Chromatography Problem Set

Go over the concepts of partition coefficient, retention time, dead time, capacity factor, relative retention factor.

1. Retention time can be used to identify a compound in a mixture using gas chromatography. Which one of the following will not affect the retention time of a compound in a gas chromatography column?
   A. concentration of the compound
   B. nature of the stationary phase
   C. rate of flow of the carrier gas
   D. temperature of the column

Questions 2-4 answer as true or false

2. All analytes in a chromatographic separation spend the same amount of time in the mobile phase.

3. Doubling the length of the column doubles the retention time of analytes and doubles the number of theoretical plates.

4. Doubling the length of the column doubles the retention time of analytes and doubles the resolution.

5. Describe how the “A” term of the van Deemter equation contributes to band broadening.

6. Describe how the “B/u” term of the van Deemter equation contributes to band broadening. Why is it inversely proportional to mobile phase flow rate?

7. Describe how the “Cu” term of the van Deemter equation contributes to band broadening. Why is it directly proportional to mobile phase flow rate?

8. The resolution term in chromatographic separations is proportional to ______________.

9. The plate height in chromatography is best described as ____________________.

10. Generally, it is thought by many chromatography dilettantes that twice the column length will give you twice the separation “power”. Comment on why this is false.
11. Gas chromatography was used to separate two similar compounds, methylcyclohexane and methylcyclohexene. A 40.0 cm packed column with OV-1 stationary phase yielded the following results:

<table>
<thead>
<tr>
<th></th>
<th>Retention time</th>
<th>Base width</th>
</tr>
</thead>
<tbody>
<tr>
<td>air (unretained)</td>
<td>1.9 min</td>
<td></td>
</tr>
<tr>
<td>methylcyclohexane</td>
<td>10.0 min</td>
<td>0.76 min</td>
</tr>
<tr>
<td>methylcyclohexene</td>
<td>10.9</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Calculate the relative retention (selectivity) factor for the two compounds.

12. A chromatographic separation of four compounds gave the following results,

<table>
<thead>
<tr>
<th>Analyte</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal</td>
<td>0.072</td>
<td>0.046</td>
<td>0.061</td>
<td>0.041</td>
</tr>
<tr>
<td>Retention Time</td>
<td>120 sec.</td>
<td>255</td>
<td>310</td>
<td>608</td>
</tr>
<tr>
<td>Peak Width at Base</td>
<td>28 sec.</td>
<td>70</td>
<td>55</td>
<td>98</td>
</tr>
</tbody>
</table>

The dead time ($t_m$) is 16.7 sec.

a. What is the capacity factor for analyte 4?

b. What are the number of plates for the column based on analyte 4?

c. What is the resolution factor for analytes 3 and 4?

Gas Chromatography

13. The separation efficiency of capillary columns over packed columns in GC is attributable to

14. The most common mobile phases in GC are _____________________________

15. Split injections are required in GC capillary columns because _______________________

16. The major contribution to the band broadening in gas chromatography is

(a) mass transport within the stationary phase
(b) longitudinal diffusion
(c) column packing size
(d) multiple paths within the column
(e) MW of the carrier gas

17. Assume that we using a very nonpolar stationary phase in GC we can guess that elution times for the four following analyte species can be ranked in terms of shortest to longest as:

I] Benzene       II] Isopropanol       III] Ethanol
18. The thermal conductivity detector, flame-ionization detector, and electron capture detector are respectively sensitive to 18

19. What is an FID and how does it work? What types of analytes does the FID respond to? 19

20. Why do capillary columns predominate in analytical GC? 20

21. What is temperature programming in GC? How does it gain an advantage over single T separations? 21

22. What is the electron capture detector? Explain its basis for operation, why is N2 necessary? What types of species are detected with the ECD? 22

23. A GC separation was conducted on a sample containing a pesticide analyte, compound X. This sample was treated with an internal standard of Q, giving a concentration of 15.0 ppm. A 1.0 µL injection onto the GC gave an FID response of 1012 for Q and 3411 for X. A 1.0 µL standard solution of 30.0 ppm Q with 15.0 ppm of X was injected giving a response of 899 and 2791 respectively.
What is the concentration of Q in the sample? 23

24. A GC-FID analysis was conducted on a soil sample containing pollutant X. The following separations were conducted:

<table>
<thead>
<tr>
<th>Injection 1</th>
<th>21.1 ppm Toluene Internal Standard</th>
<th>t,(minutes)</th>
<th>peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33.4 ppm X</td>
<td>10.11</td>
<td>36,242</td>
</tr>
<tr>
<td>Injection 2</td>
<td>21.1 ppm Toluene Internal Standard</td>
<td>14.82</td>
<td>45,997</td>
</tr>
<tr>
<td></td>
<td>unknown concentration X</td>
<td>14.77</td>
<td>39,115</td>
</tr>
</tbody>
</table>

What is the concentration of X in the sample? 24

25. In gas chromatography, what is the main advantage of a FID over a TCD? 25

(a) It is more sensitive to most organic compounds.

