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B.M.S. College of Engineering, Bengaluru-560019

Autonomous Institute Affiliated to VTU

August 2024 Supplementary Examinations

Programme: B.E. **Semester: V**
Branch: Biotechnology **Duration: 3 hrs.**
Course Code: 19BT5DCBAT **Max Marks: 100**
Course: Bioanalytical Techniques

Instructions: 1. Answer any FIVE full questions, choosing one full question from each unit.
2. Missing data, if any, may be suitably assumed.

UNIT - I

1 a) Two analytes A and B were separated on a 25 cm long column. The observed retention times were 7 min 20 s and 8 min 20 s, respectively. The base peak width for analyte B was 10 s. When a reference compound, which was completely excluded from the stationary phase under the same elution conditions, was studied, its retention time was 1 min 20 s. **10**

i. What was the resolution of the two analytes?

ii. What is the length of the column required to double the resolution if there is an analytical need to increase the separation?

b) The relative molecular mass (M_r) of a protein was investigated by exclusion chromatography using a Sephacryl S300 column and using aldolase, catalase, ferritin, thyroglobulin and blue dextran as standards. The following elution data were obtained. **10**

Protein	Molecular Weight (M_r)	Elution Volume in cm^3 (V_e)
Aldolase	158000	22.5
Catalase	210000	21.4
Ferritin	444000	18.2
Thyroglobulin	669000	16.4
Blue Dextran	2000000	13.6
Unknown	?	19.5

i. What is the relative molecular weight (M_r) of the unknown protein?

ii. Why does the elution volume decreases with increase in the molecular weight of proteins?

iii. If the same mixture is resolved on a column, which has a different diameter and different length, will the elution volume of all components remain the same? Give your reasons.

UNIT - II

2 a) SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) is a discontinuous electrophoretic system developed by Ulrich K. Laemmli which is commonly used as a method to separate proteins with molecular masses between 5 and 250 kDa. **10**

i. X-ray crystallographic studies of a given protein have conclusively proved that the protein consists of four peptide chains in association with each other. Yet SDS-PAGE of the protein gives just one band. How many explanations can you provide to justify the above observation?

ii. The following table shows the distance moved in an SDS-PAGE by a series of marker proteins of known relative molecular mass (Mr). A newly purified protein (X) run on the same gel showed a single band that had moved a distance of 45 mm. What was the Mr of protein X?

Protein	$M_{r\,(Da)}$	Distance moved (mm)
Transferrin	78000	6.0
Bovine serum albumin	66000	12.5
Ovalbumin	45000	32.0
Glyceraldehyde-3-phosphate dehydrogenase	36000	38.0
Carbonic anhydrase	29000	50.0
Trypsinogen	24000	54.0
Soyabean trypsin inhibitor	20100	61.0
β-Lactoglobulin	18400	69.0
Myoglobin	17800	69.0
Lysozyme	14300	79.0
Cytochrome c	12400	86.5

b) Conventional agarose gel electrophoresis can't separate linear double stranded DNA molecules that have a radius of gyration which is larger than the pore size of the gel. These large linear duplex DNA molecules migrate through agarose gels at the same rate irrespective of their size. **10**

Identify the quick and reliable procedure for the separation of large (>30 kb) DNA molecules. Compare the different variants of this procedure.

UNIT - III

3 a) Ultracentrifuge is a sophisticated and advanced centrifuge that operates at an extremely high speed and separates smaller molecules that cannot be separated from the traditional centrifuges. **10**

i. How do you use the ultracentrifugation for the determination of a molecular mass a particle based on sedimentation velocity?

ii. In a dilute buffer solution at 20 °C, rabbit muscle aldolase has a frictional coefficient of 8.74×10^{-8} g s^{-1}, a sedimentation coefficient of 7.35 s, and a partial specific volume of 0.742 cm^3 g^{-1}. Calculate the molecular mass of aldolase assuming the density of the solution to be 0.998 g cm^{-3}.

b) Calcium signaling is a common mechanism, since the ion, once it enters the cytoplasm, exerts allosteric regulatory effects on many enzymes and proteins. How can intracellular calcium be monitored with the use of spectroscopic technique? **05**

c) A biochemist is interested in determining molecular weight, dimension, and transport properties of a macromolecule using scattering of light. Identify and explain the principle of suitable spectroscopic techniques that can be used for molecular weight determination and give the equation for the calculation of molecular weight. **05**

OR

4 a) MALDI-TOF mass spectrometry is a versatile analytical technique to detect and characterize mixtures of organic molecules. In microbiology, it is used as a rapid, accurate, and cost-effective method for identifying microorganisms. **10**

i. Explain the principle and methodology of MALDI-TOF MS

ii. Is MALDI-TOF better for gram-negative or gram-positive bacteria?

iii. Why is the thorough cleaning of the testing plate important?

