Chem 231 Final Exam
Solutions
April 9, 2007

Short Answer Section (each answer worth 5 points unless stated otherwise)

1. Solid phase microextraction (SPME) can be performed on liquid samples directly and on the head space above liquid samples. List characteristics of sample (characteristic of either the analytes or other components of the sample) for which headspace SPME would be generally preferred.

Sample Characteristic 1) analytes are relatively volatile; 2) sample matrix is non-volatile or thermally labile

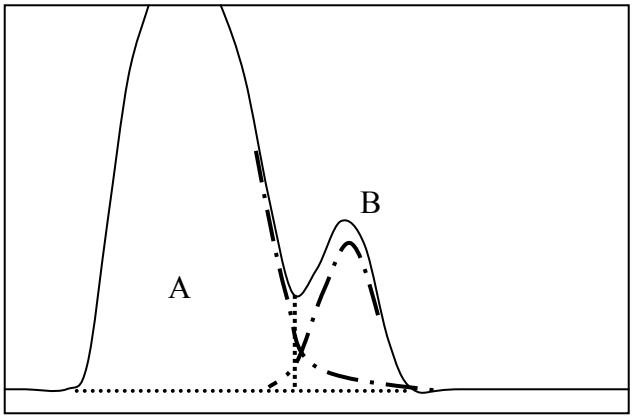
2. List or describe a method for trapping and analyzing volatile compounds in solid samples: SPME or passing gases in a closed container containing solid through a sorbent trap

3. Biodiesel is analyzed for residual glycerol. Glycerol is very polar while the major compound class of biodiesel is fatty acid methyl (or ethyl) esters. Which of the following stationary phases would be most useful in using SPE cartridges to trap and concentrate glycerol from biodiesel:
a) C18 b) C8 c) graphitic carbon **d) amino**

4. List an application for using low pressure column chromatography (gravity or flash) for which HPLC is often too expensive or time consuming to set up runs:
Common application for column chromatography = preparative separation

5. A compound in a complex sample is being analyzed by derivatization then HPLC. There is concern that extraction and conversion during derivatization is not very efficient. It would be a good idea to add a/an _____ to the original sample:
a) external standard b) internal standard **c) a recovery standard**
d) deuterated solvent

6. The following questions concern the chromatogram to the right, integrated as shown by the dashed lines. Assume the peaks are roughly Gaussian in shape. (Each worth 3 points)
a) Which peak has the largest percent integration error? **Peak B**



b) Is true area in this peak larger or smaller?
True Area is smaller (see plot)
Dash/dot line shows expected curves if no overlap

7. When running the Agilent 1100 using a long sequence, it is common to use an exit method. List an important task that the exit method performs.
Task = clean needle, purge column, shut off pump, turn off detector, turn off column heater (not in any order of importance)

8. Give the name of a detector for GC that is a selective detector and indicate what compounds it is selective for (3 points each)
Name = 1) ECD, 2) NPD, 3) MS (Selective Ion Mode), 4) FPD, 5) SCD
Class of compounds = 1) halogenated, 2) N or P containing, 3) all types, 4) S or P containing, 5) S containing

9. List one disadvantage of LC-MS relative to GC-MS (excluding efficiency advantages of GC over LC): cost, ionization of a smaller class of compounds, less fragmentation (for qualitative purposes)

Problem Section

1. (13 points) A research scientist isolates a compound (compound X) from a plant and conducts the tests using liquid – liquid extraction to optimize its extraction. Using water and 1-octanol, the following distribution coefficients , D (= $[X]_{octanol}/[X]_{water}$) are calculated as a function of pH:

pH	2	4	6	8	10	12
D	0.008	0.8	39	76	76	75

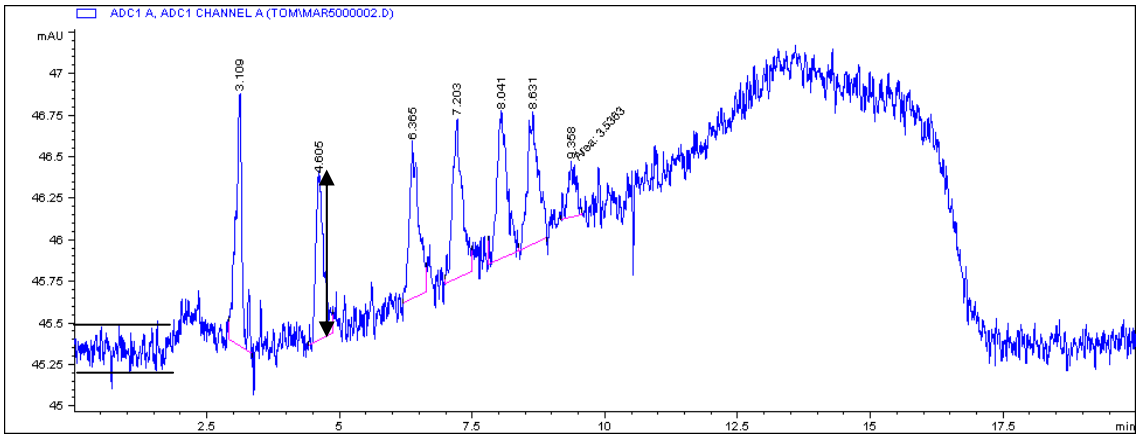
a) Does compound X appear to have acidic functional groups, basic functional groups or both types of groups?
Acid: HA – at low pH, exists as HA so D ~ K_{ow} = constant → doesn't match data
Base: B – at high pH, exists as B so D ~ K_{ow} = constant → matches data
Both groups – at low pH, exists as H_2L^+; at high pH as L^- and as HL at intermediate pH (but may be zwitterion) so highest D value at intermediate pH → doesn't match data

b) Suggest a pH for isolation of compound X from an aqueous extract of the plant material using a liquid – liquid extraction with water and octanol.
Any pH between 8 and 12 should work

c) If compound X is analyzed using HPLC with a C18 column, what would be an optimal pH if relatively strong retention is needed along with a method that will not cause degradation of the column? What happens if the pH is higher than or lower than the optimal pH?
A pH of 7 (6 to 8 o.k.) is optimal. At lower pH, retention is not so great, at higher pH the column will degrade

d – bonus 3 pts) Using information in the table, calculate a pK_a value for compound X.
From a) we know we have a weak base and $D = [B]_o/([B] + [BH^+])_{aq}$ and $K_a = [B][H^+]/[BH^+]$. Combining these, we get $D = K_{ow}/(1 + [H^+]/K_a)$.
At high pH, $[H^+] << K_a$ so $D \sim K_{ow} = 76$. By plugging in D values and $[H^+]$ for pH = 2, 4, or 6, and solving for K_a, we find $K_a = 1.05 \times 10^{-6}$ or ***$pK_a = 5.98$***.

2. (18 points). The chromatogram shown below was for sugars present at a concentration of 0.4 μg mL^{-1}. These sugars were separated using a **normal phase** column and a gradient program with an increasing % water in acetonitrile. Peaks corresponding to compounds were eluted at times of 4.60, 6.36, 7.20, 8.04, and 8.63 min. Neglect the peak at 9.36 min.



a) Estimate the detection limit for the sugar eluting at 4.60 min. assuming that the minimum detectable signal is defined as a signal to noise (peak to peak) of 2.
Noise is estimated by bands at beginning of chromatogram ~ 45.50 – 45.20 = 0.30
Signal (peak height) is estimated by arrow near peak at 4.6 min, as 46.45 – 45.4 = 1.05
S/N = 1.05/0.30 = 3.5
By proportion, 0.4 μg mL^{-1}/3.5 = LOD/2 where S/N = 2 in LOD
LOD = 0.2 μg mL^{-1}.

b) Does the sensitivity appear to improve or get worse for later eluting compounds? Is this normally expected?
Peak height decreases somewhat while the noise stays about the same so sensitivity gets worse. How sensitivity changes may depend on the compound and the detector, but in general, peaks get broader and shorter later in elution, so ***yes, this normally is expected****.*