(b) It is more sensitive to H2O and CO2.

(c) It is selective for compounds with electronegative groups.

(d) It is less sensitive to gradient elution.

(e) It is non-destructive
26. Assume that we using a very nonpolar stationary phase in GC we can guess that elution times for the four following analyte species can be ranked in terms of shortest to longest as: 

<table>
<thead>
<tr>
<th>I. Benzene</th>
<th>II. Isopropanol</th>
<th>III. Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) I, II, III</td>
<td>b) II, I, III</td>
<td>c) I, III, II</td>
</tr>
<tr>
<td>d) III, II, I</td>
<td>e) III, I, II</td>
<td></td>
</tr>
</tbody>
</table>

HPLC

27. High performance liquid chromatography (HPLC) separations are generally characterized as having a higher efficiency than gas chromatography (GC) separations.

28. When considering the van Deemter equation, why does HPLC require small column packing particles?

29. What is gradient elution and how does this differ from an isocratic one? What advantage does gradient elution have over isocratic separations? When using a C-18 stationary phase is it more beneficial to increase or decrease m.p. polarity during elution?

30. What is meant by a bulk property detector? Give an example of an HPLC detector that is based on bulk properties and one that is not.

31. In reverse phase HPLC, molecules with ______ polarity elute first, and molecules with ______ polarity elute last.

   (a) lower, higher
   (b) varies with solutes
   (c) higher, lower
   (d) same

32. Predict the elution order of the following solutes in reversed phase HPLC.

   a] \[\text{OH} \quad \text{Cl} \]
   b] \[\text{OH} \quad \text{OH} \]
   c] \[\text{C}_6\text{H}_5 \]
   d] \[\phi \]
33. The elution order for the following solutes in a liquid chromatography system consisting of a toluene mobile phase and a silica stationary phase would be

   a) benzene, tetrahydrofuran (C\textsubscript{4}H\textsubscript{8}O), methanol
   b) methanol, benzene, tetrahydrofuran (C\textsubscript{4}H\textsubscript{8}O)
   c) tetrahydrofuran (C\textsubscript{4}H\textsubscript{8}O), methanol, benzene
   d) methanol, tetrahydrofuran (C\textsubscript{4}H\textsubscript{8}O), benzene
   e) benzene, methanol, tetrahydrofuran (C\textsubscript{4}H\textsubscript{8}O)

34. In modern HPLC’s, it is customary to separate complex mixtures using a _______ elution and a _______ column.

   (a) isocratic, normal-phase
   (b) gradient, reverse-phase
   (c) isocratic, reverse-phase
   (d) gradient, normal phase
   (e) isothermal, capillary

35. What would be the relative advantages and disadvantages of using FT-IR as a HPLC detector? Discuss at least 2 advantages and 2 disadvantages. Comment on the universality (or lack of) of the detector.

36. How does the capillary column configuration achieve its advantages over the packed column setup in gas chromatography?

37. What is pulsed flow in HPLC, why does it occur, and why is this not a desirable feature?

38. An HPLC analysis was conducted for caffeine on “Super-Extra-Energy Formula 2.2 with Hyper-Drive Now!” sports drink. A 10.1 ppm methanol IS standard was introduced both into the sample and a standardized solution of 304 ppm of caffeine. The measured by a diode-array detector at each \( \lambda_{\text{max}} \) for the absorptions for methanol and for caffeine are summarized in the table below. What is the concentration of caffeine in that sports drink?

<table>
<thead>
<tr>
<th>IS Caffeine</th>
<th>Sample</th>
<th>23141</th>
<th>52777</th>
</tr>
</thead>
<tbody>
<tr>
<td>304 ppm Caffeine standard</td>
<td>28441</td>
<td>77313</td>
<td></td>
</tr>
</tbody>
</table>
39. In a HPLC, a RI detector is a ______ detector and is limited to ______ elution.

(a) universal, gradient
(b) selective, gradient
(c) selective, isocratic
(d) universal, isocratic
(e) temperature-sensitive, gradient

Capillary Electrophoresis

40. Explain electroosmotic effect necessary for flow and separation in CE. What are the migration time trends for cations, anions, and neutral species?

