CM 4710, Biochemical Processes **Fall 2007**
Homework #8 **Fri. 09 Nov., 2007**

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1. Adsorption Separation of Immunoglobulin G using Modified Dextran (adapted from Belter, Cussler, & Hu, pg 153).

The equilibrium between immunoglobulin G and a modified dextran (the adsorbent) can be described by the Langmuir isotherm. Dextran will adsorb up to 7.8×10^{-6} moles of immunoglobulin G per cm^3 of adsorbent and the Langmuir constant, K_L, is 1.9×10^{-5} moles/liter. The adsorbent density is 1 gram of adsorbent per cm^3 of adsorbent and the void fraction of a column packed with this adsorbent is 0.40.

a) What is the total adsorption capacity of a column packed with modified dextran if the feed concentration of immunoglobulin G is 2×10^{-5} moles/liter? (use column data from b))
b) What is the mean retention time for immunoglobulin G in a column of diameter equal to 5 cm with a feed flow rate of 1 liter per minute, feed concentration of 2×10^{-5} moles/liter, and a length of 1 meter?
c) How many columns would have to be employed to recover immunoglobulin G from a feed tank of 10,000 Liter volume assuming the same feed concentration?

2. Travel Distance of Solutes A and B in a Chromatographic Column.

Problem 11.6 of the text, parts a and b. Assume that $C_A = 0.10$ mg/ml and $C_B = 0.05$ mg/ml in the liquid phase of the column at equilibrium with the adsorbed solute.

3. Determining Time to Elute a Solutes A and B from a Chromatographic Column.

Problem 11.7 of the text

Due Fri. 16 Nov., 2007.

Problem 1

Adsorption of Immunoglobulin G by Modified Dextran

Langmuir Adsorption Isotherm:

$$C_S^* = \frac{C_{S,max}^* \, C_L^*}{K_L + C_L^*}$$

$C_{S,max}^*$ = maximum adsorption capacity of dextran $\left(\frac{\text{moles IG}}{\text{g dextran}}\right)$.

$$= \left(7.8\times10^{-6}\ \frac{\text{moles IG}}{\text{cm}^3\ \text{dextran}}\right)\left(\frac{1\ \text{cm}^3\ \text{dextran}}{1\ \text{g dextran}}\right)$$

$$= 7.8\times10^{-6}\ \frac{\text{moles IG}}{\text{g dextran}}$$

C_L^* = equilibrium conc. of IG in solution $\left(\frac{\text{moles IG}}{\ell}\right)$

K_L = Langmuir constant = $1.9\times10^{-5}\left(\frac{\text{moles IG}}{\ell}\right)$

a) Total Adsorption Capacity of a Column.

$$C_S^* = \frac{(7.8\times10^{-6}\ \text{moles IG/g dextran})\left(2\times10^{-5}\ \frac{\text{moles IG}}{\ell}\right)}{\left(1.9\times10^{-5}\ \frac{\text{moles IG}}{\ell}\right) + \left(2\times10^{-5}\ \frac{\text{moles IG}}{\ell}\right)}$$

$$\boxed{= 4.0\times10^{-6}\ \frac{\text{moles IG}}{\text{g dextran}}}$$

Assuming a column described in part b,

$D = 5$ cm = column diameter.

$L = 1$ m $= 10^2$ cm

V = column volume $= \pi \frac{D^2}{4} \cdot L$

$= \pi \frac{5^2 \, cm^2}{4} \cdot 10^2 \, cm = 1{,}963.5 \, cm^3$

$= 1.964 \, l$

Capacity for Adsorption Only: Cap_A

$Cap_A = M_A \cdot C_S^*$ where M_A = mass of adsorbent

$M_A = \left(1 \frac{g \text{ dextran}}{cm^3 \text{ dextran}}\right)\left((1-\varepsilon) \frac{cm^3 \text{ dextran}}{cm^3 \text{ column}}\right)\left(1{,}963.5 \, cm^3 \text{ column}\right)$ (with ε = .4)

$= 1{,}178.1$ g dextran in column

$Cap_A = (1{,}178.1 \text{ g dextran})\left(4.0 \times 10^{-6} \frac{\text{moles IG}}{\text{g dextran}}\right)$

$= 4.71 \times 10^{-3}$ moles IG in column

Capacity for Adsorption + Dissolved in Macropores

$Cap_{A+D} = (4.71 \times 10^{-3} \text{ moles IG}) + (\varepsilon)(1{,}963.5 \, cm^3) \, C_L^*$

