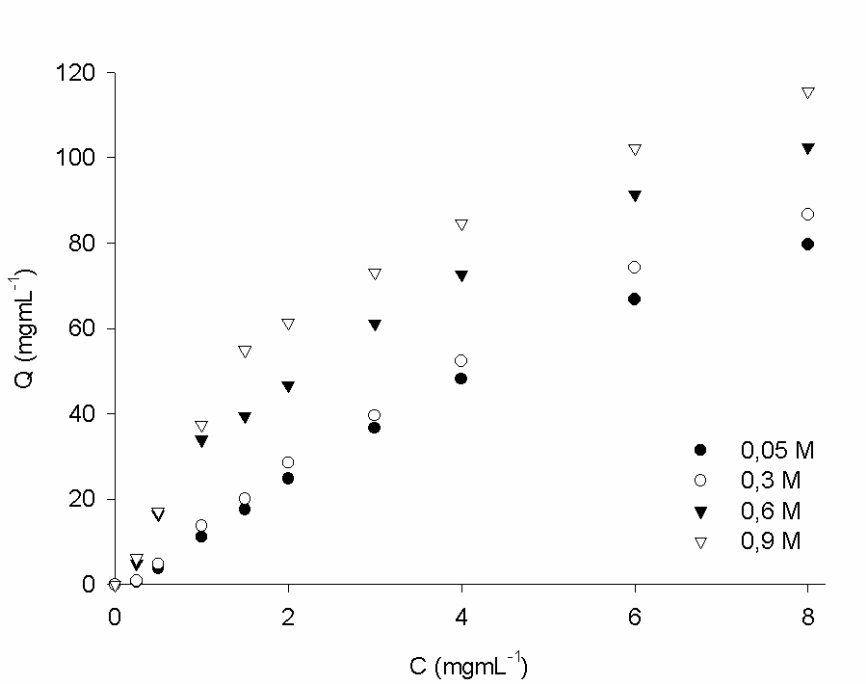
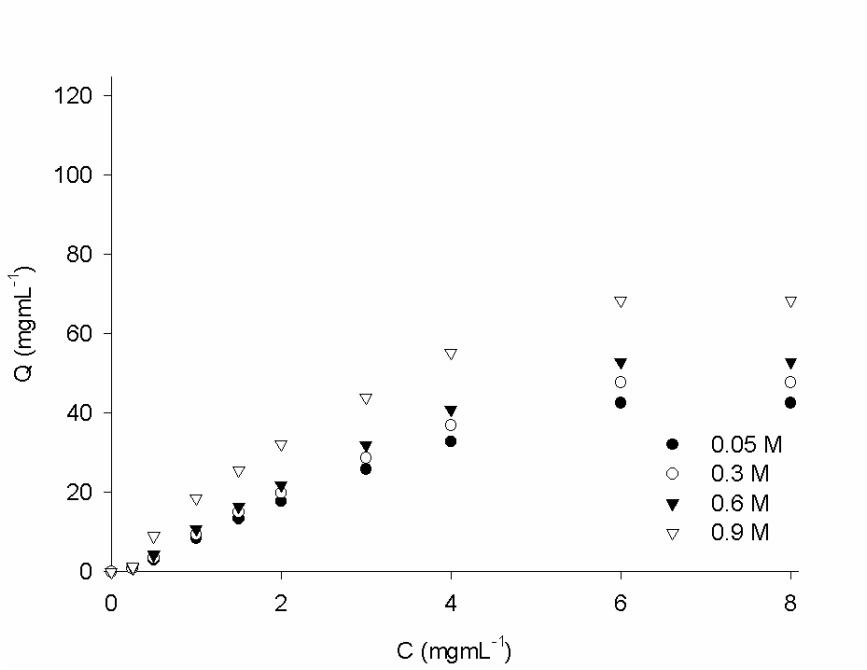


Figure 1: Isotherms of BSA interacting with Streamline Phenyl at different Na_2SO_4 concentrations. (A) 283.15 K, (B) 293.15 K, (C) 303.15 K, (D) 313.15 K.

(A)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C (mgmL-1)
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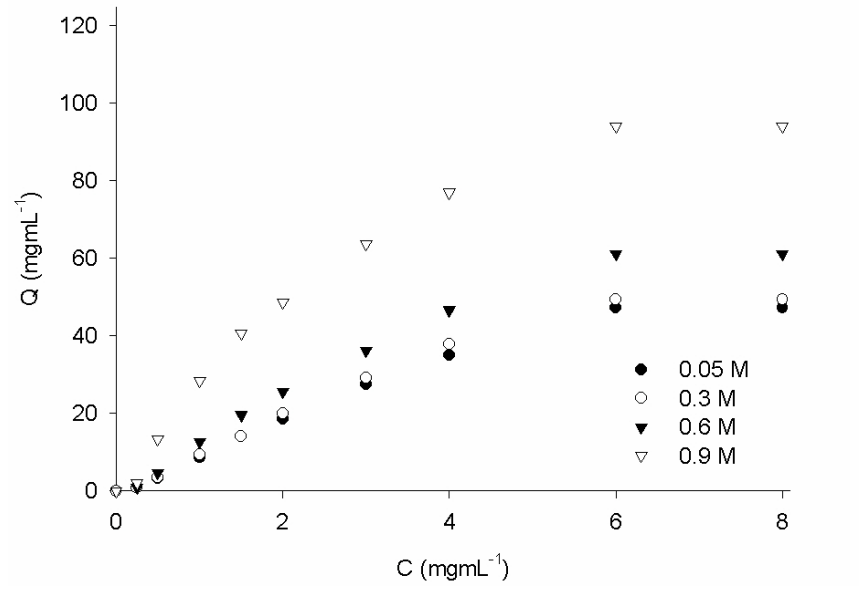
0.3 M
0.6 M
0.9 M

(B)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20
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2
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6
8
C (mgmL-1)
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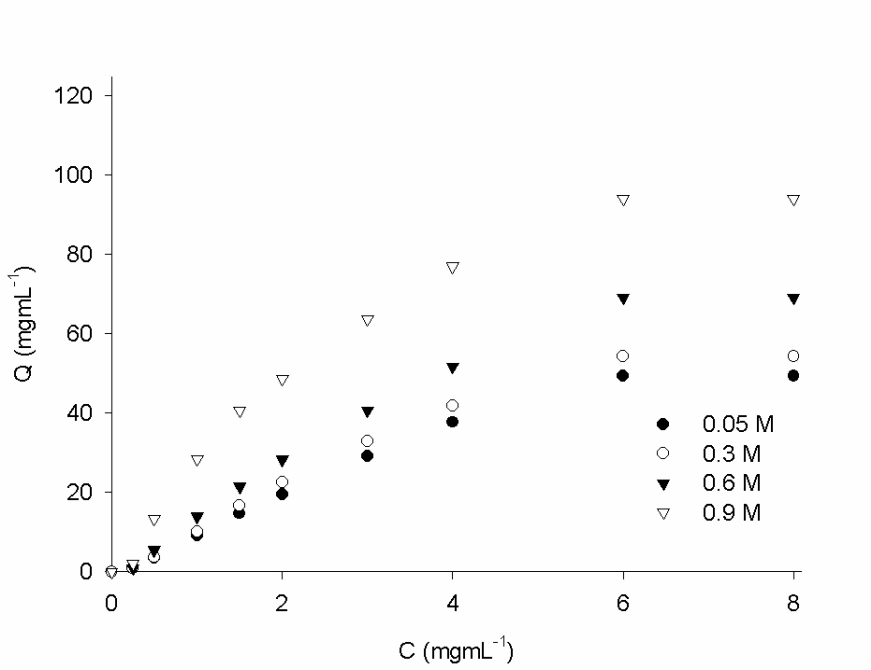
0.3 M
0.6 M
0.9 M

(C)



(D)

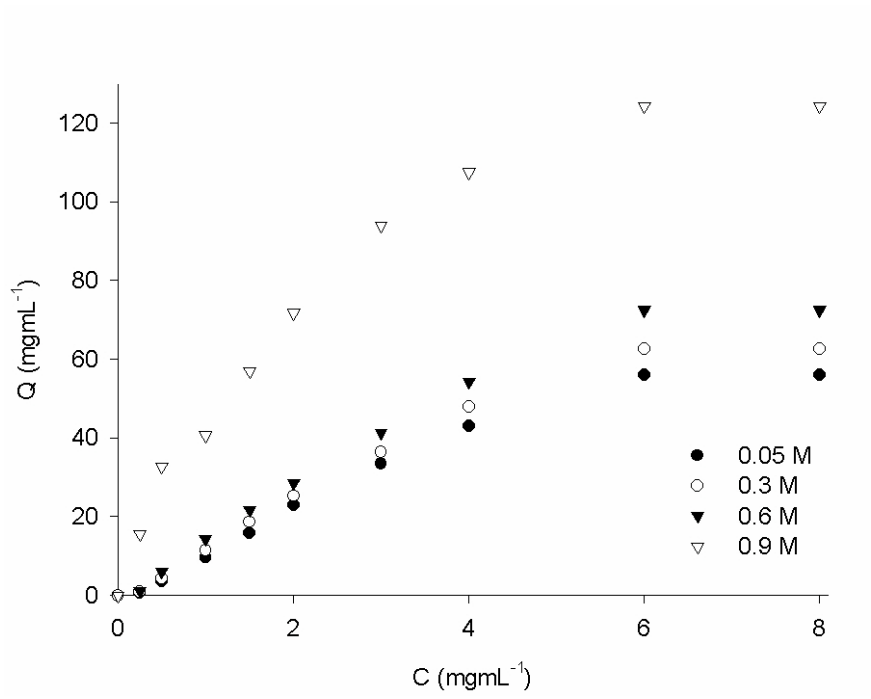


Figure 2: Isotherms of β-lg interacting with Streamline Phenyl at different Na_2SO_4 concentrations. (A) 283.15 K, (B) 293.15 K, (C) 303.15 K, (D) 313.15 K.

The isotherms show that the influence of salt concentration on adsorption is more significant for β-lg, mainly at a temperature of 313.15 K where the difference of the amount adsorbed in 0.9 M and 0.6 M is larger. Only at temperature of 313.15 K and salt concentration of 0.9 M, the amount of β-lg adsorbed is larger than BSA.

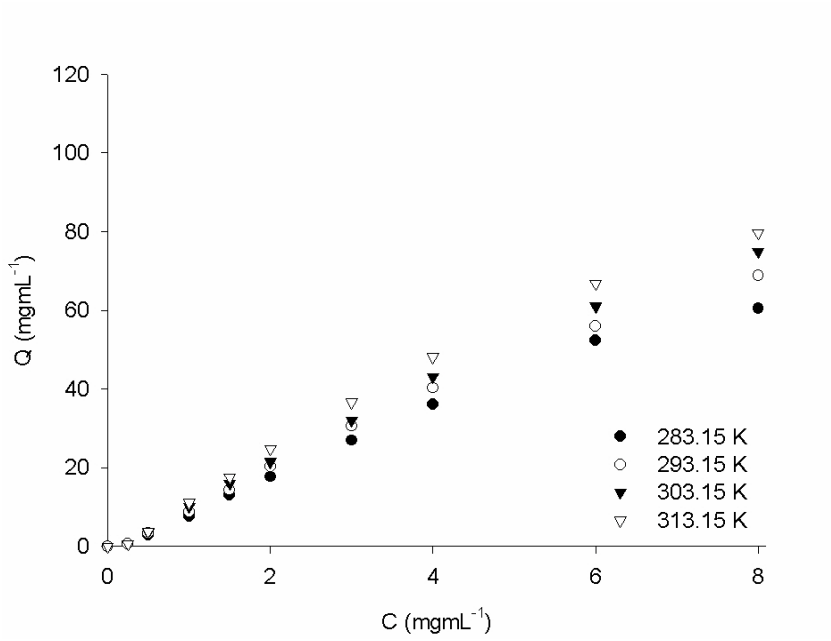
4.2 Effect of temperature in the adsorption equilibrium

The effects of temperature on the interaction of the proteins studied onto Streamline Phenyl at same Na_2SO_4 concentration are shown in Figure 3, for BSA, and Figure 4, for β-lg.

The structure of folded protein can be partially destroyed at high temperatures, exposing the inner hydrophobic core. Also, the increased hydrophobic interaction between proteins and hydrophobic ligands may induce irreversible thermal inactivation (DILL, 1987).

Increasing temperature will proportionally increase the amount of adsorbed protein in all salt concentrations, according to results provided by XIE et al (1995) and GOHEEN and GIBBINS (2000). The authors suggested that the reduced solution polarity and the decreased stability of the protein structure increase the exposure of the hydrophobic residue of the inner protein core to aqueous solution at higher temperature. FANG et al (1996) have also reached a similar conclusion by showing that hydrophobic interactions contribute significantly for the interaction of horse heart cytochrome C with the cation exchanger at the highest temperatures.

(A)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Q (mgmL-1)
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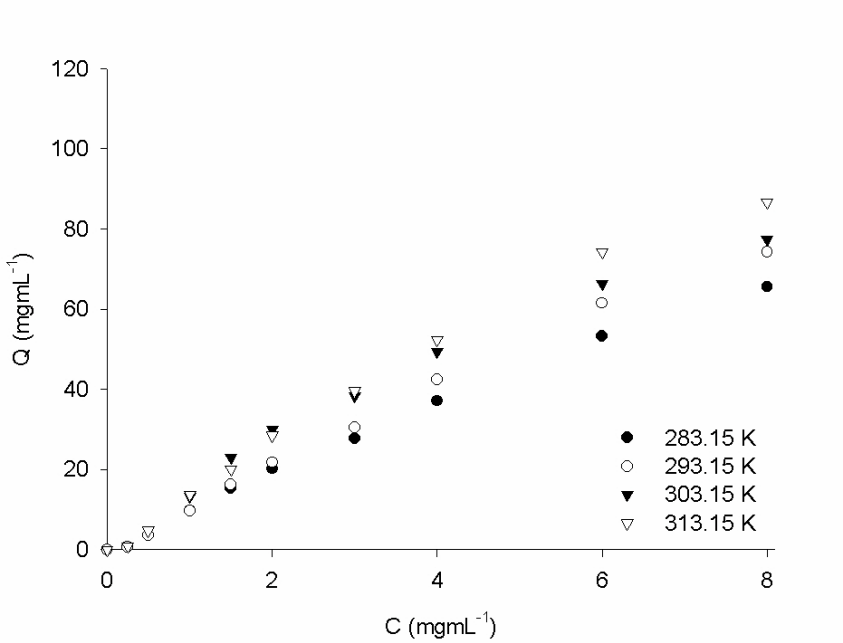
C (mgmL-1)
283.15 K
293.15 K
303.15 K
313.15 K

(B)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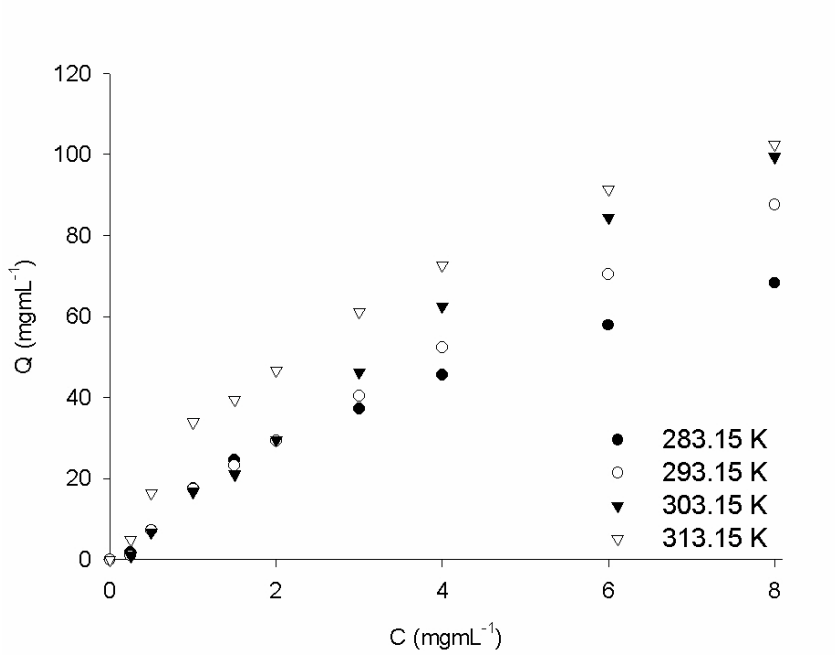
C (mgmL-1)
283.15 K
293.15 K
303.15 K
313.15 K

(C)



(D)

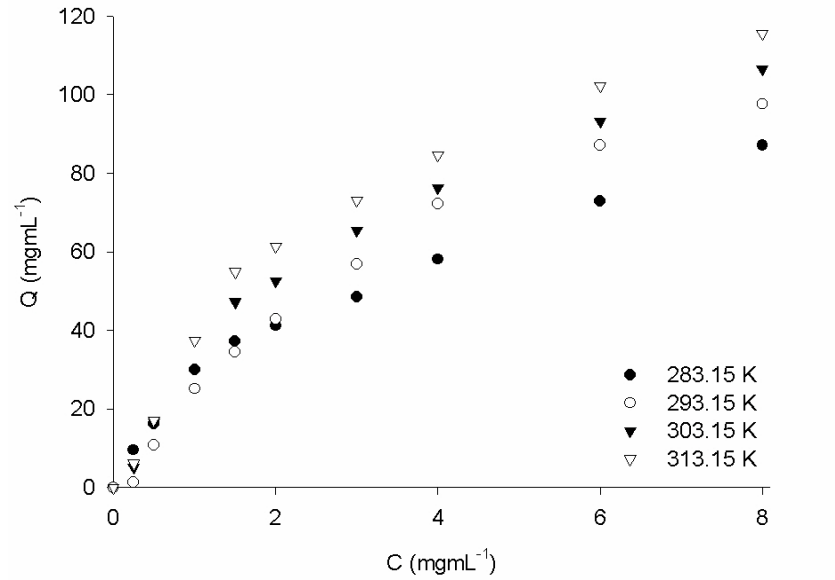
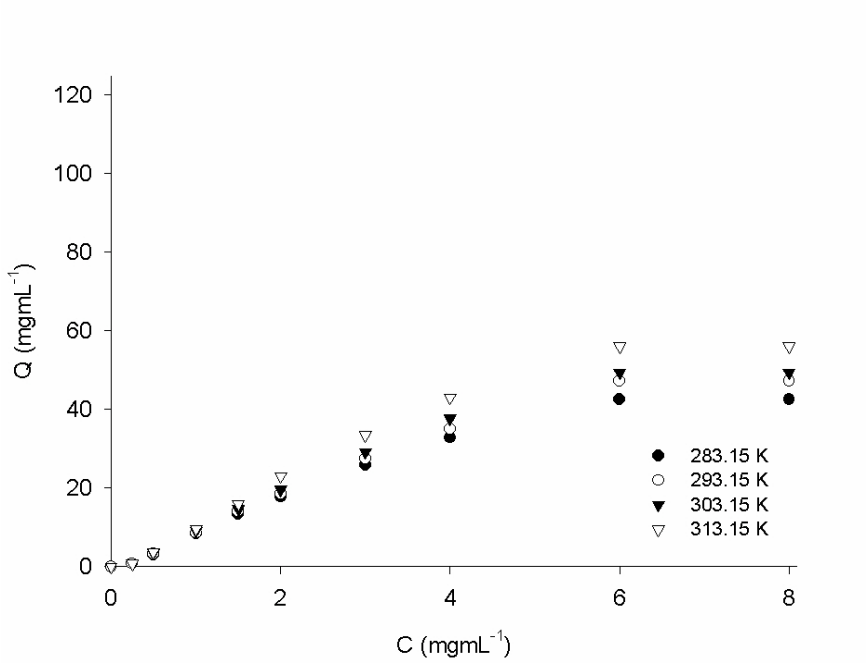


Figure 3: Isotherms of BSA interacting with Streamline Phenyl at different temperatures. (A) 0.05 M, (B) 0.3 M, (C) 0.6 M, (D) 0.9 M.

