

Separation of Bovine Serum Albumin Using Chromatographical Column: Parameters and Simulation

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Abstract

A liquid-solid chromatography of Bovine Serum Albumin (BSA) on (diethylaminoethyl-cellulose) DEAE-cellulose adsorbent is worked experimentally, to study the effect of changing the influent concentration of (0.125, 0.25, 0.5, and 1 mg/ml) at constant volumetric flow rate $Q=1\text{ml/min}$. And the effect of changing the volumetric flow rate (1, 3, 5, and 10 ml/min) at constant influent concentration of $C_0=0.125\text{mg/ml}$. By using a glass column of (1.5cm) I.D and (50cm) length, packed with adsorbent of DEAE-cellulose of height (7cm). The influent is introduced in to the column using peristaltic pump and the effluent concentration is investigated using UV-spectrophotometer at 300C and 280nm wavelength. A spread (steeper) break-through curve is gained at lean feed concentration of 0.125mg/ml, while the flow rate greater than (3ml/min) is almost the same. So it is better to work at low volumetric flow rate between (1-3) ml/min. The equilibrium-dispersion model of liquid-solid chromatography for a binary mixture, related with Langmuir isotherm correlation is used in the modeling of this work. The resulting model is solved numerically by using MATLAB V.6.5 program.

Introduction

Chromatography is a very special separation process for a multitude of reasons! First of all, it can separate complex mixtures with great precision. Even very similar components, such as proteins that may only vary by a single amino acid. Second, chromatography can be used to separate delicate products since the conditions under which it is performed are not typically serving (Hubble, 2004).

A mixture of various components enters a chromatography process and is allowed to come into contact with two phases, the stationary phase is contained in a column where the mobile phase moves in a controlled manner relative to the stationary phase, carrying with it any material that may prefer to mix with it. To determine the differences in the concentration of mobile phase (solute), a detector is used which can give a curve of the separated substance. So, the name chromatography stems from this, where each separated

substance has different color on the stationary phase and gives different curve from the detector (Stock, 1974, Encyclopedia, 1992 and Hubble, 2004).

Frontal chromatography analysis has widely used for large scale separations of industry. It is made in which conditions are chosen that the desired component very quickly saturates the column and emerges from the column shortly after the feed is saturated. Impurities, on the other hand, are strongly retained by the adsorbent and feeding is continued until the front of the adsorbed impurities approaches the column outlet (Simpson, 2001). Ion-exchange technique of chromatography is used to represent the frontal analysis. That was because of the simplicity of this technique where separation take place according to charge of both solid adsorbent and the separated molecules. There can be two types of functional groups of exchangers, covalently attached to the support beads. These are called anion exchangers (resin with positive functional groups) or cation

exchangers (resin with negative functional groups) (Raply and Walker, 1998, and Freifelder, 1998). Representing the frontal analysis with Ion-exchange chromatography by a mathematical model is an important feature not only for experimental data but, also for the process design and optimization. Experiments need to be carried out to get the mass-transfer parameters for modeling and simulation and comparisons with experimental results indicated that the prediction was acceptable.

Experimental Work

A system of Bovine Serum Albumin (BSA) protein adsorbed onto DEAE-cellulose was chosen to study the frontal analysis and compare it with the theoretical work (Alias, 2007).

Bovine Serum Albumin protein of 66430 molecular weight from Sigma Chemical Company dissolved in (5mg/ml) Tris-HCl buffer of 98% purity mead by BBH Company to produce the mobile phase of the chromatography system at (pH 7). DEAE-cellulose anion-exchanger from Sigma Chemical Company was used as stationary phase of the chromatography system. Five samples of different weights of DEAE-cellulose was prepared to evaluate the equilibrium isotherm, each one of them is put in a beaker with (30ml) of BSA solution of concentration (0.12mg/ml) and pH of 7. The samples were shaken at (30oC) for about (26 hours) then the concentrations were calculated using UV-spectrophotometer. The amount of solute adsorbed into DEAE-cellulose q in (mg/g DEAE-cellulose) was found according to the equilibrium relation:

$$q = \frac{(C_o - C)V_L}{W_A} \tag{1}$$

The adsorption isotherm was obtained by plotting q vs. C . A glass beaker of (250ml) capacity was prepared for batch experiment. A (50ml) of BSA-solution was added to the beaker containing (3.37mg) of DEAE-cellulose. At zero time, DEAE-cellulose of certain weight was added. Samples were taken every 10 minutes during the experiment and concentration is measured using UV-spectrophotometer.

