**Chem 230, Fall, 2014**
**Homework Set # 2 - SOLUTIONS**

**Longer Problems**

1. The table below shows the retention times and peak widths for 3 compounds separated by reversed-phase HPLC using a 250 mm length x 4.6 mm inside diameter column with an eluent of 45% acetonitrile, 55% water:

| Compound | Retention time (min.) | Peak Width at half height (min.) | $pK_a$ |
|---|---|---|---|
| phenol | 4.72 | 0.21 | 9.98 |
| 2-nitrophenol | 5.68 | 0.25 | 8.39 |
| 2-methylphenol | 5.91 | 0.26 | 10.09 |

Note: $w_b = 1.70 * w_{1/2}$

a) If the flow rate is 1.00 mL/min and the column is 62% by volume packing material (including stationary phase), calculate the time it takes for mobile phase to flow through the column.

*$V_{column} = \pi d^2 L/4 = [(3.14159)(0.46\ cm)^2(25\ cm)/4](1\ mL/cm^3) = 4.1548\ mL$*

*$V_m = [(100 - 62\%)/100]V_{column} = 1.58\ mL$*

*$t_m = V_m/F = 1.58\ mL/1.00\ mL/min =$* ***1.58 min.***

b) Calculate the capacity factor for phenol.

*$k(phenol) = (t_r(phenol) - t_m)/t_m = (4.72 - 1.58)/1.58 =$* ***1.99***

c) Calculate the plate number for the column using the last eluting compound.

*$N = 5.54(t_r/w_h)^2$ [eq. 2.20] $= 5.54(5.91/0.26)^2 =$* ***2860***

*can also use eq. 2.19 by switching $w_h$ to $w_b$: $w_b = 1.699 w_h = 0.44$; N =* ***2860***

d) Calculate the resolution for the two least well resolved peaks.

*Looking at the retention times, it is clear that the two o-phenols elute closest and will be the critical pair.* *$R_s = 2(5.91 - 5.68)/[1.699(0.25 + 0.26)] =$* ***0.53***

e) Calculate the separations factor for the two least well resolved peaks.

*$\alpha = t'_r(2\text{-methylphenol})/t'_r(2\text{-nitrophenol}) = (5.91 - 1.58)/(5.68 - 1.58) =$* ***1.056***

2. An open tubular GC column is 0.25 mm in diameter and 30 m long. The column wall is coated by a film that is 0.25 μm thick. The flow rate is 1.0 mL/min.

a) How long does it take He (an unretained gas to flow through the column)?

We can calculate $V_{column}$ as in prob. 1, but we will ignore the volume of the stationary phase. *$V_{column} = \pi d^2 L/4 = [(3.14159)(0.025\ cm)^2(3000\ cm)/4](1\ mL/cm^3) = 1.47\ mL$*

*$t_m = V_m/F = 1.47\ mL/1.00\ mL/min =$* ***1.47 mL***

b) An unknown gas takes 12.1 min to elute from the column. What is the distribution constant ($K_C$) for this gas? What is its retention factor?

*retention factor = (12.1 – 1.47 min)/1.47 min =* ***7.22***

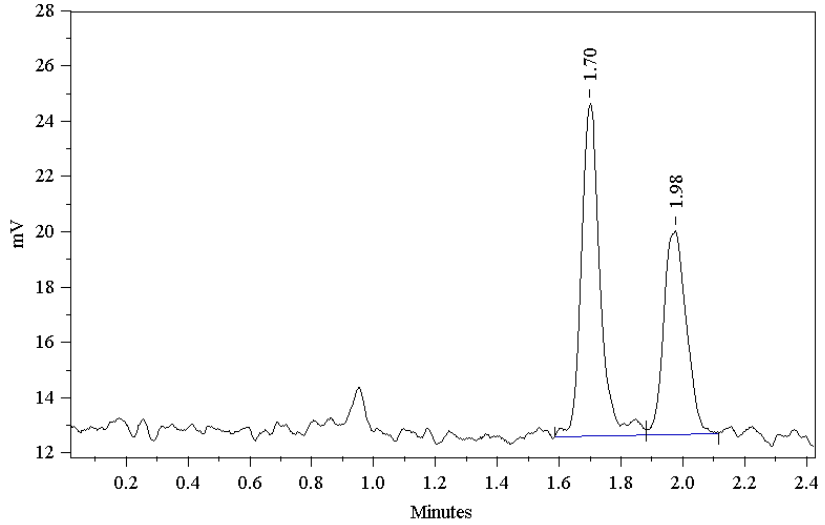
$K_C = k(V_s/V_m)$, $= V_s$ = volume of thin cylinder wall. This can be determined by determining the volume of two cylinders: one with diameter 0.25 mm and one with diameter 0.25 mm – 2(0.00025 mm).