(A)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Q (mgmL-1)
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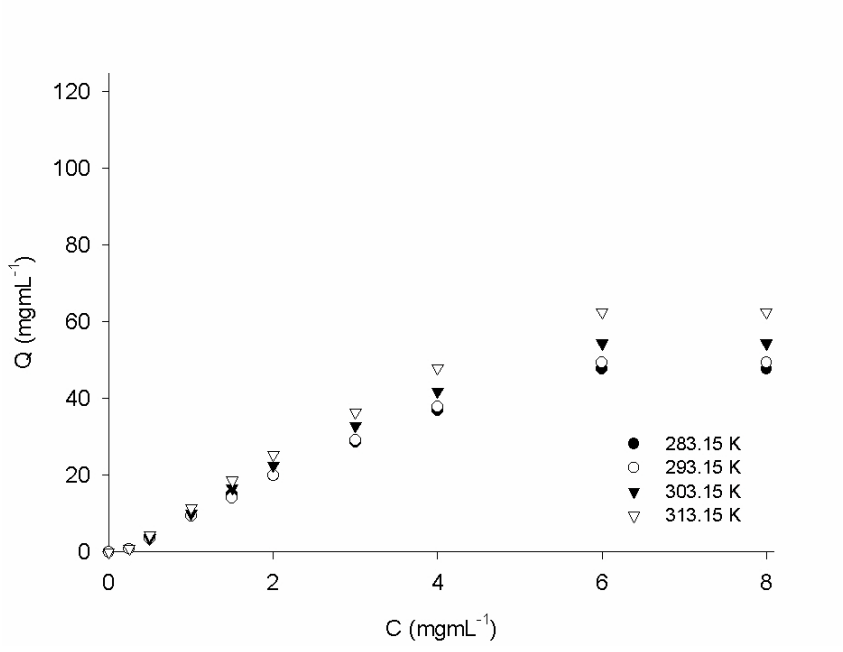
6
8
C (mgmL-1)
283.15 K
293.15 K
303.15 K
313.15 K

(B)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8
C (mgmL-1)
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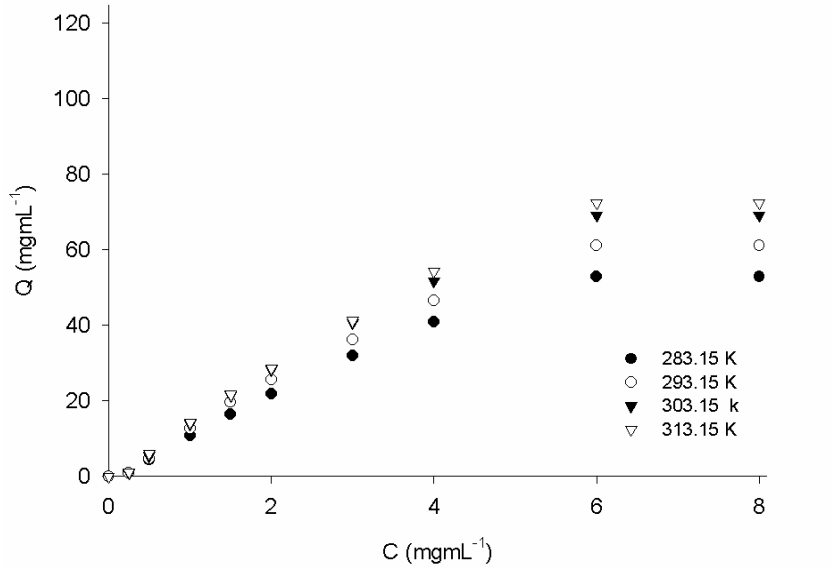
293.15 K
303.15 K
313.15 K

(C)



(D)

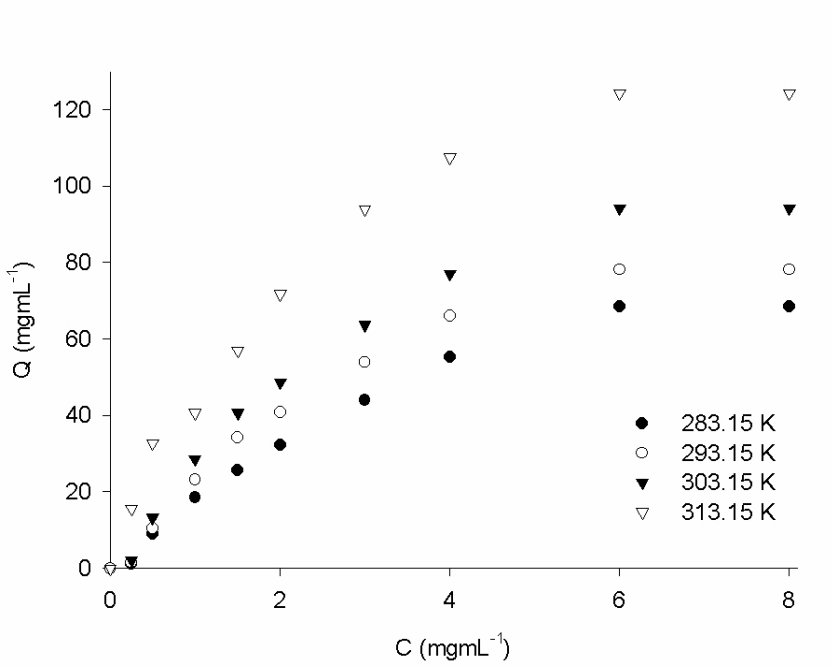


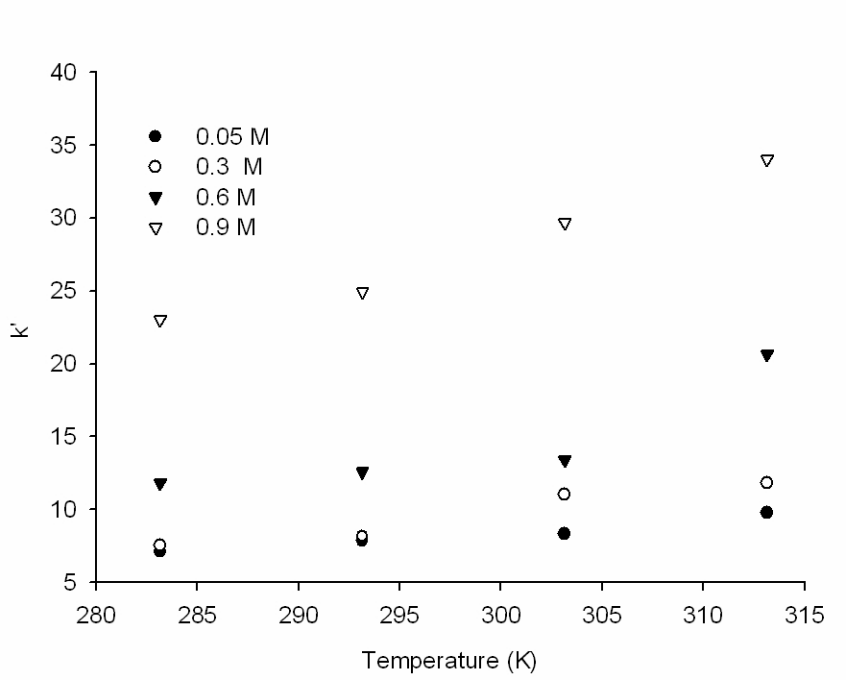
Figure 4: Isotherms of β-lg interacting with Streamline Phenyl at different temperatures. (A) 0.05 M, (B) 0.3 M, (C) 0.6 M, (D) 0.9 M.

4.3. Thermodynamic analysis

The retention behavior of BSA and β-lg on the Streamline Phenyl was investigated as a function of salt concentration and temperature. Equilibrium isotherms were determined from measurements made at four concentrations of Na_2SO_4. Then the parameter k' was determined from the obtained Langmuir isotherms and Equation 7 as seen previously.

As shown in Figure 5, k' increases in a non-linear fashion with an increase in temperature and this is more evident when the salt concentration increases. The same results were presented by DIAS-CABRAL et al (2003) for BSA adsorption on PPG-Sepharose using as modulator ammonium sulfate and sodium sulfate. According to WU et al (1986), the non-linearity is in part due to changes in protein conformation, which results in an increase in the conformational entropy at higher temperature.

(A)



(B)

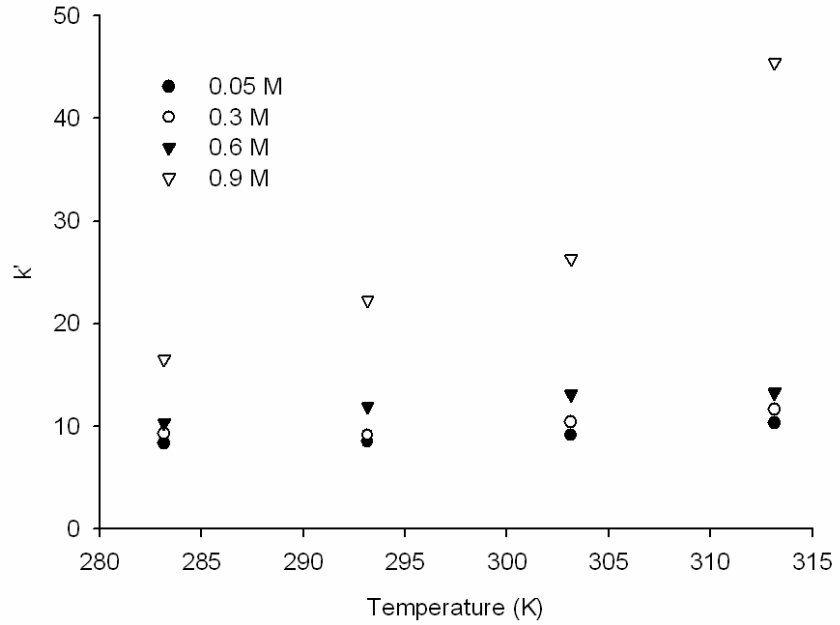


Figure 5: Plots of k' versus temperature for protein adsorption on Streamline Phenyl at various salt concentrations. (A) BSA and (B) β-lg

The change of protein retention with solvent composition can evaluate changes in contact area. The Z value, which is a measure of protein conformation, is obtained through Equation 4. Figure 6 shows the change in Z value as a function of temperature. For both proteins, Z value increases with temperature and there is a sharp change at approximately 303 K. According to DIAS-CABRAL et al (2003) and WU et al (1986) this fact can indicate a conformational change in the proteins. The Z values found in this work are smaller than that found by DIAS-CABRAL et al (2003) for BSA on PPG-Sepharose using as modulator Na_2SO_4.

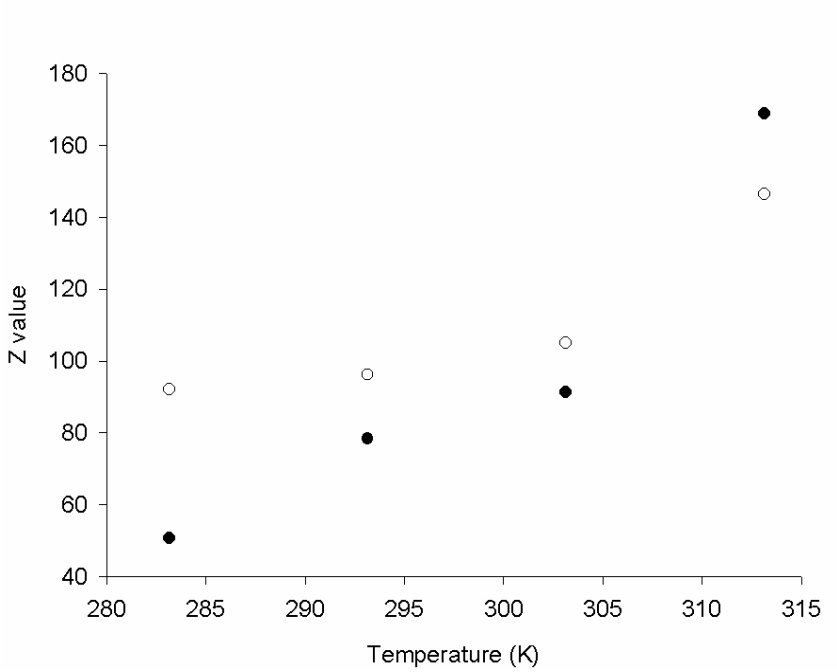


Figure 6: Plot of Z values of proteins versus temperature. (○) BSA and (●) β-lg

Retention data obtained for BSA and β-lg on Streamline Phenyl as a function of temperature are shown in Figures 7 and 8, respectively, showing a non -linear relationship. The results obtained by ESQUIBEL-KING et al (1999), presented the same relationship for adsorption of BSA on an epoxy-$(CH_2)_4$ Shepharose support and using $(NH_4)_2SO_4$ at four concentrations. DIAS-CABRAL et al (2003) adjusted the Equation (8) up to the quadratic term $(1/T^2)$, for retention data obtained for BSA on PPG-Sepharose at different concentrations of Na_2SO_4 and $(NH_4)_2SO_4$ at pH 7.0, with good results.

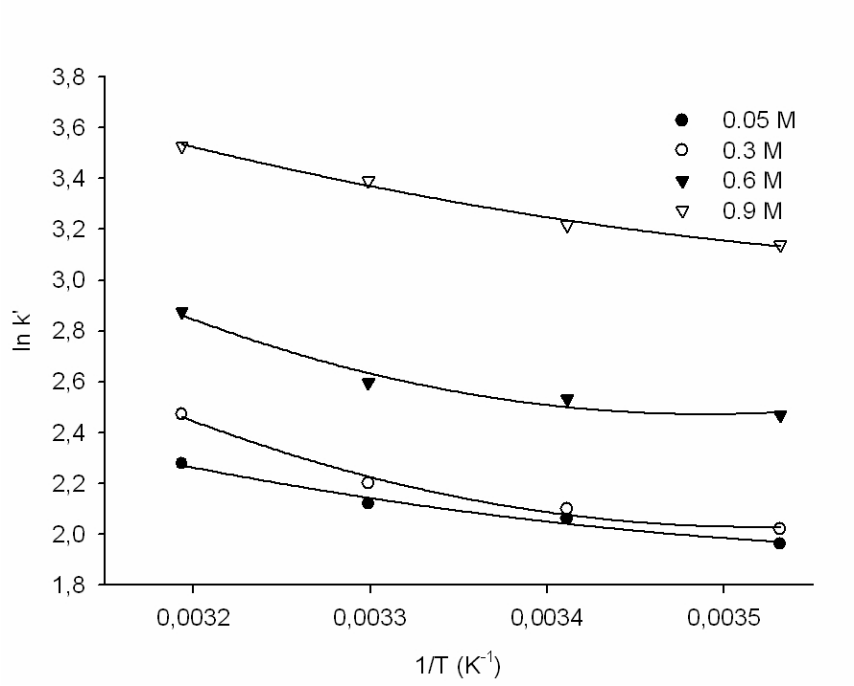


Figure 7: Van't Hoff plots for the retention of BSA on Streamline Phenyl at different concentrations of Na_2SO_4.

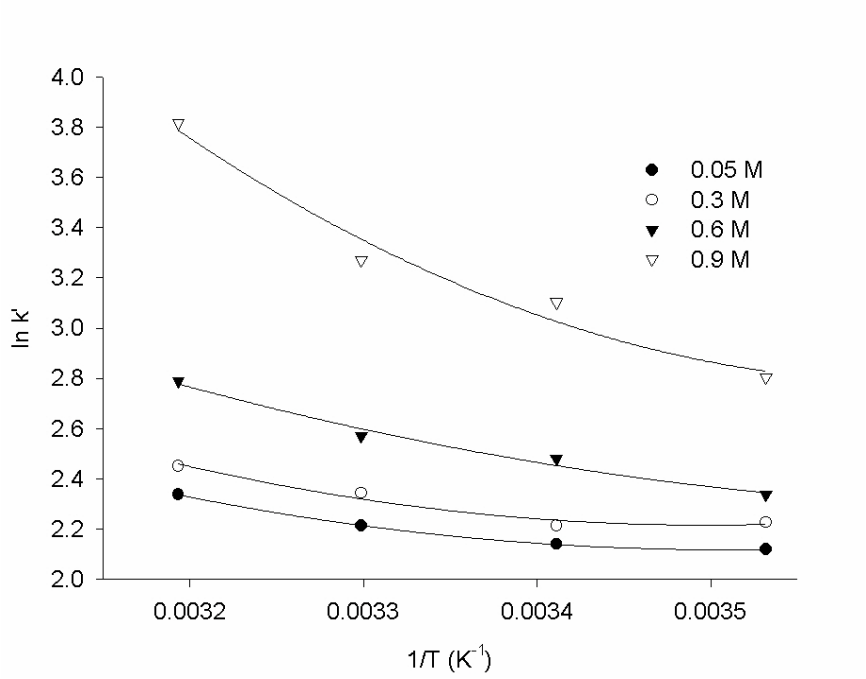


Figure 8: Van't Hoff plots for the retention of β-lg on Streamline Phenyl at different concentrations of Na_2SO_4.

The solid curves in the figures represent the predicted values using three terms of Equation (8). In all cases higher values of the determination coefficients ($R^2 > 0.989$) were obtained. This fact was observed by BOYSEN et al (1999) for the adsorptive behavior of the polypeptides with immobilized lipophilic compounds and by PURCELl et al (1992) for the adsorptive behavior of the hormonal polypeptides β-endorphin, glucagons and bovine insulin at different temperatures with immobilized n-butyl and n-octadecyl groups. The adjusted parameters of the van't Hoff equation obtained are shown in Table 1.

Table 1: Adjusted parameters of the van't Hoff equation

Protein	Concentration of Na_2SO_4 (M)	Parameters (Equation 8)		
		a_1	a_2	a_3
BSA	0.05	20.55	-10098.15	1369193.32
	0.3	52.64	-28763.95	4086296.06
	0.6	56.70	-31079.89	4453156.58
	0.9	24.80	-11608.18	1549193.88
β-lg	0.05	29.29	-15498.52	2209278.75
	0.3	34.42	-18418.40	2633061.20
	0.6	-20.67	14550.06	-2274806.80
	0.9	74.74	-39752.62	5490080.20

From the data of Table 1 and using the Equations (9), (10) and (12) the thermodynamic quantities for the retention of BSA and β-lg on Streamline Phenyl were determined. The results are shown in Tables 2 and 3 and Figures 9-11, for both proteins.

