



NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF APPLIED BIOLOGY AND BIOCHEMISTRY

BACHELOR OF SCIENCE HONOURS DEGREE

Analytical Biochemistry SBB 2203

MAY 2011

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. In multiple choice questions, some questions may have more than one correct answer and in such cases, negative marking will apply to incorrect answers. **Illustrate your answer where appropriate with large, clearly labelled diagrams**

1. (a) From the list below choose the parameters on which the sedimentation of a particle in a centrifuge is dependent.
 - (i) Radius of the rotor arm.
 - (ii) Gravity
 - (iii) Mass of particle
 - (iv) Temperature
 - (v) Density of particle
 - (vi) Mass of particle being greater than the mass of solvent displaced.
 - (vii) Density of solution
 - (viii) Charge of protein(4 marks)
- (b) Describe how you would prepare a nuclear and mitochondrial fraction using a named centrifugation method. [Volumes used not required]. (15 marks)
- (c) If the centrifuge used in the above fractionation has a radius of 8 cm, calculate the speed you would set the centrifuge to effect the fractionation of the mitochondrial fraction. (6 marks)

$$\text{RCF} = \omega^2 r \times g^{-1} \qquad g = 981 \text{cms}^{-2}$$

$$= r \text{ p m} \times 2 \pi \times 60^{-1}$$

2. (a) Two aliquots of a protein solution were analyzed by SDS-PAGE and cation-exchange chromatography. A single band was observed on the electrophoretogram whilst two peaks were observed on the chromatogram. Explain these results and draw conclusions concerning the composition of the original protein solution. (8 marks)

(b) Write notes on principles of “salting in” and “salting out”. (5 marks)

(c) A mixture of 5 proteins was analyzed by gel permeation chromatography. The molecular weight of the proteins were as follows:

Protein:	A	B	C	D	E
MW (kD):	16.91	222.6	13.37	524.8	23.24

(i) Predict the sequence of elution if the fractionation range was 5-40 kD and hence sketch the elution profile. (10 marks)

(ii) Explain why the first protein to be eluted runs at that position. (2 marks)

3. (a) The following proteins were separated by isoelectric focusing.

Protein	MW(kD)	pI
A	45	5.4
B	13	10.6
C	17	7.0
D	69	4.8
E	90	5.9

(i) Give the order of their distribution between the positive (+) and negative (-) ends of the gel. (5 marks)

(ii) Explain your answer to 3(a) (i). (3 marks)

(b) The relative electrophoretic mobilities of a number of SDS-protein complexes in a polyacrylamide gel are shown in the table below.

Protein	Molecular Weight	Relative Mobility
Myoglobin	17 200	0.95
Trypsin	23 300	0.82
Aldolase	40 000	0.59
Fumarase	49 000	0.50
Carbonic anhydrase	29 000	0.73

The relative mobility of glyceraldehyde-3-phosphate dehydrogenase was 0.65.

(i) Determine the molecular weight of glyceraldehyde-3-phosphate dehydrogenase graphically. (5 marks)

(ii) Using ultracentrifugation, the molecular weight of glyceraldehyde-3-phosphate dehydrogenase was shown to be 140 000. Explain what this means concerning the molecular structure of the enzyme. (6 marks)

- (c) Account for the fact that solubility of globular proteins is a function of pH and solubility is at a minimum when pH of solution equals the isoelectric point (pI) of the proteins.

(6 marks)

4. Describe the methods available for cell disruption or tissue homogenization.

5. (a) Distinguish between;

(i) Isothermal chromatography and isocratic elution.

(2 marks)

(ii) Chromatogram and chromatograph.

(2 marks)

(b) Choose those method(s) listed below that are **NOT** sufficiently specific to isolate a particular protein from a complex mixture of proteins in one step.

(i) Electrophoresis

(ii) Gel filtration

(iii) Salting out

(iv) Immunoprecipitation

(v) Isoelectric focussing

(vi) Affinity chromatography

(vii) Ion-exchange chromatography

(viii) Zonal ultracentrifugation

(6 marks)

(c) You are supplied with four unlabelled analgesic tablets each containing aspirin, paracetamol, caffeine and codeine as active ingredients. Describe an experiment (as done in class) that you would carry out to distinguish or identify the tablets. In your answer, illustrate results you would expect to obtain. (Exact amounts of reagents used are not required).

(15 marks)

6. Discuss the principles, practice and application of enzyme linked immunosorbent assay (ELISA).

END OF EXAMINATION

