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RESEARCH ARTICLE

Design of continuous fixed bed column for adsorption of Bovine Serum Albumin on nanostructured alumina based on Batch adsorption studies.

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Abstract

This paper presents the design of fixed bed adsorption column based on the adsorption of Bovine Serum Albumin (BSA) protein on nanostructured alumina by conducting batch adsorption and continuous adsorption studies. The obtained experimental data is used to fit the Freundlich mathematical model, on the basis of results obtained from batch adsorption studies continuous column studies were carried out using a trial experimental packed column to a known bed height with nanostructured alumina as bioadsorbent. The adsorption zone height, overall mass transfer coefficient, residence time and number of mass transfer units has been determined from column studies based on the breakthrough curve.

INTRODUCTION

The protein separations using adsorption phenomena on to the solid surface have wide application in industries. The adsorption of the proteins on to the solid surfaces depends on the interaction of proteins. The high concentration of proteins from the crude protein can be separated by ion exchange chromatography, gel filtration, membrane filtration, and adsorption chromatography. In adsorption chromatography the separation of proteins depends on the specific surface area of the adsorbent used and iso electric point (pI) of the adsorbent and the desired proteins. The application of adsorbents having large surface area plays an important role in the adsorption chromatography in a relatively inexpensive and very effective. Nanostructured ceramic particles possess high specific surface area and good mechanical strength, which can be employable as adsorbent material. The adsorption of proteins can be performed by a batch method and continuous mode. The effectiveness of adsorption depends on the pI of adsorbent, adsorbate and concentration of crude protein. The batch method is easily applied in laboratory research and in practice for adsorption of proteins in small volumes. The column performance is convenient for industrial scale applications where large volumes of proteins to be separated in less time. Lots of experimental methods are available for adsorption of proteins on solid surface using continuous column mode of adsorption, Avci et.al.2000 investigated adsorption of BSA to the weak anion exchanger DE52 in packed columns, Skidmore (Skidmore et.al.1990) used packed bed adsorption using cation exchanger S. Sepharose FF as a adsorbent to separate bovine serum albumin (BSA) and lysozyme,. Melter et.al. 2008 characterized the retention behaviour of a monoclonal antibody on a weak cation exchanger, Fractogel EMD COO−(s). Packed-bed experiments were performed by McCreath et.al. 1997 using the cation-exchanger SP-PVA-FEP to purify lysozyme from egg white, and the anion-exchanger Q-PVA-FEP to purify G6PDH from a clarified homogenate of bakers' yeast. Kim et.al.2002 studied the separation of the two variants of BLG (A and B) using a cellulose-based anion exchanger bearing water soluble polycations. The specificity of column performance is that it allows for multiple repetitions of service and regeneration cycles, which means multiple use of the same amount of alumina. Therefore, the continuous column

performance is widely used in protein adsorption. Successful design and operation of a continuous fixed bed column requires an experimental breakthrough curve, therefore a continuous column experiments were conducted for adsorption of BSA on nanostructured alumina using a trail experimental column and from the results obtained a continuous column is designed using mass transfer coefficient.

Materials

Nanostructured alumina ceramic structure was synthesized at BHEL/EPD using spary pyrolysis method was used as a adsorbent, BSA procured from HIMEDIA is used as a standard protein.

Experimental

Preparation of Bovine Serum Albumin (BSA)

The standard solution of Bovine Serum Albumin (1mg/ml) was prepared by adding 100 mg of BSA protein available in powder form to 100 ml of phosphate buffer solution at pH 7.0.

Batch Adsorption of BSA

Known amount of nanostructured alumina were taken in different centrifuge tubes and 10 ml of BSA protein solution is added to each of centrifuge tubes respectively at pH 7.0. The contents in the centrifuge tubes were mixed well by continuously shaking the tubes for 45 min and centrifuged at 10000 rpm for 20 in at room temperature. The supernatant collected was subjected for Lowry's method to determine the total protein adsorbed.

Similarly the experiment is repeated for different pH to know the adsorption at optimum pH and for different adsorbent concentration at optimum pH.

Continuous column studies

The continuous adsorption is carried out in a packed bed adsorption column of dia 30 mm and length 100 mm at an ambient temperature. The column was filled with dried nanostructured alumina for a bed depth of 2 mm. A BSA solution of initial concentration 1.0 mg/ml at pH 7.0 was continuously fed from top of the column at a flow rate of 60ml /h until break through point was reached. During the column run the effluent samples were intermittently collected by the fraction collector for every 5 minutes and analyzed for concentration of protein in the effluent.