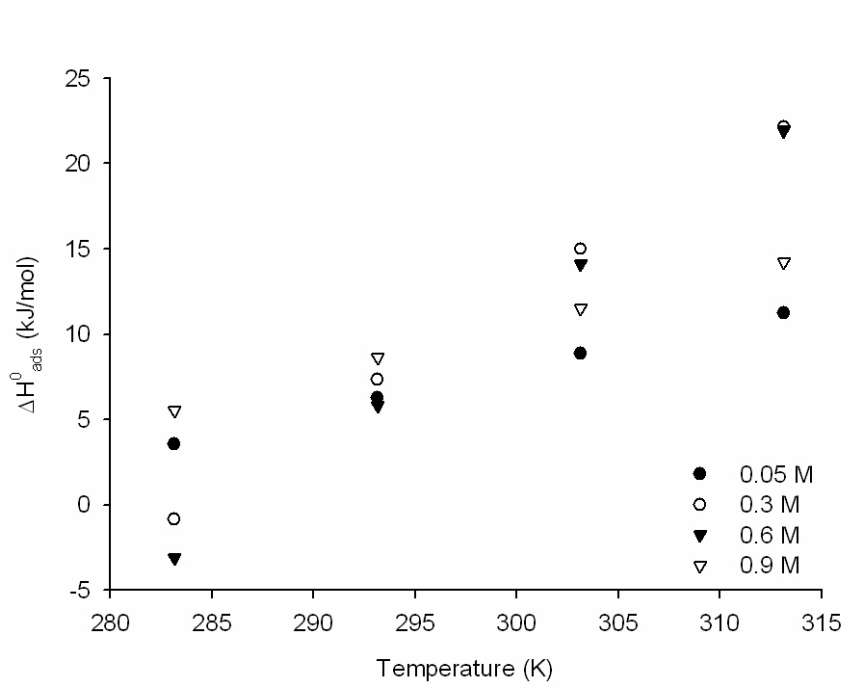
Table 2: Thermodynamic quantities for the retention of BSA on Streamline Phenyl.

Temperature (K)	Concentration of Na_2SO_4 (M)	ΔH^0_{ads} ($kJmol^{-1}$)	ΔS^0_{ads} ($Jmol^{-1}K^{-1}$)	ΔG^0_{ads} ($kJmol^{-1}$)
283.15	0.05	3.55	31.53	-5.37
293.15		6.29	41.29	-5.81
303.15		8.85	49.51	-6.15
313.15		11.25	57.56	-6.77
283.15	0.3	-0.82	16.57	-5.51
293.15		7.36	45.26	-5.90
303.15		15.00	70.48	-6.35
313.15		22.16	94.01	-7.27
283.15	0.6	-3.11	12.21	-6.57
293.15		5.80	43.55	-6.95
303.15		14.13	70.92	-7.36
313.15		21.93	96.65	-8.32
283.15	0.9	5.53	48.31	-8.14
293.15		8.63	58.88	-8.62
303.15		11.53	68.92	-9.35
313.15		14.24	77.51	-10.02

Table 3: Thermodynamic quantities for the retention of β-lg on Streamline Phenyl.

Temperature (K)	Concentration of Na_2SO_4 (M)	ΔH^0_{ads} ($kJmol^{-1}$)	ΔS^0_{ads} ($Jmol^{-1}K^{-1}$)	ΔG^0_{ads} ($kJmol^{-1}$)
283.15	0.05	-0.88	17.17	-5.75
293.15		3.54	32.55	-6.00
303.15		7.67	46.39	-6.39
313.15		11.54	58.975	-6.92
283.15	0.3	-1.49	15.91	-5.99
293.15		3.78	33.97	-6.18
303.15		8.70	50.88	-6.72
313.15		13.31	65.59	-7.22
283.15	0.6	5.65	42.06	-6.26
293.15		9.20	54.67	-6.82
303.15		12.52	65.35	-7.29
313.15		15.63	75.75	-8.09
283.15	0.9	8.09	54.59	-7.36
293.15		19.09	93.62	-8.35
303.15		29.37	126.75	-9.05
313.15		38.98	158.90	-10.77

(A)



(B)

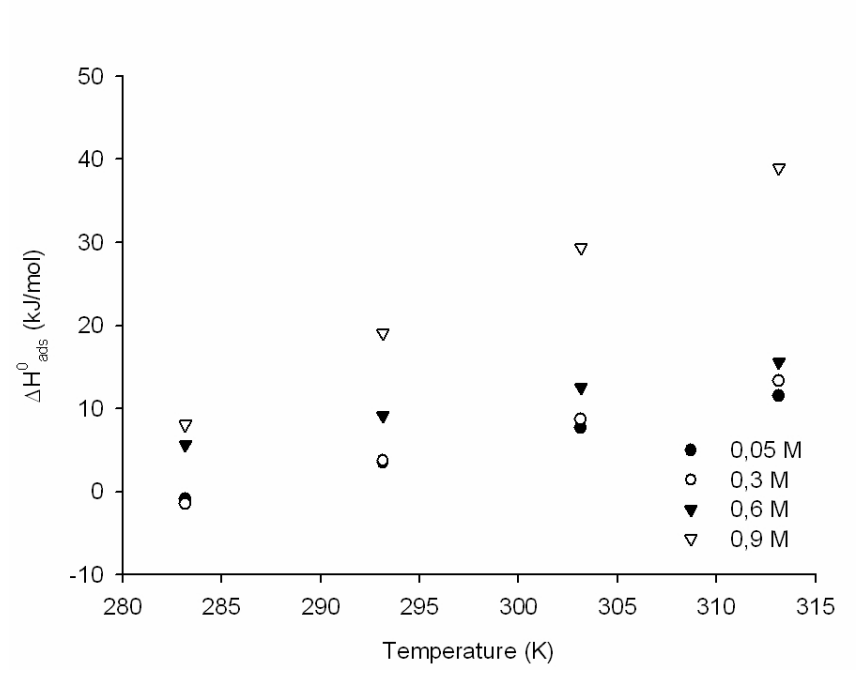
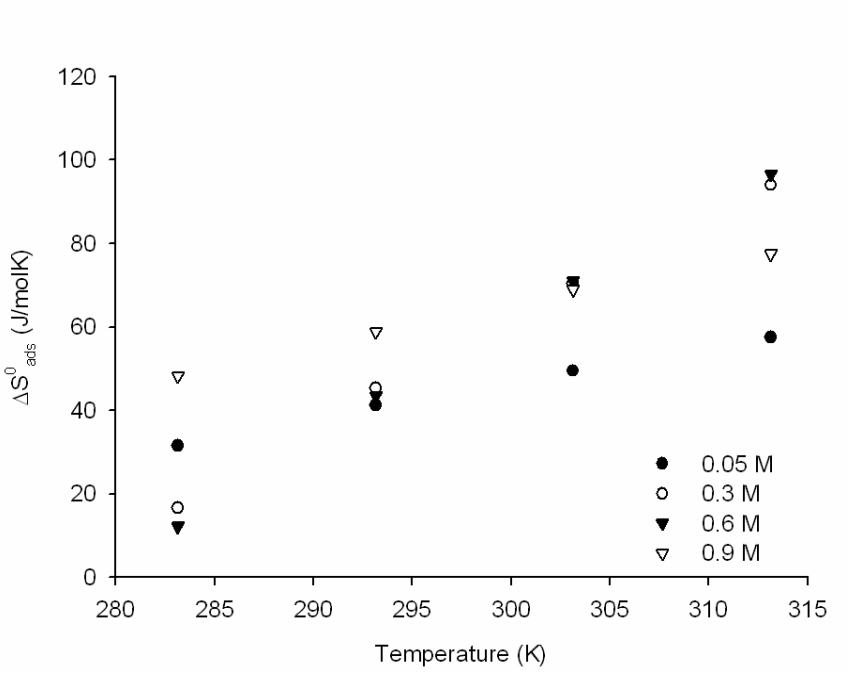


Figure 9: Enthalpy change of the proteins interacting with Streamline Phenyl at different concentrations of Na_2SO_4. (A) BSA and (B) β-lg.

(A)



(B)

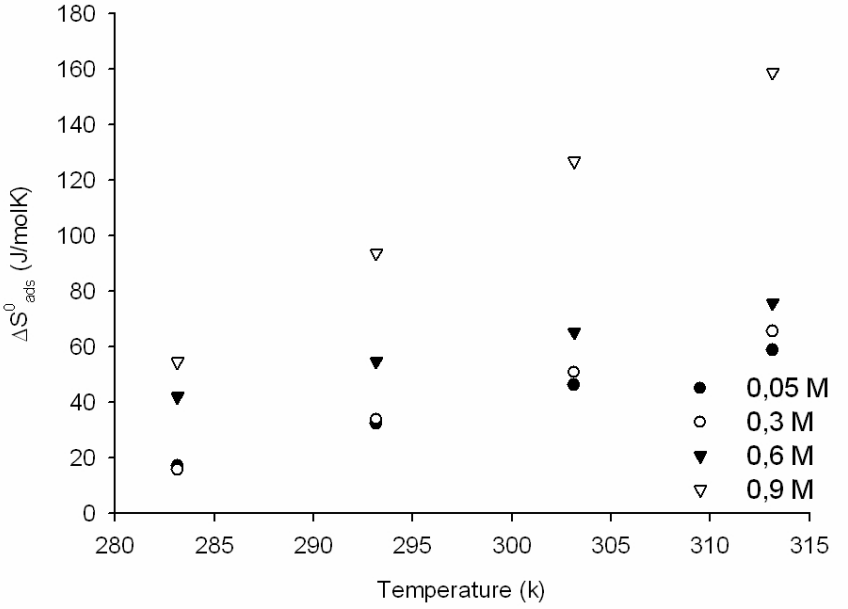
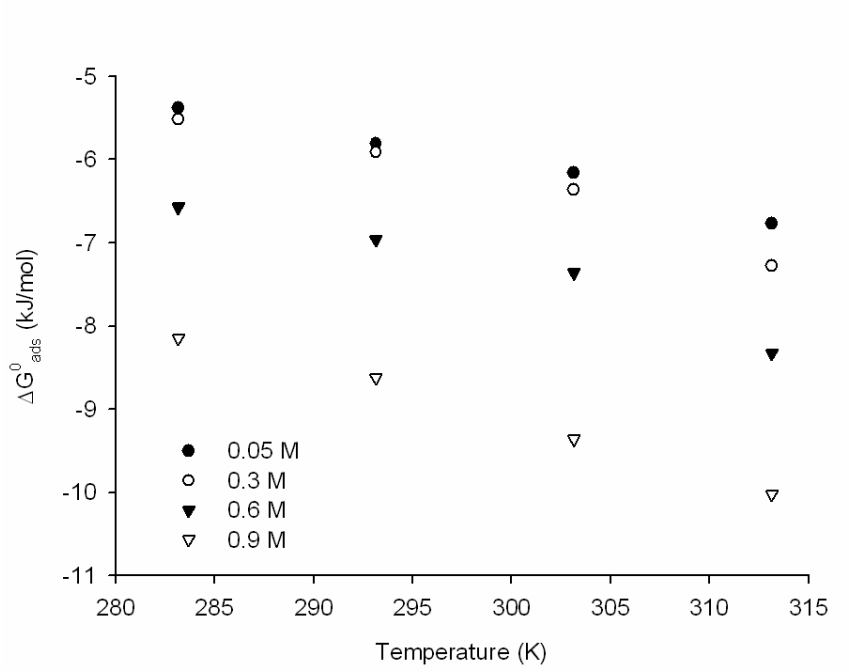


Figure 10: Entropy change of the proteins interacting with Streamline Phenyl at different concentrations of Na_2SO_4. (A) BSA and (B) β-lg.

(A)



(B)

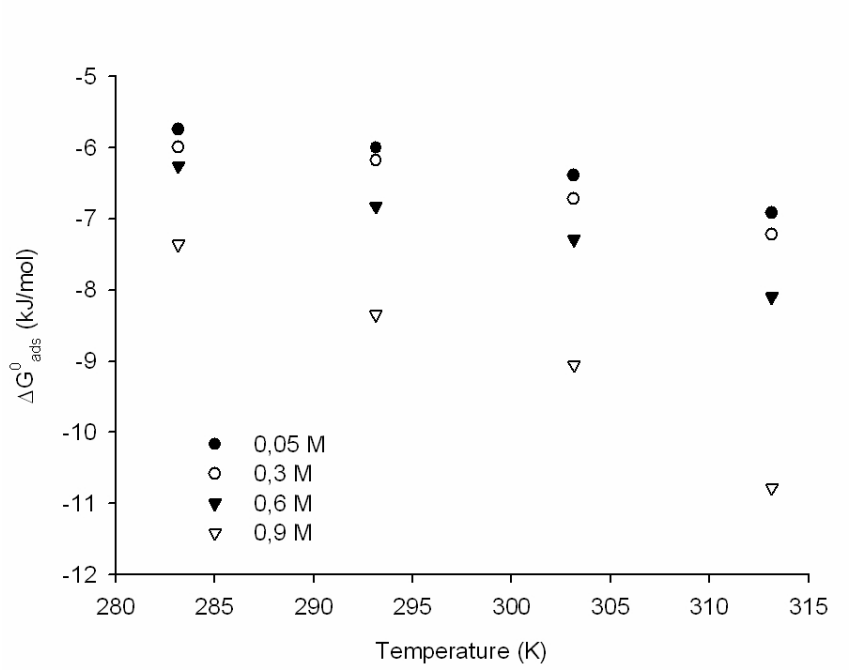


Figure 11: Free energy change of the proteins interacting with Streamline Phenyl at different concentrations of Na_2SO_4. (A) BSA and (B) β-lg.

The results presented in Figures 9 and 10 show that the adsorption enthalpy and adsorption entropy changes increase with increase in temperature for both proteins in all salt concentrations. The adsorption process of both proteins tends to be more enthalpically unfavorable with the increase in temperature. CHEN et al (2003) studied the adsorption process of myoglobin, lysozyme and α-amylase on butyl-Sepharose and octyl-Sepharose and observed that the binding procedure varied from exothermic to endothermic in most cases, indicating that entropy had a significant influence on hydrophobic interaction systems under high temperatures. The data of Figures 9 and 10 also show that there is a linear dependence of ΔH^0 and ΔS^0 on temperature, for all salt concentrations.

In all cases, the values of ΔH^0 and ΔS^0 increased with the increase of salt concentration. From the above results, it can be observed that the influence of salt concentration at higher temperature is larger for β-lg than BSA. The slope of plots increase as the salt concentration is increased. The values of ΔH^0 and ΔS^0 of β-lg varied from 11.54 $kJmol^{-1}$ to 38.98 $kJmol^{-1}$and 58.97 $Jmol^{-1}K^{-1}$ to158.90 $Jmol^{-1}K^{-1}$, respectively, at temperature of 313.15 K as the salt concentration was increased. It was observed that the increase on enthalpy and entropy was higher at 0.9 M than at other salt concentrations. This fact can be associated to a conformational change in protein structure, i.e., at high salt concentration, the protein has its hydration capacity decreased and its hydrophobicity increased, allowing a larger interaction with the adsorbent. According to DIAS-CABRAL et al (2003), endothermic values are observed at high salt concentrations due to stronger hydrophobic interactions.

According to TSAI et al (2002), the adsorption of soft proteins, such as BSA, onto the adsorbents is driven by the entropy of the system, with chaos being controlled by the rearrangement of protein structure and the variation of system dehydratio. In

contrast, the “hard” proteins, such as β-lg, are more stable compared to BSA, due to the presence of the disulfate bond. However, in this work it was observed that the change of β-lg is greater than for BSA under the same conditions.

Tables 2 and 3 and Figure 11 show that the Gibbs free energy decreases with temperature, for all the cases. Besides, the results indicated that the ΔG^0 value for β-lg is more negative than for BSA. Therefore, the adsorption process of the proteins BSA and β-lg are favorable and driven by entropy under all the conditions studied.

5. CONCLUSIONS

This study investigated the binding characteristics between proteins and hydrophobic adsorbents. The results showed that the adsorption process of BSA and β-lg on Streamline Phenyl is dependent on salt and temperature for the two proteins. The ΔH^0, ΔS^0 and ΔG^0 values presented for the process studied showed that such process is driven by entropy and is favorable for both proteins.

NOMENCLATURE

a	Henry's constant of adsorption
a_1, a_2, a_3	Parameters of Equation 8
b	Adsorption equilibrium constant
$[\%B]$	Concentration of water (volume fraction)
F	Phase ratio
I	Contains a number of constants related to the affinity of a protein to the HIC column
k'	Retention factor
K	Equilibrium constant
L_d	Hydrated ligands in salt solution
n'	Number of ligand interactions with a protein molecule
Q	Amount of compound adsorbed
q_s	Saturation capacity
R	Universal gas constant
T	Temperature
t_R	Retention time dead time
t_0	Dead time
V_a	Volume of adsorbent in the column
V_0	Column void volume
$V_{F, i+1}$	Retention volume at the inflection point
Z	Number of moles of water displaced per mole of protein adsorbed on the bonded phase surface

ΔG^0	Gibbs free energy change
ΔH^0	Enthalpy change
ΔS^0	Entropy change

Greek symbols

φ	Phase ratio
Φ	System constant depending on the phase ratio in the column
ε	Total porosity of the column

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MODELING THE ADSORPTION EQUILIBRIUM AND SIMULATION OF THE ADSORPTION PROCESS OF PROTEINS

ABSTRACT

Single-component adsorption isotherm data were determined by frontal analysis (FA) for bovine serum albumin (BSA) and β-lactoglobulin (β-lg) using Streamline Phenyl, a hydrophobic adsorbent. The experimental data obtained were modeled using five different isotherm models and the results were compared in terms of the R^2 values and the confidence intervals of the parameters. The influence of different isotherm models on simulated breakthrough curves in fixed bed columns was studied. The Langmuir isotherm model was the best isotherm model used to simulate adsorption for both proteins, with small confidence intervals and a good determination coefficient ($R^2 > 0.97$).

Keywords: modeling, adsorption, proteins, isotherm, breakthrough.

1. INTRODUCTION

Large-scale chromatography is increasingly finding new biotechnology applications due to its high separating power, selectivity and versatility. High feed concentrations are used in preparative chromatography, in contrast to analytical chromatography, leading to solute competition for adsorbent sites resulting in a non-linear effect (SPIEKER et al, 1998). Thus, the use of chromatographic processes for biological molecules can become very expensive and complex. A solution to this problem is obtained through mathematical modeling of the process. According to SPIEKER et al (1998) although some experiments will be required, their number can be greatly reduced through computer simulation results.

Mathematical modeling and numerical analysis of fixed-bed adsorption/desorption operations, such as frontal, displacement and elution, have received considerable attention since the late 1960s. Various mathematical models with different complexities have been proposed and can be basically classified into the following three major categories: a) staged equilibrium models, b) interference theory and c) rate equation model (GU, et al, 1990, RUTHVEN, 1984). Among them, the general non-linear multi-component rate equation model is the most realistic model for all kinds of multi-component adsorption/desorption column processes (GU et al 1990).

The word rate, as in rate equation model, refers to the rate expression or rate equation for the mass transfer between the mobile phase and the stationary phase. A rate model usually consists of two sets of differential mass balance equations, one for the bulk fluid phase, and the other for the particle phase (GU et al, 1993). When the mass transfer resistances are small and have a minor influence on the profiles, the equilibrium-dispersive model is recommended (KACZMARSKI et al, 2001).

The most important element in the modeling of the adsorption process is the equilibrium data, since no prediction of the column's behavior can be made without the adsorption isotherms for each component of the solution.

The aims of this work were to model the equilibrium data using five different models, to model the adsorptive process in fixed bed column and to compare the observed and predicted breakthroughs in terms of the isotherms tested.

