U.S.N.										

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

Autonomous Institute Affiliated to VTU

June 2025 Semester End Main Examinations

Programme: B.E. **Semester: V**
Branch: Biotechnology **Duration: 3 hrs.**
Course Code: 23BT5PCBAT/22BT5PCBAT **Max Marks: 100**
Course: Bioanalytical Techniques

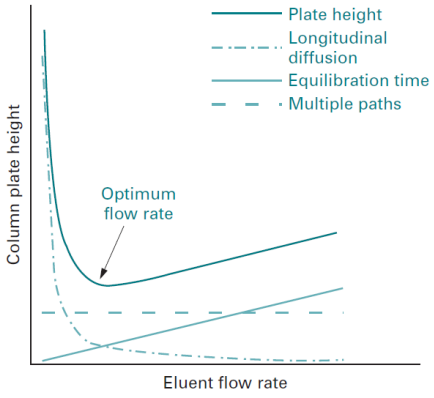
Instructions: 1. Answer any FIVE full questions, choosing one full question from each unit.
2. Missing data, if any, may be suitably assumed.

Important Note: Completing your answers, compulsorily draw diagonal cross lines on the remaining blank pages. Revealing of identification, appeal to evaluator will be treated as malpractice.

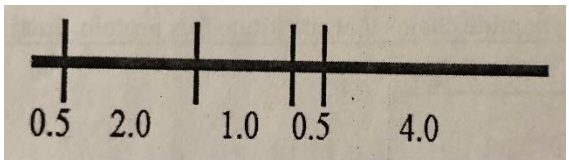
		UNIT - I	CO	PO	Marks
1	a)	Two analytes A and B were separated on a 25 cm long column. The observed retention times were 7 min 20 s and 8 min 20 s, respectively. The base peak width for analyte B was 10 s. When a reference compound, which was completely excluded from the stationary phase under the same elution conditions, was studied, its retention time was 1 min 20 s. 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?	*CO1*	*PO 1,5*	**10**
	b)	Reproduced below are the R_f values of some amino acids in two different solvents. The chromatography was carried out using Whatman No.1 paper and the mode was ascending chromatography.	*CO1*	*PO 1,5*	**10**

Amino acid	R_f Values x 100	
	n-Butanol-Acetic Acid-Water (4:1:5)	n-Butanol-Pyridine-Water (1:1:1)
Aspartic acid	23	20
Alanine	30	37
Glycine	23	29
Histidine	11	24
Leucine	70	60
Lysine	12	13
Tyrosine	45	60

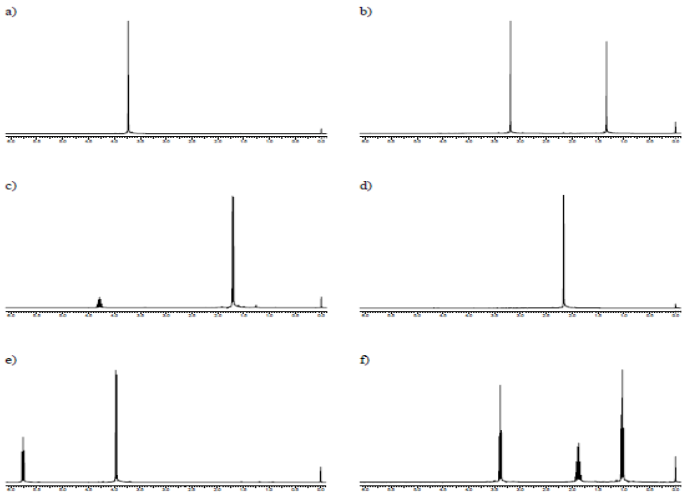
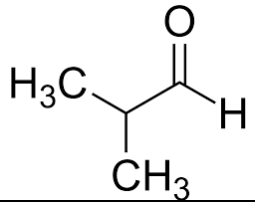
i. If the above stated seven amino acids were given to you in a mixture, will you be able to separate them satisfactorily using n-butanol-acetic acid-water (4:1:5) as the solvent system? Give reasons to your answers.
ii. Can you resolve the mixture satisfactorily using the n-butanol-pyridine-water (1:1:1) as the solvent system? Give reasons to your answers.
iii. Using only these solvent systems how can you separate the mixture of these seven amino acids satisfactorily?

