Name Answer Key

**CEM 434**

**Exam I (100 points)**

**October 5, 2016**

**Read each question carefully. Write neatly. I will not guess at grading illegible writing. Use the correct number of significant figures.**

**1 (10 pts). Describe the three undesirable effects of band broadening that influence the plate height, H, in the van Deemter equation.**

$H = A + B/u + Cu$ desire H be a small #!

$N = L/H$

A = eddy diffusion – differences in path length for molecules transported through a packed stat. phase due to tortuous pathways.

B = longitudinal diffusion – diffusion longitudinally of an injected volume on column. Movement from high conc to low conc. Broadening is greater at lower flow velocities

C = Mass transport of analyte in and out of the thick and viscous stat. phase. Similar to stagnant mobile phase mass transfer. Broadening is greater at higher flow velocities.

**2. (10 pts). Describe what the general elution problem is in general and how it is solved in gas and liquid chromatography. Be specific.**

Problem of separating analytes with widely different distribution coefficients or retention factors with one set of separation conditions.

Solved in GC through temperature programming

Solved in LC through mobile phase adjustments – gradient elution

**3. (10 pts). Compare and contrast reversed- and normal-phase liquid chromatography in terms of the stationary phases, mobile phases and elution order for analytes A (most polar), B and C (least polar).**

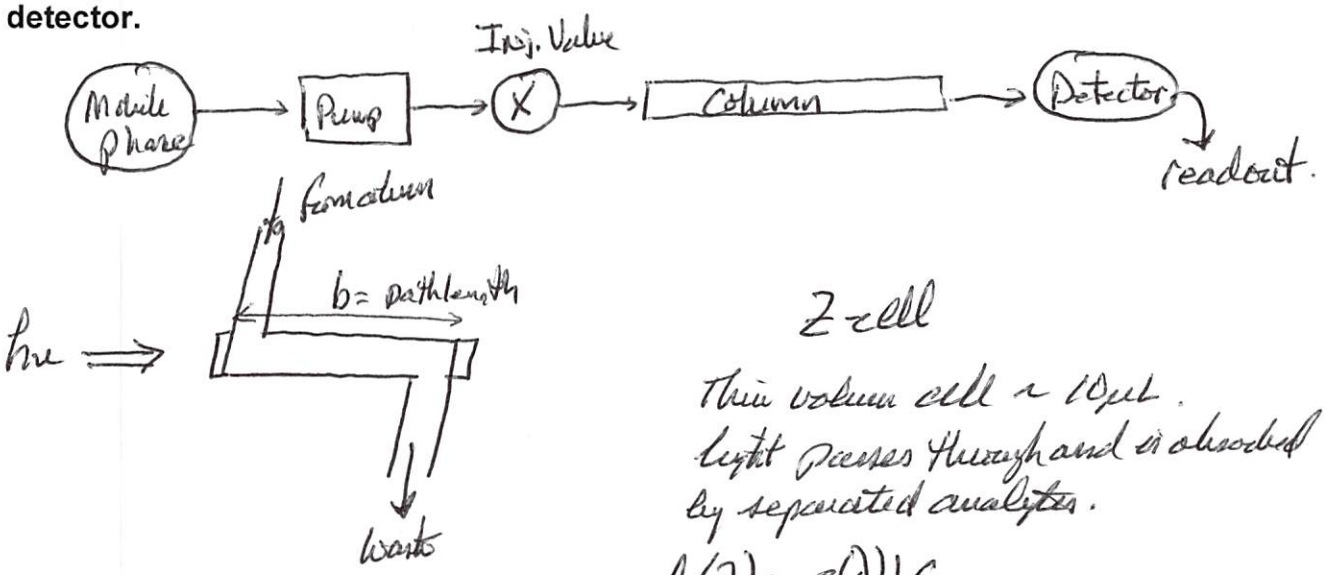
Reversed-phase: nonpolar stat. phase ($C_{18}$) + polar mobile phase ($H_2O$ / $CH_3CN$)

Elution order: A → B → C → time


Normal-phase: polar stat. phase (C-OH) + nonpolar mobile phase (hexane)

Elution order: B → B → A → time


**4. (10 pts). Sketch the design of a typical HPLC system with a UV/Vis absorbance detector.**




**5. (15 pts). The following chromatogram was obtained using a C18 column optimized for use in separating proteins and peptides. This separation was performed with a flow rate of 1.0 mL/min. Calculate the retention factor for each peak in the chromatogram and create a results table that might be used in a report. What is the resolution between Val-Tyr-Val and Met-Enk**

answer assumes use of times given at peaks.