2. THEORY

2.1. Model

Consider a fixed-bed adsorption column packed with uniform, porous, spherical, solid adsorbents. The process is isothermical and the concentration gradients in the radial direction of the bed are negligible. Suppose also that the local equilibrium exists for each component between the pore surface and the stagnant fluid phase in the macropores. According to GU et al (1993), assuming that the diffusional and mass transfer coefficients are constant and independent of the mixing effects of the components and based on the other assumptions, the following equations can be obtained from the differential mass balances for each component in the bulk-fluid and the particle phases.

$$-D_{bi}\frac{\partial^2 C_{bi}}{\partial Z^2}+v\frac{\partial C_{bi}}{\partial Z}+\frac{\partial C_{bi}}{\partial t}+\frac{3k_i(1-\varepsilon_b)}{\varepsilon_b R_p}\left(C_{bi}-C_{pi,R=R_P}\right)=0 \tag{1}$$

$$(1-\varepsilon_p)\frac{\partial C^s_{pi}}{\partial t}+\varepsilon_p\frac{\partial C_{pi}}{\partial t}-\varepsilon_p D_{pi}\left[\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2\frac{\partial C_{pi}}{\partial R}\right)\right]=0 \tag{2}$$

with the initial and boundary conditions

$$t=0,\quad C_{bi}=C_{bi}(0,Z),\qquad C_{pi}=C_{pi}(0,R,Z) \tag{3, 4}$$

$$Z=0,\quad \frac{\partial C_{bi}}{\partial Z}=\frac{v}{D_{bi}}\left(C_{bi}-C_{fi}(t)\right)\qquad Z=L,\quad \frac{\partial C_{bi}}{\partial Z}=0 \tag{5, 6}$$

$$R=0,\quad \frac{\partial C_{pi}}{\partial R}=0\qquad R=R_p,\quad \frac{\partial C_{pi}}{\partial R}=\frac{k_i}{\varepsilon_p D_{pi}}\left(C_{bi}-C_{pi,R=R_p}\right) \tag{7, 8}$$

In the Equation (2), C_{pi}^{s} is the concentration of component i in the solid phase of the adsorbents based on the unit volume of the solids, excluding pores.

The partial differential equation system above can be transformed into the following dimensionless forms.

$$-\frac{1}{Pe_{Li}}\frac{\partial^2 c_{bi}}{\partial z^2}+\frac{\partial c_{bi}}{\partial z}+\frac{\partial c_{bi}}{\partial \tau}+\xi_i\left(c_{bi}-c_{pi,r=1}\right)=0 \tag{9}$$

$$\frac{\partial}{\partial \tau}\left[\left(1-\varepsilon_p\right)c_{pi}^{s}+\varepsilon_p c_{pi}\right]-\eta_i\left[\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c_{pi}}{\partial r}\right)\right]=0 \tag{10}$$

Initial condition

$$\tau=0,\quad c_{bi}=c_{bi}(0,z),\qquad c_{pi}=c_{pi}(0,r,z) \tag{11, 12}$$

Boundary condition

$$z=0,\quad \frac{\partial c_{bi}}{\partial z}=Pe_{Li}\left(c_{bi}-C_{fi}(\tau)/C_{0i}\right) \tag{13}$$

For frontal adsorption $C_{fi}(\tau)/C_{0i}=1$

For elution $C_{fi}(\tau)/C_{0i}=\begin{cases}1 & 0\le\tau\le\tau_{imp}\\ 0 & else\end{cases}$

$$z=1,\qquad \frac{\partial c_{bi}}{\partial z}=0 \tag{14}$$

$$r=0\qquad \frac{\partial c_{pi}}{\partial z}=0\qquad\qquad r=1,\qquad \frac{\partial c_{pi}}{\partial r}=Bi_i\left(c_{bi}-c_{pi,r=1}\right) \tag{15, 16}$$

where: $c_{bi} = \frac{C_{bi}}{C_{0i}}$; $c_{pi} = \frac{C_{pi}}{C_{0i}}$; $c_{pi}^{s} = \frac{C_{pi}^{s}}{C_{0i}}$; $r = \frac{R}{R_p}$; $z = \frac{Z}{L}$; $\tau = \frac{vt}{L}$; $Pe_{Li} = \frac{vL}{D_{bi}}$;

$$Bi_i = \frac{k_i R_p}{\varepsilon_p D_{pi}}; \quad \eta_i = \frac{\varepsilon_p D_{pi} L}{R_p^2 v}; \quad \xi_i = \frac{3Bi_i \eta_i (1-\varepsilon_b)}{\varepsilon_b}.$$

2.1.1. Isotherm Models

The equilibrium isotherm defines the amount of the solute in the stationary phase, Q, as a function of the amount of the solute in the mobile phase, C (GUIOCHON, 2002). Langmuir, Bi-Langmuir, Toth, Jovanovic, and Exponentially Modified Langmuir are some of the isotherm models used for one component adsorption.

a. Langmuir isotherm

This model was proposed by Langmuir for adsorption in a gas-liquid system in 1916. A constant heat of adsorption and finite number of surface adsorption sites were assumed. With these assumptions, maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface (LANGMUIR, 1916; JACOBSON et al, 1984). It is written as:

$$Q = q_s \frac{bC}{1+bC} \tag{17}$$

In this model, q_s is the monolayer saturation capacity of the adsorbent and b is the equilibrium constant of adsorption.

b. Bi-Lagmuir isotherm

The Bi-Langmuir isotherm was first suggested by GRAHAM (1953) to account for adsorption behavior on certain non-homogeneous surfaces. The surface is assumed to be covered with two different types of chemical domains, which behave

independently. (JACOBSON et al, 1991). Then the equilibrium isotherm results from the addition of two independent local Langmuir isotherms:

$$Q = q_{s,1}\frac{b_1 C}{1 + b_1 C} + q_{s,2}\frac{b_2 C}{1 + b_2 C} \tag{18}$$

In this model, there are two saturation capacities, $q_{s,1}$ and $q_{s,2}$, corresponding to each one of the two types of sites. The total saturation capacity of the adsorbent is the sum of each saturation capacity ($q_{s,1}$ and $q_{s,2}$) and b_1 and b_2 are the equilibrium constants (GRITTI et al, 20003).

c. Toth isotherm

This model was presented by Toth in 1971. Like the Langmuir isotherm, originally derived for the study of gas-solid equilibrium, it can be applied to the case of liquid-solid equilibrium. The model has three parameters and it is used to account for the experimental adsorption isotherms obtained on non-homogeneous adsorbents (TOTH, 1971).

$$Q = q_s \frac{bC}{\left[1 + (bC)^n\right]^{1/n}} \tag{19}$$

where q_s and b have the same meaning as in the Langmuir isotherm model and n is the heterogeneity parameter (0< n <1). The parameters b and n permit independent adjustment of the initial slope and curvature of the isotherm (GUIOCHON et al, 1994 and GRITTI et al, 2003).

d. Jovanovic model

This isotherm model was derived for adsorption on a homogeneous solid surface, considering the non-localized phenomenon, without lateral interactions and covering the surface with a monolayer of solute (QUIÑONES and GUIOCHON, 1996a). The single-component model can be written as (HUANG and HORVÁTH, 1987; QUIÑONES and GUIOCHON, 1996a and 1996b):

$$Q = q_s[1 - \exp(-b \cdot C)] \tag{20}$$

where q_s is the surface concentration at saturation and b is the appropriate binding constant.

e. Exponentially Modified Langmuir isotherm

The Langmuir isotherm provides a non satisfactory description of the protein adsorption to hydrophobic adsorbents for two reasons: a) the binding of most proteins to hydrophobic adsorbents is based on multivalent interactions (JENNISSEN, 1978), b) protein adsorption to hydrophobic media is highly affected by salt concentration, but the Langmuir equation cannot express this behavior and the model parameters are implicit functions of salt concentration (MELANDER et al, 1984, OSCARSSON and KARSNAS, 1998 and CHEN and SUN, 2003). To overcome this problem, an exponentially modified Langmuir isotherm has been proposed to bring salt contribution in protein adsorption isotherms (ANTIA and HORVATH, 1989):

$$Q = \frac{\lambda b \exp(-kC_s)C}{1 + b\exp(-kC_s)C} \tag{21}$$

where λ, b and k are equation parameters, C_s is salt concentration in liquid phase and C, protein concentration.

3. EXPERIMENTAL

3.1. Materials

The proteins were purchased from Sigma (St. Louis, MO, USA). BSA is a globular ellipsoid protein with a molecular mass of 69000 Da, and isoelectric point (pI) of 4.7. The β-lg has a molecular mass of 32000 Da, when in a dimmer form (pH 3.5 to 8.0), and isoelectric point of 5.3 (CAYOT and LORIENT, 1996). The HIC support was Streamline Phenyl® purchased from Amersham Biosciences (Uppsala, Sweden) and packed in a column HR 5/5 purchased from Amersham Biosciences (Uppsala, Sweden). Sodium phosphate (monobasic and dibasic) and sodium sulfate were of analytical grade.

3.2. Apparatus

Frontal chromatography was carried out using an Äkta Purifier System (MOD 10X) from Amershan Pharmacia Biotech (Uppsala, Sweden) with a UV detector fixed in 280 nm and at flow rate fixed in 2.0 mL/min. The equipment was controlled using the software Unicorn v.1.0 (Amersham Biosciences). The system temperature was controlled by immersion of the column in a thermostatic bath (Quimis, precision of ± 0.1 K).

3.3. Procedures

3.3.1. Determination of equilibrium data

The fastest, most convenient and accurate method for isotherm determination in fixed bed column is frontal analysis (FA) (JACOBSON et al, 1984, GUIOCHON et al, 1994 and GUIOCHON, 2002). The adsorbed amount Q_{i+1} is given by:

$$Q_{i+1} = Q_i + \frac{\left(C_{i+1} - C_i\right)\left(V_{F,i+1} - V_0\right)}{V_a} \tag{22}$$

where Q_i and Q_{i+1} are the amounts of compound adsorbed by the column packing after the i^{th} and the $(i+1)^{th}$ step, in equilibrium with the concentrations C_i and C_{i+1},

respectively, $V_{F,i+1}$, is the retention volume at the inflection point of the $(i+1)^{th}$ breakthrough curve, V_0 is the column void volume, and V_a is the adsorbent volume in the column.

A column HR 5/5 packed with Streamline Phenyl® was initially equilibrated with 50 column volumes (CV) of the carrier buffer (20 mM phosphate, pH 7.0) containing various concentrations of sodium sulfate (50, 300, 600 and 900 mM) and at varying temperatures (283.15 K, 293.15 K, 303.15 K and 313.15 K). The experiments were arranged in a 4x4 factorial design. Single component solutions at concentrations of (0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0) mg mL^{-1}, for each protein, were perfused through the column, until the complete formation of the breakthrough curve.

At the end of each experiment, the column was regenerated with 30 CV of a buffer (20 mM phosphate, pH 7.0).

3.3.2. Model parameter estimate

The parameter mass transfer resistance was calculated according to the equation used by TRUEI et al (1992):

$$Sh = \frac{k_i 2R_p}{D_{pi}} = 2 + 1.45\,\mathrm{Re}^{1/2}\,Sc^{1/3} \tag{23}$$

where the Schmidt number, Sc, is given by

$$Sc = \frac{\mu}{\rho D_{pi}} \tag{24}$$

The effective diffusivity, D_{pi}, was derived from the following correlation cited by KACZMARSKI et al (2001):

$$D_{pi} = 8.31x10^{-8}\,\frac{T}{\mu M_i^{1/3}} \tag{25}$$

The axial dispersion coefficient can be calculated using empirical correlation presented by CHUNG and WEN (1968), where Peclet number, Pe, is calculated as:

$$Pe_L = \frac{vL}{D_b} = \frac{L}{2R_p\varepsilon_b}\left(0.2 + 0.011\,\mathrm{Re}^{0.48}\right) \tag{26}$$

with the Reynolds number (Re)

$$\mathrm{Re} = \frac{2R_p\varepsilon_b v\rho}{\mu} \tag{27}$$

3.3.3. Model Simulation

A computational program written in Visual Basic was developed for model simulation. The finite differences method was applied to discretize the Equation (9) while Equation (10) was discretized using the orthogonal collocation method, applying symmetric polynomials (Finlayson, 1980). The resultant algebraic equations system was solved by applying the Gauss-Siedel method.

4. RESULTS AND DISCUSSION

4.1. Adsorption Isotherms

The isotherm parameters were determined using the SAS® package (SAS Institute Inc., 1989) with the corresponding values being shown in Tables 1 and 2, for BSA and β-lg, respectively. The results show that for some isotherm models, the 95% confidence interval exceeds the parameter values by some orders of magnitude. In this work the coefficient of determination values were larger than 0.97. These results are similar to that found by KALTENBRUNNER and JUNGBAUER (1996) for the adjustment of the maximum capacity parameter of a number of different adsorption isotherms.

Table 1: Adjusted parameters for the tested isotherm models to adsorption of BSA on Streamline Phenyl at different temperatures.

Langmuir model

T (K)	C_S (M)	q_s (mg/ mL)	CI 95%	b (mL/mg)	CI 95%	R^2
283.15	0.05	304.50	±99.39	0.03	±0.01	0.99
	0.3	320.05	±56.05	0.03	±0.01	0.99
	0.6	123.33	±7.69	0.15	±0.02	0.99
	0.9	121.66	±10.12	0.26	±0.04	0.98
293.15	0.05	354.38	±89.37	0.03	±0.01	0.99
	0.3	470.95	±148.48	0.02	±0.01	0.99
	0.6	261.71	±26.14	0.06	±0.01	0.99
	0.9	175.48	±11.61	0.16	±0.02	0.99
303.15	0.05	438.77	±111.85	0.02	±0.01	0.99
	0.3	194.94	±22.27	0.08	±0.01	0.99
	0.6	381.58	±80.63	0.04	±0.01	0.99
	0.9	152.21	±8.63	0.26	±0.03	0.99
313.15	0.05	329.26	±70.50	0.04	±0.01	0.99
	0.3	325.26	±57.6	0.04	±0.01	0.99
	0.6	161.31	±8.78	0.21	±0.02	0.99
	0.9	162.83	±8.40	0.28	±0.03	0.99

Toth model

T (K)	C_S (M)	q_s (mg/ mL)	CI 95%	b (mL/mg)	CI 95%	n	CI 95%	R^2
283.15	0.05	304.50	$\pm 1.1 \times 10^3$	0.03	±0.10	1.00	±1.76	0.99
	0.3	320.05	±610.60	0.03	±0.05	1.00	±0.94	0.99
	0.6	143.40	±67.28	0.13	±0.04	0.86	±0.35	0.99
	0.9	7611.99	$\pm 4.4 \times 10^4$	0.09	±0.15	0.15	±0.18	0.99
293.15	0.05	354.38	±994.30	0.03	±0.08	1.00	±1.36	0.99
	0.3	470.95	$\pm 1.7 \times 10^3$	0.02	±0.08	1.00	±1.68	0.99
	0.6	386.52	±473.47	0.04	±0.04	0.79	±0.50	0.99
	0.9	175.48	±67.87	0.16	±0.03	1.00	±0.41	0.99
303.15	0.05	438.77	$\pm 1.3 \times 10^3$	0.02	±0.07	1.00	±1.36	0.99
	0.3	194.94	±170.26	0.08	±0.05	1.00	±0.66	0.99
	0.6	381.58	±778.57	0.04	±0.08	1.00	±1.17	0.99
	0.9	188.35	±73.60	0.25	±0.04	0.77	±0.31	0.99
313.15	0.05	329.26	±707.80	0.04	±0.08	1.00	±1.17	0.99
	0.3	325.26	±551.31	0.04	±0.07	1.00	±0.98	0.99
	0.6	223.62	±101.78	0.18	±0.04	0.71	±0.27	0.99
	0.9	162.83	±38.54	0.28	±0.03	1.00	±0.34	0.99

Jovanovic model

T (K)	C_S (M)	q_s (mg/ mL))	CI 95%	b (mL/mg)	CI 95%	R^2
283.15	0.05	163.52	±45.88	0.06	±0.02	0.99
	0.3	175.13	±27.06	0.06	±0.01	0.99
	0.6	82.02	±4.30	0.21	±0.02	0.99
	0.9	89.59	±6.76	0.30	±0.04	0.97
293.15	0.05	191.12	±42.08	0.05	±0.01	0.99
	0.3	249.65	±70.55	0.04	±0.01	0.99
	0.6	154.28	±13.45	0.10	±0.01	0.99
	0.9	116.72	±4.90	0.22	±0.01	0.99
303.15	0.05	234.37	±53.08	0.04	±0.01	0.99
	0.3	117.90	±10.32	0.13	±0.01	0.99
	0.6	212.58	±36.99	0.08	±0.01	0.99
	0.9	110.77	±5.49	0.32	±0.03	0.98
313.15	0.05	181.72	±32.58	0.07	±0.01	0.99
	0.3	182.45	±26.63	0.08	±0.01	0.99
	0.6	113.44	±5.33	0.27	±0.02	0.99
	0.9	119.07	±5.03	0.34	±0.03	0.98

Bi-Langmuir model

T (K)	C_S (M)	q_{s1} (mg/ mL)	CI 95%	b_1 (mL/mg)	CI 95%	q_{s2} (mg/mL)	CI 95%	b_2 (mL/ mg)	CI 95%	R^2
283.15	0.05	42.16	±24.33	4.28	±43.46	32.70	±216.09	4.28	±56.02	0.99
	0.3	154.17	$\pm 1.3 \times 10^{3}$	30.97	±267.87	150.33	$\pm 1.3 \times 10^{3}$	30.98	±274.70	0.99
	0.6	174.86	±216.09	4.28	±43.46	32.70	±216.09	4.28	±56.02	0.99
	0.9	41.42	±24.33	0.89	±0.60	8.90	$\pm 1.6 \times 10^{10}$	5.1×10^{3}	$\pm 3.3 \times 10_{14}$	0,99
293.15	0.05	175.39	±221.22	32.64	±181.75	178.98	±221.22	32.63	±178.10	0.99
	0.3	234.66	$\pm 1.1 \times 10^{3}$	41.71	±222.92	236.28	$\pm 1.1 \times 10^{3}$	41.72	±221.20	0.99
	0.6	$1.9 \times 10_{7}$	$\pm 8.0 \times 10^{1}_{2}$	1.8×10^{6}	$\pm 1.5 \times 10^{12}$	89.24	$\pm 1.1 \times 10^{3}$	7.44	±50.69	0.99
	0.9	79.11	±414.15	6.15	±56.63	96.36	±414.15	6.16	±46.48	0.99
303.15	0.05	218.46	$\pm 4.4 \times 10^{8}$	38.13	$\pm 1.9 \times 10^{7}$	220.30	$\pm 4.4 \times 10^{8}$	38.13	$\pm 1.8 \times 10_{7}$	0.99
	0.3	89.70	±491.24	11.91	±94.23	105.23	±491.24	11.91	±80.30	0.99
	0.6	184.64	$\pm 1.1 \times 10^{3}$	21.94	±159.61	196.93	$\pm 1.1 \times 10^{3}$	21.95	±149.61	0.99
	0.9	95.56	±32.60	2.2517	±0.96	2.3×10^{9}	$\pm 4.6 \times 10^{8}$	6.1×10^{8}	$\pm 4.2 \times 10_{8}$	0.99
313.15	0.05	160.54	±848.23	24.42	±159.48	168.71	±848.23	24.41	±151.6	0.99
	0.3	157.17	$\pm 4.4 \times 10^{8}$	21.36	$\pm 1.2 \times 10^{6}$	168.08	$\pm 4.4 \times 10^{8}$	21.36	$\pm 1.1 \times 10_{6}$	0.99
	0.6	67.53	± 233.18	2.20	±4.98	170.92	±323.29	19.23	±133.28 61	0.99
	0.9	133.86	±51.33	2.81	±1.23	1.1×10^{9}	$\pm 6.2 \times 10^{8}$	6.0×10^{8}	$\pm 1.1 \times 10_{9}$	0.99

