

Chem 230, Fall, 2014
Homework Set # 3
Long Problem Solutions

Longer Problems – to be turned in

1. One μL of isooctane (density = 0.69 g/mL) is injected into a GC (whole sample on column). If isooctane is quickly volatilized in the GC under the conditions of 180°C and 1.8 atm ,

a) What is the volume of the isooctane gas? The ideal gas law ($PV = nRT$) will be needed to calculate the density of isooctane gas.

$$\text{mass } 1.0 \mu\text{L of isooctane} = (1.0 \mu\text{L})(0.69 \text{ mg}/\mu\text{L}) = 0.69 \text{ mg}$$

$$\text{moles isooctane} = (0.69 \text{ mg})(1 \text{ mmol}/114.2 \text{ mg})(1 \text{ mol}/1000 \text{ mmol})$$

$$\text{mol isooctane} = 6.04 \times 10^{-6} \text{ mol}$$

$$PV = nRT \text{ or } V = nRT/P$$

$$V = (6.04 \times 10^{-6} \text{ mol})(0.0821 \text{ L atm/mol K})(273.2 + 180\text{K})/(1.8 \text{ atm}) = 1.25 \times 10^{-4} \text{ L}$$

$$\mathbf{V = 0.125 \text{ mL}}$$

b) If the flow inside the column is 2 mL/min , what is the minimum width in seconds that the peak could possibly have (assuming no broadening).

$$t = V/F = 0.125 \text{ mL}/(2 \text{ mL/min})(60 \text{ s/min}) = \mathbf{3.8 \text{ s}}$$

c) What reduces this as a problem in split injection, and what is required to keep this from becoming a problem in splitless injection?

In split injection, only a fraction of the volume would be injected, meaning the width of the peak could be smaller. In splitless injection running a gradient could result in narrower peak widths due to trapping on an initially cold column.

2. A gas bubble meter is used to determine the flow rate for a 0.32 mm (open tubular), 30 m column. If the column effluent takes 136 s for a bubble to travel 10.0 mL in the meter, calculate:

a) the flow rate at the bubble meter (mL/min)

$$F = V/t = (10.0 \text{ mL}/136 \text{ s})(60 \text{ s/min}) = \mathbf{4.41 \text{ mL/min}}$$

b) the linear velocity (m/s)

$$u = F/A \text{ (where } A = \text{cross-sectional area)}$$

$$u = (4.41 \text{ cm}^3/\text{min})/(\pi d^2/4) = \{(4.41 \text{ cm}^3/\text{min})/[\pi(0.032 \text{ cm})^2/4]\}(1 \text{ m}/100 \text{ cm})(1 \text{ min}/60 \text{ s})$$

$$\mathbf{u = 0.91 \text{ m/s}}$$

c) the time expected for unretained solute to elute

$$t = L/u \text{ (easy way)} = 30 \text{ m}/(0.91 \text{ m/s}) = \mathbf{32.8 \text{ s} = 0.55 \text{ min.}}$$

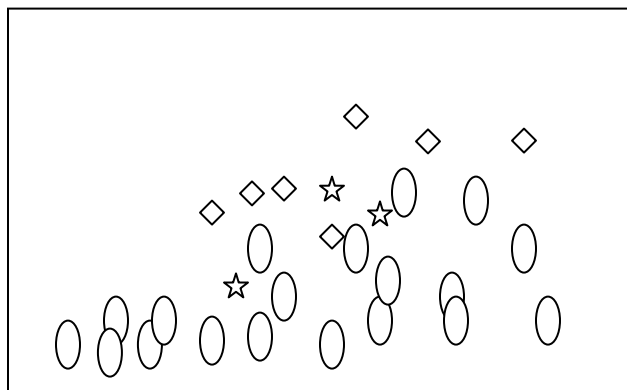
$$\text{or } t = V/F \text{ (harder way)} \quad V = \pi d^2 L/4 = 2.41 \text{ mL}$$

$$t = (2.41 \text{ mL})/(4.41 \text{ mL/min}) = 0.55 \text{ min}$$

For this question, you can ignore changes in temperature and pressure as the gas flows through the column.

3. It is desired to characterize the aromatic and oxygenated compounds in gasoline. Because of the complexity of gasoline, GC x GC is used with a non-polar 1st column, a polar 2nd column, and flame ionization detection. Looking at the 2D chromatogram (below),

2nd Column Ret.
Time



1st column Ret. Time

Note: **aromatic compounds** shown by diamonds (◇), **oxygenated compounds** shown by stars (☆) and **other compounds** shown by ovals (○). (10 points)

a) Did the GC x GC provided added resolution for the desired compounds over an expected 1D run? Explain.

Yes. One can see where if the chromatogram had been reduced down to the 1st dimension (1st column retention time), that there would be co-eluting peaks

b) Based on the chromatogram, rate the three types of compounds based on "average" polarity (least to most polar).