b) A biochemist is interested in studying the dynamic behavior of a protein molecule like Spectrin. Identify a suitable technique and explain its working principle. **05**

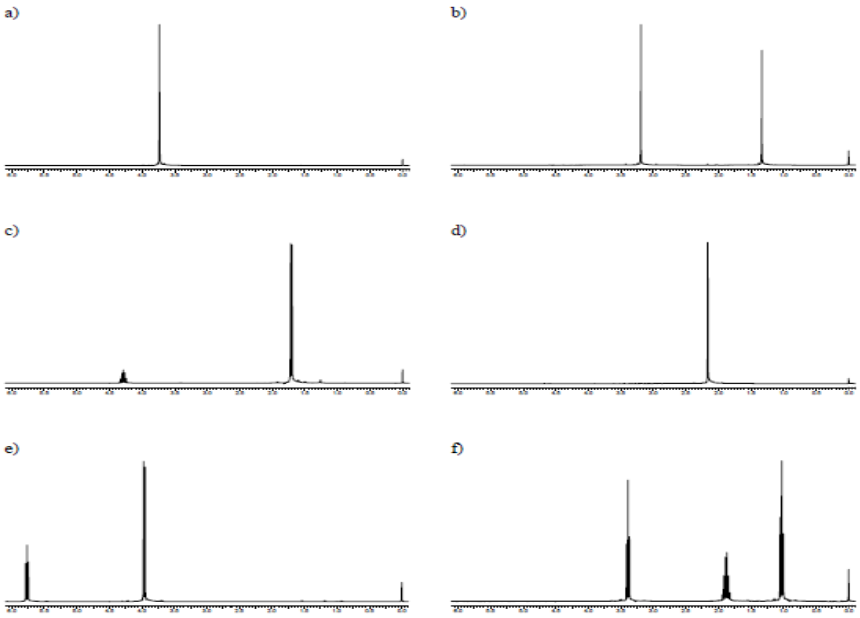
c) DNA from different bacterial sources has been obtained to determine the molecular weight. Identify a suitable hydrodynamic technique used for determining the molecular weight and explain its working principle. **05**

UNIT - IV

5 a) You have purified a recombinant protein and wonder whether it adopts a folded structure. How might you address this problem? **05**

b) A researcher would like to select a suitable technique for the structural analysis of a sample. He has information that his sample contains unpaired electrons. Identify a suitable technique that can be used and explain its principle. **05**

c) The spectra below are of acetone, 1,2-dichloroethane, 1,1,2-trichloroethane, 2,2-dimethoxypropane, 1-bromopropane and 2-bromopropane. Assign them to corresponding spectra. **10**

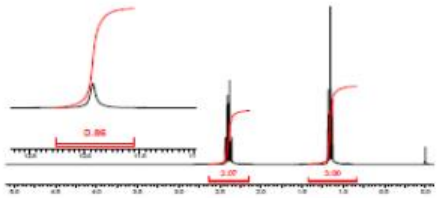


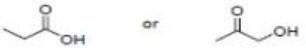
OR

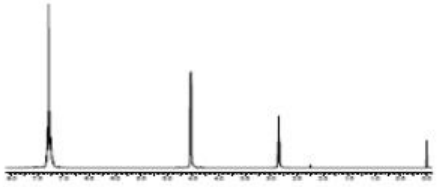
6 a) What is the difference between Raman spectroscopy and Infrared spectroscopy? Write any two of their applications. **05**

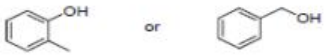
b) XAFS is a group of techniques that interrogate materials for a variety of chemical/structural information. What is the principle of XAFS? Add a note of its applications. **05**

c) Referring to each spectrum given below, chose between the alternative compounds. Justify your answers with reasons. **10**









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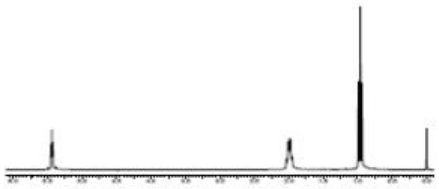
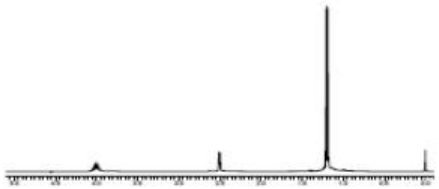


iv



UNIT - V

7 a) There are three commonly used methods for detection and measurement radioactivity. Examine the solid and liquid scintillation counting methods for the measurement of radioactivity. Apprise the advantages and disadvantages of scintillation counting. **10**

i

b) The half-life of ^{32}P is 14.2 days. How long would it take a solution containing 42,000 d.p.m. to decay to 500 d.p.m.? **05**

c) What are the rules to be followed while handling radioisotopes? **05**

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