Exponentially Modified Langmuir Model								
T (K)	**C_S (M)**	**λ (mg/mL)**	**CI 95%**	**b (mL/mg)**	**CI 95%**	**k**	**CI 95%**	**R^2**
283.15	0.05	304.50	$\pm 1.9 \times 10^3$	0.16	$\pm 5.5 \times 10^3$	32.81	$\pm 6.6 \times 10^5$	0.99
	0.3	320.05	$\pm 1.1 \times 10^3$	0.19	$\pm 3.2 \times 10^3$	5.88	$\pm 5.6 \times 10^4$	0.99
	0.6	123.33	±42.85	0.48	$\pm 2.0 \times 10^3$	1.96	$\pm 7.0 \times 10^3$	0.99
	0.9	121.66	±38.63	0.75	$\pm 4.3 \times 10^3$	1.17	$\pm 6.4 \times 10^3$	0.98
293.15	0.05	354.38	$\pm 1.8 \times 10^3$	0.16	$\pm 4.2 \times 10^3$	33.33	$\pm 5.2 \times 10^5$	0.99
	0.3	470.95	$\pm 3.8 \times 10^3$	0.16	$\pm 5.7 \times 10^3$	6.36	$\pm 1.1 \times 10^5$	0.99
	0.6	261.71	±291.18	0.31	$\pm 2.2 \times 10^3$	2.68	$\pm 1.1 \times 10^4$	0.99
	0.9	175.48	±61.21	0.58	$\pm 2.4 \times 10^3$	1.42	$\pm 4.7 \times 10^3$	0.99
303.15	0.05	438.77	$\pm 2.6 \times 10^3$	0.14	$\pm 4.2 \times 10^3$	34.79	$\pm 5.7 \times 10^5$	0.99
	0.3	194.94	±194.07	0.31	$\pm 2.5 \times 10^3$	4.38	$\pm 2.7 \times 10^4$	0.99
	0.6	381.57	$\pm 1.1 \times 10^3$	0.26	$\pm 4.4 \times 10^3$	2.92	$\pm 2.7 \times 10^4$	0.99
	0.9	152.21	±32.34	0.75	$\pm 3.0 \times 10^3$	1.14	$\pm 4.4 \times 10^3$	0.99
313.15	0.05	329.26	$\pm 1.1 \times 10^3$	0.18	$\pm 3.7 \times 10^3$	30.58	$\pm 3.9 \times 10^5$	0.99
	0.3	325.26	±820.74	0.23	$\pm 3.3 \times 10^3$	5.30	$\pm 4.9 \times 10^4$	0.99
	0.6	161.31	±38.30	0.58	$\pm 2.2 \times 10^3$	1.67	$\pm 6.5 \times 10^3$	0.99
	0.9	162.83	±30.15	0.77	$\pm 2.9 \times 10^3$	1.10	$\pm 4.1 \times 10^3$	0.99

C_S = Salt concentration.

Table 2: Adjusted parameters for the isotherm models tested for adsorption of β-lg on Streamline-Phenyl at different temperatures.

Langmuir Model						
T (K)	**C_S (M)**	**q_s (mg/ mL)**	**CI 95%**	**b (mL/mg)**	**CI 95%**	**R^2**
283.15	0.05	91.08	±14.81	0.12	±0.03	0.98
	0.3	102.91	±16.95	0.12	±0.03	0.98
	0.6	113.19	±18.01	0.12	±0.03	0.98
	0.9	121.13	±11.58	0.18	±0.03	0.98
293.15	0.05	110.97	±20.77	0.10	±0.03	0.98
	0.3	112.65	±21.21	0.11	±0.03	0.97
	0.6	130.54	±20.14	0.12	±0.03	0.98
	0.9	151.07	±11.32	0.24	±0.03	0.98
303.15	0.05	112.56	±20.95	0.11	±0.03	0.97
	0.3	119.50	±21.12	0.12	±0.03	0.97
	0.6	152.56	±24.42	0.11	±0.03	0.98
	0.9	151.07	±11.32	0.24	±0.03	0.98
313.15	0.05	128.76	±25.70	0.11	±0.03	0.97
	0.3	142.36	±26.32	0.11	±0.03	0.97
	0.6	166.03	±28.62	0.11	±0.02	0.98
	0.9	175.76	±9.49	0.35	±0.04	0.98
Toth model						

T (K)	C_S (M)	q_s (mg/ mL)	CI 95%	b (mL/mg)	CI 95%	n	CI 95%	R^2
283.15	0.05	91.08	±96.08	0.13	±0.09	1.00	±0.98	0.97
	0.3	102.91	±110.62	0.12	±0.09	1.00	±0.99	0.97
	0.6	113.19	±116.84	0.13	±0.09	1.00	±0.96	0.97
	0.9	121.13	±63.58	0.18	±0.05	1.00	±0.60	0.98
293.15	0.05	110.67	±144.60	0.10	±0.10	1.00	±1.11	0.97
	0.3	112.66	±144.38	0.11	±0.10	1.00	±1.12	0.97
	0.6	130.54	±130.65	0.12	±0.08	1.00	±0.93	0.97
	0.9	151.07	±56.08	0.24	±0.04	1.00	±0.48	0.98
303.15	0.05	112.57	±142.65	0.11	±0.10	1.00	±1.11	0.97
	0.3	119.50	±139.62	0.12	±0.09	1.00	±1.06	0.97
	0.6	152.56	±162.57	0.11	±0.08	1.00	±0.96	0.98
	0.9	151.07	±56.08	0.24	±0.04	1.00	±0.48	0.98
313.15	0.05	126.76	±175.65	0.11	±0.11	1.00	±1.19	0.97
	0.3	142.36	±178.93	0.11	±0.10	1.00	±1.10	0.97
	0.6	166.03	±196.09	0.11	±0.09	1.00	±1.03	0.97
	0.9	175.76	±39.84	0.36	±0.04	1.00	±0.36	0.98

Jovanovic model

T (K)	C_S (M)	q_s (mg/ mL))	CI 95%	b (mL/mg)	CI 95%	R^2
283.15	0.05	57.32	±6.10	0.19	±0.03	0.98
	0.3	64.61	±6.99	0.19	±0.03	0.98
	0.6	71.30	±7.42	0.19	±0.03	0.98
	0.9	81.93	±4.55	0.26	±0.02	0.99
293.15	0.05	67.82	±8.75	0.16	±0.03	0.98
	0.3	69.49	±8.84	0.17	±0.03	0.98
	0.6	82.34	±8.34	0.19	±0.03	0.98
	0.9	106.41	±4.34	0.31	±0.02	0.99
303.15	0.05	69.46	±8.74	0.17	±0.03	0.98
	0.3	74.55	±8.74	0.18	±0.03	0.98
	0.6	95.33	±10.20	0.18	±0.03	0.98
	0.9	106.41	±4.34	0.31	±0.02	0.99
313.15	0.05	79.16	±10.71	0.17	±0.03	0.97
	0.3	87.92	±10.99	0.17	±0.03	0.98
	0.6	102.60	±12.06	0.17	±0.03	0.98
	0.9	132.40	±4.19	0.40	±0.03	0.99

Bi-Langmuir model

T (K)	C_S (M)	q_{s1} (mg/ mL)	CI 95%	b_1 (mL/ mg)	CI 95%	q_{s2} (mg/ mL)	CI 95%	b_2 (mL/m g)	CI 95%	R^2
283.15	0.05	41.98	±444.29	7.92	±136.02	49.40	±444.29	7.9269	±116.25	0.97 4

	0.3	47.45	±502.89	8.04	±137.59	55.46	±502.89	8.04	±117.78	0.97
	0.6	52.11	±540.45	7.92	±133.22	61.08	±540.45	7.92	±113.69	0.97
	0.9	54.91	$\pm 1.6x10^{8}$	5.35	$\pm 3.8x10^{4}$	66.22	$\pm 1.6x10^{8}$	5.31	$\pm 3.1x10^{4}$	0,98
293.15	0.05	50.79	±548.92	9.42	±156.86	59.87	±548.92	9.42	±133.05	0.97
	0.3	51.61	±584.09	8.91	±157.96	61.04	±584.09	8.91	±133.52	0.97
	0.6	60.06	$\pm 2.4x10^{8}$	7.92	$\pm 1.2x10^{5}$	70.47	$\pm 2.4x10^{8}$	7.92	$\pm 1.0x10^{5}$	0.97
	0.9	68.62	±513.50	4.15	±62.505	82.45	±13.503	4.15	±52.001	0.98
303.15	0.05	51.56	$\pm 3.0x10^{8}$	8.91	$\pm 1.7x10^{5}$	61.00	$\pm 3.0x10^{8}$	8.91	$\pm 1.4x10^{5}$	0.97
	0.3	55.24	±612.59	8.30	±147.14	64.25	±612.59	8.30	±126.58	0.97
	0.6	69.49	$\pm 2.6x10^{8}$	8.44	$\pm 4.4x10^{5}$	83.06	$\pm 2.6x10^{8}$	8.44	$\pm 3.7x10^{5}$	0.97
	0.9	68.62	±612.59	4.15	±62.505	82.45	±513.50	4.15	±52.001	0.98
313.15	0.05	59.79	±702.40	9.00	±165.09	68.96	±702.40	9.00	±143.18	0.97
	0.3	65.19	±726.53	8.88	±155.18	77.17	±726.53	8.88	±131.09	0.97
	0.6	75.86	±779.09	9.05	±144.98	90.17	±779.09	9.05	±121.88	0.97
	0.9	80.04	$\pm 1.7x10^{8}$	2.80	$\pm 2.2x10^{5}$	95.71	$\pm 1.1x10^{8}$	2.80	$\pm 1.8x10^{5}$	0.98

Exponentially Modified Langmuir Model

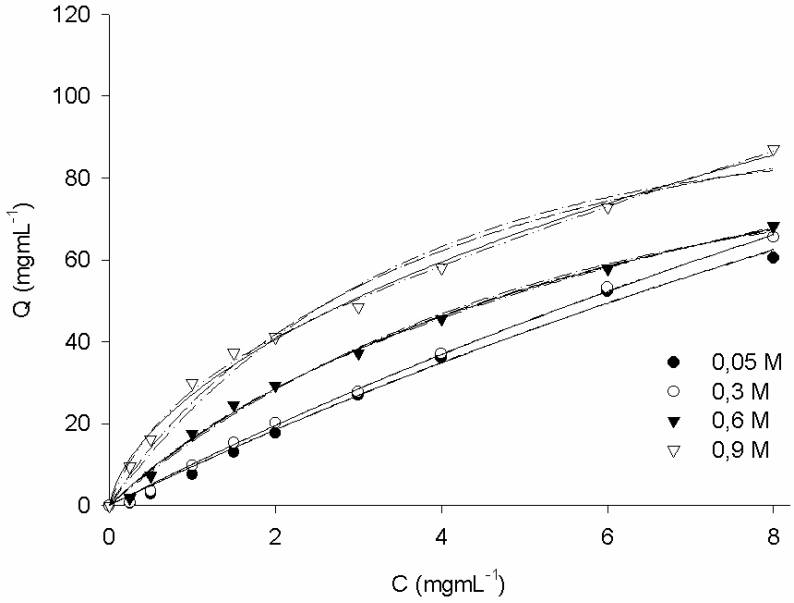
T (K)	C_S (M)	λ (mg/mL)	CI 95%	b (mL/mg)	CI 95%	k	CI 95%	R^2
283.15	0.05	91.08	±93.93	0.35	$\pm 4.3x10^{3}$	20.91	$\pm 2.3x10^{5}$	0.97
	0.3	102.91	±108.70	0.37	$\pm 4.4x10^{3}$	3.72	$\pm 3.9x10^{4}$	0.97
	0.6	113.19	±114.21	0.44	$\pm 4.7x10^{3}$	2.09	$\pm 1.7x10^{4}$	0.97
	0.9	121.13	±54.97	0.62	$\pm 3.9x10^{3}$	1.33	$\pm 6.9x10^{3}$	0.98
293.15	0.05	110.67	±150.36	0.32	$\pm 4.5x10^{3}$	22.34	$\pm 2.7x10^{5}$	0.97
	0.3	112.65	±147.11	0.33	$\pm 4.6x10^{3}$	21.88	$\pm 2.7x10^{5}$	0.97
	0.6	130.54	±127.72	0.35	$\pm 4.0x10^{3}$	20.93	$\pm 2.2x10^{5}$	0.97
	0.9	151.07	±45.61	0.55	$\pm 3.2x10^{3}$	16.60	$\pm 1.1x10^{5}$	0.98
303.15	0.05	112.59	±145.35	0.33	$\pm 4.6x10^{3}$	21.88	$\pm 2.7x10^{5}$	0.97
	0.3	119.50	±138.71	0.37	$\pm 4.7x10^{3}$	3.77	$\pm 4.2x10^{5}$	0.97
	0.6	152.56	±162.47	0.34	$\pm 4.0x10^{3}$	21.45	$\pm 2.3x10^{5}$	0.98
	0.9	151.07	±45.61	0.55	$\pm 3.2x10^{3}$	16.60	$\pm 1.1x10^{5}$	0.98
313.15	0.05	128.76	±179.63	0.33	$\pm 4.9x10^{3}$	21.94	$\pm 2.9x10^{5}$	0.97
	0.3	142.36	±182.11	0.36	$\pm 4.7x10^{3}$	3.88	$\pm 4.3x10^{4}$	0.97
	0.6	166.03	±200.95	0.41	$\pm 4.7x10^{3}$	2.20	$\pm 1.9x10^{4}$	0.97
	0.9	175.76	±30.00	0.84	$\pm 3.6x10^{3}$	0.96	$\pm 4.7x10^{3}$	0.98

C_S = Salt concentration

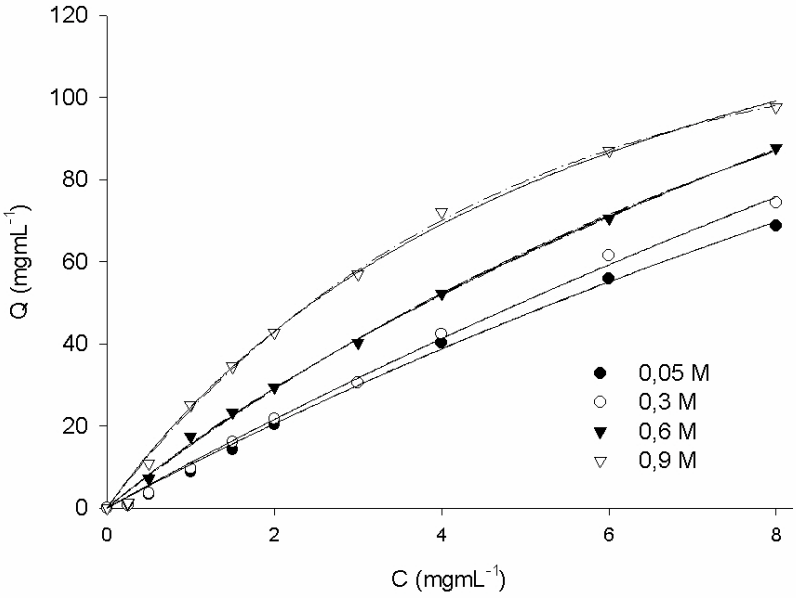
According to GRITTI and GUIOCHON (2004), the validity of the models to the simulation is also supported by the agreement between the experimental and simulated data. Figures 1 and 2 present the equilibrium isotherms (experimental and predicted) at the temperature studied, for BSA and β-lg, respectively. It can be observed that the

isotherms are favorable for all the conditions studied. In Figures 1 and 2, it is shown that all the models can satisfactorily predict experimental data under a wide range of temperatures and salt concentrations for both proteins.

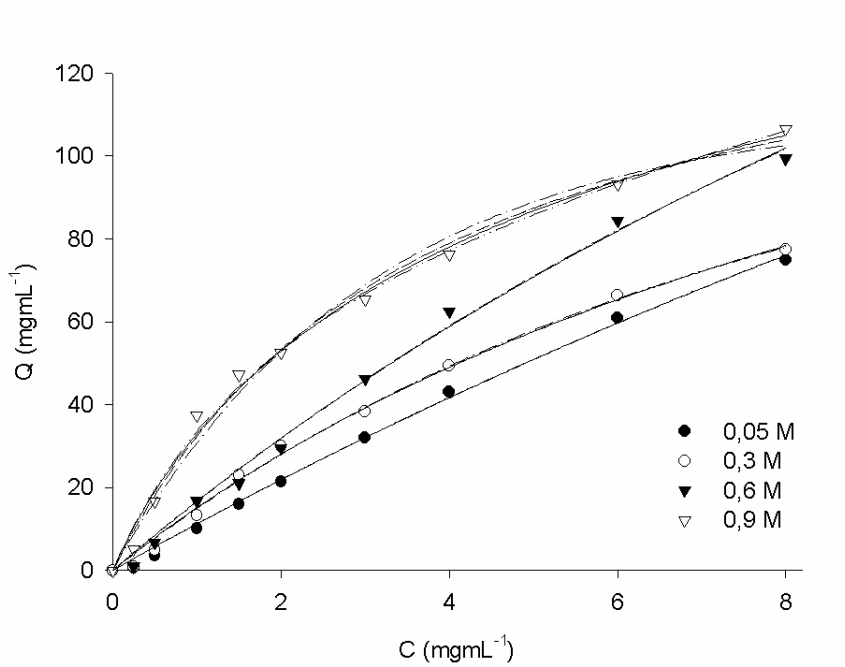
(A)



(B)



(C)



(D)

