Uva Wellassa University, Sri Lanka

BTech. Science and Technology

End Semester Examination – December 2008
BIO 319-2: Instrumental Methods in Biology

Answer four questions only
All questions carry equal marks
All symbols carry standard meanings
Time permitted 2 hrs

Question 1 (100 marks):

(a). List three types of errors that encounter in instrumental analysis *(15 marks)*.

(b). Define terms "precision" and "accuracy" *(15 marks)*.

(c). Density of copper was determined experimentally and was found to be 9.54, 9.55 and 9.56 g/cm^3 in triplicate analysis. The accepted value of the density of copper is 8.96 g/cm^3. Calculate percentile error in each case. What is the error type/s associated with these data? (*Hint*: percentile error = $(d_m-d_t)/d_t] \times 100$ where subscripts *m* and *t* denote measured and true values) *(40 marks)*.

(d). What are authenticated samples? Name a source from where you would get these samples *(15 marks)*.

(e). *"Volume of a vial is expressed as (2.16 ± 0.05) cm^3 at the 95% confidence level"* What do you mean by this expression ? *(15 marks)*

Question 2 (100 marks):

(a). Define atomic absorption, emission and fluorescence. *(20 marks)*

(b). Draw block diagrams to depict essential components of atomic absorption, atomic emission and atomic fluorescence spectrometers. *(20 marks)*

(c). What is lock and key effect? Explain this using the answer given in *section a.* *(20 marks)*

Question 2 contd.

(d). The following values of absorbance (A) were obtained for a series of standard zinc (II) solutions.

A	0.0	0.152	0.298	0.450	0.600
Zn (II) mg dm^{-3}	0	2	4	6	8

Plot the calibration curve and determine the concentration of an unknown solution with absorbance 0.225. *(40 marks)*

Question 3 (100 marks):

(a). List three chromatographic separation methods *(10 marks)*

(b). List essential components in a high performance liquid chromatography (HPLC) *(20 marks)*

(c). Differentiate between reversed phase and normal phase HPLC *(20 marks)*.

(d). In a reversed phase HPLC column, a solute was found to have a retention time of 31.3 min. while an unretained species required 0.48 min. for elution when the mobile phase was 30% methanol and 70 % water. Calculate the retention factor (k'). [Hint: $k' = (t_R - t_M)/t_M$ symbols carry standard meanings]. *(50 marks)*

Question 4 (100 marks):

(a). Define following terms in chromatography *(20 marks)*:

(i). Mobile phase (ii). Retention time

(iii). Selectivity factor (iv). Distribution coefficient

(b). List two assumptions made in the theoretical plate model used in chromatography *(20 marks)*.

(c). Substances A and B were found to have retention times of 16.40 and 17.63 min. respectively on a 30.0 cm column. An unretained species passed through the column in 1.30 min. The peak widths (at base) for A and B were 1.11 and 1.21 min., respectively. Calculate (a). Column resolution, (b). Average number of theoretical plates in the column (c). Plate height $Hint: N = 16\left(\frac{t_R}{W}\right)^2$ *(40 marks)*.

(d). Write van Deemter equation and define each term in it *(20 marks)*.

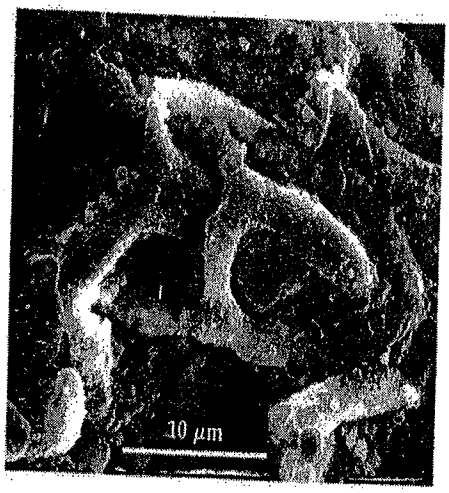
Question 5 (marks 100):

(a) State important interactions that occur when an electron beam strikes the specimen. Which of these interactions are important in Scanning Electron Microscopy (SEM)? *(20 marks)*

(b). What is a monochromatic electron beam? How it is generated? Note: a labeled diagram of electron column is required in the answer. *(20 marks)*

(c). Assume the CRT display of a SEM is 20 cm wide. What scan width would be required to achieve a magnification of 40 000? *(20 marks)*

(d). Fungi sample was imaged by SEM. Both images represent the same fungi.



A

B

i) For morphological analysis of fungi which image is recommended? Why? *(20 marks)*

ii) For compositional analysis of fungi which image is recommended? Why? *(20 marks)*

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Question 6 (100 marks):

(a). What is electrophoresis? (write in a sentence). *(10 marks)*

(b). Discuss two advantages of capillary electrophoresis over slab electrophoresis. *(10 marks)*

(c). Draw essential features of the electric double layer that is formed at the column surface and the solution. *(30 marks)*

(d). Define *(20 marks)*

(i). Electrophoretic mobility

(ii). Iso-electric point

(e). What is SDS-PAGE (sodium **d**odecyl **s**ulfate-**p**oly**a**crylamide **g**el **e**lectrophoresis) method used for protein separation? What is the function of SDS in this method? *(30 marks)*