

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 2005

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) A pebble tied to a piece of string is swung in a circular motion at high speed.
- (i) Describe the forces acting on the pebble. (4 marks)
 - (ii) If the piece of string snaps, how would the pebble appear to move (2 marks)
- (b) You are required to prepare a subcellular fraction at 900g on a bench centrifuge. If the average radius of the angle rotor is 8 cm. What speed will the centrifuge need to be set at to effect this fractionation. (5 marks)
- $$\text{RCF} = \frac{m\omega^2 r}{g} \quad g = 981 \text{ cm/s}^2 \quad \omega = \frac{\text{rpm} \times 2\pi}{60}$$
- (c) Draw a diagram to indicate the minimum and maximum radius of 8 cm on a fixed angle rotor. (4 marks)
- (d) Give an account on the theory and practice of differential centrifugation. (10 marks)
- 2.(a) Explain the following terms used in chromatography.
- (i) HETP
 - (ii) N
 - (iii) T_R
 - (iv) K_d
 - (v) V_e (5 marks)
- 2.(b) A molecular exclusion column (20 cm i.d x 90 cm) is run at a flow rate of 40 ml/hr.
- (i) In what volume would you expect to see the elution of a protein which is fully excluded from the gel? (3 marks)
 - (ii) If a peak elutes at $V_e > V_0$, what does this indicate? (2 marks)

- 2.(c) Draw a fully labeled schematic diagram of a Gas Liquid Chromatograph and describe the function of each component. (15 marks)
- 3.(a) You are supplied with a blood sample of a subject who has a salt imbalance. You are required to analyze the level of sodium in his blood. Describe an experiment (as done in class) that you would carry out to determine the level of the element. (In your answer include the block diagram and theory of the instrument used). (10 marks)
- (b) (i) Describe the principle of the technique known as SDS-PAGE, explaining how it may be used to estimate MWs. (10 marks)
(ii) What is the purpose of the stacking gel? (5 marks)
- 4.(a) Distinguish between the following terms as used in spectrophotometry:
- (i) Absorbance and transmission (2 marks)
 - (ii) Chromophore and auxochrome (2 marks)
 - (iii) Hyperchromic shift and hypsochromic shift (2 marks)
 - (iv) Natural band width and spectral band width (2 marks)
 - (v) Absorption spectrum and radiation spectrum (2 marks)
- (b) Briefly compare and contrast the principles and applications of visible spectrophotometry and U.V. spectrophotometry. (10 marks)
- (c) A standard protein solution (0.5mg/ml) gave an absorbance of 0.60 when determined by the Lowry method. A test sample of unknown protein concentration was diluted 1 in 20 and gave an absorbance of 0.40. Calculate the concentration of the original test sample. (5 marks)
5. Write an essay on methods that are available for tissue disruption and homogenization. (25 marks)
- 6.(a) Write short notes on the information that can be extracted from a GLC chromatogram. (5 marks)
- (b) The following five proteins were separated by SDS-PAGE

PROTEIN	MW (Daltons)	pI
A	45 000	5.4
B	13 400	10.6
C	17 000	7.0
D	69 000	4.8
E	90 000	5.9

- Give the order of their migration from the point of sample application of the gel to the other end of the gel. (5 marks)
- (c) Write short notes on three (3) types of ELISA. (15 marks)

END OF EXAMINATION