Chem 230, Fall, 2014
Homework Set # 3 – Short Answer SOLUTIONS

1. List two advantages of temperature programming in GC.
a) Allows separation of solutes with widely varying retention factors in a reasonable time.
b) Allows one to use splitless or SPME injections without excessive broadening of peak widths
c) Can reduce the total time needed for a chromatogram
d) Results in narrow peak widths for later eluting peaks
e) Results in greater peak heights and sensitivity for later eluting peaks

2. What van Deemter term is nearly zero with OT GC columns?
The A term (since there is not much difference in paths).

3. Why would it normally not make sense to use a 0.53 mm diameter column with a thin film thickness (e.g. 0.25 μm) in GC?
To reduce the C-term, we need to have fast mass transport. It does not make sense to have very fast transport in the liquid phase by using a thin film thickness if the gas phase transport is slow (due to the wider diameter of the 0.53 mm tube). In this case, all of the C-term value would be from the gas phase diffusion. The disadvantage from going with a thin film is reduced capacity, which in the case of a thin film with a wide tube, is not needed.
Note: if one were only worried about too much capacity (e.g. for solute eluting near the maximum column temperatures), then it might make sense to use a thin film to reduce the retention factor.

4. List three important criteria for detector performance.
a) sensitivity of detector (e.g. detection limit)
b) range of useful (e.g. linear) operation
c) response time (fast enough to not broaden any peaks)
d) classes of analytes detector responds to (how universal or selective)
e) destructive vs. non-desctructive
f) more practical criteria: cost, if additional gases or high power is required, size of detector

5. A polar compound is analyzed on an OV-1 column and a carbowax column using GC. If the column dimensions and flow rates are the same, on which column will the retention time be greater?
Greater retention on the polar carbowax column would be expected (if run at the same temperature).

6. What type of GC detector could be used if comprehensive detection of hydrocarbons in the sample is desired?
Flame ionization detector would be the most commonly used. One could also use TCD or MS detectors with some limitations.

7. For what type of samples would you want to use a fixed loop injector in GC?
Gas samples.

8. It is desired to analyze for small to moderate sized (C2 to C10) linear alcohols in water. Water cannot be directly injected into the GC. What other methods could be used to inject the alcohols? How could this method bias the sample?
One could use headspace (volume above water layer in a closed container) injection. This would bias toward the smaller alcohols as they have higher concentrations in the gas phase due to greater volatility. By bias I mean a bigger peak area for a given aqueous concentration would occur. By running standards, there would be no bias in the measurements, but analysis of the largest sized alcohols could be difficult due to minimal volatility unless the sample was heated.
One could use headspace SPME injections. This would bias toward the intermediate size alcohols. The smallest are the most volatile but will not tend to stick in the SPME solid phase, while the largest are not volatile enough to get into the headspace.
Once could use direct SPME injection. One would want to use a solid phase of weak to moderate polarity (to decrease the amount of water injected while still injecting alcohols). Bias would depend on the polarity of the SPME fiber with less polar fibers tending to give larger peaks for the larger alcohols (which are less polar). Even moderately polar fibers would bias against the smallest alcohols as those are very water soluble and polar.
One could use purge and trap injection techniques (not discussed much in class). In this case a gas purges alcohols out of the water and into a trap, which later is desorbed for injection. This may lead to a bias similar to that observed for headspace injection.

9. Imagine that you have a sample containing various low molecular weight chlorinated hydrocarbons at concentrations in the percent range. What type of column (packed or capillary) would you use? Now imagine that the concentrations were in the parts per billion ranges. What type of column would you use (specify the phase)?
For low % range, you would want to use a packed column or possibly a megabore column to avoid overloading the column (without needing to dilute samples). For concentrations in the parts per billion range, a capillary column would make more sense since this would result in less broadening. It would make sense to use a non-polar stationary phase (because chlorinated hydrocarbons tend to be pretty non-polar) unless one wants to use lower temperatures or faster chromatograms.

10. When the results from a sample obtained using GCxGC are plotted (in 3D), most of the peaks are found to lie close to a diagonal through the 1st and 2nd dimension retention times of the plot. Is GCxGC effective for this sample? Why or why not?
No. If the peaks lie along the diagonal, it means retention is very similar for the sample compounds in each dimension. This means the additional peak capacity given by the second column is not used very efficiently (otherwise, more of the 3D chromatogram space would be used up). While the separation my have been successful, most likely, a single dimension method would also have worked for this sample.

11. List two pieces of additional equipment needed for GCxGC.
Additional equipment (vs. 1D GC): 1) modulator, 2) 2nd column,3) 2nd oven for 2nd column, and 4) software to transform data into three dimensional array. One may also need to replace the detector and digitizer since 2D GC requires somewhat faster detectors and data collection to avoid broadening of narrow peaks.

