**NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY**
DEPARTMENT OF APPLIED CHEMISTRY
BACHELOR OF SCIENCE HONOURS DEGREE
END OF SECOND SEMESTER EXAMINATIONS – APRIL 2014
CHROMATOGRAPHIC SEPARATIONS – SCH 4292
TIME: 3 HOURS

INSTRUCTIONS TO CANDIDATES
Answer **ANY FOUR** questions. Each question carries 25 marks.
Total Marks – 100

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1. a. Define the following terms as they apply in chromatography.

    i.   Capacity factor
    ii.   Selectivity factor
    iii.   Resolution
    iv.   Eluate
    v.   Gradient elution   [10]

    b. Compare and contrast the role of the mobile phase in GC with that in ordinary

    LC. Include a description of the important properties of the mobile phase in

    each separation and its impact on the quality of a separation.   [8]

    c. What is a guard column? And why is it is necessary to use a guard column in an

    HPLC than in a GC.   [7]

2. a. The questions below refer to the two chromatograms shown below:
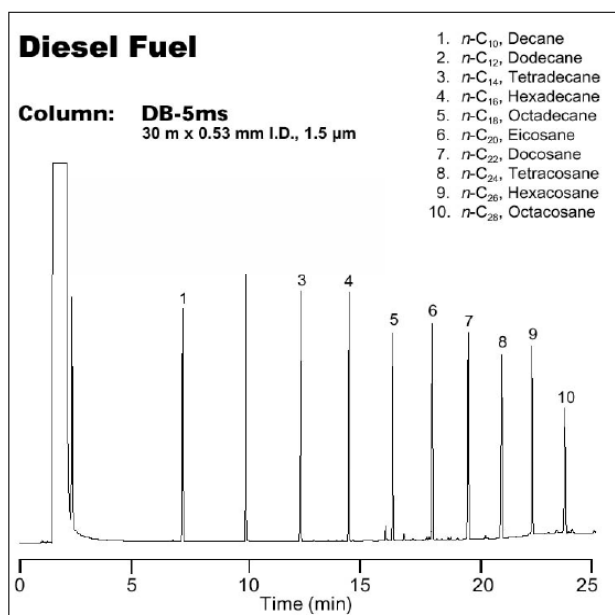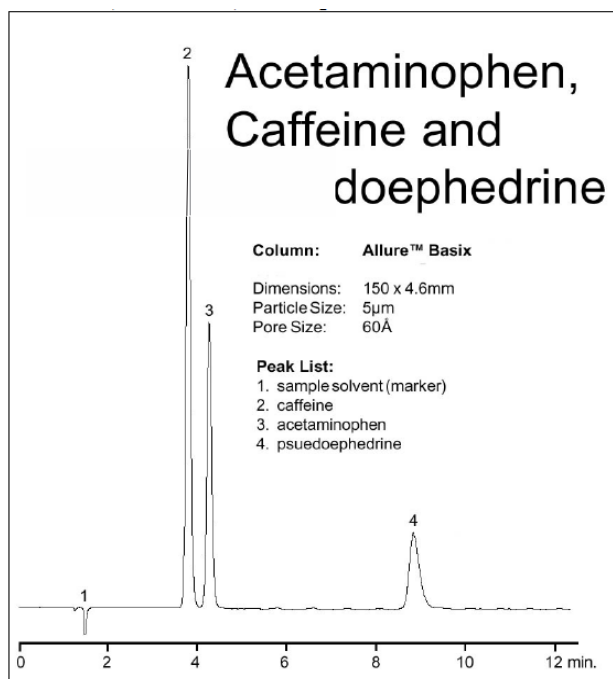
i. One of the chromatograms was recorded with capillary gas chromatography, the other with high performance liquid chromatography. **Assign** each chromatogram with the correct technique that was used for the separation. **Explain** in detail how you arrived at your conclusion. [5]

ii. Between capillary GC and HPLC. Which technique normally has the lowest plate height (*H*) and which technique normally has the largest number of theoretical plates (*N*). [4]

iii. Calculate the theoretical plate height, *H*, and the number of theoretical plates, *N*, for **peak 4 of the first chromatogram**. *It is ok to estimate the values of the variables you need for the calculation from the axes of the chromatogram.* The length of the column was 15.0 cm. [6]

iv. We discussed resolution as a measure of the separation of two peaks. **Sketch** two peaks that are not completely resolved and give a typical value of the resolution for these peaks. [4]

v. What is the value for the resolution of two peaks that have baseline resolution? In other words, what is the minimum value of the resolution for two peaks to be considered completely resolved? [2]

vi. Explain in detail two factors that one can apply to improve the separation of peaks that are co-eluting to have baseline resolution. [4]

3. The van Deemter equation relates the column efficiency (measured as *H*) to the mobile phase flow rate (μ):

$$H = A + \frac{B}{\mu} + C\mu$$

a. Sketch a typical van Deemter plot that shows how *H* depends on *μ*. **Clearly label the axes and the graphs.** [7]

b. There are three terms in the van Deemter equation: the *A* term, the *B* term, and the *C* term, each describing a different type of contribution to the band broadening.
Explain with the aid of diagrams what each term means and the type of band broadening described by **each of the 3 terms.** [12]

c. Show through the use of a diagram how the van Deemter curve for HPLC differs when using 10 μm particles and 3 μm particles. **Explain** why these curves are different. [6]