3.12.8 Fixed bed absorber

The fixed bed adsorption column is designed based on the theory explained by K A Gawhane (Mass transfer operation II) and MS patil et.al.2012 for adsorption of proteins using nanostructured ceramic materials based on the values obtained by the trial experimental setup. Consider an ideal break through curve where the concentration of BSA in the effluent rises steeply with time, and then reaches to the initial concentration of BSA when the bed of the adsorbent is saturated with adsorbate.

The total solute-free solvent is plotted on the x-axis and on the solute concentration in the effluent is plotted on y - axis. Let Ls be the mass flow rate of solute-free solvent entering the bed per unit cross sectional area, with initial solute concentration Co mass solute/mass solvent. The break point concentration C_b is usually selected as 10% of Co, and the adsorption bed is considered to be saturated when the effluent concentration C_e is increased to 90% of initial concentration Co, W_a is the solute free effluent up to break point, mass/area, W_e is the solute free effluent up to exhaustion of bed, mass/area, W_a is the total effluent accumulated during the appearance of the breakthrough curve, mass / area, Z_a is the height of the adsorption zone cm, which is a part of the bed in which the concentration changes from C_b to C_e at any time, θa is the time required for the adsorption zone to move its own height down the column, after establishment of the zone, min.

$$\theta a = \frac{Wa}{Ls} - 1$$

If Θ_e is the time required for the adsorption zone to move out of the bed, min.

$$\theta e = \frac{We}{Ls} - 2$$

Z is the height of the initial height of the bed cm

Θ_f is the time required for the formation of the adsorption zone, min.

$$Za = Z\frac{\theta a}{\theta e - \theta f} - 3$$

The quantity of solute removed from the solution in the adsorption zone from the breakpoint to saturation is U gm of solute per cross sectional area of bed. This is given by the shaded area of Fig. 3.0,

$$U = \int_{Wb}^{We} (Co - C)dw - 4$$

If all the adsorbent in the adsorption zone were saturated, it would contain CoWa mass of solute/area as a result at the breakpoint, when the zone is still within the column, the fractional ability of the adsorbent in the zone still to adsorb solute is given by

$$f = \frac{U}{C0Wa} = \frac{\int_{Wb}^{We}(Co - C)dw}{CoWa} - 5$$

Limiting values of f (f = 0, all the adsorbent in the zone is completely saturated with solute
f =1, all the adsorbent in the zone are without adsorbate) any value of f between 0 to 1 corresponds to a real adsorption rate and the value of (1-f) gives the time that the amount of the solute retained in the bed which reaches saturation when the zone is moving. These limiting conditions, at least, are described by

$$\theta f = (1 - f)\theta a - 6$$

Comparing the equations 3 and 6

$$Za = Z\frac{Wa}{We - (1 - f)Wa} - 7$$

The above equation (equation 7) is used to determine the height of the adsorption bed zone, to total solids actually adsorbed can be calculate by

$$\text{Percentage of saturaton} = \frac{Z - fZa}{Z} - 8$$

The rate and amount of adsorption in the adsorption zone can be analyzed using the concept of transfer units. To determine the transfer units imagine that the solid adsorbent is moving in the upwards through the column countercurrent to the flow of adsorbent at a rate such that the adsorption zone remain stationery within the column (Fig.3). Here the adsorbent leaving the top of the column will be in equilibrium with the entering solution, the solution leaving from the bottom of the column is free from solute and equilibrium with the adsorbent entering the column.

This suggests considering an infinitely tall column, but we consider only the inlet and outlet concentration of the adsorption zone.

In the present work mass transfer coefficient is determined by using the equation described by NN. Vukojevic Medvidovlc et.al.2013 is used based on the result from the breakthrough curve. Mass transfer rate of solute from solution through the column at different bed depths, dh, is given by the following equation.

$$Gw.dc = Kya.(C - C^*)dH - 9$$

Where $Gw = is\ the\ flux\ of\ solution, \frac{kg}{min\ m^2}$

$Kya = Overall\ mass\ transfer\ coefficient\ including\ the\ diffusion\ through\ pores\ \frac{kg}{min\ m^3}$

$C^* = Equlibrium\ concentration\ of\ solute\ in\ solution\ corresponding\ to\ the\ adsorbed\ concentration\ \frac{mg}{l}$

(C - C*) is the driving force for adsorption, by integrating the equation 1 between the range from C_B to C_E and CB to C, Where C_B is the concentration of solute at breakthrough point, and C_E is the concentration of solute at exhaustion point, the height of the mass transfer zone can be evaluated.

$$hz = \frac{Gw}{Kya}\int_{Cb}^{Ce}\frac{dc}{C - C^*} - 10$$

Where h_Z is the height of the mass transfer zone in m.

