

NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY

FACULTY OF APPLIED SCIENCES

BACHELOR OF SCIENCE HONOURS DEGREE EXAMINATIONS

DEPARTMENT OF APPLIED BIOLOGY AND BIOCHEMISTRY

THEORY: ANALYTICAL BIOCHEMISTRY SBB 2203

MAY 2003

3 HOURS (100 marks)

INSTRUCTIONS

Answer Four (4) Questions. Each question carries 25 marks. Where a question contains subdivisions, the mark value for each subdivision is given in brackets. Illustrate your answer where appropriate with large, clearly labelled diagrams

- 1(a) Write notes on first order and second order derivative spectra. (5 marks)
- (b) Compare and contrast a conventional single beam spectrophotometer and a double in time spectrophotometer. (10 marks)
- (c) In the NUST 2001/2002 Enzymology class, a spectrophotometric NADH-340nm system was used with lactate and serum to estimate the activity of lactate dehydrogenase. An effective absorbance of 0.14 was obtained. What was the concentration of the lactate in the assay. (5 marks)
Molar Absorbancy NADH at 340.nm=6.22x10³
- (d) Write notes on how fluorescence comes about. (5 marks)
- 2(a) Write an essay on the principles, practice and application of isopycnic centrifugation in biochemical analysis. (15 marks)
- (b) A swing-out bench centrifuge can attain a maximum speed of 7000 revolutions per minute. If the radius of the rotor is 4cm and the length of the tube is 8cm, calculate the maximum relative centrifugal field that can be attained.
$$RCF = \frac{m\omega^2 r}{g}$$
 where $\omega = \frac{rpm \cdot 2\pi}{60}$; $g = 981 \text{ cm/s}^2$
- (c) Draw a swing-out rotor and a fixed angle rotor to show the maximum radii respectively. (5 marks)
- Draw a fully labeled schematic diagram of a Gas Liquid Chromatograph and describe briefly the function of each component. (10 marks)
- (b) Compare and contrast flame ionization and Electron Capture as detection methods in Gas Liquid Chromatography. (5 marks)
- (c) Explain the following terms used in chromatography (5 marks)
- i) HETP
 - ii) N
 - iii) T_R
 - iv) K_d
 - v) V_c
- (d) Compare and contrast normal phase and reverse phase chromatography. (5 marks)

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- (a) Write notes on
- i) Ion exchange chromatography (5 marks)
 - ii) Affinity chromatography (5 marks)
 - iii) Size exclusion chromatography (5 marks)
- (b) A mixture containing proteins A B C D E and F was analyzed by gel permeation chromatography protein molecular weights of the proteins were as follows:
A = 16.91 kD, B = 222.6 kD, C=13.37 kD, D=524.8 kD, and E=23.24 kD
Predict the sequence of elution if the protein fractionation range was 5-40 kD hence sketch the elution profile. (10 marks)
- 5(a) Distinguish between "equilibration" of a TLC tank and that of ion exchange resins (8 marks)
- (b) Describe an ELISA method you would use to measure antibody levels from serum. Give an example of its application. (10 marks)
- (c) The binding constant for the binding of a given antigen to an antibody is 10^{-9} m and the rate constant for its binding is 10^{-8} M^{-1} . Calculate the rate constant for the dissociation of the antigen from the antibody. (7 marks)
- 6(a) Two proteins A (MW 3000) and B (MW 9200) were used as molecular weight markers on an SDS page system. The relative electrophoretic mobility obtained were 0.80 and 0.41 respectively. Determine the molecular weight of a protein whose relative mobility was 0.62 on this gel (5 marks)
- (b) Describe the technique of SDS page for separation of proteins. How would you visualize the protein after separation? (20 marks)

END OF EXAMINATION

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