Frontal chromatography experiments were carried out to find the breakthrough curve of BSA adsorbed to DEAE-cellulose bed. To study the effect of changing the initial concentration (0.125, 0.25, 0.5, and 1 mg/ml) at constant volumetric flow rate of (1ml/min) and changing the volumetric flow rate (1, 3, 5, and 10 ml/min) at constant initial concentration of (0.125mg/ml). The procedures of all run experiments are as follows:

DEAE-cellulose was poured into the column at (7cm) length.

BSA-solution was prepared at the desired concentration for each run using Tris-HCl buffer.

The solution was introduced to the top of the column using peristaltic pump at a certain flow rate.

Samples were collected directly from the bottom of the column using tubes for every (1ml) and then the concentration was measured using UV-spectrophotometer at 280 nm wavelength as in (fig. 1).

The resulting breakthrough curves were determined by plotting effluent concentration (C/C_o) against dimensionless time τ . (Alias, 2007).

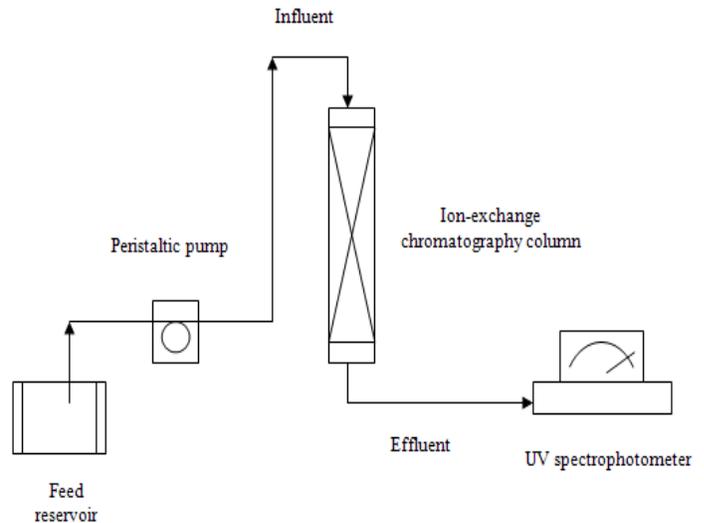


Fig.1 the experimental apparatus of BSA adsorbed in DEAE-cellulose bed.

Modeling and Simulation

Batch Adsorber

Batch adsorber analyses are important to calculate the external mass transfer coefficient k_f (m/s) of batch experiment and pore diffusivity D_p (m^2/s). The formulation of batch adsorber based on (Ahmed, 2006). For batch adsorber, the mathematical model with pore diffusion model is:

- Mass balance in fluid-bulk phase:

$$V_L \frac{dC_i}{dt} + \frac{3W_A}{\rho_p R_p} k_f (C_{bi} - C_{Pr=R_p}) = 0 \tag{2}$$

Where:

V_L = volume of fluid in the batch adsorber

W_A = mass of adsorbent

- Mass balance inside particle phase:

$$\varepsilon_p \frac{\partial C_p}{\partial t} + \rho_p \frac{dq}{dt} = \varepsilon_p D_p \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial C_p}{\partial r} \right) \quad (3)$$

- Initial conditions and boundary conditions

$$t=0 \quad C_b = C_o: \quad C_p = 0 \quad q = 0$$

$$r=0: \quad \left(\frac{\partial C_p}{\partial r} \right)_{r=0} = 0 \quad \left(\frac{dq}{dr} \right)_{r=0} = 0$$

$$r=R_p \quad \varepsilon_p D_p \left(\frac{\partial C_p}{\partial r} \right)_{r=R_p} = k_f [C_b - C_{p,r=R_p}]$$

- In case of using Langmuir isotherm correlation:

$$q = q_m \frac{C_p b}{1 + C_p b} \quad (4)$$

Where C_b and C_p are the solute concentration in the fluid-bulk and particle phase, respectively.

The external mass transfer coefficient for the solute adsorbed at certain particle size and optimum agitation speed, can be obtained by the analytical solution for equation (2.29) where at $t = 0$, $C_{p,r=R_p} = 0$ and $C_b = C_o$, hence:

$$k_f = -\frac{R_p \rho_p V_L}{3W_A t} \ln \left(\frac{C_t}{C_o} \right) \quad (5)$$

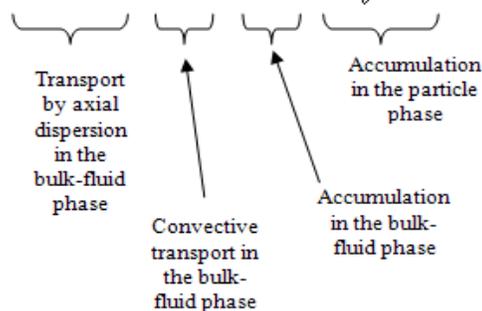
Where C_o , C_t are the solute concentration at time zero and time (t) and obtained from the typical concentration decay curve.

