NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY

FACULTY OF APPLIED SCIENCES

DEPARTMENT OF APPLIED BIOLOGY AND BIOCHEMISTRY

BACHELOR OF SCIENCE HONOURS DEGREE IN APPLIED BIOLOGY AND BIOCHEMISTRY

ANALYTICAL BIOCHEMISTRY SBB 2203

EXAMINATION PAPER

MAY 2017

This examination paper consists of 3 pages

Time Allowed:	**3 hours**
Total Marks:	**100**
Special Requirements:	**None**

INSTRUCTIONS TO CANDIDATES

1. **Answer Four (4) Questions. Each question carries 25 marks.**
2. **Where a question contains subdivisions, the mark value for each subdivision is given in brackets.**
3. **In multiple choice questions, some questions may have more than one correct answer and in such cases, negative marking will apply to incorrect answers.**
4. **Illustrate your answer, where appropriate, with large clearly labeled diagrams.**

1.(a) Explain the relationship between:

(i) Absorbance and concentration. (2 marks)

(ii) Transmitance and concentration. (2 marks)

(b) If 25% of light is absorbed by a homogenous solution, determine:

(i) Percentage transmittance. (1 mark)

(ii) Absorbance. (2 marks)

(c) Given that the molecular mass of myoglobin is 17.8 kD and that molar absorptivity is 15 000 L/mol/cm, calculate the absorbance of a 1 mg/ml solution in a standard cuvette. (3 marks)

(d) Compare the instrumentation and application of visible spectrophotometry and UV spectrophotometry. (15 marks)

2.(a) Indicate which of the following statements are **True** and which are **False**.

The sedimentation of a particle in a centrifuge is dependent on:

(i) The radius of the rotor arm.

(ii) The mass of the particle.

(iii) The charge of the particle

(iv) The density of the particle.

(v) The gravity of the earth. (5 marks)

(b) With the aid of a diagram, indicate the forces acting on a particle undergoing centrifugation. (4 marks)

(c) (i) Calculate the speed that a centrifuge with an 8 cm rotor has to be set in order to prepare a subcellular fraction at 600g.

$$RCF = \frac{m\omega^2}{g} \qquad g = 981 cm/s^2$$

$$\omega = \frac{rpm \times 2\pi}{60}$$

(5 marks)

(ii) Name the subcellular fraction that will be obtained. (1 mark)

(d) Describe the principles and practice of isopycnic centrifugation. (10 marks)

3.(i) Discuss the theory and practice of salt fractionation. Include in your discussion the definition of salting-in and salting out, the effect of different salts and the effect of dilutions. (10 marks)

(ii) 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 concentrations. Assume no precipitation occurs at overlapping concentrations. (10 marks)

(iii) Describe how you would estimate the molecular mass of a native enzyme. (5 marks)

4. State the importance of cell disruption and homogenization and discuss the methods that are available for cell disruption and homogenization.

5.(a) Indicate which of the following statements are **True** and which are **False**, regarding the immobilization of proteins on polystryrene plates.

(i) Immobilzation is achieved through passive adsorption of the protein to the plastic plate.
(ii) The immobilization process occurs through hydrophilic interactions between plastic and protein residues.
(iii) The protein solution is dissolved in an acidic buffer.
(iv) The protein solution is dissolved in an alkaline buffer.
(v) The plate is left to incubate for several hours or overnight at 4^{o}C to 37^{o}C.

(5 marks)

(b) Compare the principle, practice and use of the Sandwich ELISA method and the Indirect ELISA method. (20 marks)

6.(a) Write short notes on how you would equilibrate:
(i) A thin layer chromatographic plate. (5 marks)
(ii) An ion exchange column. (5 marks)

(b) Compare and contrast reversed phase chromatography with normal phase chromatography. (10 marks)

(c) Give a brief account of how a Flame Ionization Detector (FID) works. (5 marks)

END OF EXAMINATION