41. How would the addition of an anionic surfactant above the critical micelle concentration affect the elution order of species in CE?

(a) will not affect that order
(b) will cause a reversal of that order
(c) will not affect the order of ionic species but cause the separation of neutrals
(d) will increase the selectivity of the cationic species
(e) will increase the selectivity of the anionic species

42. What is the electro-osmotic effect in capillary electrophoresis? Why do all species migrate to one electrode? Do they migrate to the cathode or the anode? What is the general order of migration time for cations, anions, and neutrals? Why are neutrals poorly resolved?
Answers

1 Retention time of each species in the mixture depends on relative attraction to the stationary and mobile phases, and is independent of species concentration. Temperature, flow rate of carrier gas, the nature of the stationary phase and temperature all impact on the interactions of the species with the stationary phase and thus retention.

2 True. All analytes spend $t_0$ (the dead time) amount of time in the mobile phase. Differences in retention are due solely to the amount of time that different analytes spend in the stationary phase.

3 True.

4 False. Although the number of theoretical plates will be doubled in this example, the master resolution equation indicates that resolution only increases as the square of the number of theoretical plates. Thus, resolution in this example will only increase by the square root of 2, or a factor of 1.4. This result is why increasing column length is not the best strategy for increasing resolution, especially considering one will sacrifice analysis time.

5 Multiple paths – read notes

6 Longitudinal diffusion – please read notes

7 mass transfer effects – read notes

8 $L^{1/2}$

9 variance per unit length

10 Remember that $Rs \propto L^{1/2}$. See chapter 26 pages 776-782. So 2x the column length increase resolution by 1.4. Also remember that $B/u$ effects increase with separation time and 2x will increase t by 2x. Also, using a longer column uses more m.p. and decreases experimental throughput.

11 $\alpha = (t_{r2} - t_m) / (t_{r1} - t_m) = 10.9 - 1.9 / 10 - 1.9 = 1.11$

12 a) $k' = (t_r - t_m) / t_m = (608-16.7) / 16.7 = 35.4$
   b) $N = 16(t_r/w)^2 = 16(608/98)^2 = 616$
   c) $Rs = \Delta t_r / W_{avg} = (608-310) / 0.5(98+55) = 3.9$

13 $B/u$ effects

14 $H_2$, He, and $N_2$

15 they are limited in terms of sample loading

16 b
all species, organics, and electron withdrawing organics

The flame ionization detector (FID) for GC is based on the formation of organic radicals, CH and CHO within a flame. These radicals are reduced at a cathode and the current flow is proportional roughly to the number of organic carbons in the analyte. The flow or effluent from the separation column is fed to a flow air and fuel (H₂) where the analytes are combusted. A cathode is further upstream from the flame. The FID is responsive only to organic carbons. Again this gets back to the v-D eqn. The B/u, longitudinal diffusion term contributes most to band broadening in the gas phase. Capillary columns allow for the unobstructed and therefore faster flow of the gaseous m.p. over their packed counterparts.

By going from colder to warmer temperatures, it is possible to add another dimension separation of solutes beyond the chromatographic ones. This is based on boiling point differences. Generally the initial T is below that of the solutes species and slowly ramped up. See also problem 27-3.

See http://www.instrumentalchemistry.com/gasphase/pages/ecd.htm Nickel-63 source emits energetic electrons collides with N₂ (introduced as make-up gas or can be used as carrier gas)
Ni-63 \Rightarrow e^-

e^- + N_2 \Rightarrow 2e^- + N_2^+

The result is a constant current that is detected by the electron collector (anode).

As an analyte flows through past the Ni-63 source electron capture is possible by electron-withdrawing species:

\[ A + e^- \Rightarrow A^- \]

Current decreases as a result of e- capture by analyte. This is one of the few instances in which a signal is produced by a decrease in detectable phenomenon.

Sensitive to electron withdrawing groups especially towards organics containing –F, -Cl, -Br, -l also, -CN, NO

\[
\frac{A_s}{[X]} = F \frac{A_q}{[Q]}
\]

\[
\frac{2791}{[30.0]} = F \frac{899}{[15.0]}
\]

\[ F = 1.55 \]

\[
\frac{3411}{[X]} = 1.55 \frac{1012}{[15.0]}
\]

\[ [X] = 32.6 \text{ ppm} \]

\[ 39,115 \times (36,242/39,115) \times (33.4 \text{ppm}/45,997) = 26.3 \text{ ppm} \]

False. Efficiency is generally related as the number of theoretical plates, and GC separations generally exhibit an order of magnitude higher number of theoretical plates than HPLC separations.

\[ H = A + B/u + Cu \]

In the v-D equation the MT effects predominate, i.e. Cu. Increasing the surface area/bulk ratio of the s.p. is a way to great improve the MT between the two phases.
This requires small diameter supports for the s.p. The cost is the pressure required to squeeze the m.p. through the space between the smaller diameter particles.

