CHEM 230
Exam 2 – Oct. 14, 2014
SOLUTIONS

Short Answer/Multiple Choice Section:
Each Question worth 3 points

1. The most common way that the stationary phase in reversed-phase HPLC is "attached" to a column is:
a) a coating on the wall of an open tubular column
b) a coating chemically bonded to packing material
c) the surface of polar packing material
d) a free liquid with a strong affinity for the surface of the packing material

2. In instrument-based chromatographs, a chromatogram is a plot of _*response*_ on the y-axis and _*time*_ on the x-axis.

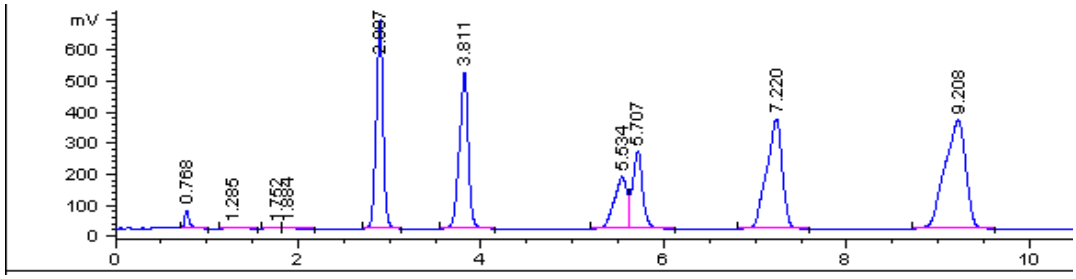
3. GC is being used to detect small chain alcohols present in hexane solvent. A non-polar stationary phase is not desired because:
a) the alcohols may elute close to the solvent peak with a non-polar stationary phase
b) adsorption and tailing is more likely with a non-polar stationary phase
c) the non-polar stationary phase will result in a slow chromatogram
d) the non-polar stationary phase will increase solute-solute interaction in the mobile phase

4. At higher than optimal flow rates in a chromatographic separation, further increasing the flow rate will tend to result in decreased separation efficiency because of:
a) increased longitudinal diffusion
b) increased differences in molecule paths in the mobile phase
c) increased dispersion from mass transport to and within the stationary phase
d) all of the above

5. List a change to an HPLC column that would result in an increase in N (the plate number). Change = *_1) increase column length, 2) decrease diameter of packing material, 3) switch to superficially porous packing particles__*

6. In a normal phase HPLC separation using a hexane/2-propanol mobile phase, two peaks are not fully resolved. In order to increase retention to improve separation, one should:
a) decrease the flow rate
b) decrease the temperature
c) increase the percent of 2-propanol
d) increase the percent of hexane

Longer Answer Questions:
Questions 1-7 below refer to the following chromatogram and data table which show the separation of linear fatty acids (C18:3, C18:2, C18:1, C16:0, C17:0, and C18:0 – where the first number gives the length of the fatty acids in number of carbons and number after the colon gives the number of double bonds – all in *cis* isomer). All of the fatty acids have pK_a values of around 4.8. The separation was performed on a C18 (reversed phase) column with an eluent of 0.001 M trifluoroacetic acid (TFA = strong acid) in water (8%) and 92% acetonitrile. W_b in table is the baseline width.



Compound	Retention (min.)	Area	W_b (min.)
Unretained	0.768	NA	NA
C18:3	2.887	3611.5	0.127
C18:2	3.811	3389.3	0.156
C18:1	5.534	1656	0.222
C16:0	5.707	1908.9	0.180
C17:0	7.22	4182.1	0.266
C18:0	9.208	5552.7	0.358

1. Calculate the retention factor (k) of C16:0. (4 pts)
k = (5.707 – 0.768)/0.768 = ***6.43***

2. Based on the elution order, what effect does the number of double bonds have on the compound's "polarity"? (4 pts)
The only difference in the C18:X compounds is the number of double bonds. It is clear that increasing the number of double bonds (from 0 to 3) results in earlier elution. Early elution in reversed phase HPLC is expected for the more polar compounds. Thus the more double bonds, the more polar the compound is.