Modeling of Liquid-Solid Chromatographical Column

The formulation of Chromatographical column is Based on (Volker, 1999, and Eggers, 2003), the governing equations can be obtained from differential mass balances of the bulk-fluid phase and the particle phase, respectively, for component i.

- Continuity equation in the bulk-fluid phase:

$$-D_{bi} \frac{\partial^2 C_{bi}}{\partial Z^2} + v \frac{\partial C_{bi}}{\partial Z} + \frac{\partial C_{bi}}{\partial t} + \frac{1-\varepsilon_b}{\varepsilon_b} \frac{\partial q_i}{\partial t} = 0 \quad (6)$$



Using C_{pi} , the concentration in the stagnant fluid-phase (in the macropores), and writing the expression of interfacial flux leads to:

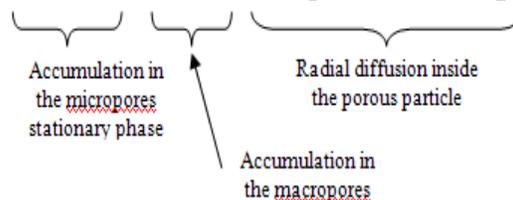
$$\frac{\partial q_i}{\partial t} = \frac{3k_{fi}}{R_p} (C_{bi} - C_{pi,R=R_p}) \quad (7)$$

$$-D_{bi} \frac{\partial^2 C_{bi}}{\partial Z^2} + v \frac{\partial C_{bi}}{\partial Z} + \frac{\partial C_{bi}}{\partial t} + \frac{3k_{fi}(1-\varepsilon_b)}{\varepsilon_b R_p} [C_{bi} - C_{pi,R=R_p}] = 0 \quad (8)$$

- Continuity equation inside the macropores:

The particle phase continuity equation in spherical coordinates is:

$$(1-\varepsilon_p) \frac{\partial C_{pi}^*}{\partial t} + \varepsilon_p \frac{\partial C_{pi}}{\partial t} - \varepsilon_p D_{pi} \left[\frac{1}{R^2} \frac{\partial}{\partial R} \left(R^2 \frac{\partial C_{pi}}{\partial R} \right) \right] = 0 \quad (9)$$



- Initial and boundary conditions

The initial and boundary conditions may be represented by the following equations:

Initial condition ($t = 0$):

$$C_{bi} = C_{bi}(0, Z) = 0 \quad (10)$$

$$C_{pi} = C_{pi}(0, R, Z) = 0 \quad (11)$$

Boundary conditions:

$$Z = 0: \frac{\partial C_{bi}}{\partial Z} = \frac{v}{D_{bi}}(C_{bi} - C_{oi}) \quad (12)$$

$$Z = L: \frac{\partial C_{bi}}{\partial Z} = 0 \quad (13)$$

$$R = 0: \frac{\partial C_{pi}}{\partial R} = 0 \quad (14)$$

$$R = R_p: \frac{\partial C_{pi}}{\partial R} = \frac{k_{fi}}{\varepsilon_p D_{pi}}(C_{bi} - C_{pi,R=R_p}) \quad (15)$$

Model Parameters of Fixed-Bed Chromatography Column

a) Correlations to estimate (molecular diffusivity) D_m : Polson proposed the equation for high molecular weight solutes:

$$D_m = 9.4 \times 10^{-15} \left(\frac{T}{\mu M_w^{1/3}} \right) \quad (16)$$

Where:

T is temperature (K), μ is the viscosity of the solution (Pa-s) and M_w is the molecular weight of the solute (Polson, 1950).

b) Correlation to estimate mass transfer coefficient k_f in fixed-bed adsorption:

$$Sh = 2.0 + 1.45Sc^{1/3} Re^{0.5} \quad (Re < 100) \quad (17)$$

Where:

$Sh = 2R_p k_f / D_m$ (Sherwood number), $Sc = \mu / (\rho D_m)$ (Schmidt number), and $Re = 2R_p \rho v / \mu$ (Reynolds number). μ is the fluid (water) viscosity, and ρ is the fluid (water) density (Jian, 2004, and Montesinos, 2005).

c) Correlation to estimate D_b the axial dispersion coefficient can be estimated from the following correlation:

$$D_b = \frac{2R_p v \varepsilon}{2.0 + 0.011 Re^{0.48}} \quad (Re < 10) \quad (18)$$

Where:

v is the interstitial velocity along the column $= 4Q / \varepsilon \pi d^2$ (Q is the volumetric flow rate) and $Re = 2R_p \rho v / \mu$ (Jian, 2004, and Montesinos, 2005).