Gradient elutions vary the m.p. solvent composition and polarity during separation. This has an advantage over isocratic separations where solvent compositions are kept constant. A gradient elution will allow for the separation of a large variety of species with a broad spectrum of polarities with a much shorter times than isocratic ones. Generally it’s best that polarity decrease during separation when using a C-18 s.p. If a non-polar m.p. is used at the beginning of the elution, there will be no retention between the solutes and the C-18 s.p.

See problem 28-7 h. Measure a physical property of the m.p. Example – UV-vis absorbance, fluorescence techniques are examples of bulk property detectors. Electrochemical detectors are not, since they are based on redox exchanges with solutes near the electrode surface.

Advantages: Collection of the entire IR spectrum of analytes is possible. FT-IR data acquisition is rapid so it works well with a flowing system such as HPLC.

It is nearly universal in its response to analytes. A few have no IR active modes, but most especially larger molecules have some sort of IR active vibration.

Disadvantages: FT-IR detection is difficult in mobile phases that are highly polar such as water and alcohols that have intense absorptions.
The limit of detection is high relative to other HPLC detectors. Molar absorptivity for IR transitions is low compared with electronic ones.

There are other possibilities for both advantages and disadvantages. I’ll have to read consider your answers.

Starting with the van Deemter equation: \( H = A + \frac{B}{u} + Cu \) we should consider all three terms.

A – the dispersion of peak area due to multiple paths in the column is not a consideration in the capillary column as only one path predominates.

B/u – this is the longitudinal diffusion term which tells us that the longer the time the analyte band spends in the column the more dispersion. This is the most significant of the three terms
in the band broadening characteristics of the van Deemter equation. Flow of the mobile phase through a capillary column is relatively unimpeded when in comparison with a packed column. This allows for a faster mobile phase flow rate through the column.

Cu – The mass transfer between the m.p. the s.p. term is of less importance in the considerations of the GC capillary column however, the s.p. is made as thin as possible to accommodate facile kinetics between the s.p. and the m.p.

37 Pulsed flow is the rhythmic flow pattern that occurs due to the cycles within a reciprocating pump. This is not a desirable feature as it causes remixing of the solutes in HPLC. Note – it is partially addressed by pulse dampers.

38 First normalize caffeine peaks with the response by the IS.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IS</th>
<th>Caffeine</th>
<th>Caffeine/IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>304 ppm Caffeine standard</td>
<td>28441</td>
<td>77313</td>
<td>2.7184</td>
</tr>
<tr>
<td>23141</td>
<td>52777</td>
<td></td>
<td>2.2807</td>
</tr>
</tbody>
</table>

Assume that

\[ y = mx + b \]

with \( y \) the normalized detector response and \( x \) the concentration. This one point analysis assumes that \( b = 0 \).

\[ 2.7184 = m(304) \]

\[ m = 8.927e-3 \]

Now calculate the analyte concentration.

\[ 2.2807 = 8.927e-3 (x) \]

\[ x = 255 \text{ ppm} \]

see [http://wilstar.com/caffeine.htm](http://wilstar.com/caffeine.htm) for typical caffeine concentrations.

Also using Formula 5-19 from Harris:

\[ \frac{A_x}{C_x} = F \left( \frac{A_s}{C_s} \right) \]

\[ \frac{77313}{304 \text{ ppm}} = F \left( \frac{28441}{10.1 \text{ ppm}} \right) \]
For the sample:

\[ \frac{52777}{C_x} = 9.031 \times 10^{-2} \text{(23141/10.1)} \]

\[ C_x = 255 \text{ ppm} \]

The electroosmotic flow is based on the flow of adsorbed cations to the \(-\text{O}^{-}\) sites on the surface of the silica capillary. These cations will migrate towards the cathode dragging along with it a solvation sphere of water molecules (and thus neutrals) along with solvated anions. All three major types are pulled to the cathode with migration time trends of

\[ t(\text{cations}) < t(\text{neutrals}) < t(\text{anions}) \]

What is the electro-osmotic effect in capillary electrophoresis? Why do all species migrate to one electrode? Do they migrate to the cathode or the anode? What is the general order of migration time for cations, anions, and neutrals? Why are neutrals poorly resolved?

http://en.wikipedia.org/wiki/Capillary_electrophoresis