

2022

**MICROBIOLOGY — HONOURS**

**Paper : DSE-B-3**

**(Instrumentation and Biotechniques)**

**Full Marks : 50**

*The figures in the margin indicate full marks.*

*Candidates are required to give their answers in their own words as far as practicable.*

Answer **question no. 1** and **any three** questions from the rest (2–6).

1. Answer **any ten** questions :

2×10

- (a) Your protein has a net negative charge at pH 7. Which ion exchange chromatography will you use for its purification using a buffer of pH 7? Justify your answer.
  - (b) State any one chief limitation associated with Gas chromatography which is not present in case of liquid chromatography.
  - (c) Why is a protein's solubility minimum at its isoelectric pH?
  - (d) What is the unit of absorbance? Explain.
  - (e) What are red shift and blue shift?
  - (f) What is isosbestic point?
  - (g) What is electrophoretic mobility? What is its mathematical expression?
  - (h) What is empty magnification?
  - (i) What are the affinity ligands? Give examples.
  - (j) Why loading dye is added to the sample in gel electrophoresis?
  - (k) Can chromatography be used to purify volatile substances? Justify.
  - (l) What is the advantage of fluorescence microscopy over electron microscopy?
  - (m) Why is bromothymol blue incorporated into the mobile phase in paper chromatography?
  - (n) What are the advantages of using 2D gel electrophoresis over 1D protein gel electrophoresis?
  - (o) Which conditions can result in deviations from Beer's law when the path length is constant?
2. (a) How does High Performance Liquid Chromatography (HPLC) work?
- (b) For HPLC, the column needs to be made of steel or glass lined metal tubing. Give a brief idea about the types of columns used in HPLC. Why C18 column is considered better for separating compounds such as long-chain fatty acids as compared to relatively small organic compounds.

**Please Turn Over**

- (c) Which detector is used in GLC? Is GLC adsorption or partition?  
(d) What are the advantages of HPLC over GLC? 3+(2+2)+(1+1)+1
3. (a) What is the principle of operation of SEM?  
(b) Why TEM is the preferred method to image any nanoparticle sample over SEM?  
(c) Compare SEM and TEM with respect to resolution, magnification and image dimension.  
(d) Discuss how the TEM and SEM images of the same organism will differ. Why these microscopes do not naturally produce color images? 2+2+3+(2+1)
4. (a) What is the difference between preparative and analytical centrifugation? What are the different types of preparative centrifugation?  
(b) What is the unit of sedimentation coefficient? Write down the factors which influence the sedimentation rate.  
(c) Ultracentrifuges have variety of parts and components which perform different functions. Discuss the working principle and different parts of it. (1+2)+(1+2)+(2+2)
5. (a) What is the difference between a chromophore and fluorophore?  
(b) How can you determine the concentration of a sample using the Lambert-Beer's law?  
(c) What are extrinsic and intrinsic fluorescence? Give examples.  
(d) What types of electronic transitions are involved from a bonding orbital in the ground state to a non-bonding orbital in the higher energy level? 2+3+3+2
6. (a) Explain the role of the following compounds in PAGE :  
(i) Ammonium Persulphate  
(ii) TEMED  
(iii) SDS.  
(b) Which method of protein electrophoresis would be suitable to resolve a mixture of large number of proteins into individual species? Why?  
(c) Enlist the factors which affect the electrophoretic mobility of a particle.  
(d) What is the principle of Pulse field gel electrophoresis? Give an application. 3+2+3+2
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