Simulation Program

The model equations must be solved numerically. The numerical method of lines (MOL) is used in order to solve the coupled system of PDE obtained either from

batch adsorber or chromatography column. The bulk-fluid phase and the particle equations are first discretized using the FE (finite element) and the OC (orthogonal collocation) methods, respectively. The resulting ODE system is solved using an existing ODE solver provided by MATLAB v-6.5. The code of MATLAB m-files for both batch adsorber and chromatography column are presented in (Ahmed, 2006). This program is used for both single and multicomponent systems after changing some parameters. The model parameters used in the simulation program are listed in tables 1 and 2.

Results and Discussion

Isotherm Determinations and Constants

The adsorption isotherm of BSA adsorbed onto DEAE-cellulose of 0.35mm-particle size at (30°C and 5mg/ml Tris-HCl buffer pH 7) is shown in (fig. 2), where the initial concentration of BSA-solution was (0.12 mg/ml). By fitting the equilibrium data with Langmuir isotherm correlation, the results were good ($q_m = 2.022$ mg/g) and ($b = 13.083$ ml/mg).

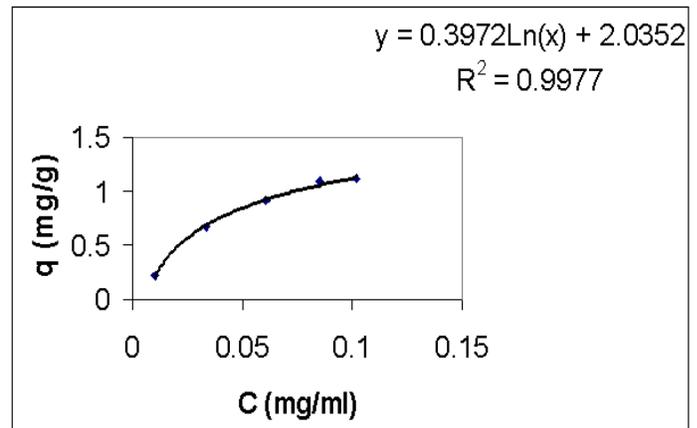


Fig. 2: Adsorption isotherm of BSA onto DEAE-cellulose at (30°C)

Intraparticle Diffusivity coefficient D_p Estimations

In order to find Intraparticle Diffusivity coefficient D_p , it was needed to find the mass transfer coefficient k_f in batch adsorption process and then the numerical solution of batch adsorber model is applied.

The external mass transfer k_f coefficient in batch adsorber was computed from initial rate data, (i.e. from the concentration decay curve fig. 3) using equation (5):

$$k_f = - \frac{R_p \rho_p V_L}{3W_A t} \ln \left(\frac{C_t}{C_o} \right) \quad (19)$$

Where C_o , C_t are the solute concentration at time zero and time t . For accurate estimation of k_f , samples were taken after 10, 20, and 30 minutes and analyzed immediately. The average calculated value of k_f was 1.0683×10^{-6} m/s. The pore diffusivity coefficient was derived from the typical concentration decay curve by iterative search technique predicted on the minimization of the difference between experimental and predicted data from pore diffusion model (Ahmed, 2006). The results are shown in (fig. 3). The pore diffusivity coefficient for BSA solute according to (fig 3) is 0.078×10^{-10} m²/s.

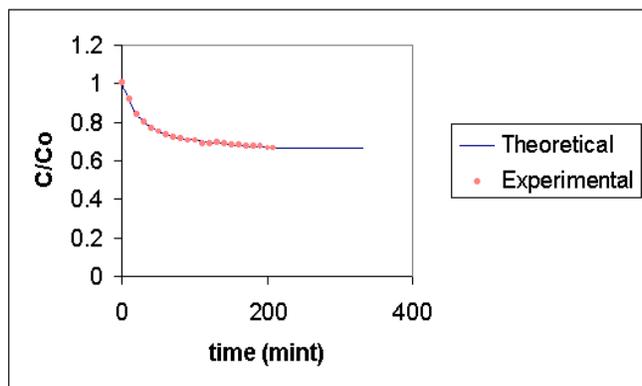


Fig. 3 Comparison of the measured concentration-time data at (30°C) with that predicted by pore diffusion model in batch adsorber.