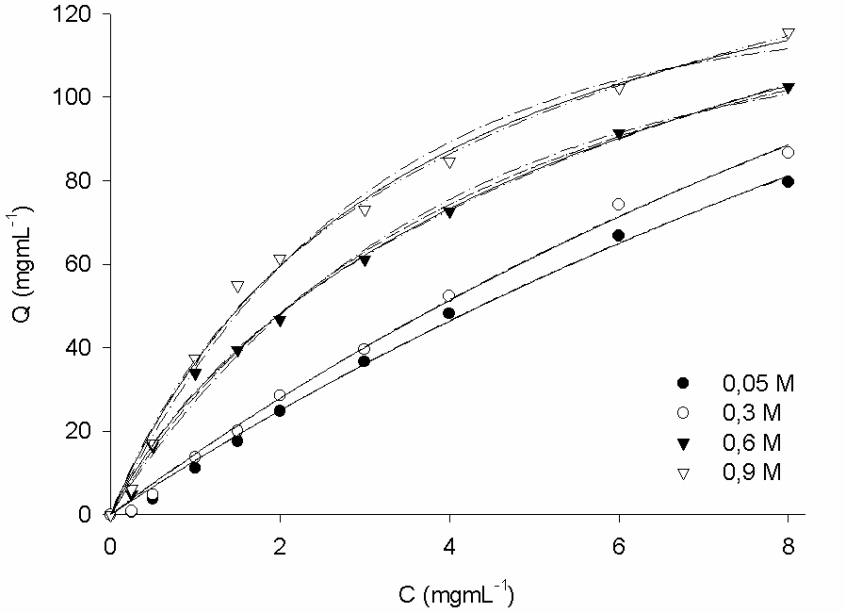
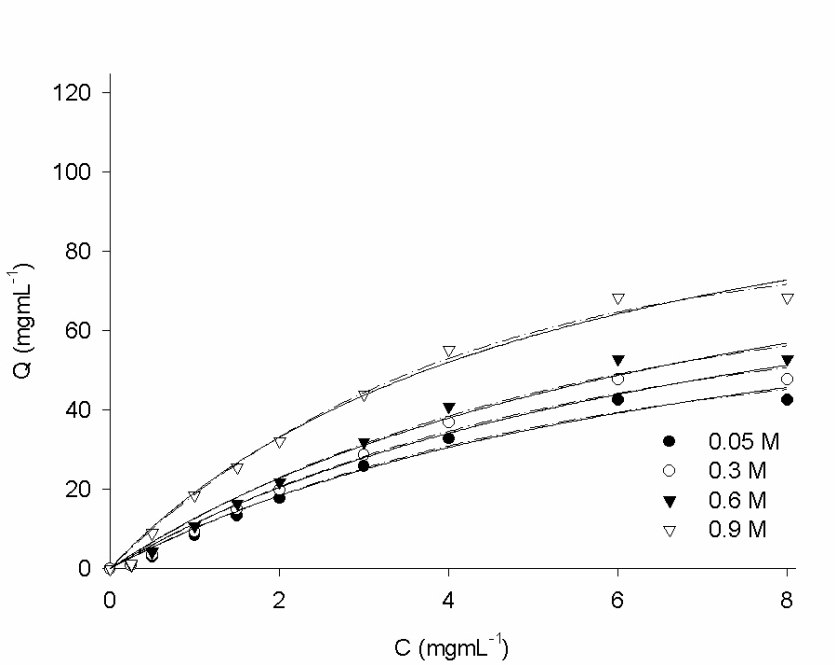
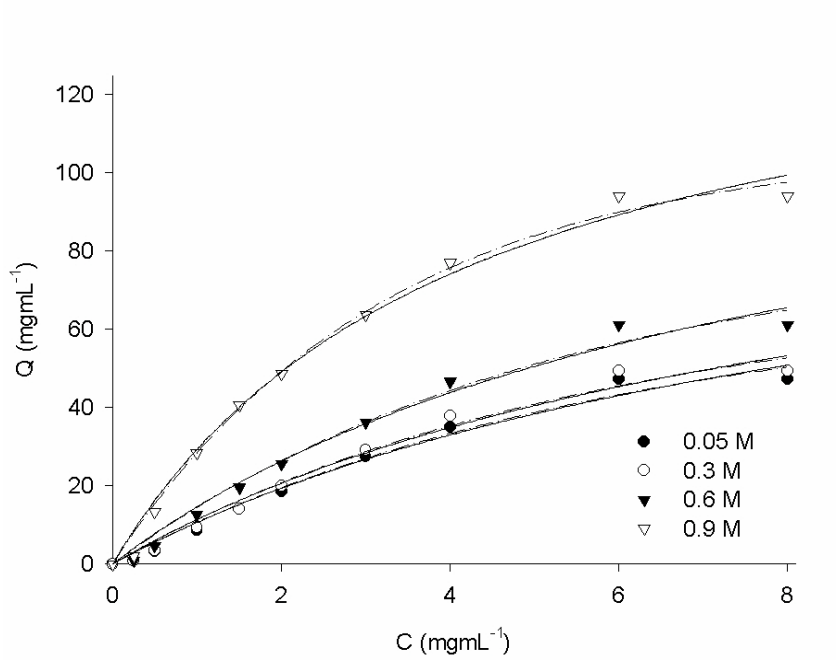


Figure 1: Adsorption isotherms of BSA at four salt concentrations (Na_2SO_4) and temperatures and adjusted curves obtained to the Toth (—), Langmuir (----), Exponentially Modified Langmuir(….), Bi-Langmuir (-···) and Jovanovic (-·-·-) model.Protein concentration plot in the stationary phase (Q) versus protein concentration in the mobile phase at equilibrium. (A) 283.15 K, (B) 293.15 K, (C) 303.15 K and (D) 313.15 K.

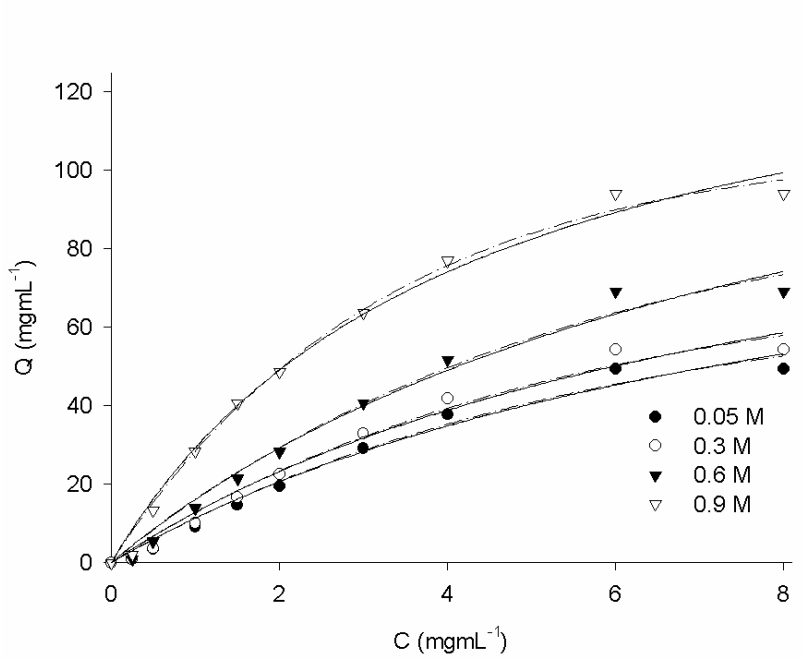
(A)


120
100
80
60
40
20
0
Q (mgmL-1)
0
2
4
6
8
C (mgmL-1)
0.05 M
0.3 M
0.6 M
0.9 M

(B)


120
100
80
60
40
20
0
Q (mgmL-1)
0
2
4
6
8
C (mgmL-1)
0.05 M
0.3 M
0.6 M
0.9 M

(C)



(D)

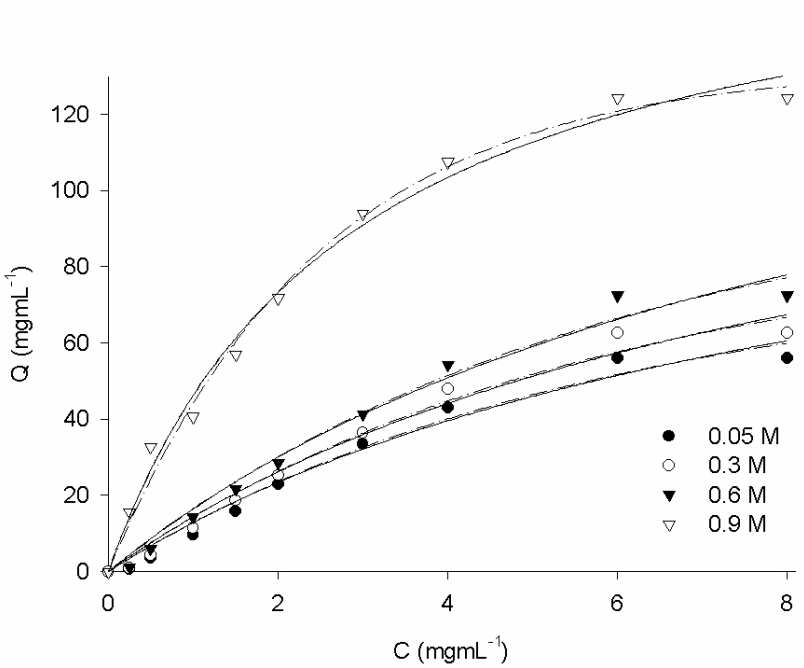


Figure 2: Adsorption isotherms of β-lg with Streamline Phenyl at four salt concentrations and temperatures and adjusted curves obtained to the Toth (—), Langmuir (----), Exponentially Modified Langmuir(….), Bi-Langmuir (-···-) and Jovanovic (-·-·-) model. Protein concentration plot in the stationary

phase (Q) versus protein concentration in the mobile phase at equilibrium (A) 283.15 K, (B) 293.15 K, (C) 303.15 K and (D) 313.15 K.

4.3. Column Experiments

Figures 4 and 5 show the experimental and simulated breakthrough curves for BSA and β-lg, respectively. The shapes of the experimental breakthrough curves for BSA and β-lg are very similar. The column saturation time for BSA was smaller than for β-lg. Breakthrough simulation for both proteins was accomplished using the adjusted parameter values shown in Tables 1 and 2 and *SimuCromWin software*. Since the adjusted parameter of the Bi-Langmuir model is inconsistent and does not have a small confidence interval, the simulation for this model was not carried out.

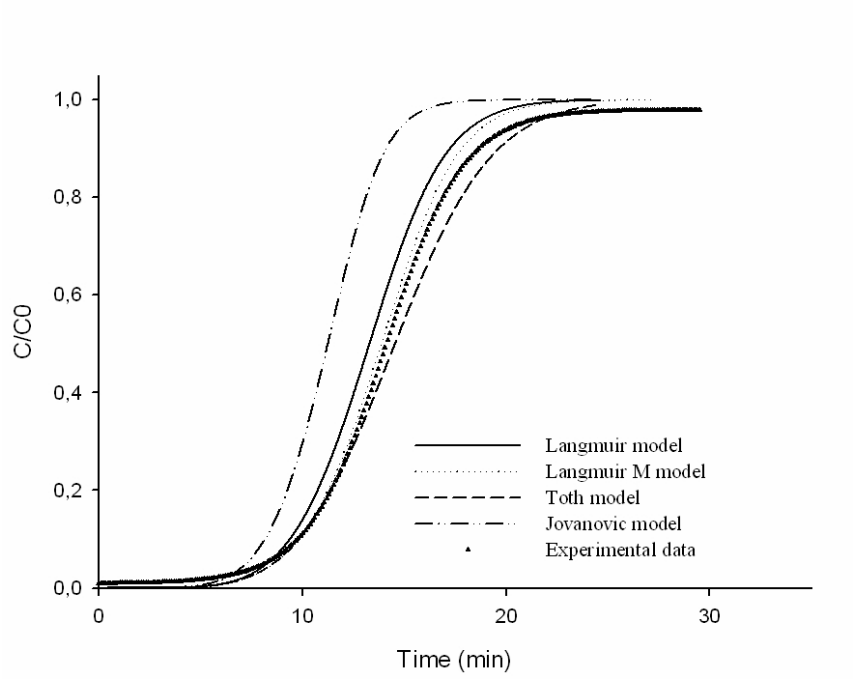


Figure 4: Comparison between the experimental breakthrough curve and numerical solutions of the general rate model using different isotherm models for BSA. (C_0 = 1.5 mg/mL, salt concentration 0.9 M and temperature = 313.15 K)

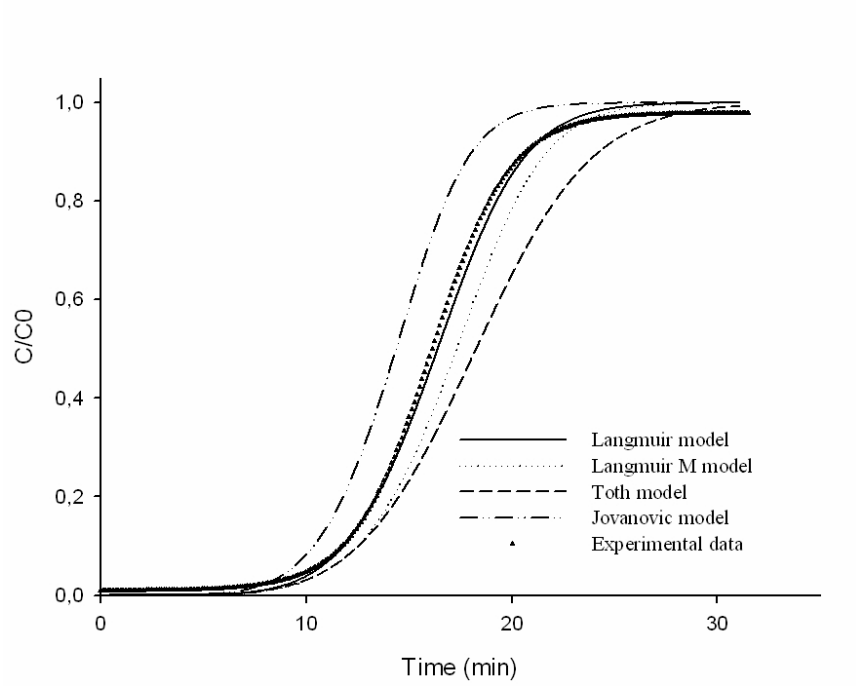


Figure 5: Comparison between the experimental breakthrough curve and numerical solutions of the general rate model using different isotherm models for β-lg. (C_0 = 1.5 mg/mL, salt concentration 0.9 M and temperature = 313.15 K)

The BSA result shows that the numerical solutions using the Langmuir, Toth and Exponentially Modified Langmuir isotherm models had a good agreement with the experimental data. Although the estimative parameters had a small confidence interval, the Exponentially Modified Langmuir model had the best agreement. The Langmuir and Toth models had a good agreement with the first part of the experimental breakthrough curve, which is of major importance in industrial processes. All the Langmuir and Toth model parameter estimates had small confidence intervals and the R^2 value was satisfactory for the condition studied, as it can be seen in Table 1.

In the simulations, the Pe, Bi and η values, calculated using the Equation 17, were kept in 500, 9.620 and 1.415, respectively, for all the experiments with BSA. For the β-lg protein, the values were 500, 8.990 and 1.828.

The β-lg results were similar to that obtained for BSA. The Langmuir model had the best agreement along the entire experimental breakthrough curve. The validity of the

Langmuir isotherm model is supported by the good agreement between the experimental breakthrough curve and those calculated using this isotherm and the general rate model of chromatography. Therefore, despite the satisfactory performance the other models had in the adsorption process simulation, the Langmuir model was superior to the others due to its simplicity, besides being thermodynamically consistent.

The simulation times of the BSA and β-lg adsorption processes at 0.9 M of salt concentration and 313.15 K are shown in Table 4 for four isotherm models. The results show that for both proteins, the Langmuir model had a smaller simulation time than the Toth and Exponentially Modified Langmuir models.

Table 3: Adsorption process simulation times.

	Simulation Time (s)	
Isotherm model	BSA	B-lg
Langmuir	86	96
Toth	118	134
Jovanovic	78	94
Exponentially Modified Langmuir	110	124

5. CONCLUSIONS

There is a good agreement between experimental and calculated breakthrough curves for all isotherm models for both proteins. This result shows the adsorption process validity of the Toth, Langmuir, Jovanovic and Exponentially Modified Langmuir models. The Langmuir model has a small simulation time and a good determination coefficient for BSA and β-lg, thereby being the first choice to simulate the studied process. The Jovanovic model underestimates the column adsorptive capacity.

NOMENCLATURE

b, b_1, b_2	Adsorption equilibrium constant
Bi	Biot number for component i
C_{bi}	Bulk phase concentration of component i
C_{fi}	Feed concentration profile of component i
C_{oi}	Concentration used for non-dimensionalization max $\{C_{fi}(t)\}$
C_{pi}	Concentration of component i in the stagnant fluid phase inside particle macro-pores
C^s_{pi}	Concentration of component i in the solid phase of the adsorbents based on the unit volume of the solids, excluding pores
C_s	Salt concentration of mobile phase
C_i	Concentration at equilibrium
c_{bi}	C_{bi}/C_{0i}
c^s_{pi}	C^s_{pi}/C_{0i}
c_{pi}	C_{pi}/C_{0i}
D_{bi}	Axial dispersion coefficient of component i
D_{pi}	Effective diffusivity of component i
k	Parameter of Exponentially Modified Langmuir model
L	Column length
n	Heterogeneity parameter of the Toth isotherm
Pe_{Li}	Peclet number for component i
Q	Amounts of compound adsorbed
q_s, q_{s1}, q_{s2}	Saturation capacity
R	Radial coordinate for particle
Re	Reynolds number

R_p	Particle radius
r	R/R_p
Sc	Schmidt number
Sh	Sherwood number
t	Time
v	Interstitial velocity
V_a	Volume of adsorbent in the column
V_0	Column void volume
$V_{F,\ i+1}$	Retention volume at the inflection point
Z	Axial coordinate variable
z	Z/L

Greek letters

ε_b	Bed void volume fraction
ρ	Density of the mobile phase
λ	Exponentially Modified Langmuir isotherm parameter
μ	Viscosity of mobile phase
ε_p	Particle porosity
η_i	Dimensionless constant for component i
ξ_i	Dimensionless constant for component i
τ	Dimensioless time
τ_{imp}	Dimensionless time duration for the restangular pulse of the sample

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OPTIMIZATION OF THE ADSORPTION AND ELUTION PROCESSES OF WHEY PROTEIN IN HYDROPHOBIC INTERATION CHROMATOGRAPHY BY SURFACE RESPONSE ANALYSIS

ABSTRACT

In this paper, the response surface technique was used for optimization of the adsorption and elution steps, in a fixed bed column, packed with a hydrophobic ligand (Streamline Phenil). The solutes used were the whey proteins bovine serum albumin (BSA) and β-lactoglobulin (β-lg). The results shown that the values 0.85 M and 313.15 K of salt concentration and temperature, respectively, are the best for adsorption process and separation of BSA and β-lg.

Keywords: adsorption, hydrophobic interaction, optimization, surface response, proteins.

1. INTRODUCTION

The development of techniques and methods for the separation and purification of proteins has been essential for many of the recent advancements in biotechnology research. The global aim of a protein purification process is not only the removal of unwanted contaminants, but also the concentration of the desired protein and their transfer to an environment where it is stable and in a form ready for the intended application (QUEIROZ et al, 2001).

Among the protein purification techniques, the chromatography has dominated due to their high resolving power. According to ESQUIBEL-KING et al (1999), the hydrophobic interaction chromatography (HIC) is the chromatography technique that has gained much attention for the downstream purification of proteins. The separation in

HIC are achieved quickly, with little product degradation and low solvent requirements, with very good levels of purification (QUEIROZ et al, 2001). The selectivity in this technique derives from the hydrophobic interactions between the hydrophobic resin and non-polar hydrophobic patches on the solute surfaces (DIAS-CABRAL et al, 2003). The selectivity changes with the temperature and hydrophobicity of the column and with the composition of the mobile phase.

Therefore, the study of the adsorption and elution conditions, temperature and salt concentration, is very important in the separation processes of proteins. This paper is aimed at the optimization of the adsorption and elution processes of the whey proteins bovine serum albumin (BSA) and β-lactoglobulin (β-lg) by surface response analysis. The adsorption conditions are accounted by experimental data and the elution conditions by experimental equilibrium data and simulation using the *software SimuCromWin*.