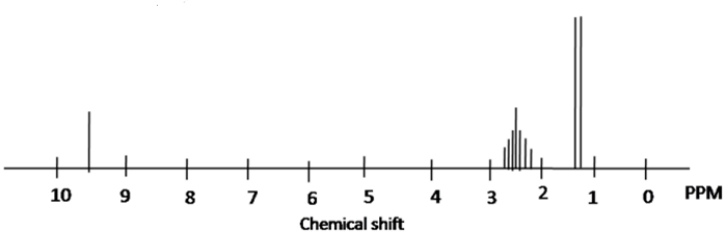
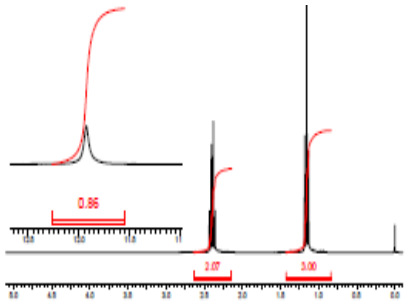
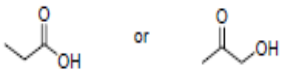
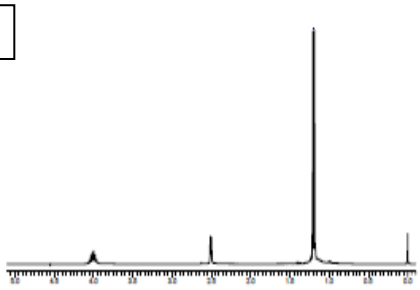
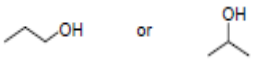
			OR			
	2	a)	The relative molecular mass (M_r) of a protein was investigated by exclusion chromatography using a Sephacryl S300 column and using aldolase, catalase, ferritin, thyroglobulin and blue dextran as standard. The following elution data were obtained. Protein | Molecular Weight (M_r) | Elution 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 decrease 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.	*CO1*	*PO 1,5*	**10**
		b)	Critically examine those factors which are influenced by the flow rate of the eluent through the column taking into consideration van Deemeter plot  	*CO1*	*PO 1,5*	**05**
		c)	i. How will you separate the following mixture of amino acids using ion exchange chromatography? a. Alanine (pI = 6.01) and Glutamic acid (pI = 3.22) b. Glutamic acid (pI = 3.22) and Aspartic acid (pI = 2.77)	*CO1*	*PO 1,5*	**05**

<table>
<tr><td></td><td></td><td></td><td>UNIT - II</td><td></td><td></td><td></td></tr>
<tr><td></td><td>3</td><td>a)</td><td>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.
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?
<table><tr><th>Protein</th><th>M_r</th><th>Distance moved (mm)</th></tr><tr><td>Transferrin</td><td>78000</td><td>6.0</td></tr><tr><td>Bovine serum albumin</td><td>66000</td><td>12.5</td></tr><tr><td>Ovalbumin</td><td>45000</td><td>32.0</td></tr><tr><td>Glyceraldehyde-3-phosphate dehydrogenase</td><td>36000</td><td>38.0</td></tr><tr><td>Carbonic anhydrase</td><td>29000</td><td>50.0</td></tr><tr><td>Trypsinogen</td><td>24000</td><td>54.0</td></tr><tr><td>Soyabean trypsin inhibitor</td><td>20100</td><td>61.0</td></tr><tr><td>β-Lactoglobulin</td><td>18400</td><td>69.0</td></tr><tr><td>Myoglobin</td><td>17800</td><td>69.0</td></tr><tr><td>Lysozyme</td><td>14300</td><td>79.0</td></tr><tr><td>Cytochrome c</td><td>12400</td><td>86.5</td></tr></table></td><td>CO2</td><td>PO 1,5</td><td>10</td></tr>
<tr><td></td><td></td><td>b)</td><td>The bands are greatly sharpened by an ingenious technique known as discontinuous pH or disc electrophoresis, which requires a two-gel system and several different buffers. What is the mechanism of disc electrophoresis which produces high resolution and good band definition?</td><td>CO2</td><td>PO 1,5</td><td>05</td></tr>
</table>

