CHEM 230
Exam 3 – SOLUTIONS

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

1. The main advantage in using open tubular columns (vs. packed columns) in GC is:
a) increased analyte capacity
b) high efficiency (plate number)
c) better selectivity
d) lower cost
[a) and b) are false and c) just depends on the chemistry of the coating – so should be independent of column type]

2. Dicarboxylic acids ($HO_2C(CH_2)_nCO_2H$) can not be analyzed easily directly by GC. However, by converting them to diesters ($CH_3O_2C(CH_2)_nCO_2CH_3$), good GC separation and detection is possible. What is a major effect of this derivatization that leads to improvement? **1) increased volatility, 2) decreased polarity**

3. What is the purpose of flow restrictor in supercritical fluid chromatography. The flow restrictor restricts the flow rate and causes a pressure drop. Purpose = **the flow restrictor, put after the column or detector, keeps the fluid above the critical pressure so that it remains a supercritical fluid (as opposed to becoming a gas)**

4. Supercritical fluid chromatography is particularly good for preparative separations because:
a) one can use open tubular columns
b) efficiency and/or flow rates typically are much higher than HPLC
c) a large variety of mobile phases are used unlike with HPLC
d) all of the above
[open tubular is very poor for prep work due to low flow rates. there typically are not a lot of mobile phases used (CO_2 dominates) in SFC]

5. List a change to the mobile phase that will decrease retention in anion exchange chromatography if the stationary phase is the strong anion exchanger type.
Change = **1) increase concentration of anion exchanger or 2) switch to stronger anion exchanger**

6. A biochemist analyzing triglycerides by normal phase HPLC wants to perform a gradient using the eluents hexane and methylene chloride. The % of which solvent should increase in the gradient run? **increase methylene chloride** (more polar, stronger solvent)

Longer Answer Questions:
1. Alkyl amines, such as those listed below, are difficult to separate by HPLC using silica based C18 columns. The amine groups are relatively polar and the **pK$_a$ values are given for their conjugate acids**. (9 pts)

HO
NH$_2$
ethanolamine pK$_a$ = 9.50
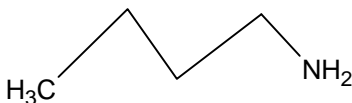
H$_3$C
NH$_2$
butylamine pK$_a$ = 10.64

a) Indicate why separations are difficult with standard silica-based C18 columns.
Compounds need to be in their uncharged states. For bases, this occurs at pH near to or above pK$_a$. This would require a pH of at least 9 (to weakly retain ethanolamine for example). However, silica columns are stable only up to about pH 8.

b) Indicate an alternative way of using a type of liquid chromatography to separate them - indicate the type of column and the type of eluent needed (including pH) for successful separation.
*1) use cation exchange with a cation exchanger (e.g. HNO$_3$ in water) and a pH under 8.*
2) use the above C18 column with an ion-pairing reagent such as octyl sulfate (must be negative charge) in a water – acetonitrile eluent.
3) use a hybrid silica C18 column that is stable at higher pH values and use an acetonitrile - pH 11 aqueous buffer as eluent
4) use a polymeric reversed phase column and use an acetonitrile - pH 11 aqueous buffer as eluent

2. A researcher is analyzing sediments that have been contaminated by various solvents. He is particularly interested in quantifying halogenated solvents using GCxGC with flame ionization detection on sediment extracts. The first column is non-polar while the second column is polar. The following 2D chromatogram is produced where stars show location of halogenated solvents and circles show locations of non-halogenated solvents. (12 pts)

column 2
retention time

column 1 retention time
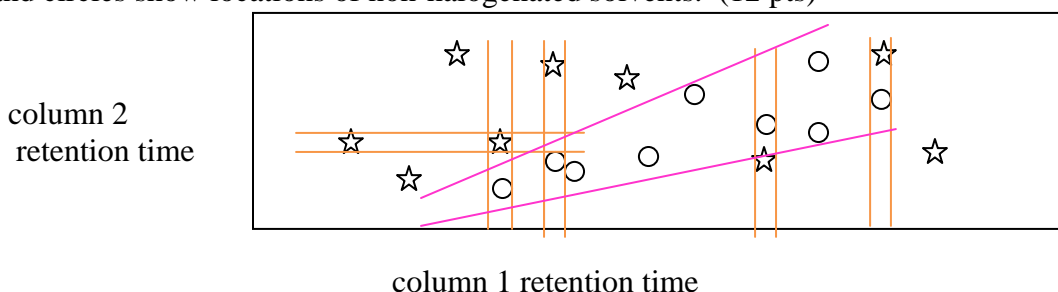
a) Assuming the peaks had widths shown by the circles or stars, was 2D GC needed to separate out all observed compounds (vs. a 1D separation using either column)?
*Yes. By condensing the data into a 1D separation, overlap clearly occurs (see orange lines)*

