

CHUKA UNIVERSITY

THIRD YEAR EXAMINATION FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN FOOD SCIENCE AND TECHNOLOGY AND HUMAN NUTRITION & DIETETICS

CHEM 304: ANALYTICAL CHEMISTRY

INSTRUCTIONS

Answer question **One** (Compulsory) and any other **Two** questions.

QUESTION ONE (30 MARKS)

a) Differentiate between the following:

- (i) Isocratic elution and gradient elution. (2 marks)
- (ii) Normal-phase chromatography and reversed-phase chromatography. (2 marks)

b) The concentration of caffeine in soft drinks can be determined by a reversed-phase HPLC separation using a mobile phase of 20% acetonitrile and 80% water and a nonpolar C₁₈ column. Results for a series of 10-mL injections of caffeine standards are as shown in the table below.

[Caffeine] (mg/L)	Peak area (arb. units)
50.0	230,000
100.0	450,000
125.0	550,000
250.0	1,090,000

- (i) State the purpose of mobile phase. (1 marks)
 - (ii) State the purpose of caffeine standards. (2 marks)
 - (iii) Determine the concentration of caffeine in a sample if a 10-mL injection gives a peak area of 420,000. (3 marks)
- c) During a chromatographic separation of components A and B, the distance travelled by solvent is 4.7 cm and that of sample A and B are 4.1cm and 1.8 cm respectively. calculate the retention factor for each component. (2 marks)
- d) Describe the principle of size exclusion chromatography. (3 marks)
- e) (i) Distinguish between accuracy and precision. (2 marks)

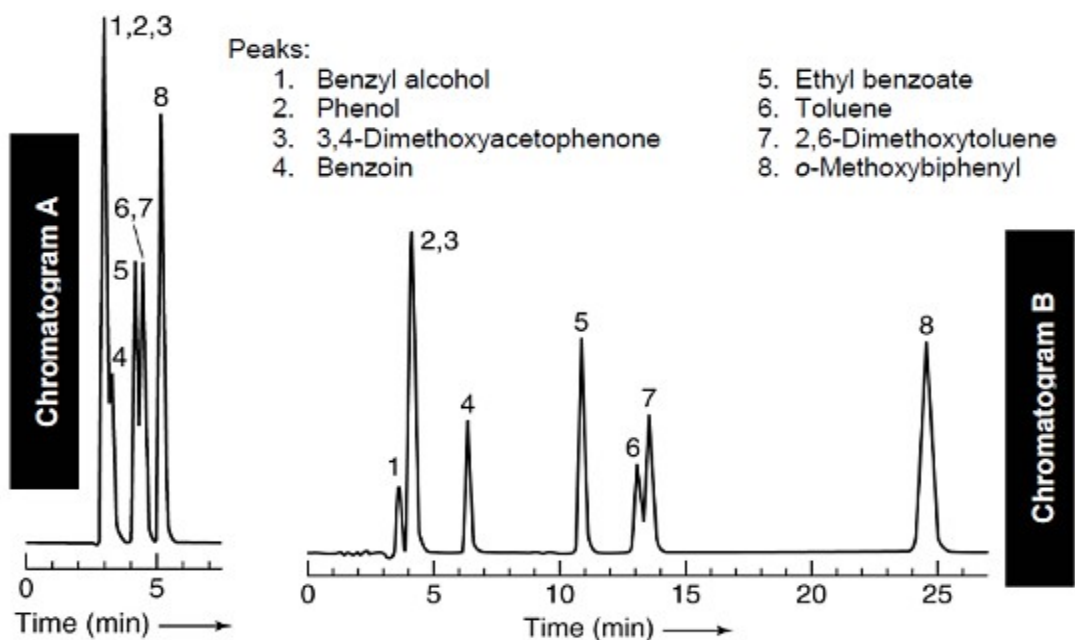
- (ii) Riboflavin (vitamin B₂) was determined in a cereal sample by measuring its fluorescence intensity in 5% acetic acid solution. A calibration curve was prepared by measuring the fluorescence intensities of a series of standards of increasing concentrations. The following data were obtained. Use the method of least squares to obtain the best straight line for the calibration curve and to calculate the concentration of riboflavin in the sample solution. The sample fluorescence intensity was 15.4. (5 marks)

Riboflavin, $\mu\text{g/ml}$	Fluorescence intensity
0.000	0.0
0.100	5.8
0.200	12.2
0.400	22.3
0.800	43.3

- f) Describe the basic difference between atomic emission and atomic absorption spectroscopy. (2 marks)
- g) Describe how the method of standard additions would correct or compensate for the physical effects and interferences in AAS. (2 marks)
- h) Explain why atomic emission methods with an ICP source are better suited for multielement analysis than flame atomic absorption methods. (1 mark)
- i) State the advantages of plasma sources compared with flame sources for emission spectrometry. (3 marks)

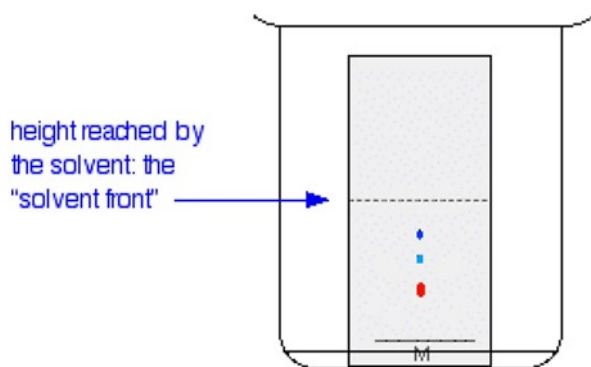
QUESTION TWO (20 MARKS)

- a) Consider the two chromatograms shown below.