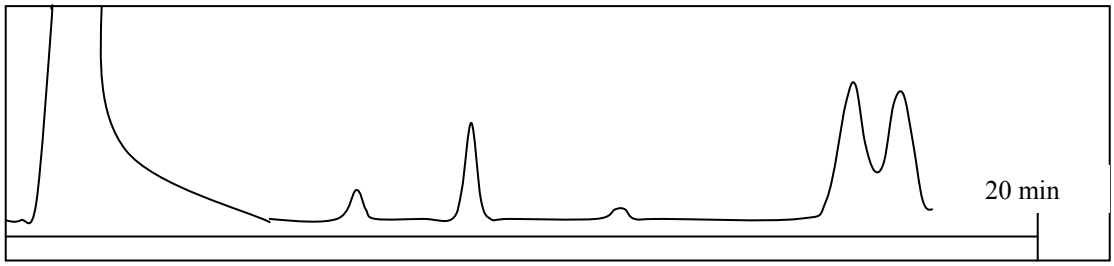
c) Based on the column type and retention times, which peak corresponds to the most polar compound?
Since this is normal phase, the most polar compounds will stick most strongly to a polar column and elute latest. ***The most polar compound is in the peak at 8.63 min.***

3. (15 points) A method was developed to test for the analysis of fatty acids in particulate matter in the air in order to determine if meat grilling is a significant source of particulate matter. Air was passed through a filter at a flow rate of 17 L/min for 12 hours with the particulate matter trapped on the filter. The filters were extracted in a mixture of methanol and dichloromethane to remove the fatty acids. This extract, containing fatty acids, was then reacted to fatty acid methyl esters (FAMEs). The FAMEs were extracted into hexane using hexane and water. The hexane was collected and evaporated to less than 1 mL and then diluted to 1.0 mL in hexane with nonane added as an internal standard added so that nonane was present at a concentration of 25.0 μg mL^{-1}. A standard containing FAMEs and nonane with each component present at 25.0 μg mL^{-1} was also prepared. These samples were analyzed by GC with the data shown below. Given atomic weights, **calculate the concentration of stearic acid ($CH_3(CH_2)_{16}COOH$) in the air in units of ng L^{-1}**. Stearic acid methyl ester is $CH_3(CH_2)_{16}COOCH_3$. AWs: C = 12.01, H = 1.008, and O = 16.00 (all in units g/mol).

	Peak Areas	
	Nonane	Stearic Acid Methyl Ester
Standard	32,061	26,931
Sample	47,133	15,031

V_{air} = (17 L/min)(12 hours)(60 min/hr) = 12240 L
$A_X/A_{IS} = FC_X/C_{IS}$
Standard $F = (A_X/A_{IS})/(C_X/C_{IS})$ = (26931/32061)/(25/25) = 0.840
Sample C_X (methyl ester) = $(A_X/A_{IS})(C_{IS}/F)$ = (15031/47133)(25.0/0.84) = 9.491 μg mL^{-1}
Mass FAME = (9.491 μg mL^{-1})1.0 mL = 9.491 μg mL^{-1}
MW(FA) = 284.5 g/mol; MW(FAME) = 298.5 g/mol
So Mass FA = (9.491 μg)(284.5 g/mol/298.5 g/mol) = 9.007 μg
Concentration in ng/L = (9.007 μg)(1000 ng/μg)/(12240 L) = ***0.74 ng/L***

4. (7 points) The following chromatogram is obtained by GC using a 30m x 0.25 mm DB-5 column (non-polar) column. When looking at the mass spectrum, it is seen that the compounds corresponding to the last two peaks have very different polarities.
a) Indicate how separation of the last two peaks can be improved without changing the column.
b) Indicate how separation of the last two peaks can be improved by switching to a different type of column.



a) Run at lower temperatures (or possibly lower flow rates)

b) Use a more polar column