Chromatography Experiments

- Figures (4 to 7) were obtained from the adsorption of BSA solution on DEAE-cellulose adsorbent at different concentrations of influent C_o that at $Q = 1$ ml/min. As follows:

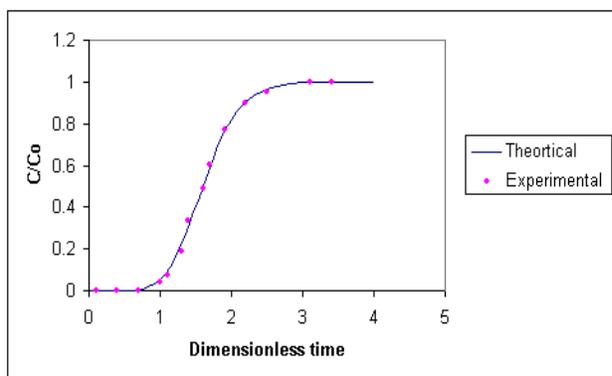


Fig. 4 Break-through curve of BSA on DEAE-cellulose at $C_o = 0.125$ mg/ml

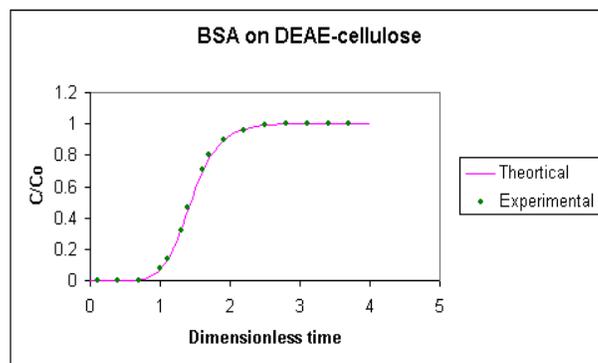


Fig. 5 Break-through curve of BSA on DEAE-cellulose at $C_o = 0.25$ mg/ml

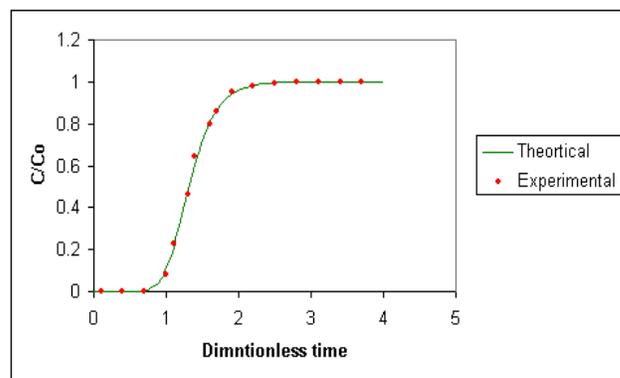


Fig. 6 Break-through curve of BSA on DEAE-cellulose at $C_o = 0.5$ mg/ml

Figure 8 represents the effect of changing the initial concentration at (0.125, 0.25, 0.5, and 1 mg/ml). From this figure, the change in inlet solute concentration markedly affects the shape and position of the break-through curve. The higher the solute concentration the faster the break-through. However, the quantity of solution to be supplied is large when the solute concentration is low to arrive the optimum value.

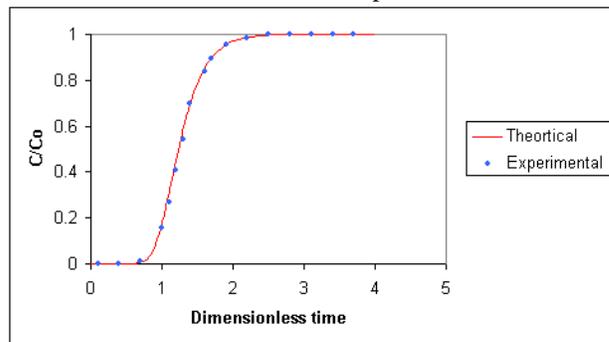


Fig. 7 Break-through curve of BSA on DEAE-cellulose at $C_o = 1$ mg/ml

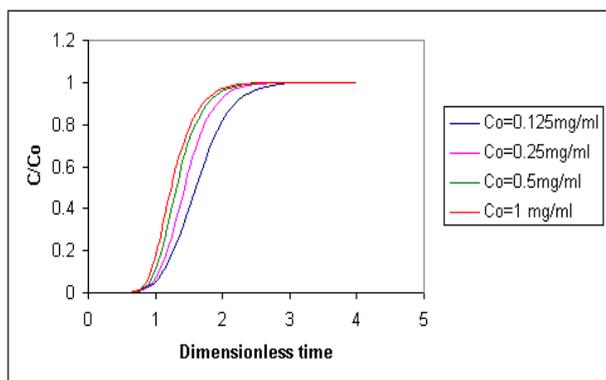


Fig. 8 Break-through curve of BSA on DEAE-cellulose at different initial concentrations

