Chromatography Problem Set

From Exam 2 2002

9] Describe how the "A" term of the van Deemter equation contributes to band broadening.

10] 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?

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

12] The separation efficiency of capillary columns over packed columns in GC is attributable to

13] The most common mobile phases in GC are ________________________________

14] Split injections are required in GC capillary columns because ___________________________

17] The resolution term in chromatographic separations is proportional to _____________________

18] The plate height in chromatography is best described as ___________________________

19] 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

20] The thermal conductivity detector, flame-ionization detector, and electron capture detector are respectively sensitive to

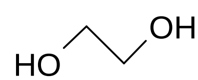
Answers

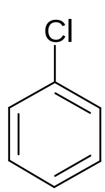
9] *Multiple paths – read notes*
10] *Longitudinal diffussion – please read notes*
11] *MT – read notes*
12] *B/u effects*
13] *H_2, He, and N_2*
14] *they are limited in terms of sample loading*
17] $L^{1/2}$
18] *variance per unit length*
19] *III, II, I*
20] *all species, organics, and electron withdrawing organics*

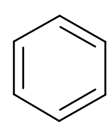
From Exam 3 2005

8] Predict the elution order of the following solutes in reversed phase HPLC. (5 points)

a] 

b] 

c] 

d] 

10] 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?

Answers To Exam 3 2005

8} B > A > C > D

10}

$$\frac{A_x}{[X]} = F\frac{A_q}{[Q]} \qquad \frac{2791}{[30.0]} = F\frac{899}{[15.0]}$$

F = 1.55

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

[X] = 32.6 ppm

From Exam 3 2006

1] 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 (20 points)

3] How does the capillary column configuration achieve its advantages over the packed column setup in gas chromatography? (10 points)

4] What is pulsed flow in HPLC, why does it occur, and why is this not a desirable feature? (10 points)

5] What is an FID and how does it work? What types of analytes does the FID respond to? (10 points)

6] Explain electroosmotic effect necessary for flow and separation in CE. What are the migration time trends for cations, anions, and neutral species? (10 points)

7] 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 λ_{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? (20 points)

	IS	Caffeine
Sample	23141	52777
304 ppm Caffeine standard	28441	77313

Answers

1]

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.

3]

Starting with the van Deemter equation: $H = A + B/u + Cu$ 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.

4]

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.

5]

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 (H_2) where the analytes are combusted. A cathode is further upstream from the flame. The FID is responsive only to organic carbons.

6]

The electroosmotic flow is based on the flow of adsorbed of cations to the $–O^-$ 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

$$t\ (cations) < t\ (neutrals) < t\ (anions)$$

7]

First normalize caffeine peaks with the response by the IS.

	IS	Caffeine	Caffeine/IS
Sample	23141	52777	2.2807
304 ppm Caffeine standard	28441	77313	2.7184

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 = 8.92$\underline{7}$e-3 *Now calculate the analyte concentration.*

2.2807 = 8.92$\underline{7}$e-3 (x)

x = 255 ppm *see http://wilstar.com/caffeine.htm for typical caffeine concentrations.*

Also using Formula 5-19 from Harris:

$A_x/C_x = F\,(A_s/C_s)$

77313/304ppm = F (28441/10.1ppm)

F = 9.031e-2

For the sample:

$52777/C_x = 9.031e\text{-}2\ (23141/10.1)$

$C_x = 255$ ppm

From Exam 3 2008

2] 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?

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

4] 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. ~~composition~~ polarity during elution?

5] 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.

6] Why do capillary columns predominate in analytical GC?

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

8] What is the electron capture detector? Explain its basis for operation, why is N_2 necessary? What types of species are detected with the ECD?

9] 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.

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

		t_r(minutes)	peak area
Injection 1	21.1 ppm Toluene Internal Standard	10.11	36,242
	33.4 ppm X	14.82	45,997
Injection 2	21.1 ppm Toluene Internal Standard	10.05	38,774
	unknown concentration X	14.77	39,115

What is the concentration of X in the sample?

ANSWERS to **Exam 3 2008**

2] 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

3]

$H = A + B/u + Cu$

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.

4]

Gradient elutions vary the m.p. solvent composition and polarity during separation. This has an advantage over isocratic separations where solvent compostions 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.

5]

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.

6]

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.

7]

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.

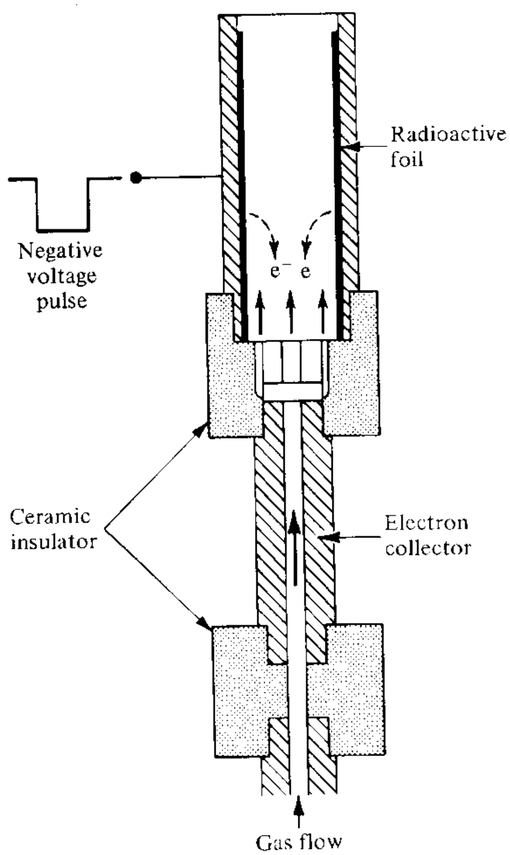
8]

See http://www.instrumentalchemistry.com/gasphase/pages/ecd.htm Nickel-63 source emits energetic electrons collides with N_2 (introduced as make-up gas or can be used as carrier gas) producing more electrons:

Ni-63 => e-

FIGURE 18.10

Electron capture detector. (Courtesy of Varian Associates.)



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

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, -I also, -CN, NO_2

9]

Remember that Rs α $L^{1/2}$. 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.

10]

39,115(36,242/39,115)*(33.4ppm/45,997) = 26.3 ppm*