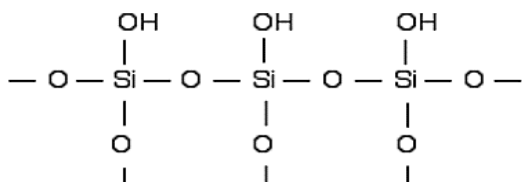


These chromatograms were recorded using reverse phase HPLC.

- State an example of a mobile phase that might be used for this type of separation (i.e., reverse phase). (1 mark)
 - State an example of a detector that might be used for these compounds. (1 mark)
 - State an example of a stationary phase that might be used for these separations. (1 mark)
 - Chromatogram B was recorded on the same column and with the same sample as chromatogram A, but the separation is very different. This is because the mobile phase was changed between the two separations. Explain how the mobile phase was changed to affect the separation so dramatically. (2 marks)
- b) Explain the ways in which a chromatographer performs a gradient separation in Liquid chromatography (LC) and gas chromatography (GC). (4 marks)
- c) This diagram below shows the results of a simple thin layer chromatography (TLC) experiment.



- (i) Describe briefly the procedure that would have been followed in order to obtain the above TLC chromatogram, assuming that a suitable thin layer chromatography plate was provided. (2 marks)
- (ii) Briefly explain why the beaker should be covered (1 mark)
- (iii) Describe briefly how the R_f value for each of the spots on the above chromatogram would be determined. (1 mark)
- (iv) The R_f value for a particular component in a mixture is only constant if you carefully control certain variables during the experiment. State three variables which would have to be controlled. (3 marks)
- (v) TLC technique can be used to identify particular amino acids in a mixture of amino acids. However, the chromatograms obtained are colourless. State a staining reagent that can be used to spray the plate to make the spots visible. (1 mark)
- (vi) The diagram shows the surface of silica gel structure.



A plate coated with silica gel was used as stationary phase with propanone as the solvent for thin layer chromatography. If the mixture being identified contained compound P, which could form strong hydrogen bonds, compound Q, which formed hydrogen bonds, but not as strongly as P and compound R, which was polar, relying on dispersion forces and dipole-dipole interactions for its intermolecular attractions. Describe how the positions of the spots for compounds A, B and C would be in the chromatogram. (3 marks)

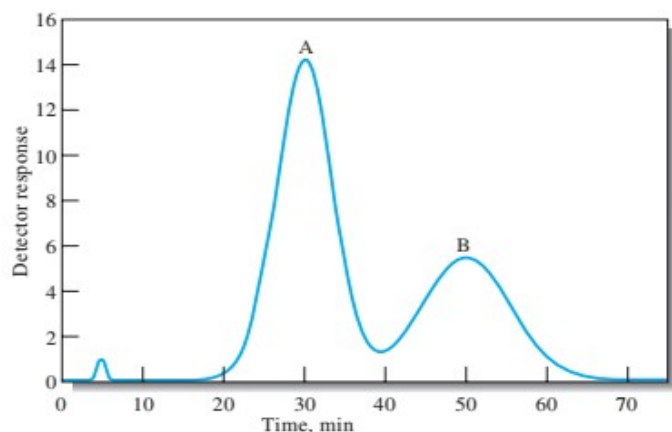
QUESTION THREE (20 MARKS)

- a) Explain why atomic emission is more sensitive to flame instability than atomic absorption. (2 marks)
- b) Explain why a high-resolution monochromator is needed for AES while AAS requires a monochromator having relatively low-to-moderate resolving power. (4 marks)
- c) Briefly explain the properties of the ICP torch that have allowed it to become the dominant source for AES (3 marks)
- d) Describe three ways of introducing an analyte into an ICP torch. (3 marks)

- e) State four (4) common methods used for decomposing and dissolving samples for atomic absorption methods. (2 marks)
- f) Briefly explain the principle of Gas chromatography (2 marks)
- g) (i) State four (4) characteristics of an ideal GC detector. (2 marks)
(ii) Describe how flame ionization detector works, stating the type of samples it can analyze (2 marks)

QUESTION FOUR (20 MARKS)

- a) A chromatogram of a two-component mixture on a 25-cm packed liquid chromatography column is shown in the figure below. The flow rate was 0.40 mL/min.



- (i) Find the times that components A and B spend in the stationary phase. (1 mark)
- (ii) Find the retention times for A and B. (2 marks)
- (iii) Determine the retention factors for the two components. (2 marks)
- (iv) Find the full widths of each peak and the full width at half maximum values. (2 marks)
- (v) Find the resolution of the two peaks. (3 marks)
- (vi) Find the average number of plates for the column. (3 marks)
- (vii) Find the average plate height. (2 marks)

- b) From the data given below, determine the correlation coefficient between the amount of toxin produced by a fungus and the percent of yeast extract in the growth medium. (5 marks)

Sample	% Yeast Extract	Toxin (mg)
(a)	1.000	0.487
(b)	0.200	0.260
(c)	0.100	0.195
(d)	0.010	0.007
(e)	0.001	0.002