- Figures (9 to 12) were obtained from the adsorption of BSA solution on DEAE-cellulose adsorbent at different volumetric flow rates Q that at $C_o = 0.125\text{mg/ml}$ initial concentration. As follows:

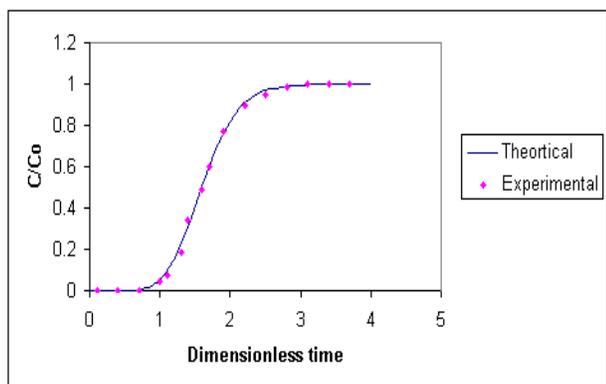


Fig. 9 Break-through curve of BSA on DEAE-cellulose at $Q = 1\text{ml/min}$

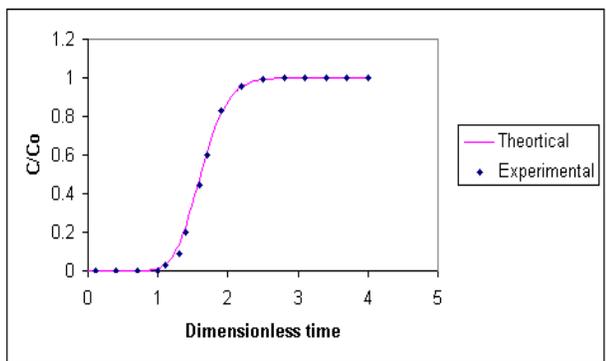


Fig. 10 Break-through curve of BSA on DEAE-cellulose at $Q = 3\text{ml/min}$

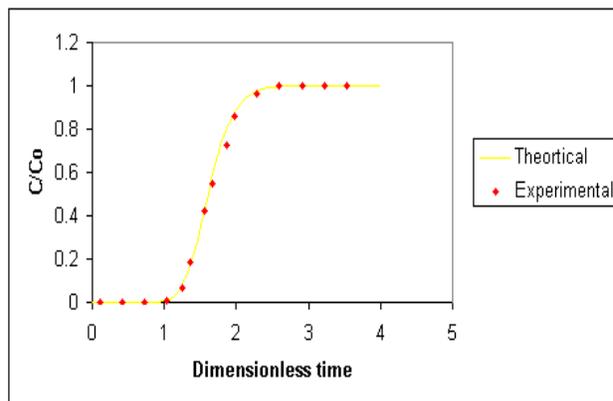


Fig. 11 Break-through curve of BSA on DEAE-cellulose at $Q = 5\text{ml/min}$

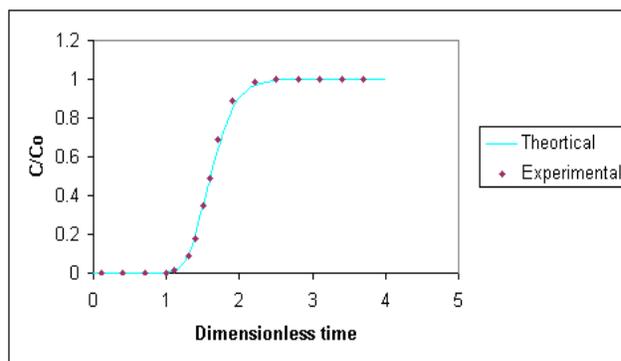


Fig. 12 Break-through curve of BSA on DEAE-cellulose at $Q = 10\text{ml/min}$

Figure 13 represents the effect of changing the volumetric flow rate of the mobile phase for (1, 3, 5, and 10 ml/min). Flow rate affects the film mass transfer coefficient. If R_p is unchanged, for correlation 17, k_f is proportional to $v^{0.5}$. The flow rate also affects the axial dispersion coefficient according to correlation 18. For higher flow rates, Break-through is faster and poor adsorption efficiencies will result. When the flow rate is low, an increase in the spreading of the break-through curve will occur and an increase in the time that the solute is in contact with the stationary phase, allowing more time for adsorption and permitting near-local equilibrium conditions. That the break point is unchanged which is about 0.6 of initial concentration.