For the value of h less than h_Z then h value lies between the C_b to C

$$h = \frac{Gw}{Kya}\int_{Cb}^{C}\frac{dc}{C - C^*} - 11$$

dividing equation 3/2

$$\frac{h}{hz} = \frac{Gw}{Kya}\int_{Cb}^{C}\frac{dc}{C - C^*} - -12$$

$$\frac{h}{hz} = \frac{V - Vb}{Ve - Vb} - -13$$

V_b = Volume at breakthrough point in liters

Ve = Volume at exhaust point in liters

V = Volume of the solution between V_b and V_e

Mass transfer zone can be calculated by substituting the values of K_{ya}, and G_w in equation 3, to calculate the overall mass transfer coefficient, Ka, in the continuous adsorption column for the known bed height can be calculated by the following equation

$$Ka = \frac{N * Gw}{H} --14$$

Where: $Gw\ is\ the\ mass\ flux\ of\ solution, \frac{kg}{min\ m^2}$

$Ka\ is\ the\ overall\ mass\ transfer\ coefficient\ \frac{kg}{minm^3}$

$N\ is\ the\ number\ of\ mass\ transfer\ units$

$H\ is\ the\ bed\ height\ in\ meter$

The Mass flux of solution in the packed column can be evaluated by the following relation:

$$Gw = \frac{(Q.\rho)}{(A.\epsilon)} --15$$

Where $Q\ is\ the\ flow\ rate\ of\ solution\ through\ the\ column \frac{m^3}{min}$

$\rho\ is\ the\ density\ of\ the\ liquid\ solution\ passing\ through\ the\ column\ \frac{kg}{m^3}$

$\epsilon\ is\ fixed\ porosity\ of\ the\ bed$

$A\ is\ the\ area\ of\ the\ column\ m^2$

To calculate the number of transfer units N from the results of the breakthrough curve Vukojevic Medvidovlc et al., used a graphical dependency of ln C^*/C_0 vs 1+ ln C^*/C_0 based on the assumption that film diffusion controls the mass transfer in the column. The value of 1+ ln C^*/C_0 at 0.05 ln C^*/C_0 is evaluated (Fig 8) and used in the following equation to evaluate N

$$1 + \frac{C^*}{C_0} = N(\tau - 1) --16$$

Where τ can be evaluated by knowing the break point and exhaust point in the breakthrough curve.

Results and discussion

Table 1: Conditions inside the trial experimental column maintained

Experimental conditions	Values
Bed void fraction	0.466
Bed void density kg/m^3	74
Bed depth mm	2
Flow rate l/hr	.06
Initial cationic concentration mg/ml	1.0

Effect of adsorbent concentration on adsorption of coagulant protein

10ml of BSA solution is taken in five centrifuge tubes and the quantity of nanostructured alumina is varied from 0.02 to 0.1 g. The contents in the tubes were mixed well for 45mins (Optimum time) at pH 7.0 (Optimum pH) and then centrifuged for 20 min at 10000 rpm. The supernatant collected was estimated by standard Lowry's method using spectrophotometer at 660 nm.