The compounds coming out higher are the more polar (more retained on polar column). Based on that, the most polar compounds are the aromatics, then the oxygenated compounds, with the other compounds being the least polar.

4. It is desired to determine wax composition by GC. A DB-5 (non-polar) 0.32 mm diameter x 0.5 µm film thickness x 30 m column is being used. The wax molecules consist of esters made from medium chain (6 to 12 carbon) alcohols attached to long chain (12 to 18 carbon) fatty acids (with no, one or two double bonds). Initial runs show that the column must be at the maximum **temperature** of 325°C for 45 minutes to elute all compounds. Separation of each wax molecule is good except if the only difference is in the number of double bonds.

a) List two changes in the column (dimensions or stationary phase) that would allow faster elution of the wax molecules.

1) Go to shorter column, 2) go to thinner stationary phase thickness or wider column to decrease V_s/V_m , 3) go to more polar column, or 4) go to column with greater maximum temperature. The wax molecules are highly non-polar (probably similar polarity as the DB-5 stationary phase) so a shift to a moderately polar to strongly polar column would

significantly decrease retention. Note, that it might also reduce the maximum column temperature which could increase the run time, instead.

b) What change might improve separation of wax molecules with the same number of carbons but different number of double bonds?

Using a more polar column should improve separation based on the number of double bonds as double bonds increase compound polarity.

5. A scientist is using a 4.6 x 150 mm, 5 μm particle size C18 HPLC column to carry out a separation. The isocratic separation occurs at a flow rate of 1.0 mL/min (50% methanol, 50% water) with a back pressure of 160 bar, takes 18 min., and gives a resolution of 1.8 for the two least well resolved peaks (with k values of 2.7 and 2.8). The unretained time is 1.5 min. He wants to speed up the separation by buying a similar column with 3 μm particles.

a) If the maximum pressure of the HPLC is 300 bar, what length (to the nearest 25 mm) should he purchase so that he can still run at 1.0 mL/min?

If the purchase is based on running just under the maximum pressure, he should calculate the length by reflecting on both changes to particle diameter and length on pressure.

Pressure is proportional to column length and proportional to the inverse square of the particle diameter, or $P = kL/d_p^2$ (where k is a generic constant)

As a proportion, this becomes $P_{\text{new}}/P_{\text{old}} = (L_{\text{new}} \cdot d_{p,\text{old}}^2)/(L_{\text{old}} \cdot d_{p,\text{new}}^2)$

$L_{\text{new}} = (P_{\text{new}}/P_{\text{old}})(d_{p,\text{new}}^2/d_{p,\text{old}}^2)L_{\text{old}} = (300 \text{ bar}/160 \text{ bar})[(3 \mu\text{m})^2/(5 \mu\text{m})^2](150 \text{ mm})$

*$L_{\text{new}} = 101 \text{ mm}$ or rounding to the nearest 25 mm, **a 100 mm column would be needed.***

Note that it might be impractical to run at 100 mm as this could be too close to the maximum operating pressure, since column back pressure tends to increase as they get used.

b) Estimate how long it will take to perform the separation at the length found in a) assuming the % methanol stays the same and K_C and β remain the same (β will probably increase some).

Assuming K_C and β stay the same, k will be the same. Since the flow rate is the same, the run time will decrease in proportion to the decrease in the length of the column.

$\text{time}(\text{new}) = \text{time}(\text{old})(L_{\text{new}}/L_{\text{old}}) = 18 \text{ min.}(100 \text{ mm}/150 \text{ mm}) = \mathbf{12 \text{ min.}}$

c) Estimate how long it will take to perform the separation at the length found in a) with % methanol optimized so that resolution remains the same. Hint: indicate how you expect N to be changed, then adjust k so that resolution remains the same, and assume the same % change occurs to the k value for the last eluting peak.

To solve this problem, we want to calculate the new k_B value (for the second eluting peak initially with a k of 2.8) to keep the resolution at 1.8, but using the new column which will result in narrower peaks.

Initially (old column), $R_s = 1.8 = [(N)^{0.5}/4][(\alpha - 1)/\alpha][k_B/(1 + k_B)]$ where $\alpha = (2.8/2.7) = 1.037$ and $k_B = 2.8$

$1.8 = [(N)^{0.5}/4][(1.037 - 1)/1.037][2.8/(1 + 2.8)]$

or $N^{0.5} = (1.8)(4)/[(0.0357)(0.737)] = 274$ or $N = 74,900$ (this value is unrealistically large for this type of column because the instructor gave unrealistic numbers for this problem).