$= \left[4.71 \times 10^{-3} + (.4)(1{,}963.5 \, cm^3)\left(2 \times 10^{-5} \frac{\text{moles IG}}{l}\right)\right]$ $\left(\frac{10^{-3} \, l}{cm^3}\right)$

$= 4.73 \times 10^{-3}$ moles IG

* most of the capacity (>99%) is due to adsorption!

b) Mean Retention Time of IG, $\bar{t}_{IG}$

$$\bar{t}_{IG} = \frac{L}{u_i}\left[1 + \rho_p \frac{(1-\varepsilon)}{\varepsilon} f'(C_L^*)\right]$$

$$u_i = \text{interstitial velocity} = \frac{u_s}{\varepsilon} = \frac{F/A}{\varepsilon}$$

$$= \frac{(1\ \ell/\text{min})/(\pi \frac{5^2\ cm^2}{4})}{(.40)\left(\frac{1\ \ell}{10^3\ cm^3}\right)} = 127.32\ cm/min = 2.12\ \frac{cm}{s}$$

$$f'(C_L^*) = \frac{\partial C_s^*}{\partial C_L^*} = \frac{\partial}{\partial C_L^*}\left(\frac{C_{s,max}^* C_L^*}{K_L + C_L^*}\right)$$

$$= C_{s,max}^*\left(\frac{1}{K_L + C_L^*} - \frac{C_L^*}{(K_L + C_L^*)^2}\right)$$

$$= C_{s,max}^*\left(\frac{C_L + K_L}{(K_L + C_L^*)^2} - \frac{C_L^*}{(K_L + C_L^*)^2}\right)$$

$$= \frac{C_{s,max}^* K_L}{(K_L + C_L^*)^2}$$

$$= \frac{\left(7.8\times10^{-6}\ \frac{\text{moles IG}}{\text{g dextran}}\right)\left(1.9\times10^{-5}\ \frac{\text{moles IG}}{\ell}\right)}{(1.9\times10^{-5} + 2\times10^{-5})^2\left(\frac{\text{moles IG}}{\ell}\right)^2}$$

$$= .097\ \frac{\ell}{\text{g dextran}}$$

$$\bar{t}_{IG} = \frac{(1\ m)\left(\frac{10^2\ cm}{m}\right)}{2.12\ \frac{cm}{s}}\left[1 + \left(\frac{1\ \text{g dextran}}{cm^3\ \text{dextran}}\right)\left(\frac{1-.4}{.4}\right)\left(.097\ \frac{\ell}{\text{g dextran}}\right)\left(\frac{10^3\ cm^3}{\ell}\right)\right]$$

$$= 6{,}863.2\ \text{sec} = 114.4\ \text{min} = 1.91\ \text{hr}$$

c) Number of Dextran Columns, N

Total Volume to Treat $= 10^4 \ell$ of IG soln.

Volume Treated per Column $= F \bar{t}_{IG}$

$$= \left(1 \frac{\ell}{min}\right)(114.4\ min) = 114.4\ \ell$$

$$N = \frac{10^4 \ell}{114.4 \ell} = \boxed{87.4 \text{ columns} \sim 88 \text{ columns}}$$

Alternative Solution

$$\text{Total Moles of IG} = (10^4 \ell)\left(2 \times 10^{-5} \frac{\text{moles IG}}{\ell}\right)$$

$$= 0.2 \text{ moles IG}$$

$$\text{Total Columns} = \frac{0.2 \text{ moles IG}}{4.71 \times 10^{-3} \frac{\text{moles IG}}{\text{column}}} = 42.5$$

$$\boxed{\sim 43 \text{ columns}}$$

Why is there a difference?

I can't explain it --- any insights by students?

Problem 2

Chromatographic Separation of A from B

Data: $C_A = 0.10 \frac{mg\,A}{ml}$ at equilibrium in liquid

$C_B = 0.05 \frac{mg\,B}{ml}$ " " " "

$M = 3g/150\ ml$ bed volume $= 0.02 \frac{g\ sorbent}{ml\ bed\ volume}$

$\varepsilon = 0.35$

A = bed cross-sectional area (empty) = $6\ cm^2$

$\Delta V = 50\ ml$ of solvent added to column

Design Eqn:

$$\Delta x = \frac{\Delta V}{A[\varepsilon + M f'(C_L)]}$$

$$f_A = \frac{k_1 C_A}{k_2 + C_A}$$

$$f_A'(C_A) = \frac{d}{dC_A}\left(\frac{k_1 C_A}{k_2 + C_A}\right) = \frac{k_1 k_2}{(k_2 + C_A)^2}$$

$$= \frac{(.2\ mg\,A/mg\ sorbent)(.1\ mg\,A/ml\ liquid)}{[(.1\ mg\,A/ml\ liquid) + (.1\ mg\,A/ml\ liq.)]^2}$$

$$= 0.5 \frac{ml\ liquid}{mg\ sorbent}$$ ✓

$$f_B'(C_B) = \frac{k_1' k_2'}{(k_2' + C_B)^2} = \frac{(.05)(.02)}{[(.02) + (.05)]^2} = .2 \frac{ml\ liq.}{mg\ sorb}$$