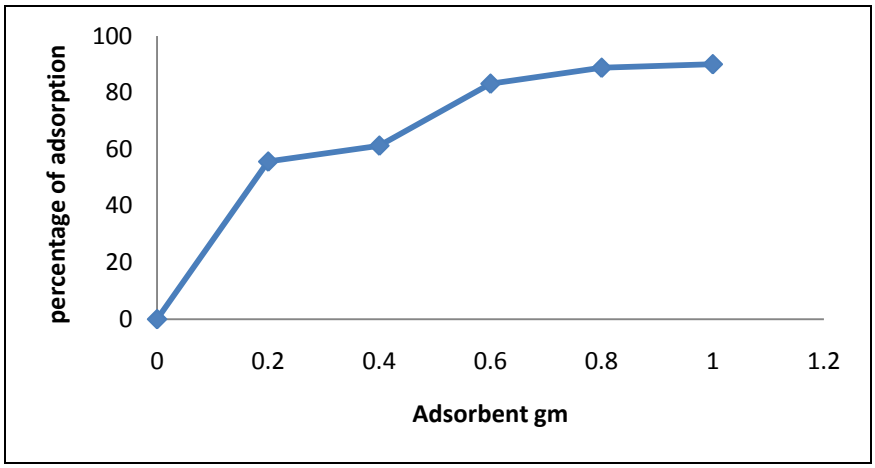

Fig1.0: Effect of adsorption concentration on alumina

The above graph (Fig 1.0) indicates there is increase in adsorption of BSA on nano nanostructured alumina with increase in adsorbent concentration. Initially the percentage of adsorption was 55% at 0.2 gm of adsorbent with further increase in the adsorbent the adsorption increased to 88% for 0.8 gm of adsorbent and finally to 90% for 1.0 gm of adsorbent.

Freundlich isotherms

The experimental data obtained from the adsorption of BSA at different adsorbent concentrations is used to fit the Freundlich isotherms models.

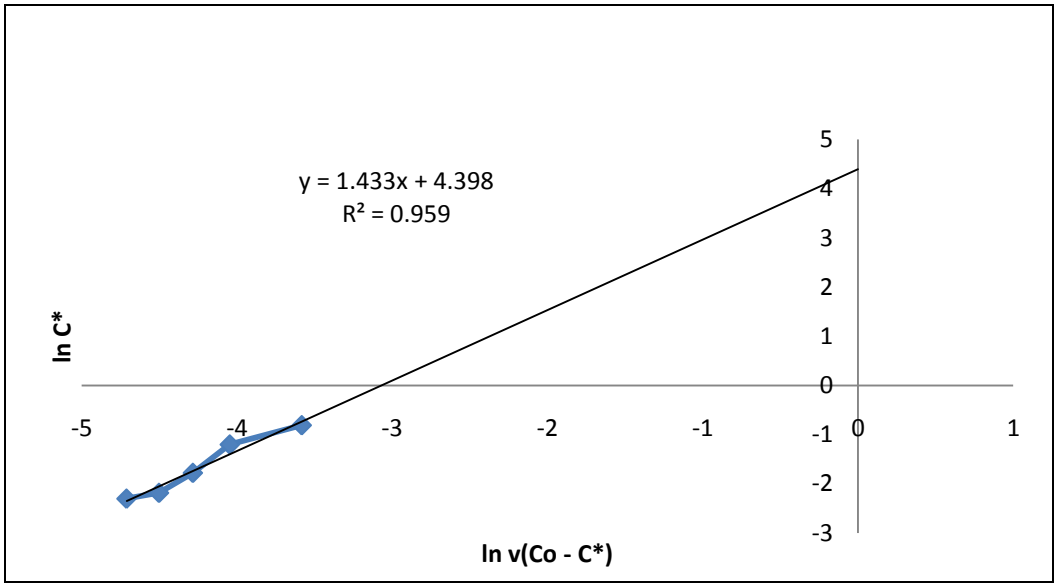

Fig 2.0: Freundlich isotherm for adsorption of alumina

The nature of the above graph (fig 3)it confirms that the adsorption of BSA on nanostructured alumina follows the freundlich isotherm model with a slope value of 1.43. The slope value of alumina suggests the alumina is suitable for adsorption of BSA.

Break through curve

Break through curve of C*/C0 V/s cumulative weight of the effluent after adsorption for the bed height of 2 mm is drawn (Fig 3.0) from the results obtained from the continuous adsorption of BSA proteins over the nanostructured alumina ceramic particles at a flow rate of 60 ml /h.