2. THEORY

2.1. Model

Consider a fixed-bed adsorption column packed with uniform porous, spherical, solid adsorbents. The process is isothermical and the concentration gradients in the radial direction of the bed are negligible. Suppose also that the local equilibrium exists for each component between the pore surface and the stagnant fluid phase in the macropores. According to GU et al (1993) assuming that the diffusional and mass transfer coefficients are constant and independent of the mixing effects of the components and based in the others assumptions the following equations can be obtained from the differential mass balances for each component in the bulk-fluid and the particle phases.

$$-D_{bi}\frac{\partial^2 C_{bi}}{\partial Z^2}+v\frac{\partial C_{bi}}{\partial Z}+\frac{\partial C_{bi}}{\partial t}+\frac{3k_i(1-\varepsilon_b)}{\varepsilon_b R_p}\left(C_{bi}-C_{pi,R=R_P}\right)=0 \tag{1}$$

$$(1-\varepsilon_p)\frac{\partial C^s_{pi}}{\partial t}+\varepsilon_p\frac{\partial C_{pi}}{\partial t}-\varepsilon_p D_{pi}\left[\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2\frac{\partial C_{pi}}{\partial R}\right)\right]=0 \tag{2}$$

with the initial and boundary conditions

$$t=0,\quad C_{bi}=C_{bi}(0,Z),\qquad C_{pi}=C_{pi}(0,R,Z) \tag{3, 4}$$

$$Z=0,\quad \frac{\partial C_{bi}}{\partial Z}=\frac{v}{D_{bi}}\left(C_{bi}-C_{fi}(t)\right)\qquad Z=L,\quad \frac{\partial C_{bi}}{\partial Z}=0 \tag{5, 6}$$

$$R=0,\quad \frac{\partial C_{pi}}{\partial R}=0\qquad R=R_p,\quad \frac{\partial C_{pi}}{\partial R}=\frac{k_i}{\varepsilon_p D_{pi}}\left(C_{bi}-C_{pi,R=R_p}\right) \tag{7, 8}$$

In the Equation (2), C^s_{pi} is the concentration of component i in the solid phase of the adsorbents based on the unit volume of the solids, excluding pores.

The partial differential equation system above can be transformed into the following dimensionless forms.

$$-\frac{1}{Pe_{Li}}\frac{\partial^2 c_{bi}}{\partial z^2}+\frac{\partial c_{bi}}{\partial z}+\frac{\partial c_{bi}}{\partial \tau}+\xi_i\left(c_{bi}-c_{pi,r=1}\right)=0 \tag{9}$$

$$\frac{\partial}{\partial \tau}\left[(1-\varepsilon_p)c^s_{pi}+\varepsilon_p c_{pi}\right]-\eta_i\left[\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c_{pi}}{\partial r}\right)\right]=0 \tag{10}$$

Initial condition

$$\tau=0,\quad c_{bi}=c_{bi}(0,z),\qquad c_{pi}=c_{pi}(0,r,z) \tag{11, 12}$$

Boundary condition

$$z=0,\quad \frac{\partial c_{bi}}{\partial z}=Pe_{Li}\left(c_{bi}-C_{fi}(\tau)/C_{0i}\right) \tag{13}$$

For frontal adsorption $C_{fi}(\tau)/C_{0i}=1$

For elution $$C_{fi}(\tau)/C_{0i}=\begin{cases}1 & 0\le\tau\le\tau_{imp}\\ 0 & else\end{cases}$$

$$z=1,\qquad \frac{\partial c_{bi}}{\partial z}=0 \tag{14}$$

$$r=0\qquad \frac{\partial c_{pi}}{\partial z}=0 \qquad\qquad r=1,\qquad \frac{\partial c_{pi}}{\partial r}=Bi_i(c_{bi}-c_{pi,r=1}) \tag{15, 16}$$

were: $c_{bi}=\frac{C_{bi}}{C_{0i}};\quad c_{pi}=\frac{C_{pi}}{C_{0i}};\quad c_{pi}^{s}=\frac{C_{pi}^{s}}{C_{0i}};\quad r=\frac{R}{R_p};\quad z=\frac{Z}{L};\quad \tau=\frac{vt}{L};\quad Pe_{Li}=\frac{vL}{D_{bi}};$

$$Bi_i=\frac{k_i R_p}{\varepsilon_p D_{pi}};\quad \eta_i=\frac{\varepsilon_p D_{pi} L}{R_p^2 v};\quad \xi_i=\frac{3Bi_i\eta_i(1-\varepsilon_b)}{\varepsilon_b}.$$

2.1.1. Isotherm Model

Langmuir isotherm

This model was proposed by Langmuir, in 1916, for adsorption in a gas-solid system. A constant heat of adsorption and finite number of surface adsorption sites was assumed. With these assumptions maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface (LANGMUIR, 1916; JACOBSON et al, 1984). It is written as:

$$Q=\frac{q_m C}{k_d+C} \tag{17}$$

In this model, q_m is the monolayer saturation capacity of the adsorbent and $k_d = \frac{1}{b}$ is the equilibrium constant of adsorption.

3. EXPERIMENTAL

3.1. Materials

BSA and β-lg were purchased from Sigma (St. Louis, MO, USA). BSA is a globular ellipsoid, with a molecular mass of 69000 Da, and isoeletric point (pI) of 4.7. The β-lg has a molecular mass of 32000 Da, when in dimmer form, and isoeletric point of 5.2 (CAYOT and LORIENT, 1997). The HIC support was Streamline Phenil® purchased from Amershan Pharmacia Biotech (Uppsala, Sweden). It was packed in a column HR 5/5 purchased from Amershan Pharmacia Biotech (Uppsala, Sweden). Sodium phosphate (monobasic), sodium phosphate (dibasic) and sodium sulfate were of analytical degree.

3.2. Apparatus

Frontal chromatography was carried out using an Äkta Purifier System (MOD 10X) from Amershan Pharmacia Biotech (Uppsala, Sweden) with a UV detector fixed in 280 nm and at flow rate fixed in 2.0 mL/min. The equipment was controlled using the software Unicorn v.1.0 (Amersham Biosciences). The system temperature was controlled by immersion of the column in a thermostatic bath (Quimis, precision of ± 0.1 K).

3.3. Procedures

3.3.1. Frontal experiments to obtain equilibrium data

The method that is fast, the most convenient and accurated for isotherm determination in fixed bed column is frontal analysis (FA) (JACOBSON et al, 1984, GUIOCHON et al, 1994 and GUIOCHON, 2002). The adsorbed amount Q_{i+1} is given by:

$$Q_{i+1} = Q_i + \frac{(C_{i+1} - C_i)(V_{F,i+1} - V_0)}{V_a} \quad (18)$$

where Q_i and Q_{i+1} are the amounts of compound adsorbed by the column packing after the i^{th} and the $(i+1)^{th}$ step, in equilibrium with the concentrations C_i and C_{i+1}, respectively, $V_{F,i+1}$, is the retention volume at the inflection point of the $(i+1)^{th}$ breakthrough curve, V_0 is the column void volume, and V_a is the volume of adsorbent in the column.

A column HR 5/5 packed with Streamline Phenil® was initially equilibrated with 50 column volumes (CV) of the carrier buffer (20 mM phospahate, pH 7,0) containing various concentrations of sodium sulfate (50, 300, 600 and 900 mM) and in the various temperatures (283.15 K, 293.15 K, 303.15 K and 313.15 K). A 4x4 factorial design was applied to developed the experiments. Single component solutions, with concentrations of (0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0) mg mL^{-1}, for each proteins, were perfused through the column, until the complete formation of the breakthrough curve.

Finished each experiment, the column was regenerated with 30 CV of a buffer (20 mM phosphate, pH 7.0).

3.3.2. Estimation of the model parameters

The mass transfer resistance parameter was calculated according to the equation used by TRUEI et al (1992):

$$Sh = \frac{k_i 2R_p}{D_{pi}} = 2 + 1.45\,\mathrm{Re}^{1/2}\,Sc^{1/3} \tag{19}$$

where the Schmidt number, Sc, is given by

$$Sc = \frac{\mu}{\rho D_{pi}} \tag{20}$$

The molecular diffusivity, D_{pi}, was derived from the following correlation cited by KACZMARSKI et al (2001):

$$D_{pi} = 8.31x10^{-8} \frac{T}{\mu M_i^{1/3}} \tag{21}$$

The axial dispersion coefficient can be calculated using empirical correlation presented by CHUNG and WEN (1968), where Peclet number, Pe, is calculated as:

$$Pe_L = \frac{vL}{D_b} \frac{L}{2R_p \varepsilon_b} \left(0.2 + 0.011 \mathrm{Re}^{0.48}\right) \tag{22}$$

with the Reynolds number (Re)

$$\mathrm{Re} = \frac{2R_p \varepsilon_b v \rho}{\mu} \tag{23}$$

3.3.3. Model Simulation

A computational program, written in Visual Basic, was developed for model simulation. The finite differences method was applied to discretize the Equation (9) while Equation (10) was discretized using the orthogonal collocation method, using symmetric polynomials (FINLAYSON, 1980). The resultant algebraic equations system was solved applying the Gauss-Siedel method.

4. RESULTS AND DISCUSSION

Process optimization, in both cases, was carried out by the surface response analysis, based on experimental factorial design and regression analysis (BOX et al, 1978).

4.1. Adsorption process

Experimental values of Q, for the equilibrium solution concentration of 8 $mg.mL^{-1}$, with its respective adsorption conditions are presented in Table 1 for BSA and β-lg.

Table 1: Experimental values of Q with its respective adsorption conditions.

		Q ($mg.mL^{-1}$)	
Salt Concentration (M)	Temperature (K)	BSA	B-lg
0.05	283.15	60.4	42.4
	293.15	68.7	47.1
	303.15	74.9	49.3
	313.15	79.5	55.9
0.3	283.15	65.5	47.6
	293.15	74.2	49.3
	303.15	77.3	54.3
	313.15	86.6	62.5
0.6	283.15	68.2	52.8
	293.15	87.6	61.0
	303.15	99.5	69.1
	313.15	102.5	72.4
0.9	283.15	87.1	68.3
	293.15	97.5	78.0
	303.15	106.5	94.0
	313.15	115.6	124.2

In systems in which variables change, there is a strong interest in assessing the effects of the factors on the behavior of measurable quantities (the response). Such an assessment is possible through regression analysis. Thus, from the above results, it is useful to use a mathematical model which is a function of the main process variables to each type response. The determined models for BSA and β-lg are presented by Equations 2 and 3, respectively.

$$Q = -185.409 + 0.847\mathrm{T} + 37.353C_s \tag{24}$$

$$Q = 10.829 + 0.1309\mathrm{T} - 481.175C_s + 65.058C_S^2 + 1.569TC_S \tag{25}$$

where: T is the temperature (K) and C_S is the salt concentration (M).

Tables 2 and 3 show the regression values, deviations and correlation coefficients for BSA and β-lg regression models, respectively, obtained by statistic package SAS (SAS Institute Inc., 1989). For BSA the F test values is 116.20 and for β-lg, 56.40. This means a confidence larger than 99% in both cases. Therefore, the

regression is more significant than the deviations, so that the adjusted models are adequate and with good determination coefficients.

Table 2: Results of regression analysis for BSA.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F test[a]
Regression	2	3706.951	1853.476	
Deviation	13	207.365	15.951	116.2
Total variation	15	3914.316		
Determination coefficient	0.975			

[a] $F_{0,99,\,2,13} = 6.70$

Table 3: Results of regression analysis for β-lg.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F test[a]
Regression	4	6280.066	1570.017	
Deviation	11	306.182	27.834	56.4
Total variation	15	6586.24		
Determination coefficient	0.974			

[a] $F_{0,99,\,4,11} = 5.67$

With the models, it is possible to generate surfaces that represent the influence of variables on the responses. Figure 1 depicts the effect of salt concentration as well as the temperature on the adsorption process of BSA onto Streamline Phenil resin. It can be observed that the adsorbed amount of BSA is affected by the increasing of temperature and salt concentration. The same result can be observed for β-lg in the Figure 2. In spite of the adsorbed amount of proteins increase with the raising of the temperature, in the experiments the highest temperature was 313.15 K due to the possibility of protein denaturation.

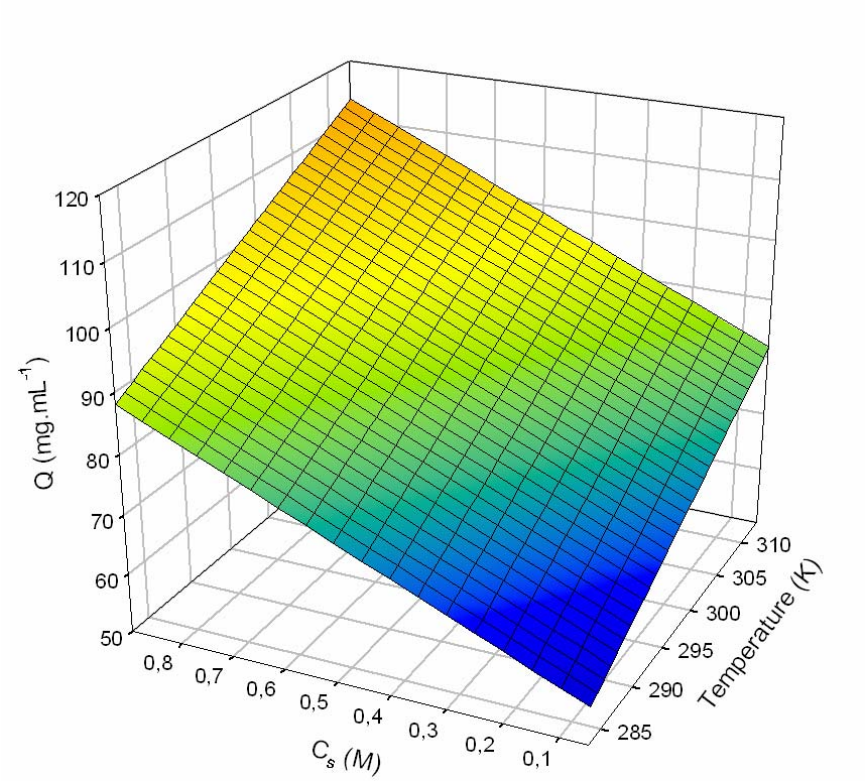

Figure 1: Effect of T and C_S on the adsorption process of BSA onto Streamline Phenil resin for C = 8.0 $mg.mL^{-1}$.

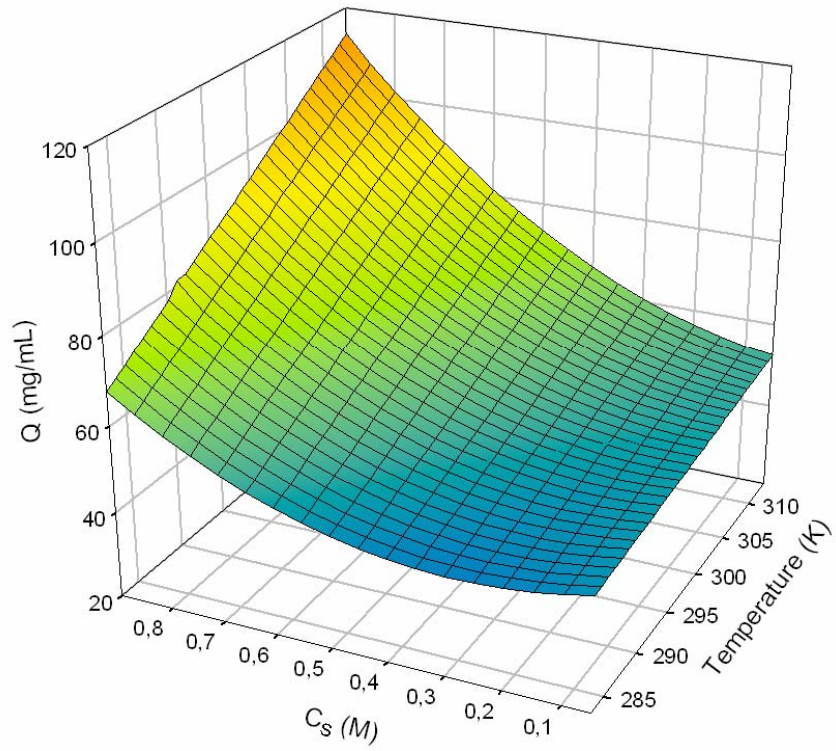

Figure 2: Effect of T and C_S on the adsorption process of β-lg onto Streamline Phenil resin for C = 8.0 $mg.mL^{-1}$.

3.2. Elution process

3.2.1. Modeling of the Langmuir isotherm parameters

The Langmuir isotherm model (Equation 17) was fitting to the experimental data using the statistical package SAS® and the estimative of the parameters were obtained.

From these estimatives, it was made a regression analysis to account the influence of the factors, temperature and salt concentration, on the behavior of the parameters q_m and k_d. The determined models for these parameters are presented by Equations 26-29, for BSA and β-lg respectively.

$$q_m = -29805.90 - 287.40\mu + 202.41T - 0.338T^2 \quad (26)$$

$$k_d = -2218.93 - 36.70\mu + 15.36T - 0.026T^2 \quad (27)$$

$$q_m = -327.78 + 48.49\mu + 1.46T \quad (28)$$

$$k_d = -6.68 + 37.15\mu - 12.07\mu^2 0.050T - 0.103T\mu \quad (29)$$

Tables 4-7 show the regression values, deviations and correlation coefficients for BSA and β-lg regression models, respectively, obtained by statistic package SAS (SAS Institute Inc., 1989). According the results of test F the confidence is larger than 99% in all cases. Therefore, the regression is more significant than the deviations, so that the adjusted models are adequate and with good determination coefficients.

Table 4: Results of regression analysis for q_m for BSA.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F test[a]
Regression	3	153239.8	51079.94	
Deviation	12	47968.95	3997.412	12.78
Total variation	15	201208.75		
Determination coefficient	0.901			

[a] $F_{0,99,\,3,12} = 5.95$

Table 5: Results of regression analysis for k_d for BSA.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F test[a]
Regression	3	2399.712	799.9042	
Deviation	12	401.0497	33.42081	23.93
Total variation	15	6586.24		
Determination coefficient	0.899			

[a]$F_{0,99,\,3,12} = 5.95$

Table 6: Results of regression analysis for q_m for β-lg.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F test[a]
Regression	2	8094.314	4047.157	
Deviation	13	536.4352	41.26425	98.08
Total variation	15	8630.7492		
Determination coefficient	0.940			

[a]$F_{0,99,\,2,13} = 6.70$

Table 7: Results of regression analysis for k_d for BSA.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F test[a]
Regression	4	59.3526	14.8381	
Deviation	11	6.5527	0.5957	24.91
Total variation	15	6586.24		
Determination coefficient	0.921			

[a]$F_{0,99,\,4,11} = 5.67$

Therefore, from the Equations the Langmuir isotherm model, for BSA and β-lg, can be written as:

$$Q = \frac{(-29805.90 - 287.40C_S + 202.41T - 0.338T^2)C}{(-2218.93 - 36.70C_S + 15.36T - 0.026T^2) + C} \tag{30}$$

$$Q = \frac{(-327.78 + 48.49C_S + 1.46T)C}{(-6.68 + 37.15C_S - 12.07C_S^2 + 0.050T - 0.103TC_S) + C} \tag{31}$$

3.2.2. Simulation

In laboratorial study and industrial application, column operations often include both adsorption and desorption. The elution stage is widely used to recover biomolecules from a dilute solution after they adsorbed onto a column (GU et al, 1991). This stage can be influenced by the process variables as salt concentration, temperature, flow rate and feed concentration. Some of these variables can be affect the values of isotherm parameters which influence directly the separation of the adsorbed compounds. In the elution process, in columns, the separation degree can be account by the resolution value, R_S. This can be calculated by the following equation (FISCHER, 1974):

$$R_s = 2\frac{(V_{R2} - V_{R1})}{(W_{H1} + W_{H2})} \tag{32}$$

where: V_{R1} is the retetion volume for pick 1, V_{R2} is the retetion volume for pick 2, W_{H1} is the widh of the pick 1 e W_{H2} is the widh of the pick 2.