For A

$$\Delta x = \frac{(50\ ml)(1\ cm^3/ml)}{(6\ cm^2)\left[(.35) + \left(20\ \frac{mg\ sorbent}{ml\ bed}\right)\left(.5\ \frac{ml\ liq.}{mg\ sorb.}\right)\right]}$$

$$= 0.81\ cm$$

For B

$$\Delta x = \frac{(50\ ml)(1\ cm^3/ml)}{(6\ cm^2)\left[(.35) + (20)(.2)\right]}$$

$$= 1.92\ cm$$

CM 4710, Biochemical Processes **Fall 2003**
Homework #7 Solution **Fri. 7 Nov., 2003**

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3. Determining Time to Elute a Solutes A and B from a Chromatographic Column.

Problem 11.7 of the text

The fundamental eqn. For gel chromatography is

$$V_e = V_o + K_D V_i$$

For A: $V_e = 20\ cm^3 + 0.5(30\ cm^3) = 35\ cm^3$

$T_A = 35\ cm^3/100\ cm^3/hr = 0.35\ hr$

For A: $V_e = 20\ cm^3 + 0.15(30\ cm^3) = 24.5\ cm^3$

$T_A = 24.5\ cm^3/100\ cm^3/hr = 0.245\ hr$

CM 4710, Biochemical Processes **Fall 2003**
Homework #8 **Fri. 4 Nov., 2005**

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3. Determining Time to Elute a Solutes A and B from a Chromatographic Column.

Problem 11.7 of the text

Due Fri. 11 Nov., 2005.

1. **Adsorption Separation of Immunoglobulin G using Modified Dextran (adapted from Belter, Cussler, and Hu, pg 153)**

Information

Langmuir isotherm

$$C_S = \frac{C_{SMAX} C_L}{K_L + C_L}$$

Dextran will adsorb up to 7.8E - 6 mol/IG/cm^3 = C_{SMAX}

$$K_L = 1.9\times10^{-5}\, mol/L$$

$$\rho_{adsorbent} = 1\frac{g}{cm^3}$$

$$\varepsilon(\text{void fraction}) = 0.40$$

Solution

a) **Adsorption capacity of the column**

$$D = 5cm$$

$$L = 1m = 100cm$$

$$C_L = 2\times10^{-5}\frac{mol}{L}$$

$$\text{Capacity of the column} = (\text{volume of adsorbent})(\text{solute concentration on adsorbent})$$

$$\text{Capacity of the column} = (1-\varepsilon)(\text{volume of the column})(C_S)$$

$$\text{Volume of the column} = \pi\left(\frac{5cm}{2}\right)^2(100cm) = 1963.5cm^3$$

$$C_S = \frac{\left(7.8\times10^{-6}\frac{mol}{cm^3}\right)\left(2\times10^{-5}\frac{mol}{L}\right)}{1.9\times10^{-5}\frac{mol}{L} + 2\times10^{-5}\frac{mol}{L}} = 4\times10^{-6}\frac{mol}{cm^3}$$

$$\text{Capacity of the column}: (1-0.40)(1963.5cm^3)\left(4\times10^{-6}\frac{mol}{cm^3}\right) = 4.712\times10^{-3}\,mol$$

b) Mean retention time

$$t = \frac{L}{U_i}\left[1+\rho\left(\frac{1-\varepsilon}{\varepsilon}\right)f^{'}(C_L)\right]$$

$$L = 100cm$$

$$F = 1\frac{L}{\min} = 1000\frac{cm^3}{\min}$$

$$\rho_{adsorbent} = 1\frac{g}{cm^3}$$

$$U_i = \frac{F}{\varepsilon A} = \frac{1000\frac{cm^3}{\min}}{0.40\left(\pi\left(\frac{5cm}{2}\right)^2\right)} = 127.324\frac{cm}{\min}$$

$$f^{'}(C_L) = \frac{d}{dC_L}\left(\frac{C_{SMAX}C_L}{K_L + C_L}\right) = \frac{C_{SMAX}K_L}{(K_L + C_L)^2}$$