		c)	The following image represents a map of a piece of DNA, where each vertical line represents a recognition site for restriction site for restriction enzyme *BamHI*. If this DNA was digested completely by *BamHI*, represent the agarose gel results that you expect to see.  	*CO 2*	*PO 1,5*	**05**
			OR			
	4	a)	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. Identify the quick and reliable procedure for the separation of large (>30 kb) DNA molecules. Compare the different variants of this procedure.	*CO2*	*PO 1,5*	**10**
		b)	Substantiate the statement "Rocket Immuno-Electrophoresis is used as a rapid way to quantitate antigen in complex samples".	*CO2*	*PO 1,5*	**05**
		c)	What will be the relative electrophoretic mobilities of Alanine (pI = 6.02), Arginine (pI = 10.76), Glutamic acid (pI = 3.22), Serine (pI = 5.68), and Tryptophan (pI = 5.88) at pH 5.68?	*CO2*	*PO 1,5*	**05**
			UNIT - III			
	5	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. 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 x 10-8 g s-1, a sedimentation coefficient of 7.35 s, and a partial specific volume of 0.742 cm3 g-1. Calculate the molecular mass of aldolase assuming the density of the solution to be 0.998 g cm-3.	*CO 1*	*PO 1,5*	**10**

		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?	*CO3*	*PO 1,5,12*	**5**
		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.	*CO4*	*PO 1,5,12*	**5**
			OR			
	6	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. 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?	*CO4*	*PO 1,5,12*	**10**
		b)	What is fluorescence recovery after photobleaching? How do you use this technique to study diffusion and interactions of macromolecules?	*CO4*	*PO 1,5,12*	**05**
		c)	Examine the principle of a physical technique used to determine the thermodynamic parameters of interactions in solution.	*CO4*	*PO 1,5,12*	**05**
			UNIT - IV			
	7	a)	You have purified a recombinant protein and wonder whether it adopts a folded structure. How might you address this problem?	*CO4*	*PO 1,5,12*	**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	*CO3*	*PO 1,5,12*	**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 (Any five).  	*CO3*	*PO 1,5,12*	**10**
			OR			
	8	a)	What is the difference between Raman spectroscopy and Infrared spectroscopy? Write any two of their applications.	*CO4*	*PO 1,5,12*	**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.	*CO4*	*PO 1,5,12*	**05**
		c)	i. What is chemical shift? How do you calculate chemical shift? ii. In ^{1}H NMR spectrum, each signal represents one kind or set of proton(s) in a molecule. In certain molecule, multiple signals (multiplet) are observed rather than a singlet. The structure and the corresponding 1H NMR spectrum have been given below. Assign the structure to the given NMR spectrum.  	*CO 3*	*PO 1,5,12*	**10**

			iii. For each ^{1}H NMR spectrum given below, choose between  the alternative compounds. Give your reasons. a   b   			
			UNIT - V			
	9	a)	Examine the solid scintillation counting methods for the measurement of radioactivity. Apprise the advantages of scintillation counting.	*CO 4*	*PO 1,5,12*	**10**
		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.?	*CO4*	*PO 1,5,12*	**05**
		c)	What are the rules to be followed while handling radioisotopes?	*CO4*	*PO 1,5,12*	**05**
			OR			
	10	a)	Demonstrate the liquid scintillation counting methods for the measurement of radioactivity. Apprise the disadvantages of scintillation counting.	*CO4*	*PO 1,5,12*	**10**

		b)	Examine the types of radioactive decay.	*CO4*	*PO 1,5,12*	**05**
		c)	Compare and contrast merits and demerits of radioisotopes used in biological sciences.	*CO4*	*PO 1,5,12*	**05**

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