3. Without the TFA present, what would happen to the retention times? (5 pts)
*Without TFA, the eluent would be at a neutral pH ~7 which is much above the pK_a values. Thus the fatty acids would go from an acid to an anion form. Anions would not be retained on the C18 stationary phase and would elute quickly (****so much shorter retentiontimes****).*

4. Calculate the N value C17:0 (using equations for assumed Gaussian peak shapes). (4 pts)
$N = 16(7.22/0.266)^2 =$ ***11800***

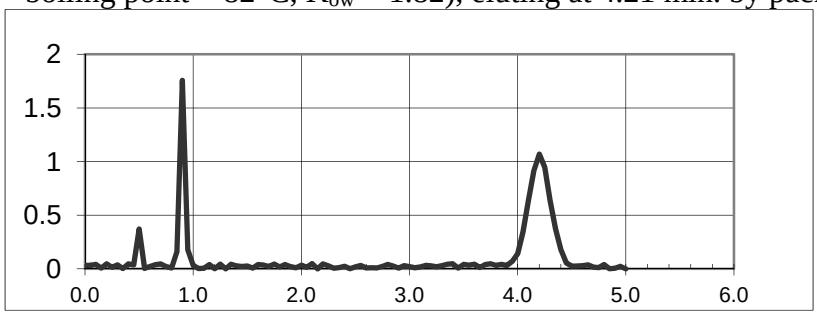
5. What is the resolution between the two least well resolved peaks. (4 pts)
Least well resolved peaks are for C18:1 and C16:0.
$R_s = \Delta t/Ave(W_b) = (5.707 – 5.534)/[1/2(0.222 + 0.180)] =$ ***0.86***

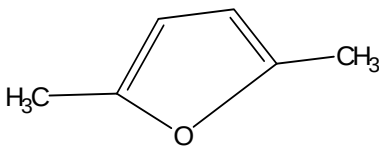
6. Would decreasing the % acetonitrile be expected to improve the resolution of the overlapping peaks? Explain your answer. (5 pts)
Decreasing the % acetonitrile, which is the strong (or less polar) solvent in reversed phase HPLC, would be expected to increase retention and improve resolution.

BONUS. Let's assume that acetonitrile (CH_3CN) can stabilize double bond-containing solutes through π electron interactions. What would happen if we switched from using acetonitrile and TFA in water to using methanol and TFA in water for the solvent? (2 pts)
If acetonitrile can stabilize double bond-containing solutes relative to methanol, a switch to methanol as the organic modifier would be expected to result in increased retention of the unsaturated fatty acids (fatty acids with double bonds, and especially those with more double bonds). Once no solvent is present allowing stabilization of the unsaturated fatty acids, those will spend more time in the stationary phase and ***elute later****. For example, the C18:1 peak may elute after C16:0 due to this change.*

7. What type of peak shape is seen (tailing, fronting, or Gaussian shaped)? Explain how you know. (4 pts)
Fronting*. The peaks are observed to rise slowly and fall quickly.*

8. The following chromatogram is observed when separating 2,5-dimethylfuran (structure below, boiling point = 93°C, K_{ow} = 182), eluting at 0.93 min, from 2-propanol ($CH_3CH_2OHCH_3$ – boiling point = 82°C, K_{ow} = 1.82), eluting at 4.21 min. by packed column GC at 100°C.





a) Based on the solutes's volatilities and polarities, would you expect the stationary phase to be polar or non polar. (4 pts)
Polar*. Despite having a lower boiling point, the more polar solute (2-propanol) elutes later. It must have greater affinity for the stationary phase than the relatively non-polar dimethylfuran.*

b) What is not "optimal" about the separation? Suggest one change to make it more optimal. (8 pts)
Given the narrow peaks, ***the chromatographic run takes too long.*** *We could 1) increase the temperature (decreasing the run time until the peaks overlap or the DMF comes out close to the unretained peak), 2) switch to a less polar column. The less polar column should retain 2-propanol less, resulting in a faster run time, 3) switch to a shorter column, 4) run at a higher flow rate, or 5) use a temperature program.*