b) What can be said of the polarity of the halogenated compounds vs. that of the non-halogenated compounds?
While most of the halogenated compounds are more polar, their polarities span a much greater range, while the non-halogenated compounds (within pink lines) all have fairly similar polarity (upper right corner is most polar lower left is least polar)

c) Suggest a way in which the halogenated compounds would be well resolved and quantified using only 1D GC with a different detector.
All of the halogenated compounds are well separated from each other along column 1. So a 1-D separation using column 1 with a detector specific to halogens (electron capture detector or MS selecting out halogenated compounds) would work well.

3. The gas chromatogram shown below was for the analysis of 1 µL of liquid sample (reaction products) injected using a split ratio of 1:4 (one part in 4 injected into the column), with separation on a 0.25 mm x 15 m open tubular column with 0.2 µm film thickness (normally a high efficiency column).
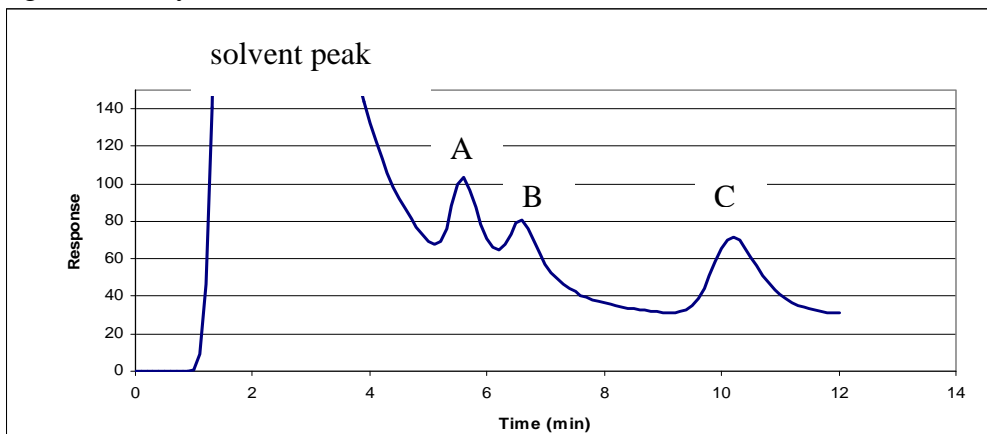
Indicate: a) A problem in how the GC was run the quality of the chromatogram.
b) A change in conditions which could result in an improved chromatogram. (8 pts)
a) The peaks are overly broad, tail and overlap with the solvent tail. This indicates column overloading.
b) The split ratio could be adjusted to vent a greater fraction of the sample (e.g. 1:100) or the sample could be diluted (not as good as solvent peak interference would still occur).

4. An HPLC separation is performed with a 4.6 mm (diameter) by 150 mm length amino column (polar stationary phase) with 5 µm diameter packing material using 60% ethyl acetate/40% methanol as eluent. To speed up the separation, the chemist wants to switch to 3.5 µm diameter packing in a 100 mm column. Assuming the flow rate, mobile phase composition, and β (volume ratio) are the same, answer the following questions. (13 pts)
a) using calculations, estimate the percent change (including direction) in the pressure.
$P = kL/d_p^2$ *or* $P_2/P_1 = (L_2/L_1)(d_{p1}^2/d_{p2}^2) = (100/150)(5/3.5)^2 = 1.36$ *or* **36% increase in P**

b) using calculations, estimate the percent change (including direction) in N.
$N = L/H$ *and* $H = kd_p$ *or* $N = kL/d_p$ *or* $N_2/N_1 = (L_2/L_1)(d_{p1}/d_{p2}) = 0.95$ *or* **5% decrease in N**

bonus) after the switch, lower than calculated N values are observed for early eluting peaks. What is the cause of this? (2 pts)
Despite the slight decrease in N, peaks are eluting earlier with the shorter column and are narrower. This causes a greater % contribution of measured widths from extra-column broadening which primarily affects the earlier eluting peaks.