WEST BENGAL STATE UNIVERSITY

B.Sc. Programme 5th Semester Examination, 2020, held in 2021

MCBGDSE01T-MICROBIOLOGY (DSE1)

Time Allotted: 2 Hours | Full Marks: 40

The figures in the margin indicate full marks.
Candidates should answer in their own words and adhere to the word limit as practicable.
All symbols are of usual significance.

Question No. 1 is compulsory and answer any *four* from the rest

1. Answer any ***four*** questions from the following: 2×4 = 8

   (a) Write down the Abbe equation. Define numerical aperture.

   (b) What do you mean by RCF?

   (c) What is the difference between an anion exchanger and a cation exchanger?

   (d) What is the function of β-mercaptoethanol and sodium dodecyl sulfate in denaturing SDS-PAGE?

   (e) What will be the order of elution of the following proteins from a Sephadex G-50 column (fractionation range: 1 – 30 kDa): catalase (222 kDa), α-chymotrypsin (21.6 kDa), concanavalin B (42.5 kDa), lipase (6.7 kDa), and myoglobin (16.9 kDa)?

   (f) What is the difference between swinging-bucket rotor and fixed-angle rotor?

   (g) Mention two applications of density gradient centrifugation.

2. (a) Briefly describe how the phase-contrast and fluorescence microscopes work. Give a specific use for each type. 6

   (b) What is the basic difference between brightfield and darkfield microscopy? 2

   OR

   (c) What is the difference between SDS-PAGE and Native Gel electrophoresis? Mention two applications of Native Gel electrophoresis. 2+2

   (d) What are the different buffers used in SDS PAGE? Mention the pH values of each. 2

   (e) An enzyme separated by gel filtration in aqueous buffer at pH 7.0 had an apparent molecular weight of 160,000Da. When examined by SDS-PAGE, a single band of apparent molecular weight of 40,000Da was observed? Explain these findings. 2

3. (a) What is the full form of R_f and what is its definition? What are the moving and stationary phases in paper chromatography? 3

(b) Compounds A and B are found in a mixture. R_f values for both compounds were calculated. Which of the following sets of R_f values would reflect the largest separation between A and B? 2

(i) R_{fA}= 0.6, R_{fB} = 0.5 (ii) R_{fA} = 0.1, R_{fB} =0.1

(iii) R_{fA} = 0.5, R_{fB} = 0.6 (iv) R_{fA} = 0.4, R_{fB} = 0.2

(c) How can you visualize amino acids in TLC? Briefly explain the process of visualization of amino acids after their separation in TLC. 1+2

4. (a) Why is two-dimensional (2D) gel electrophoresis called "two-dimensional"? What is the principle of isoelectric focusing (IEF)? 3

(b) How is the pH gradient prepared in the gel during IEF? 3

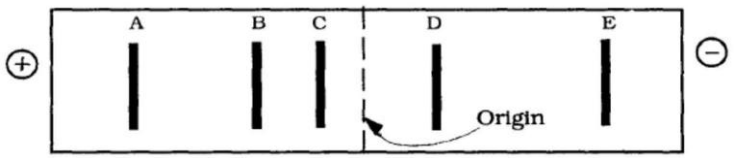
(c) What would be the relative arrangement of the following proteins after they had been subjected to isoelectric focusing (pI in bracket): insulin (5.4), cytochrome c (10.6), histone (10.8), myoglobin (7.0), and ribonuclease A (7.8)? 2

5. (a) An enzyme of Mr = 24,000 and pI = 5.5 is contaminated with a protein of similar molecular weight but with a pI = 7.0, and another protein of Mr = 100,000 and pI = 5.4. Suggest a purification strategy. 3

(b) What is zymography used for and what is its principle? 3

(c) What is the principle behind visualization of DNA in agarose gels by ethidium bromide? 2

6. (a) A mixture of five polypeptides (PP_1, PP_2, PP_3, PP_4 and PP_5) was subjected to paper electrophoresis at pH 8.5. This was stained after electrophoresis and revealed the following pattern of migration. The five polypeptides have the following isoelectric points: PP_1=9.2, PP_2=4.6, PP_3=8.0, PP_4=6.9, and PP_5=10.4. Provided that all the polypeptides have same molecular weights, identify which zone corresponds to each polypeptide. Give reasons for the answer. 4



(b) How will you separate a mixture of amino acids, Alanine and glutamic acid using ion exchange chromatography? (Hint: Alanine is a neutral amino acid and glutamic acid is an acidic amino acid.) 2

(c) Give two examples of group-specific ligands commonly used in affinity chromatography and their uses. 2

7. (a) Define sedimentation coefficient. What is its SI unit? 2

(b) A protein has a sedimentation coefficient value of 3.12×10^{-13} sec. in water. Its diffusion coefficient in water is found to be 8.2×10^{-7}cm^2/sec. Both the above values have been corrected for 20°C in water. The partial specific volume of the protein is 0.735 and the density of water at 20°C is 0.9982. Determine the molecular weight of the protein. 2

(c) Mention the factors on which the sedimentation coefficient of a macromolecule depends. 2

(d) Aliquots from the same protein-containing sample were subjected to SDS-PAGE and cation exchange chromatography. After staining SDS-PAGE reveals just one sharp band while one obtains two distinct peaks at different elution volumes in cation exchange chromatography. What are your conclusions about the composition of the protein solution? 2

8. (a) Write briefly about Ultracentrifugation. 3

(b) Calculate RCF when the angular velocity is 5000 rpm given the radial distance of the particle from the axis of rotation is 10 cm. 3

(c) The sedimentation coefficient of a protein is 2.3S. When this protein is treated with β-mercaptoethanol the value changes to 3.4S. Can you explain this change? 2

N.B. : *Students have to complete submission of their Answer Scripts through E-mail / Whatsapp to their own respective colleges on the same day / date of examination within 1 hour after end of exam. University / College authorities will not be held responsible for wrong submission (at in proper address). Students are strongly advised not to submit multiple copies of the same answer script.*

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