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RAJARATA UNIVERSITY OF SRI LANKA
FACULTY OF APPLIED SCIENCES

Bachelor of Science Honours in Microbiology
Third Year – Semester II Examination – January/ February 2023

MIB 3206 – ANALYTICAL TECHNIQUES IN MOLECULAR BIOLOGY

Time: Two (02) hours

Answer ALL questions.

1. a) Explain how the speed of a particle moving in a centrifuge tube would be different when i. that particle is close to the top and close to the bottom of the tube.

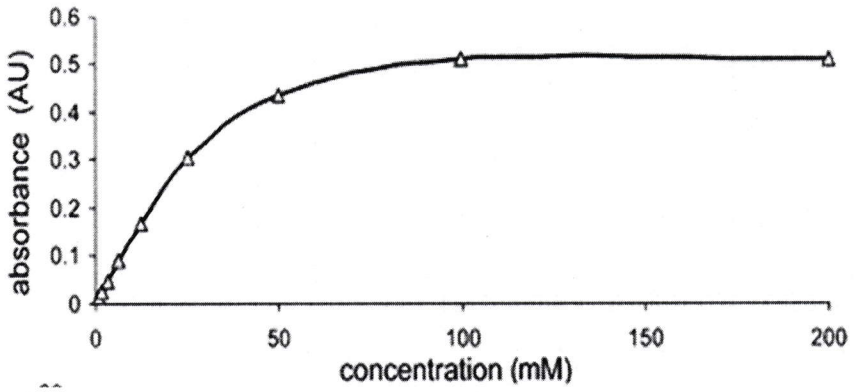
ii. the density of the medium is high and low and

iii. the viscosity of the medium is high and low. **(40 marks)**

b) Explain the forces operating on particles settled at a particular place in a centrifuge tube during isopycnic centrifugation. **(35 marks)**

c) Discuss the importance of a nomogram. **(25 marks)**

2. a) Following is a generalized graph illustrating the relationship of concentration of particles and absorbance.



The above relationship can also be explained by Beer's law.

Discuss how the shape of the graph gradually deviates from Beers law after a certain concentration. **(50 marks)**

b) The optical density of a competitive ELISA is inversely proportional to the analyte concentration. Elaborate on this statement. **(50 marks)**

3. a) Six proteins with different pI values as 3, 4, 5, 7, 11 and 12.5 are present in a plant extract. What is the expected order of elution, when a cation exchange column equilibrated at pH 8.8 is used? Explain your answer. **(40 marks)**

   b) "In the electrophoretic separation of proteins, discontinuous gel gives better results". Justify the above statement. **(30 marks)**

   c) A student working with a protein, accidently mixed it with a salt solution. Unfortunately, neither suitable chromatographic columns nor resins are available in the laboratory.
   Describe a method which could be used to remove salts from the protein sample.
   (30 marks)

4. a) Describe briefly, **two (02)** methods of extracting secondary metabolites in plants.
   (20 marks)

   b) You are provided with five plant specimens labelled as P, Q, R, S and T. Based on the knowledge on extraction and separation of plant secondary metabolites using TLC, describe a method that you would follow to screen the above samples for antimicrobial properties. **(60 marks)**

   c) While you are attempting to separate the secondary metabolites in a plant extract using silica gel as the stationary phase, a very poor separation was observed.
   Explain how you would improve the separation. **(20 marks)**

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