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

JUNE 2004

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) Distinguish between the following terms used in spectrophotometry.

(i) bathochromic shift and hypsochromic shift. (2 marks)

(ii) chromophore and auxochrome (2 marks)

(iii) natural band width and spectral band width (2 marks)

(iv) absorption spectrum and radiation spectrum (2 marks)

(v) fluorescence and phosphorescence (2 marks)

(b) You are provided with a pure extract of NADH. Calculate the mass of NADH you would need to dissolve in 100 ml in order to obtain an A_{340} of 0.311. (5 marks)

$MW_{NADH} = 665$ $\varepsilon_{NADH} = 6.22 \times 10^3$

(c) Briefly compare the principles and applications of visible spectrophotometry and U.V. spectrophotometry. (10 marks)

2.(a) Draw a fully labeled schematic diagram of a Gas Liquid Chromatograph equipped with an FID and describe briefly the function of each component. (10 marks)

(b) The chromatogram overleaf was obtained after subjecting a mixture of compounds to liquid chromatography. Silica gel 60 was the stationary phase and 50% saturated dichloromethane was used as the mobile phase at a flow rate of 1 ml per minute.

Calculate:

(i) the relative retention times for peaks 1 and 5. (2 marks)

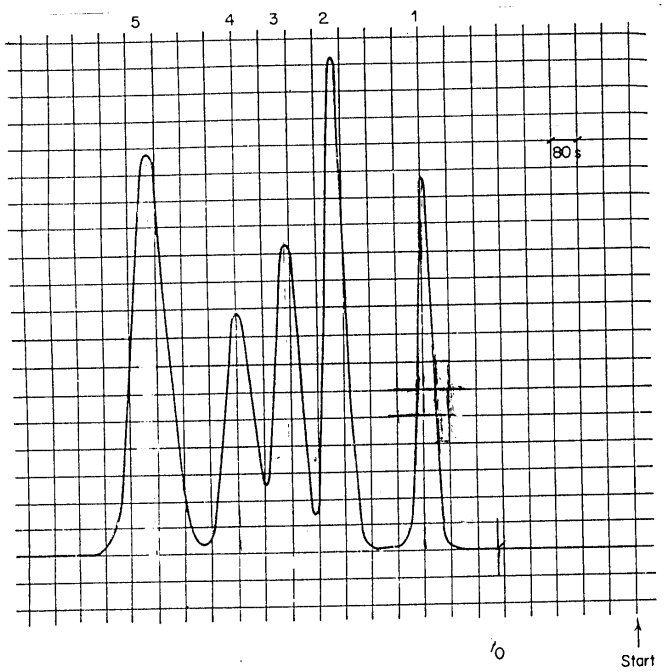
(ii) the retention times for peaks 3 and 4. (2 marks)

(iii) the plate number (N) for peak 1 given that $N = 5.54\left(\frac{t_R}{W_h}\right)^2$ (1 mark)

Where w_h is peak width at half peak height.

(c) Write short notes on the Czerny-Turner configuration as a model of a grating monochromator. (10 marks)

odd no's



3.(a) (i) A pure protein was shown to dissolve in 0.1 M $(NH_4)_2 SO_4$ solution but precipitated in 3 M $(NH_4)_2 SO_4$ solution. When the excess salt was removed by dialysis, the protein redissolved. Give an explanation for the observation that high salt concentration decreases the solubility of protein. (5 marks)

(ii) Sketch and label fully the solubility curve of a protein. (4 marks)

(b) An analytical biochemist wished to precipitate albumin from serum. He made a 75% saturated Ammonium sulphate [$(NH_4)_2 SO_4$] solution and obtained a protein precipitate. Albumin Precipitates at about 75% $(NH_4)_2 SO_4$ saturation. Criticize his way of obtaining the albumin precipitate and discuss how you would proceed with the albumin precipitation using the same salt. (6 marks)

(c) Briefly compare and contrast salt fractionation of proteins and isoelectric precipitation of proteins. (10 marks)

4.(a) Describe two basic designs of enzyme linked immunoassays. (10 marks)

(b) A monoclonal antibody solution was split into two samples. Sample 1 was electrophoresed on a Polyacrylamide gel and a single sharp band was obtained. Sample 2 was treated with β-mercaptoethanol and electrophoresed. Two bands were observed. Explain these observations. (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 $10^8 M^{-1} S^{-1}$. Calculate the rate constant for the dissociation of the antigen from the antibody. (5 marks)

5. Discuss the theory and practice of salt fractionation include in your discussion a definition of salting-in and salting-out, the effect of different salts, the effect of dilutions. Give an example of how a theoretical enzyme can be purified from a mixture of proteins by salt fractionation. In this protein mixture, some proteins tend to precipitate at lower salt concentrations, some at higher salt concentration. Assume no precipitation occurs at overlapping concentrations. How would you estimate the MW of the native enzyme? (25 marks)

6.(a) Describe the differences between rate zonal centrifugation and isopycnic centrifugation. (12 marks)

(b) You are provided with a centrifuge which has a fixed angle rotor. The maximum radius of the rotor is 10cm. A centrifugation process requires you to apply a relative centrifugal force (RCF) of 10 000g.

(i) What speed will your centrifuge have to attain to generate the required RCF. (5 marks)

$$RCF = \frac{m\omega^2 r}{g} \qquad \omega = \frac{(rpm \times 2\pi)}{60}$$

$g = 981\ cm/sec^2$ $\qquad$ $m = 1\ g$

(ii) Draw a sketch of the fixed angle rotor and indicate the radius of 10 cm on it. (2 marks)

(c) What do you understand by the following terms in centrifugation?

(i) partial specific volume
(ii) sedimentation constant
(iii) swing out rotor
(iv) isopycnic band
(v) preparative centrifugation
(vi) analytical centrifugation (6 marks)

END OF EXAMINATION

odd no's