Now, for the new column, we know that $H = kd_p$ and $N = L/H$ so $N = kL/d_p$ (where k is a generic constant)

Alternatively, we can say, $N_{\text{new}} = N_{\text{old}}(L_{\text{new}}d_{p,\text{old}})/(L_{\text{old}}d_{p,\text{new}}) = 1.11N_{\text{old}} = 83,200$

Now, we can use the new N value in the resolution equation with the same α and resolution values, and solve for the new k value.

$$1.8 = [(83,200)^{0.5}/4][(1.037 - 1)/1.037][k_B/(1 + k_B)]$$

$$[k_B/(1 + k_B)] = 0.700 \text{ or } k_B = 2.33 \text{ or a reduction of } k \text{ by a factor of } 2.33/2.80 \text{ or } 0.832$$

Now, we can see what affect this change will have on the length of time needed for the run. We can assume it took until $t_r(\text{last peak}) + w_B(\text{last peak})/2$ (until the chromatogram reached the baseline after the last peak). At 18 min, and $74,900 = 16[(18 - w_B/2)/w_B]^2$ or $74,900 = 16[(324 - 18w_B + w_B^2/4)/w_B^2]$ or $74,900w_B^2 = 5248 - 288w_B + 4w_B^2$ or $74,900w_B^2 + 288w_B - 5248 = 0$ or $w_B = 0.263 \text{ min}$ and $t_r(\text{last peak}) = 18 - 0.263/2 = 17.87 \text{ min}$. Thus $k(\text{last peak}) = (17.87 - 1.5)/1.5 = 10.91$

Now, with the new column, $t_{\text{unretained}} = (1.5 \text{ min})(100 \text{ mm})/(150 \text{ mm}) = 1.0 \text{ min}$ (due to the column now being 50% shorter with the same flow rate)

and $k_{\text{new}} = (10.91)(2.33/2.8) = 9.07$ (adjusting for the decrease seen in k_B)

$$9.07 = (t_r(\text{last peak}) - 1.0 \text{ min})/1.0 \text{ min}$$

$$\text{or } t_r(\text{last peak}) = 10.08 \text{ min.}$$

We also need to calculate $w_B(\text{last peak})$ from $83,200 = 16(t_r(\text{last peak})/w_B(\text{last peak}))^2$
 $w_B(\text{last peak})^2 = 16(10.08 \text{ min.})^2/83,200 = 0.0195$ or $w_B(\text{last peak}) = 0.140 \text{ min}$.

Finally, the time required = $t_r(\text{last peak}) + w_B(\text{last peak})/2 = 10.2 \text{ min}$.

Note: you could get essentially the same answer by assuming the retention time for the last peak = the chromatogram time which would greatly simplify the calculations. With the old column, $w_B(\text{last peak})$ becomes 0.263 min, and $k = 11.00$. Then with the new column, $k = 9.15$ or $t_r(\text{last peak}) = 10.2 \text{ min}$. (same to 3 sig figs).

6. It is desired to analyze a series of anilines (see structure below) using HPLC with a silica-based reversed phase column. If the conjugate acids of anilines (anilinium ions) have pK_a values ranging from 4 to 5, indicate to what pH the eluent should be buffered for:

a) retention of anilines

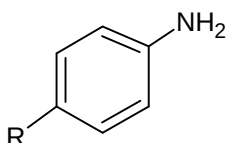
pH range of 5 to 8 would be best. pH of 5 to 6 would result in some reduction of retention due to partial ionization, while $pH > 8$ is not good for the health of the column.

b) retention of ion pairs (with the addition of the strong acid, hexanesulfonic acid to the eluent)

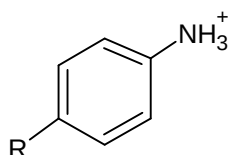
pH of 2 to 4 would be best where the compounds exist in the cation form needed to form ion pairs.

Explain your answer.

aniline



anilinium ion



7. The table below shows the retention times and peak widths for 3 compounds separated with reversed phase HPLC using an eluent of 45% acetonitrile, 55% pH 5 aqueous acetate buffer and a C18 column:

Compound	Retention time (min.)	Peak Width at base (min.)	pK _a
Unretained Peak	1.60	-	-
2,4-dichlorophenol	5.68	0.25	7.80
2-Methylphenol	5.97	0.26	10.09
3-EthylPhenol	6.72	0.30	10.05

a) Using the same column and same two eluent constituents (acetonitrile and pH 5 buffer), how can the resolution between 2,4-dichlorophenol and 2-methylphenol be improved (assuming α remains constant with % acetonitrile)?

*The resolution between the two least well resolved pair (2,4-dichlorophenol and 2-Methylphenol) is 1.14, which is not baseline resolved. Greater resolution is needed, which can be done by increasing k . Since this is reversed phase, a weaker solvent is needed. **One would increase the % pH aqueous buffer.***

b) Suggest a different pH to improve the above separation of 2,4-dichlorophenol and 2-methylphenol by increasing the separation factor (α). Discuss how you would expect elution to change.

Because the pK for 2,4-dichlorophenol is lower than for 2-Methylphenol, increasing the pH to around 7.5 to 8 would be expected to improve the separation. 2,4-Dichlorophenol readily ionizes at those pH values, leading to reduced retention and improved selectivity without significantly affecting the percent ionization for 2-methylphenol.