CHEM 3590 Instrumental Analysis University of Manitoba

Test 2, November 6 2017

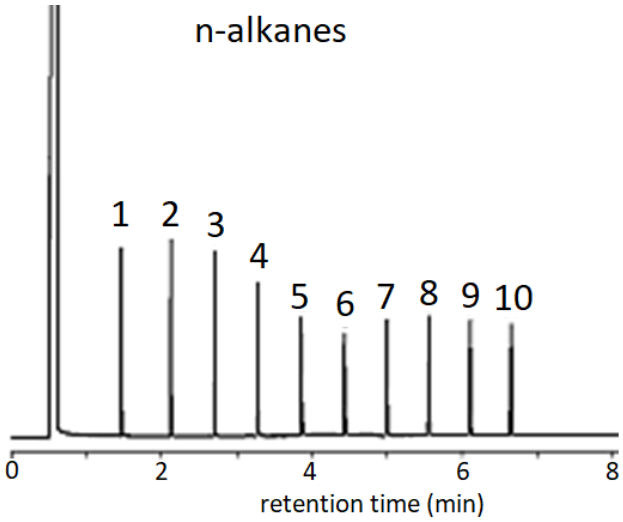
Human Ecology 108 10:30-11:30 am ***Please answer 5 questions out of 7***

Question 1:

What is the minimal resolution value for chromatographic peaks to be separated at the baseline? Find this by drawing a chromatogram and calculating the resolution using both equations available.

Question 2:

Given the gas chromatogram below obtained for n-alkanes, indicate approximate retention times for 2-ethyl butane and 3-methyl pentane and calculate their retention indices.



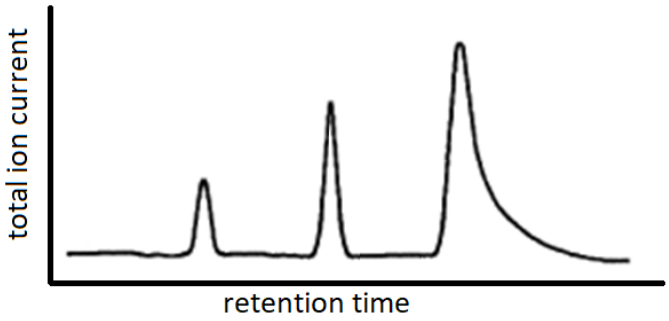
Question 3:

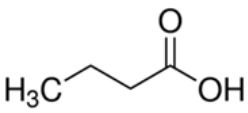
A mixture of monosaccharides (including glucose, galactose) is analyzed by GC-FID.

a) Propose a derivatization method for monosaccharides for GC.
b) How would this improve the chromatographic performance and the detection limit?

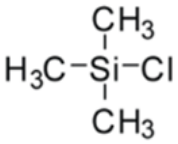
Question 4:

a) What is the main role of the GC-MS interface and how does it accomplish its task?
b) Butyric acid was derivatized with TMS-Chloride for GC-MS, but the reaction was incomplete. The reaction mixture was injected in the GC. The total ion chromatogram (below) showed three peaks. Assign a peak to each component and plot the selected $M^{+\cdot}$ selected ion chromatograms at their correct *m/z* ratios.





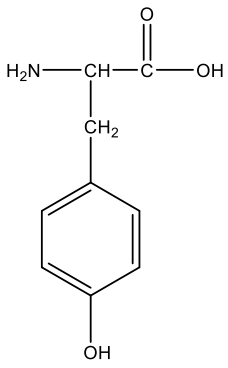
Butyric acid
MW 88



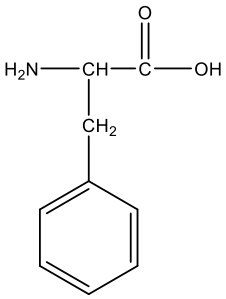
TMS-Cl
MW 108.5
(Si: 28, Cl: 35.5)

Question 5:

Two amino acids, tyrosine and phenylalanine, are being separated by anion exchange chromatography on trimethylbenzylammonium beads (pKa = 9.8).