12. List four different subtypes of liquid-based chromatography based on separation mechanism.
a) reversed phase *b) normal phase* *c) size exclusion*
d) ion exchange *e) ligand exchange* *f) affinity*

13. What change in packing material geometry (but not column dimensions) in HPLC leads to greater resolution without an increase in back pressure?
a) superficially porous packing material
b) monolith columns

14. List one advantage to using polymeric packing material in place of silica in HPLC.
a) Wider pH range without column degradation
b) Less column bleeding (if replacing bonded phase silica with straight polymeric material)

15. List a non-bonded packing material that can be used for reversed phase HPLC separations.
polymeric (styrene – divinylbenzene) or porous graphitic carbon

16. A chemist was performing a silica based normal phase HPLC separation. Her 12% 2-propanol in hexane eluent was running low, so she re-prepared her eluent. Then, she found k values shifted by about 10%. Explain the change.
This was probably due to a shift in the % water in the eluent.

17. 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?
The % of methylene chloride, which is a slightly polar molecule, should increase during the run.

18. To what pH value range should the mobile phase be adjusted for a reversed phase (silica-based C18) HPLC separation of benzoic acid?
The pH range would be from 2 (general lower limit in silica) to 4.2 (the pK_a value). As one approaches the pK_a value (e.g. 3.2 to 4.2) the retention time will decrease as the benzoic acid will start to be ionized a significant fraction of the time.

19. What is a problem in the separation of amines (weak bases) with pK_a values greater than 8 by reversed phase (silica-based C18) HPLC?
To be uncharged molecules, pH > pK_a is required (note that pK_a value is for conjugate acids). However, this mostly occurs at pH >8 where bonded silica is not stable.

Is this also a problem for the separation of anilines (aromatic amines with pK_a values of around 4)?
No. They can be analyzed at pH of 4 to 8 without problems of column degradation.

20. What is one advantage of using monolithic columns in HPLC?
Lower pressure drop for the same efficiency of conventional packed columns

21. What is one disadvantage to using polymeric packing material in HPLC?
Lower separation efficiency. Often can not withstand as high pressures as silica columns.

22. List two factors besides solvent polarity that may be important in choosing solvents for the mobile phase in HPLC.
a) selectivity. Different solvents will affect different analytes differently. A solvent that leads to co-elution of two compounds with one solvent could be changed to a solvent capable of decent separation in another solvent
b) viscosity. The lower the viscosity, the lower the back pressure, allowing one to use higher flow rates for faster separations.
c) interference with detectors. For example, with a UV detector, the solvent should not absorb light over the wavelength range used.
d) cost
e) compatibility with tubing and column packing material.

23. What aspect of the stationary phase is normally changed to affect retention in SEC?
The pore size.

24. List two ways to decrease retention in anion exchange chromatography.
Retention can be changed by 1) increasing the concentration of the anion exchanger in the buffer, 2) by using a stronger anion exchanger, or 3) by changing the pH to partially protonate analytes (if possible).

25. What affects analyte sensitivity with a refractive index detector for HPLC?
The greater the difference between the analyte and solvent refractive indices, the more sensitive the RID will be.

26. Is use of lower wavelengths (e.g. 205 nm) better for simple samples or complex samples if the goal is to quantitate individual compounds using UV detection with HPLC?
Use of lower wavelengths will detect nearly all molecules. With complex samples, there is a greater likelihood of analyte peaks overlapping with other compounds when using lower wavelengths. With simple samples, this is less likely.

27. A chemists shifts from analytical bore HPLC (4.6 mm diameter) to microbore (say 1 mm diameter):

a) Why may it be necessary to reduce the cell volume in a UV detector?

If decreasing from 4.6 mm to 1 mm, the flow rate will need to decrease by a factor of $(4.6/1)^2$, or to 21 times lower flow to keep the pressure drop constant. If using the same cell volume, it will take 21 times more time for the analyte band to flow through the detector cell. This is likely to cause significant band broadening.

b) What effect will this have on analyte sensitivity (just the change in cell volume – assuming all cell dimensions are reduced)

If the cell is rectangular in shape and all dimensions are reduced by the same factor, each dimension would need to be decreased by $(21)^{1/3}$ or by 2.8 to reduce the volume by a factor of 21 to keep the time in the cell the same as with the 4.6 mm column flow rate. This would decrease the path length (b), which is one of the dimensions, by 2.8, causing a decrease in concentration sensitivity (concentration detection limit) by a factor of 2.8. However, since the volume injected is also typically decreased (see below), the mass sensitivity is normally higher.

c) Is it normally necessary to inject less sample with microbore HPLC?

Yes. A 20 μL loop in an analytical bore at a flow rate of 1.0 mL/min would lead to an injection width of (20 μL)(60 s/min)/(1000 μL/min) = 1.2 s, vs. 24 s if operated at a flow rate of 0.050 mL/min (20 times lower).*