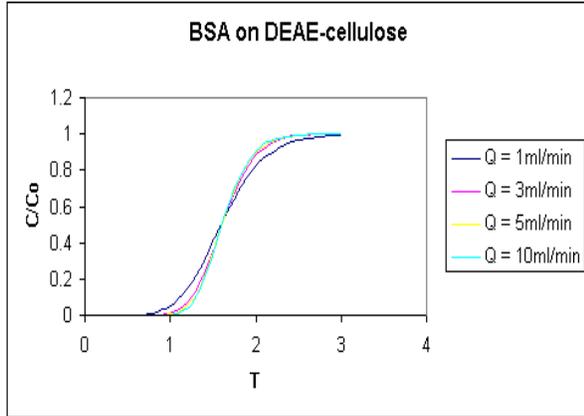


Fig. 13 Break-through curve of BSA on DEAE-cellulose at different volumetric flow rates

- All the resulting break-through curves obtained to the adsorption of BSA on DEAE-cellulose bed, are almost sharp and the break point occur about 0.557 from the initial concentration for high volumetric flow rates, and the adsorption process is faster at about (30-40) minutes. From the observations of fig. 13, the break-through curves obtained for volumetric flow rates of (3-10 ml/min) are almost the same (*i.e.* the break-through curves are almost compatible).

Simulation Data	
Parameter	Value
R_p	0.35×10^{-3} m
ε_p	0.6
b	13.083 m ³ /kg
q_m	0.002022 kg/kg
D_p	0.078×10^{-10} m ² /s
k_f	1.0683×10^{-6} m/s
ρ_p	309.5 kg/m ³
V_L	0.05×10^{-3} m ³
W_A	3.37×10^{-3} kg
C_o	0.12 kg/m ³

Table 1 Batch adsorber of BSA on DEAE-cellulose system at 30°C.

Table 2 Data used in the simulation program of chromatography column	
Parameter	Value
Column length L	0.07 m
Column diameter d	0.015 m
Particle diameter R_p	0.35×10^{-3} m
Column porosity ε	0.775
Particle porosity ε_p	0.6
Axial dispersion D_b	Correlation (18)
Pore diffusivity D_p	0.078×10^{-10} m ² /s
Molecular diffusivity D_m	Correlation (16)
External mass transfer coefficient k_f	Correlation (17)
Maximum adsorption capacity q_m	0.002022 kg/kg
Langmuir isotherm constant b	13.083 m ³ /kg
Bed density ρ_p	309.5 kg/m ³

Conclusions

1. Comparisons were made between the results obtained from the model program and that gained from experimental work data, the agreement was very good, because all the parameters used in the model program were evaluated either experimentally or by empirical relations of grate accuracy. This program is applied in all cases of liquid-solid chromatography systems with a very good accuracy.
2. For the experimental work data or literature data, Langmuir isotherm correlation gives good results. This correlation is a good assumption for the system of very low concentrations of influent.
3. From the experimental worked data it was concluded that it is preferable to work with lean feed and low volumetric flow rate. In those conditions, a steeper or spread break-through curve will be gained which lead to increase the solute recovery efficiency. Also the effect of increasing the volumetric flow rat more than (3ml/min) is not efficient.
4. Whenever the break-through curve is spread (steeper), the break-through time point will occur with less C/C_o ratio
5. Axial dispersion coefficient D_b , which is affected by the flow rate of the system is more significant at lower flow rate and is less noticeable as the rate is increased, but the pore diffusivity D_p and external mass transfer coefficient k_f is not that influential parameter because it represent the diffusivity inside the pore of particle, which its size is very small.