Tyrosine, pI = 5.66

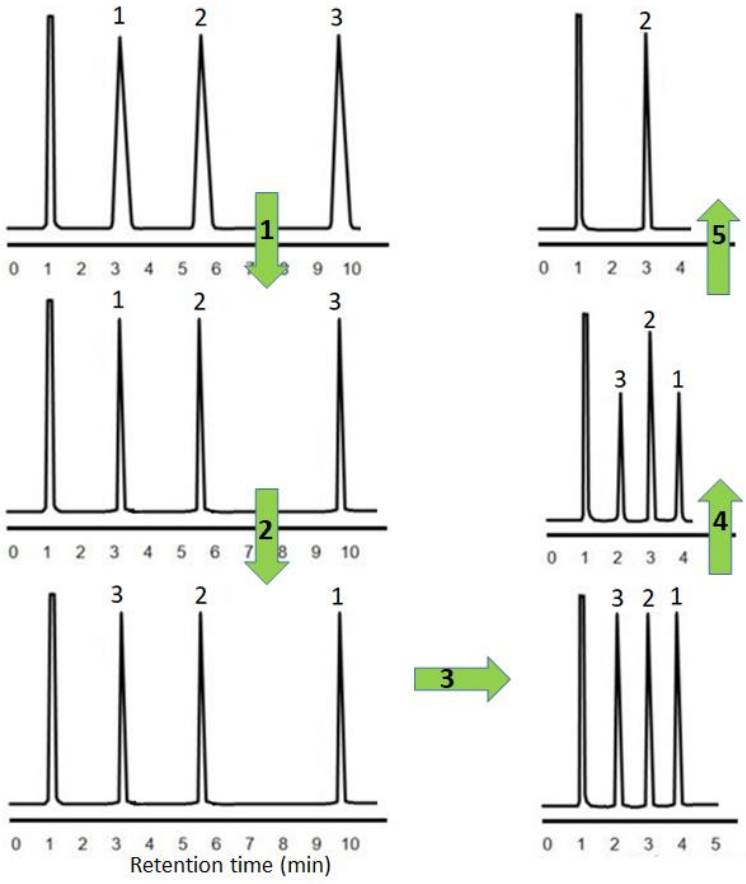


Phenylalanine, pI = 5.48

a) If the mobile phase is at pH 7, which amino acid forms the stronger interaction with the beads (and will elute second)? Justify.
b) Describe the effect of a pH gradient on this separation and justify.

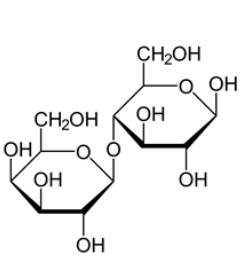
Question 6:

The following series of normal phase chromatograms (UV/fluorescence detection) was obtained by varying a single parameter at a time. Suggest a parameter for each step and justify (steps 1 to 5).

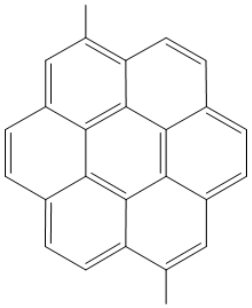


Question 7:

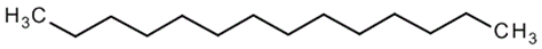
a) In a SEC experiment, a porous silica column is used to separate lactose, n-tetracosane and trimethylcoronene (see structures below). Predict their order or elution and justify.



Lactose
MW 342



Coronene
MW 342



n-tetracosane
MW 338

b) What kind of detector(s) would be useful for this separation and why?

Equations

$$I = 100 \times \left[n + \frac{t_{r(\text{unknown})} - t_{r(n)}}{t_{r(N)} - t_{r(n)}} \right]$$

$$I = 100 \times \left[n + \frac{\log(t'_{r(\text{unknown})}) - \log(t'_{r(n)})}{\log(t'_{r(N)}) - \log(t'_{r(n)})} \right]$$

$$k' = \frac{t_r - t_m}{t_m}$$

$$R = \frac{2\Delta t_r}{w_2 + w_1}$$

$$R = \frac{1}{4}\sqrt{N} \times \frac{\propto -1}{\propto} \times \frac{k'}{1 + k'}$$

$\alpha = k'_1/k'_2$

$$N = \frac{L}{H}$$

$$N = 16\left(\frac{t_r}{w}\right)^2$$