4. a. What are the Normal Phase, Reverse Phase, Ion Exchange and Size Exclusion Chromatography? What are the physical principles behind these different methods, and what properties of the molecule do they use to separate different compounds from each other. [20]

b. Consider the following 3 compounds;
   $HOCH_2CH_2OH$ (ethylene glycol); C_6H_{14} (hexane); $C_6H_5CH_3$ (toluene)
   In what order would the compounds elute in reverse phase HPLC. Explain.
   [5]

5. Answer the following questions related to the gas chromatogram below.
   Experimental conditions: Packed column (4 mm diameter x 2 m long), Carbowax
   stationary phase, 40 mL/min helium carrier gas flow rate, FID detector, column
   temperature = $100^{o}C$, injector temperature = $150^{o}C$, detector temperature = $150^{o}C$.
   Peak M corresponds to an unretained compound.
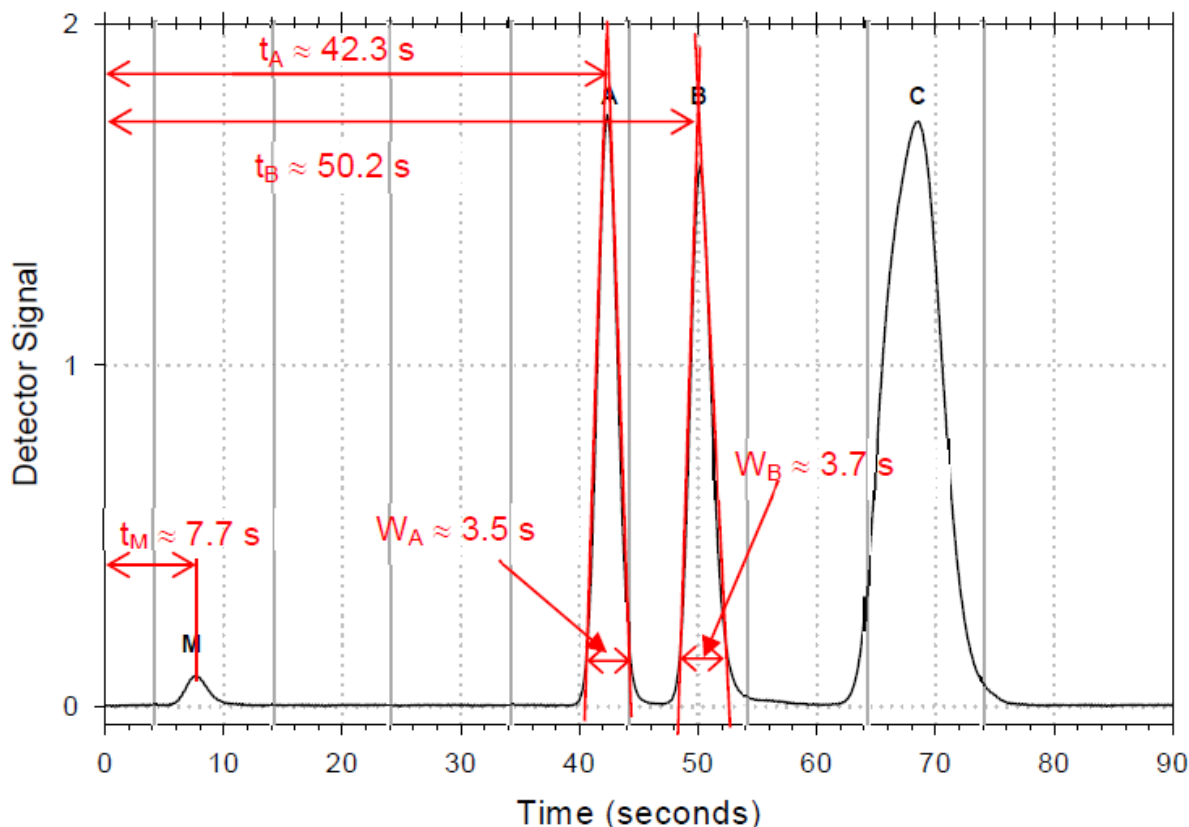
a. Caculate the selectivity factor and resolution for peaks A and B. [6]
b. Calculate the number of theoretical plates for peaks A and B. [4]
c. Based on the size of the peaks what can you say about the relative
   concentrations of components A and B. [2]
d. It appears peak C is the result of co-elution of two compounds. In GC how
   would you change experimental conditions to resolve these peaks? What effects
   are these changes likely to have on the separation of components A and B. [4]
e. What is a thermal conductivity detector? Why is a thermal conductivity detector
   a much more universal GC detector than a flame ionization detector? If the TCD
   is so much more universal, why use an FID at all? [9]

End of Examination!!!!!!!!!!!!!