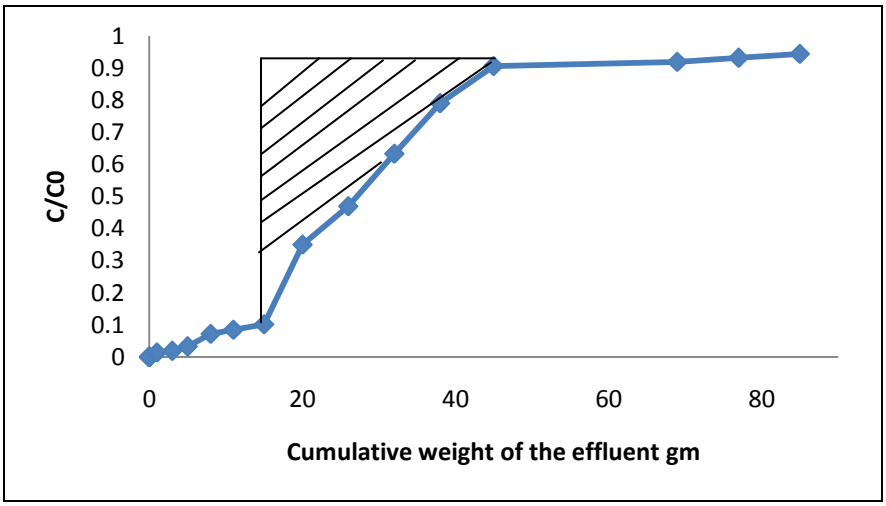

Fig 3.0: Breakthrough curve with respect to cumulative weight of effluent

From the above graph the break point is considered at 10% of (0.1mg/ml) initial concentration and exhaust point at 90% (0.9 mg/ml) of initial concentration. The breakthrough point is 15 g and exhaust point is 45g.

Calculations

1. Feed flow rate, mass per unit cross sectional area of bed, c

$$Ls = \frac{Volumeteric\ flow\ rate\ X\ Density}{Cross\ section\ area}$$

$$Ls = \frac{1\frac{ml}{min} X 1\frac{g}{cc}}{\frac{\pi d\,2}{4}}$$

Where initial dia of the column is 3.0 cm

$$Ls = \frac{1}{7.069} = 0.1414\ \frac{g}{cm2min}$$

2. The breakthrough curve is plotted for the adsorption of BSA as a standard protein over the surface of nanostructured alumina ceramic structure as a fixed bed height of 2mm in the column of dia 3.0 cm. The cumulative weight of effluent, solute-free solvent (BSA) verses solute concentration in the effluent is drawn (Fig 2.0).

   From the above graph

   W_b = 15 g and

   W_e = 45 g

3. Concentration of BSA at break point, assumed to be 10% of initial concentration

$$= 1X0.1\ \frac{mg}{ml}$$

$$= 0.1\ X\ \frac{10-3}{10-3}\frac{gS}{gSolvent}$$

$$= \frac{0.1gS}{g}solvent$$

4. Concentration of BSA at exhaustion point, assumed to be 90% of initial concentration

$$= 1X0.9\ \frac{mg}{ml}$$

$$= 0.9\ X\ \frac{10-3}{10-3}\frac{gS}{gSolvent}$$

$$= \frac{0.9gS}{g}solvent$$

5. The total effluent accumulated between the break point and exhaust point is,

$$Wa = We - Wb$$

$Wa = 45 - 25$
$Wa = 30\ g$
6. Time required to move the adsorption zone height to the downstream is

$$\theta a = \frac{Wa}{Ls}$$
$$\theta a = \frac{30}{0.1414}$$
$$\theta a = 212\ min$$
7. Time required to move the adsorption zone to out of the bed
$$\theta e = \frac{We}{Ls}$$
$$\theta e = \frac{45}{0.1414}$$
$$\theta e = 319\ min$$

8. Fraction of the area of the shaded portion of the graph (Fig) is calculated to be, f = 0.007, here f is defined as the fractional ability of the adsorbent in the zone still to absorb the adsorbate.

9. Adsorption zone height
$$Za = Z\frac{\theta a}{\theta e - \theta f}$$
$$Za = 0.2\ \frac{30}{319 - 210}$$
$$Za = 0.05\ cm$$
10. $$Degree\ of\ saturation\ of\ the\ bed = \frac{Z - fZa}{Z}$$
$$Degree\ of\ saturation\ of\ the\ bed = \frac{.2 - .007 * .005}{.2}$$
$$Degree\ of\ saturation\ of\ the\ bed = 98\%$$

Mass transfer coefficient

To determine the mass transfer coefficient the graph (Fig 3) is plotted and the value of x-axis is evaluated at the 10 % of initial concentration (0.1) of Y-axis.