$$f^{'}\left(2\times10^{-5}\frac{mol}{L}\right) = \frac{\left(7.8\times10^{-6}\frac{mol}{cm^3}\right)\left(1.9\times10^{-5}\frac{mol}{L}\right)}{\left(1.9\times10^{-5}\frac{mol}{L} + 2\times10^{-5}\frac{mol}{L}\right)^2} = 0.097436\frac{L_{SOLUTION}}{cm^3{}_{ADSORBENT}}$$

$$f^{'}\left(2\times10^{-5}\frac{mol}{L}\right) = 97.4359\frac{cm^3{}_{SOLUTION}}{cm^3{}_{ADSORBENT}}$$

$$t = \frac{100cm}{127.324\frac{cm}{\min}}\left[1+\left(1\frac{g}{cm^3}\right)\left(\frac{1-0.40}{0.40}\right)\left(97.4359\frac{cm^3{}_{SOLUTION}}{cm^3{}_{ADSORBENT}}\right)\right] = 115.574\min$$

c) Number of columns to recover product in 10000 L

$$\text{Number of columns} = \frac{(10000L)\left(2\times10^{-5}\frac{mol}{L}\right)}{4.712\times10^{-3}mol} = 42.44 \approx 43\text{ columns}$$

2. Travel distance of solutes A and B in a chromatographic column.
Information

$$\mathrm{m_i} = f_i(c) = \frac{k_{1i} c_i}{k_{2i} + c_i}$$

$i = A, B$

$k_{1A} = 0.2$ mg solute A adsorbed/mg adsorbent

$k_{2A} = 0.1$ mg solute/ml liquid

$k_{1B} = 0.05$ mg solute B adsorbed/mg adsorbent

$k_{2B} = 0.02$ mg solute/ml liquid

mass of adsorbent = 3g = 3000mg

Bed volume = 150ml

$\varepsilon = 0.35$

$\mathrm{A} = 6\mathrm{cm}^2$

$\Delta V = 50 ml$

Solution

a) Position of each band in the column

$$\Delta X = \frac{\Delta V}{A\left(\varepsilon + Mf'(C_L)\right)}$$

M is the amount of adsorbent per unit volume of column (mg/ml)

$$f'(C_L) = \frac{d}{dc_i}\left(\frac{k_{1i}c_i}{k_{2i}+c_i}\right) = \frac{k_{1i}k_{2i}}{\left(k_{2i}+c_i\right)^2}$$

For A

$$f'\left(0.1\frac{mg\text{ A}}{\text{ml}}\right) = 0.5\frac{ml}{mg_{adsorbent}}$$

For B

$$f'\left(0.05\frac{mg\text{ B}}{\text{ml}}\right) = 0.204082\frac{ml}{mg_{adsorbent}}$$

$$M = \frac{3000mg}{\left(\frac{150}{1-.35}\right)} = 13\frac{mg}{ml}$$

$$\Delta X_A = \frac{50ml}{\left(6\text{cm}^2\right)\left(0.35+\left(13\frac{mg}{ml}\right)\left(0.5\frac{ml}{mg_{adsorbent}}\right)\right)} = 1.21655cm$$

$$\Delta X_B = \frac{50m}{\left(6\text{cm}^2\right)\left(0.35+\left(13\frac{mg}{ml}\right)\left(0.204082\frac{ml}{mg_{adsorbent}}\right)\right)} = 2.77494cm$$

b)

$$\frac{L_A}{L_B} = \frac{\Delta X_A}{\Delta X_B} = 0.438$$

$$R_{fA} = \frac{\varepsilon}{\varepsilon + M\left(f'\left(0.1\frac{mg\text{ B}}{\text{ml}}\right)\right)} = 0.051$$

$$R_{fB} = \frac{\varepsilon}{\varepsilon + M\left(f'\left(0.05\frac{mg\text{ B}}{\text{ml}}\right)\right)} = 0.117$$

3. Determining Time to Elute Solutes A and B from a Chromatographic Column

Information

$K_{DA} = 0.5$

$K_{DB} = 0.15$

$V_0 = 20cm^3$

$V_i = 30cm^3$

$V_{column} = 60cm^3$

$F = 100\frac{cm^3}{h}$

Solution

Estimate exit time of A and B

$$t = \frac{V_0 + K_D V_i}{F}$$

$$t_A = \frac{20cm^3 + (0.5)(30cm^3)}{100\frac{cm^3}{h}} = 0.35h$$

$$t_B = \frac{20cm^3 + (0.15)(30cm^3)}{100\frac{cm^3}{h}} = 0.245h$$