*$V_s = [(3.14159)(0.025\ cm)^2(3000\ cm)/4] - [(3.14159)(0.025\ cm - 0.00005)^2(3000\ cm)/4]$*

*$V_s = 0.0059\ cm^3$*

*Alternatively, for a very thin wall (e.g. in this case),* $V_s = \pi dtL$ =
*(3.14159)(0.025 cm)(0.000025 cm)(3000 cm) = 0.0059* $cm^3$
$K_C = k(V_m/V_s) = 7.22(1.47\ cm^3/0.0059\ cm^3)$ = ***1800 (= 1.80 x*** $10^3$***)***

Questions 3 – 5 below deal with the following chromatogram:
This chromatogram shows the separation of glucose (1.70 min) from levoglucosan (1-6-anhydro-β-glucose) (1.98 min). The chromatogram was obtained with a 150 mm length C18 column using 90% water, 10% acetonitrile at a flow rate of 1.0 mL/min. A Table below gives peak widths and areas.




| Name | Ret. Time (min) | Conc. (mg/L) | Peak Height (mV) | Width at base (min) |
|---|---|---|---|---|
| Unretained | 0.95 | - | - | - |
| glucose | 1.70 | 1.0 | 12.05 | 0.115 |
| levoglucosan | 1.98 | 1.0 | 7.36 | 0.133 |

3. Determine retention factor (k) of glucose and the resolution ($R_S$) between glucose and levoglucosan.
$k = (1.98 - 0.95)/0.95 =$ ***1.1***
$R_s = 2(1.98 - 1.70)/(0.115 + 0.133) =$ ***2.3***

4. Based on the chromatography, which compound is more polar. Explain.
***Glucose****. The more polar compound will spend more time in the more polar eluent than in the non-polar stationary phase (for reversed phase columns as C18 is).*

5. Is the separation optimized for samples containing only these two compounds? If not, how could it be improved without changing the column?
***No.*** *An* $R_s$ *of 2.3 means that it could be sped up a little. This could be done by* ***increasing the % acetonitrile*** *(the stronger eluent).*

6. Compounds X and Y are run by GC on two open tubular columns, a DB-1 (100% methyl) and a DB-17 (50% methyl, 50% phenyl), that have identical dimensions (diameter, film thickness and length) under identical conditions. The retention times and peak widths are show in the Table below. Note: phenyl groups interact more strongly with polar groups than methyl groups.

| Compound | DB-1 Ret. time (min.) | DB-1 Peak Width (min.) | DB-17 Ret. time (min.) | DB-17 Peak Width (min.) |
|---|---|---|---|---|
| unretained | 1.11 | - | 1.12 | - |
| X | 10.21 | 0.32 | 11.07 | 0.35 |
| Y | 10.47 | 0.33 | 10.37 | 0.32 |

Peak width at baseline listed

a) Which compound is more polar? Explain your answer.

***Compound X.*** *It is more retained with the more polar column and less retained with the less polar column.*

b) What is the primary reason for improved resolution in the DB-17 column? Consider changes to N, $\alpha$, and k and explain your answer.

*Since the dimensions are identical, N would be expected to be very similar. Both $\alpha$ and k are increased, but* ***the change in $\alpha$ does more to improve resolution.***

7. An alternative fuel is to be analyzed by packed column GC. It is known to have around 10 compounds. When several standards of the 10 compounds are run by GC at 35°C (the lowest practical operating temperature), k values are found to range from 0.2 to 0.7. Is a successful analysis of this fuel likely with this column? Explain why or why not.

***No.*** *It is not likely to be a successful analysis. The k values are low, which may be o.k. when only a few compounds are present. Since 10 compounds exist, there is a good chance that several of the compounds will have overlapping peaks. Normally, that would mean decreasing the operating temperature to increase retention, but that is not possible due to currently operating the GC at the lowest stable temperatures. One would need to either lower the temperature more (through technical changes to the GC) or to use a more retentive column (e.g. thicker stationary phase film thickness).*