Nomenclature

Notation

a_i	Constant from Langmuir correlation ($q_m \cdot b$)
b_i	Langmuir isotherm constant (m^3/kg)
Bi_i	Biot number of mass transfer for component i , ($k_f R_p / \varepsilon_p D_{pi}$). (-)
C_{oi}	Concentration used for nondimensionalization, $\max\{C_{fi}(t)\}$.
C_t	Concentration of solute at any time. (kg/m^3)
C_{bi}	Bulk-Fluid phase concentration of component i , (kg/m^3)
C_{fi}	Feed concentration profile of component i a time dependant variable (kg/m^3)
C_{pi}	Concentration of component i in the stagnant fluid phase inside the particle macropores (kg/m^3)
C_{pi}^*	Concentration of component i in the solid phase of particle (kg/m^3)
c_{bi}	$= C_{bi}/C_{oi}$
(-)	
c_{pi}	$= C_{pi}/C_{oi}$
(-)	
c_{pi}^*	$= C_{pi}^*/C_{oi}$
(-)	
D_{bi}	Axial dispersion coefficient of component i (m^2/s)
D_{pi}	Pore diffusivity coefficient of component i (m^2/s)
D_m	Molecular diffusivity (m^2/s)
d	Inner diameter of a column (m)
k_{fi}	External mass transfer coefficient of component i (m/s)
L	Column length (m)
M_w	Molecular weight.
Pe_{Li}	Peclet number of axial dispersion for component i , vL/D_{bi} .
Q	Volumetric flow rate of mobile phase (m^3/s)
q	Concentration of solute in stationary phase bed (kg solute/kg sorbent)
q_m	Maximum adsorption capacity (kg solute/kg sorbent)
R	Radial coordinates for particle
Re	Reynolds number for sphere, ($2R_p \rho v / \mu$).
R_p	Particle radius (m)
r	$= R/R_p$
Sc	Schmidt number, ($\mu / \rho D_m$).
Sh	Sherwood number for sphere, ($2R_p k_f / D_m$).
t	Time (s)
T	Temperature (C)
V_L	the final volume of the sample put in beaker (ml)
z	Dimensionless axial coordinate Z/L .

Greek

ε_b	Column or bed porosity.
ε_p	Particle porosity.
v	Interstitial velocity, ($4Q/\varepsilon_b \pi d^2$).
τ	Dimensionless time.
μ	Fluid (water) viscosity (Pa.s).
ρ	Fluid (water) density (Kg/m^3).
ρ_p	Particle density (Kg/m^3).

Subscripts and superscript

P	Particle phase.
m	Mono layer.
i	i -th component.
j	j -th component.
*	Particle phase concentration

Abbreviations

BSA	Bovine Serum Albumin.
FDM	Finite Difference Method.
FEM	Finite Element Method.
OCM	Orthogonal Collocation Method.
ODE	Ordinary Differential Equation.
PDE	Partial Differential Equation.

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فصل مصّل الدم الحيواني باستخدام عمود الكروماتوكراف: المعاملات والمحاكات

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الخلاصة

كروماتوكراف الصلب-السائل لمصّل الدم الحيواني (Bovine Serum Albumin) على مادة (Diethyleaminoethiel-cellulose) او (DEAE-cellulose) قد تم اشتغاله عملياً. لدراسة تأثير التغير البتركيز الداخل الى العمود (0.125, 0.25, 0.5, 1mg/ml) and بثبوت معدل الجريان الحجمي (1ml/min) ثم دراسة تأثير التغير بمعدل الجريان الحجمي (1, 3, 5, and 10ml/min) بثبوت التركيز الداخل (0.125mg/ml). العمل التجريبي قد تم باستخدام عمود زجاجي نصف طول قطره الداخلي (1.5سم) وطوله (50 سم)، محشو بمادة DEAE-cellulose (diethylaminoethyl-cellulose) بطول (7 سم). السائل الداخل يدخل إلى العمود باستخدام مضخة من نوع peristaltic pump ، وتركيز السائل الخارج تم قياسه باستخدام جهاز الأشعة فوق البنفسجية UV-spectrophotometer بدرجة حرارة (30°C) بطول موجي (280 nm). كان تركيز السائل الداخل (0.125mg/ml) الأفضل لأنه يعطي break-through point قليلة مع break-through curve منبسط. هذا التركيز (0.125mg/ml) استخدم في تطبيق تجارب أخرى لمعدلات جريان مختلفة. هنا، معدلات الجريان الحجمي التي هي اقل من (3ml/min) تكون تقريبا متطابقة لنفس الظروف التشغيلية. لهذا من الأفضل لهذا النظام أن يعمل على معدل جريان حجمي قليل يتراوح بين (1-3ml/min) بنفس الظروف. موديلات التوازن المتشنت للكروماتوغراف السائل الصلب لخليط ثنائي المربوط بعلاقة لانكمور ايزوثيرم قد استخدم في اشتقاق الموديل الرياضي لهذا العمل. الموديل الناتج قد حل عددياً باستخدام برنامج . MATLAB v.6.5