The resolution is directly proportional to separation degree. In this work the conditions of the elution process, temperature and salt concentration, for proteins, BSA and β-lg, was optimizaded by surface response analysis. The response variable in this case was the resolution value. The process was simulated using isotherm parameters calculated through the models obtained above and the software SimuCromWin.

First, the elution were simulated according by the factorial design 2 x 2 with a central point. The conditions utilized in this stage were salt concentration (0,05 M, 0,45M and 0,2M) and temperature (283,15K, 303,15K and 293,15K). The results shown that the resolution increases with the increase of the temperature and salt concentration. Then, others experiments were done changing the temperature and salt concentration values in the range studied experimentally. With the results the regression analysis was made and a model for resolution in function of temperature and salt concentration was

obtained. The determined model and the for the regression values, deviations and determination coefficient are presented by Equation 33 and Table 8, respectively.

$$R_s = 1.9407 - 0{,}00669T - 8.3325C_S + 0{,}029TC_S \quad (33)$$

Table 8: Results of regression analysis for R_S.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F test[a]
Regression	3	0.6688043	0.2229348	
Deviation	13	0.6963486	0.05356528	4.16
Total variation	16	6586.24		
Determination coefficient	0.831			

[a] $F_{0,95,\,3,13} = 3.41$

The Figure 3 shows the generated surface from the model above. It represents the influence of the variables, temperature and salt concentration, on the resolution value. It can be seen that the resolution is strongly affected by change in salt concentration when the temperature is constant. This behavior is approximately the same when the salt concentration is constant and the temperature changes, principally to salt concentration of 0.9M. Inside the studied range the best conditions for separation of BSA and β-lg is 0.85 M and 313.15K.

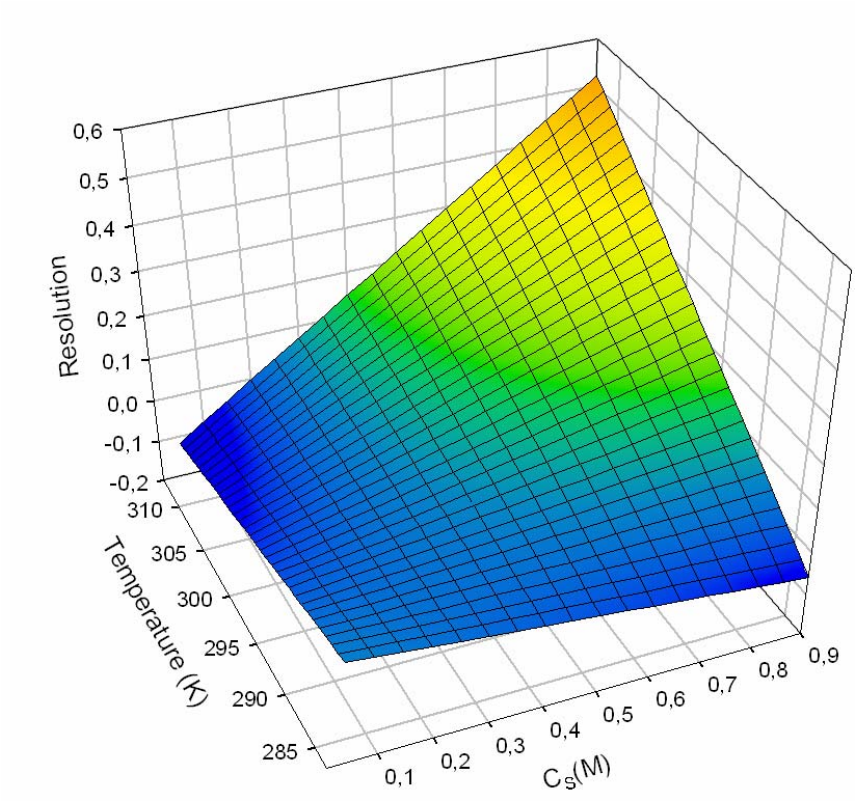

Figure 3: Effect of temperature and salt concentration on resolution.

The Figure 4 presents the result of simulation of the elution using the best elution's conditions obtained by optimization. In this case, the elution process had a good resolution (peak separation) and a total process time of, approximately, 120 min. However, this process can be interrupted at 20 min, if the desired product is only the compound of the first peak.

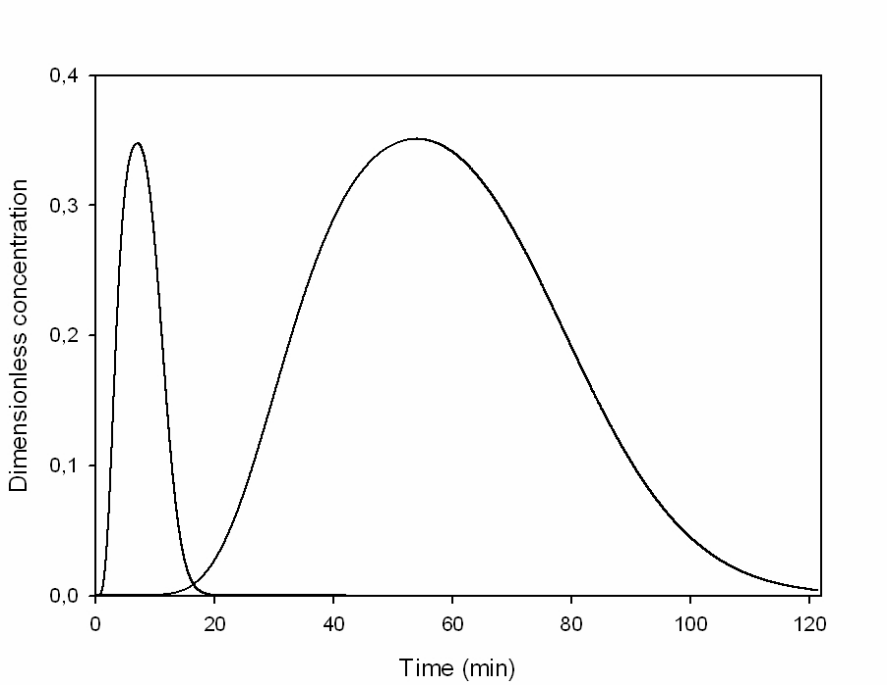

Figure 4: Two-component elution (Temperature = 313.15 K and Salt concentration = 0.85 M). First peak => BSA and Second peak => β-lg.

4. CONCLUSIONS

The surface response methodology is a powerful tool to find the best range of operating conditions. Through this procedure, it is possible to identify which variables are the most significant to lead the process to operate at high performance. The simulation is another powerful tool in process optimization due to decrease of time lost and costs. With the experimental data, the *software SimuCromWin* and the surface response analysis was possible determinate the best conditions for adsorption and dessorption processes. The values found to adsorption process were 0.9 M and 313.15 K and to dessorption process were 0.85 M and 313.15 K, for both proteins.

NOMENCLATURE

Bi	Biot number for component i
C_{bi}	Bulk phase concentration of component i
C_{fi}	Feed concentration profile of component i
C_{oi}	Concentration used for nondimensionalization, max $\{C_{fi}(t)\}$
C_{pi}	Concentration of component i in the stagnant fluid phase inside particle macropores
C_s	Salt concentration of mobile phase
C_i	Concentration at equilibrium
c_{bi}	C_{bi}/C_{0i}
c_{pi}	C_{pi}/C_{0i}
D_{bi}	Axial dispersion coefficient of component i
D_{pi}	Effective diffusivity of component i
k_d	The equilibrium constant of adsorption
L	Column length
n	Heterogeneity parameter of the Toth isotherm
Pe_{Li}	Peclet number for component i
q_m	Monolayer saturation capacity of the adsorbent
R	Radial coordinate for particle
Re	Reynolds number
R_p	Particle radius
r	R/R_P
Sc	Schmidt number
Sh	Sherwood number
v	Interstitial velocity

V_a	Volume of adsorbent in the column
V_0	Column void volume
$V_{F,\ i+1}$	Retention volume at the inflection point
Z	Axial coordinate variable
z	Z/L

Greek symbols

ε_b	Bed void volume fraction
ρ	Density of the mobile phase
μ	Viscosity of mobile phase
ε_p	Particle porosity
η_i	Dimensionless constant for component i
ξ_i	Dimensionless constant for component i
τ	Dimensioless time
τ_{imp}	Dimensionless time duration for the restangular pulse of the sample

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CONCLUSÕES GERAIS

A partir dos resultados obtidos por meio do estudo da adsorção de BSA e β-lg em uma resina de interação hidrofóbica (Streamline Phenil) foi possível observar que este processo é dependente da concentração de sal, e que o processo é espontâneo e entropicamente dirigido.

Na simulação do processo de adsorção, foi observado um bom ajuste entre as curvas de ruptura experimentais e calculadas, principalmente para os modelos de isoterma de Toth, Langmuir, Jovanovic e Langmuir Exponencialmente Modificado. Ao se utilizar o modelo de Langmuir foi obtido o menor tempo de simulação para as duas proteínas.

O modelo de taxa geral mostrou-se apropriado tanto para o estágio de adsorção como para o estágio de eluição. O *software SimuCromWin* que já era uma ferramenta rápida e eficiente para a simulação de processos cromatográficos, tornou-se mais completo após as alterações feitas neste trabalho. Foram incluídas opções para a escolha de outros modelos de isotermas além do modelo de Langmuir, e a opção para a simulação do processo de eluição. Com estas modificações, podem ser realizados estudos para a seleção do melhor modelo de isoterma para simulações em cromatografia preparativa, além da otimização dos parâmetros do processo de separação.

A metodologia de superfície de resposta e a simulação são poderosas ferramentas a serem utilizadas na otimização de processos. Com a análise de superfície de resposta e o *software SimuCromWin* foi possível determinar as melhores condições para os processos de adsorção e dessorção das proteínas de soro de queijo, BSA e β-lg, em cromatografia de interação hidrofóbica utilizando como adsorvente a resina Streamline Phenil.

ANEXO

METODOLOGIA PARA DETERMINAÇÃO DE ISOTERMAS DE ADSORÇÃO POR ANÁLISE FRONTAL

1. Adsorvente e coluna

Nos experimentos foi utilizado o adsorvente hidrofóbico Streamline Phenyl (Amershan Pharmacia Biotech, Uppsala, Sweden), com densidade e tamanho de partícula de 1,2 g/mL e 100 μm a 300 μm, respectivamente. Sua matriz possui um núcleo de quartzo cristalino, forma esférica e porosidade de 0,62 sendo estável em todos os tampões aquosos comumente usados.

A coluna utilizada foi a HR 5/5 (Amershan Pharmacia Biotech, Uppsala, Sweden), empacotada com, aproximadamente, 0,58 g de resina Streamline Phenyl, embebida em solução tampão Fosfato 20 mM, pH 7,0, por aproximadamente 8 h, a qual foi adicionada à coluna por meio de uma seringa, resultando em um leito de 6 cm de altura e 5 mm de diâmetro. A porosidade final do leito foi de 0,58.

2. Adsorvato

As proteínas albumina de soro bovino (BSA) e β-lactoglobulina (β-lg) fornecidas pela Sigma (St. Louis, MO, USA) foram utilizadas como adsorvato. BSA é uma proteína globular, com uma massa molecular de 69000 Da, e ponto isoelétrico (pI) de 4.7. A β-lg tem uma massa molecular de 32000 Da, quando na forma de dímero, e ponto isoelétrico de 5.2 (CAYOT and LORIENT, 1997).

3. Dados de equilíbrio

Os experimentos foram conduzidos em um cromatógrafo Äkta Purifier MOD 10X (Amershan Pharmacia Biotech, Uppsala, Sweden), usando um detector UV com comprimento de onda fixado em 280 nm e vazão de 2,0 $mL.min^{-1}$.

Uma coluna HR 5/5 empacotada com Streamline Phenil foi inicialmente equilibrada com 50 volumes de coluna (CV) do tampão fosfato 20 mM, pH 7,0 contendo as concentrações de sulfato de sódio (50, 300, 600 e 900 mM) e nas temperaturas (283.15 K, 293.15 K, 303.15 K e 313.15 K) pré-determinadas. Os experimentos foram desenvolvidos num esquema fatorial 4 x 4 em delineamento inteiramente casualizado. A coluna foi imersa num banho termostático com recirculação, fornecido pela Quimis (Brasil) cuja precisão é de ± 0,1 K, para que a temperatura da mesma fosse mantida constate. A figura 1 mostra o esquema experimental montado.

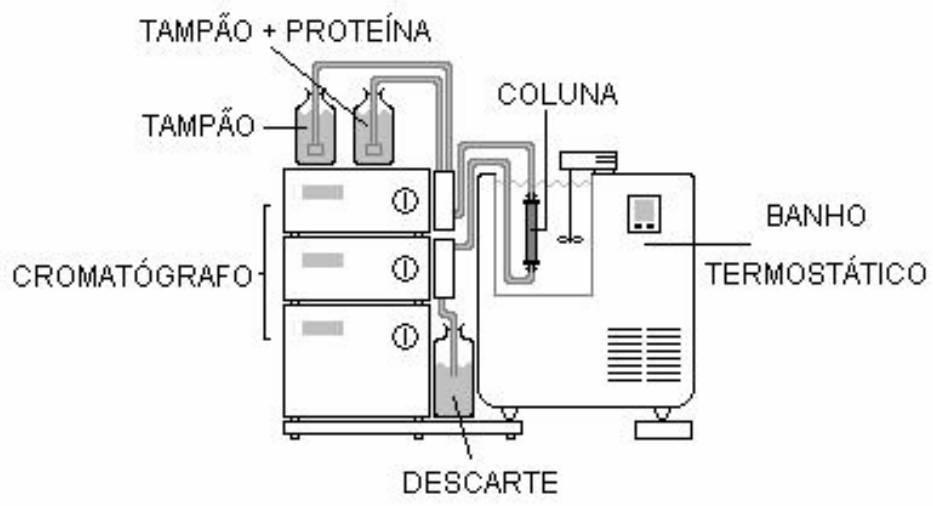

Figura 1: Representação esquemática da montagem experimental utilizada.

Soluções monocomponentes, com concentrações de (0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0) mg mL^{-1}, para cada uma das proteínas. A solução de 0,25 $mg.mL^{-1}$ foi percolada, através da coluna até a completa formação da curva de ruptura. Depois da concentração de proteína na saída da coluna estar estabilizada, a concentração da

solução de alimentação foi ajustada para o próximo valor do planejamento experimental. Esta nova solução foi percolada pela coluna até a formação de uma nova curva de ruptura. Este procedimento foi realizado sucessivamente até concentração de 8 $mg.mL^{-1}$, sendo que o perfil da curva obtida no experimento segue o comportamento descrito na Figura 2. Terminado cada experimento, a coluna foi lavada e regenerada com 30 volumes de coluna do tampão fosfato 20 mM, pH 7,0.

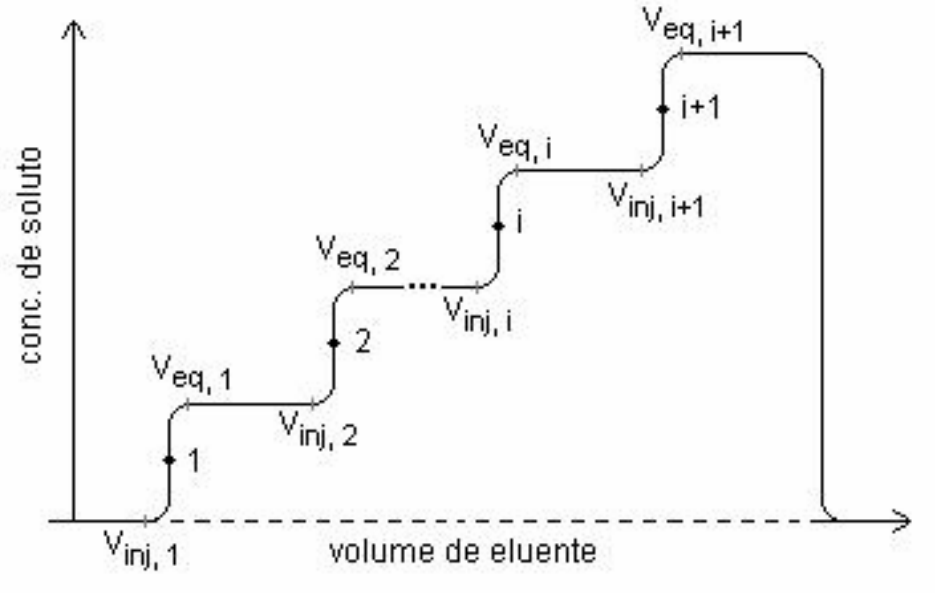

Figura 2: Perfil obtido no experimento de análise frontal.

A quantidade de proteína adsorvida, Q_{i+1} ($mg.mL^{-1}$) é obtida por:

$$Q_{i+1} = Q_i + \frac{(C_{i+1} - C_i)(V_{F,i+1} - V_0)}{V_a} \quad (1)$$

em Q_i e Q_{i+1} são as quantidade de soluto adsorvido por volume de adsorvente no *i-ésimo* e no *(i+1)-ésimo* passo, quando no equilíbrio com as concentrações C_i e C_{i+1}, respectivamente. $V_{F,i+1}$, é o volume entre o ponto de inflexão i e a *(i+1)-ésima* curva de ruptura, V_0 é o volume de vazio da coluna, e V_a é o volume de adsorvente na coluna.

Em estudos anteriores (JACOBSON et al, 1984; JACOBSON et al, 1987; GUIOCHON et al, 1994) a mudança de concentração de um passo para outro era realizada de modo manual. Neste trabalho, foi usada uma programação em gradiente,

em que a solução tampão pura e a solução tampão com proteína (no valor máximo de concentração trabalhada) eram misturadas pelo cromatógrafo, de acordo com o programa pré-estabelecido. Por exemplo, para a concentração de proteína na entrada da coluna ser igual a 4,0 mg.mL-1, a solução-tampão e a solução com proteína eram misturadas em partes iguais pelo equipamento.

Com isso, em alguns passos do experimento, o equilíbrio há muito já havia sido alcançado, e a nova concentração ainda não havia começado a ser percolada através da coluna. Este fato poderia levar a erros no cálculo da quantidade de soluto adsorvida, uma vez que o valor de $V_{F,i+1}$ estaria superestimando o valor real.

Utilizou-se então um artifício para que fosse realizada a correção desse valor. O valor de $V_{F,i+1}$ foi calculado de acordo com a seguinte expressão:

$$V_{F,i+1} = V_{i+1} - V_i - \left(V_{inj,i+1} - V_{eq,i}\right)$$

Em que $V_{F,i+1}$ é o volume ajustado percolado através da coluna do ponto *i* até o ponto *i+1*, V_{i+1} é o valor real percolado através da coluna até o ponto *i+1*, V_i é o valor real percolado através da coluna até o ponto *i*, $V_{inj,i+1}$ é o volume em que a concentração de proteína é alterada para o passo *i+1* e $V_{eq,i}$ é o volume em que a curva de ruptura do passo *i* é estabilizada (atinge o equilíbrio).

Desse modo é possível se calcular o valor da quantidade de soluto adsorvida em cada passo, determinando-se os pontos experimentais das isotermas de adsorção nas condições estudadas.