$k_1' = \frac{t_r - t_m}{t_m} = \frac{3.3 - 2.1}{2.1} = 0.57$ Gly-Tyr $\quad k_2' = \frac{4.7 - 2.1}{2.1} = 2.2$ Val-Tyr-Val

$k_3' = \frac{10.1 - 2.1}{2.1} = 3.8$ met-Enk

$R_s = \frac{2(10.1 - 6.7)}{(1.0 + 0.5)} = \underline{4.5}$

| | $k'$ |
|---|---|
| Gly-Tyr | 0.57 |
| Val-Tyr-Val | 2.2 |
| met-Enk | 3.8 |

**6. (5 pts). In the development of a GC method, list three experimental variables under the control of the analyst.**

1. Temperature of column – temperature program
2. Column or stationary phase chemistry
3. Column architecture – packed vs. open tubular.
4. Flow rate of carrier gas also.

**7. (5 pts). Use your knowledge of partition kinetics to speculate on how an analyte might behave if the column temperature in a GC analysis is set too low.**

Remember, analytes must be volatilized and in the gas phase on the column. If the column temperature is too low, the analyte might condense and be permanently retained on the column stationary phase.

$K = \frac{C_S}{C_M}$

**8. (10 pts). In a GC separation of alkanes, for which the chromatographs are shown below, what was done to sharpen peaks and shorten run times? Justify your answer.**

In the region with the exam, the overall separation time was reduced from 50 min to 8 min. Also, the peak widths, especially for the later eluting components, were dramatically reduced.

This could be accomplished in GC by temperature programming. The top chromatogram had a fixed temperature (isothermal) of 100°C while in bottom chromatogram temperature was ramped from 50-120°C.

Temperature programming.

**9. (10 pts). For the experimental data shown below, the signal intensity at 330 is indicated in table form. (a) what is the signal-to-noise ratio for these data and (b) if one completed 10 sets of these measurements, how would the signal-to-noise change?**

Test has numerical & practical data

Repeated Measurements at 330 nm

4.4
2.6
3.7
4.6
3.8
2.5
4.1
3.5

$$\frac{S}{N} = \frac{\bar{X}}{SD} = \frac{3.6}{0.77} = 4.6$$

$$\left(\frac{S}{N}\right)_{10} = \sqrt{10}\left(\frac{S}{N}\right)_{0} = \sqrt{10}(4.6)$$

$$= 14.5$$

signal $\frac{S}{N}$ by over a factor of 3x

**10 (10 pts). The following calibration data were obtained in an absorbance measurement by UV/Vis spectrophotometry.**

| Concentration (ppm) | Absorbance (AU) | Blank Measurements |
|---|---|---|
| 19.5 | 0.980 | 0.001, 0.002, 0.001, 0.003, 0.002 |
| 9.74 | 0.440 | $\bar{Y}_{blank} = 0.0018 \cong 0.002$ |
| 4.87 | 0.255 | $SD = 8.4 \times 10^{-4}$ |
| 0.974 | 0.101 | |

**Calculate the sensitivity of the measurement and the limit of detection.**

Slope of Abs.C plot = 0.0431 = m  b = 0.111

$Y = mx + b$

Slope of this response curve is sensitivity (m) = 0.0431 AU/ppm

$Y_{min} = \bar{Y}_{blank} + 3s = 1.8\times10^{-3} + 3(8.4\times10^{-4}) = 4.3\times10^{-3}$

$C_{min} = \frac{Y_{min} - \bar{Y}_{blank}}{m} = \frac{4.3\times10^{-3} - 1.8\times10^{-3}\ AU}{4.31\times10^{-2}\ AU/PPM} = 5.8\times10^{-2}\ ppm$

$= 58\ ppb$

You must use linear regression to get slope!! Cannot determine from 2 pts to confidence.

**11. (5 pts) Indicate two methods that are often very useful for improving signal-to-noise ratios in analytical signals from measurements.**

grounding - electrical connection of all components to earth ground.

Shielding - placing instrument in Faraday cage to keep stray electromagnetic radiation / stray signals from affecting detector output signal.