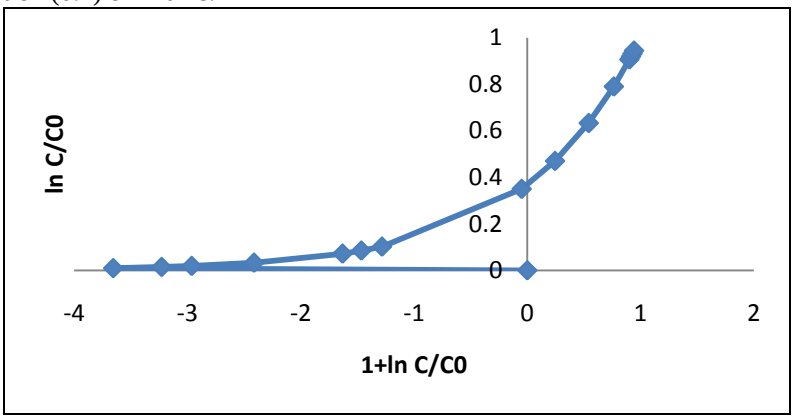

Fig 4.0: The graph of ln C/C_0 v/s 4+lnC/C_0 to determine MTC

From the graph (Fig 4.0) the value of x-axis for the 10% of initial concentration (0.1) the value of y-axis is -1.4, which is substituted in the equation to calculate the MTC. The value of y-axis at 10% concentration is chosen because in continuous adsorption the maximum adsorption takes place between breakthrough point and exhaust point, therefore the mass transfer coefficient is calculated at the breakthrough point.

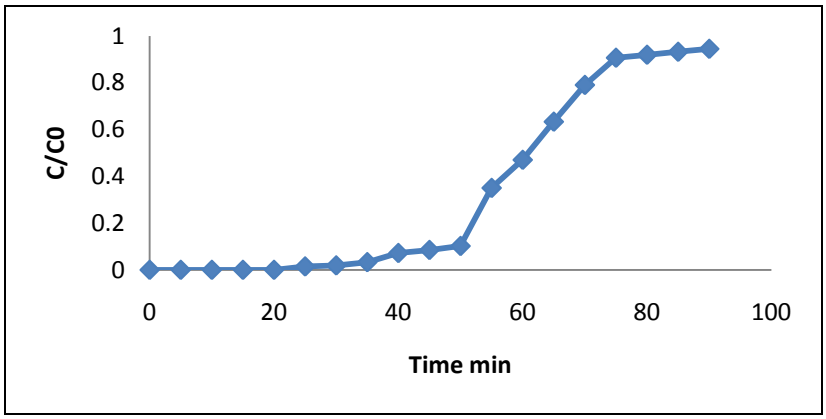

Fig 5.0: The graph of C/C_0 v/s time to determine MTC

From the breakthrough curve of C/C_0 v/s time (Fig 5.0) the adsorption increase with respect to time initially and the maximum adsorption (90%) occurs at breakthrough point and minimum adsorption takes place at exhaust point (10%) and after existence of exhaust point the adsorption become constant for some time and starts decreasing. From the above graph the breakthrough point occurs at 50min and exhaust point occurs at 75 min for the flow rate of 60ml/hr.

The residence time $\tau = t_B/t_E = 0.667$

Number of mass transfer units NTU = 3.6

Mass transfer flux $G_W = 0.0505$ Kg/s m^2

Mass transfer coefficient at 90 % adsorption = 91.06 kg/s m^3

The determined MTC, NTU and Z_a were used to design a fixed bed adsorption column.

Table 2: The calculated parameters to design a fixed bed adsorption column

S No	Properties	Values determined
1	Bed height H	$2x10^{-3}$ m
2	Break point T_B min	50 min
3	Exhaust point T_E min	75 min
4	$\tau = t_B/t_E$	0.667
5	Q	1.667E-08 m^3 /s
6	Diameter of the column	0.03 m
7	Area	0.00070695 m^2
8	Porosity	0.466
9	Density of crude protein	1000 kg/m^3
10	Value of 1+Ln C*/Co from the graph at C*/Co = 0.1	-1.2
11	Number of transfer units in the adsorption column	3.6
12	Mass transfer flux	0.050591 kg/m^2 s
13	Mass transfer coefficient $K_{La} = (N*G_W)/H$	91.06 kg/m^3s

Conclusion

The design approach, based on a batch experiment is presented. The adsorption process where nanostructured γ-alumina with low tap density and hollow morphology was used as adsorbent material adsorbs the BSA protein. Also the isotherms show that it is feasible to scale up the process. The constant wave approach was used to develop explicit equation for the breakthrough curves of fixed bed adsorption processes with the Freundlich adsorption isotherms. The column test runs for adsorption of protein were performed and from the results it was inferred that experimental and predicted breakthrough curves were in good agreement.

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