Key to Homework 3

1.
(a) High pressure is used to keep a certain flow rate in Hplc column. The Size of packing materials in a HpLC column is from 3 to 10 µm. From Darcy's equation, High pressure is needed to generate a reasonable flow rate when the packing materials are in such small sizes.

(b), (c) See teaching notes

2,3 See teaching notes

4. decrease the proportion of benzene. Gradient elution: strength of mobile phase is from weak to strong.

5. GC: Carrier gas, mobile phase has less effects on the retention of solutes,

LC: mobile phase generates a flow to carry the solutes for seperation (i.e. serve as a carrier) However, molile phase can strongly interact with the solutes. Thus it affects the retention of solutes.

SFC: similar to LC, ① serve as a carrier of solutes for seperation. ② it affects the retention of solutes in a chromatoghaphic system.

6. See teaching notes

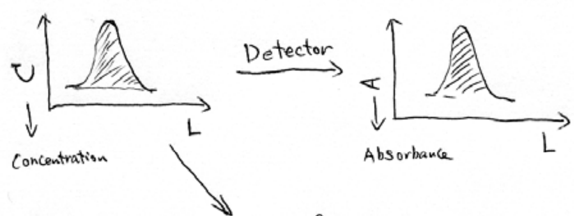
7. GC: Change stationary phase

LC. SFC: the seperation factor of solutes can be manipulateed by changing mobile phase, stationary phase, or both.

8. (a) The retention time will be reduced because the solvent strength of the mobile phase is increased

(b) 1-aminooctane will be eluted first. At pH = 3.0, 1-aminooctane is charged but octanoic acid is in a neutral form. Thus the polarity of 1-aminooctane is larger than that of octanoic acid. So, 1-aminooctane will be eluted first in reversed-phase column described in (a)

9.



The area of peak $S_{(c)} = \int C \cdot dL$, $S_{(c)}$ is the total amount of a solute.

The area of peak $S_{(A)} = \int A \cdot dL = \int \varepsilon C l \cdot dL = \varepsilon \cdot l \cdot \int C \cdot dL = \varepsilon \cdot l \cdot S_{(c)}$ ··· ①

(absorbance) (Beer's Law)

Because solute A and B have equal amout, $S_{(c),A} = S_{(c),B}$ ··· ②

$\varepsilon_A = 2.26 \times 10^4\ M^{-1} cm^{-1}$, $\varepsilon_B = 1.68 \times 10^4\ M^{-1} cm^{-1}$, $l_A = l_B$ (same detection cell)

From ①, we can get.

$S_{(A),A} = \varepsilon_A l_A \cdot S_{(c),A}$ ··· ③

$S_{(A),B} = \varepsilon_B l_B \cdot S_{(c),B}$ ··· ④

Then $\frac{S_{(A),A}}{S_{(A),B}} = \frac{\varepsilon_A}{\varepsilon_B} \times \frac{l_A}{l_B} \times \frac{S_{(c),A}}{S_{(c),B}} = \frac{2.26 \times 10^{-4}\ M^{-1} cm^{-1}}{1.68 \times 10^{-4}\ M^{-1} cm^{-1}} \times \frac{l_A}{l_B} \times \frac{S_{(c),A}}{S_{(c),B}} = 1.345$ ··· ⑤

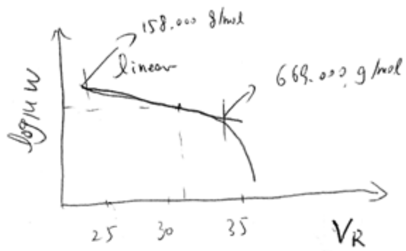
$S_{(A),A} = 1.19 \times h_A w_{1/2,A} = 1.19 \times 128\ mm \times 10.1\ mm$, $w_{1/2,B} = 7.6\ mm$

(2) $\frac{S_{(A),A}}{S_{(A),B}} = \frac{1.19 \times 128\ mm \times 10.1\ mm}{1.19 \times h_B \cdot 7.6\ mm}$ ··· ⑥

Combining ⑤ and ⑥ gives $\dfrac{1.19 \times 128\ mm \times 10.1\ mm}{1.19 \times h_B \times 7.6\ mm} = 1.345$

Then $h_B = \dfrac{1.19 \times 128\ mm \times 10.1\ mm}{1.19 \times 7.6\ mm \times 1.345} = 126.5\ mm$.

10 (a) make a plot log MW vs. V_R



(b) index from the figure gives MW = 270,000 g/mol

11. See page 4.

12. (a) Yes. At pH=4 protein will have lower net negative charge density than that at pH 8. The lower the negative charge density, the less the retention of a solute in a anion exchange column. Thus the gradient of eluent pH from pH 8 to pH4 is the order of elution from a weak mobile phase to a stronger one. Therefore, it will help the seperation

(b) Yes

13-16 teaching notes.

Homework 3 b

1.-4. teaching note

5. $N = \frac{\mu_{app} V}{2D}$, Thus the plate number will increase as V. (6400 — 25500 V)

However, when V is too large, Joule heating caused band broadening will play a significient role. (31700 V, 38,000 V)

11.

(a)

one antibody bends to two insulin

$$k_{insulin} = \frac{\text{moles in stationary phase}}{\text{moles in mobile phase}} = \frac{\text{moles of antibody} \times 2}{C_{insulin} \times V_m} \quad (1)$$

antibody

$$N_{(solid)} + \text{Insulin}_{(solute)} \rightleftharpoons N\text{-Insulin}_{(solid)}$$

$$K = \frac{[N\text{-Insulin}]}{C_{Insulin} \times [N_{(solid)}]} = \frac{1}{C_{Insulin} \times 1} = 2 \times 10^{8}\ M^{-1}$$

$$\Rightarrow C_{insulin} = 5 \times 10^{-9}\ M \quad (2)$$

put (2) into (1)

$$k_{insulin} = \frac{50 \times (10^{-9})\ mol}{5 \times 10^{-9}\ M \times 0.53 \times 10^{-3}\ L} = 38,000$$

$$k_{insulin} = \frac{t_R - t_M}{t_M} \Rightarrow t_R = t_M (k+1) = \frac{V_m}{F}(k+1) = \frac{0.53\ mL}{1\ mL/min}(38,000+1)$$

$= 20,000$ min

$\doteq$ 14 days

(b) using the same calculations as above

$k = 1.89$

$t_R = 1.53$ min