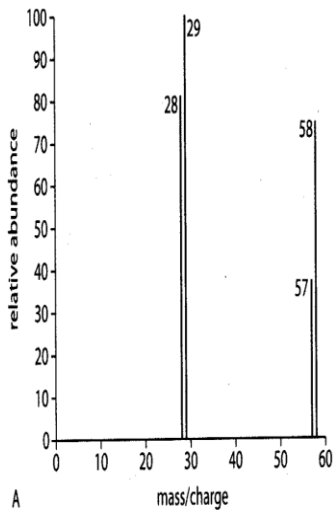# B.M.S. College of Engineering, Bengaluru-560019
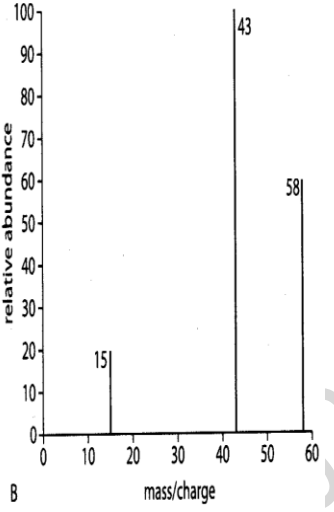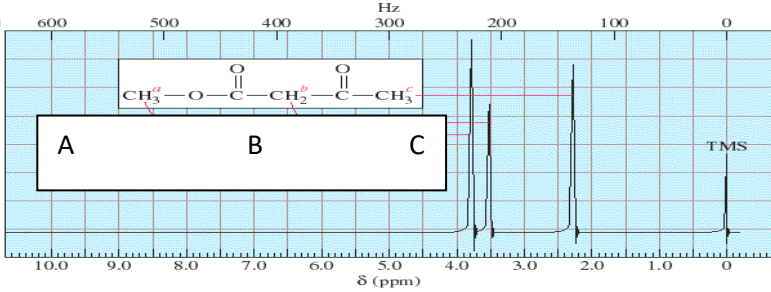
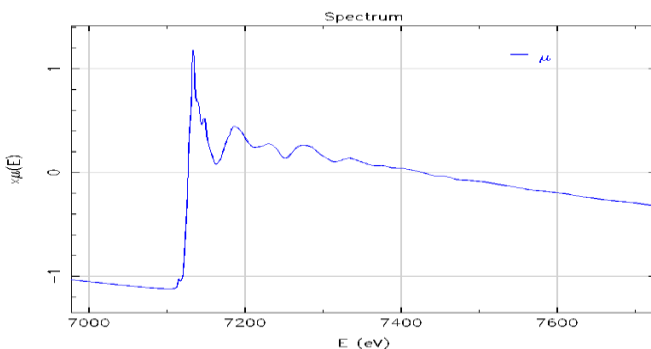**Autonomous Institute Affiliated to VTU**

January / February 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.

			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. What was the resolution of the two analytes?			**8**
	b)		Provide a simple form of the Van Demeter equation, and define the terms involved in the equation	CO1	PO 1,5	**5**
	c)		A mixture containing hemoglobin, 65,000 Daltons; myoglobin, 17,000 daltons and myosin, 180,000 daltons needs to be separated. Suggest the most suitable chromatographic technique for their separation. Explain the principle and working of the technique.	CO1	PO 1,5	**7**
			OR			
2	a)		Malodors are commonly detected in molded polymers used for general purposes such as household items, domestic appliances, plastic furniture, wheel and bumper covers in the automotive industry, and laboratory equipment. Research indicates that such strong odors are associated with the emission of harmful substances and volatile organic compounds (VOCs). Above a certain level of tolerance these volatile compounds have long-term effects detrimental to health after frequent exposure. A researcher seeks to investigate some of the compounds present in a molded polymer used by a Company C in their finished product and suggest ways to minimize or eliminate the smell without compromising other physical and chemical properties of the final polymer. (i) Identify the most suitable technique that can be used. Justify your choice. What is the principle of this technique? (ii) Give a schematic of its instrumentation and list the components involved.	CO1	PO 1,5	**8**

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.

				CO2	PO 1,5	4
		b)	6-phosphogluconate dehydrogenase has a pI of 6. Explain why the buffer used for a chromatography on DEAE-cellulose must have a pH greater than 6 but less than 9 in order to ensure the enzyme is efficiently bound to the column.			
		c)	A chromatography column with a length of 10.3 cm and an inner diameter of 4.61 mm is packed with a stationary phase that occupies 61.0% of the volume. If the volume flow rate is 1.13 mL/min, find the linear flow rate in cm/min.	CO1	PO 1,5	8
			UNIT - II			
3		a)	What is the role of APS and TEMED in SDS PAGE protocol?	CO2	PO 1,5	4
		b)	A mixture of five isoenzymes having a molecular weight range from 40kDa to 60 kDa need to be separated out .The pI value of this mixture of these isoenzymes ranges from 6.02 to 6.07. Identify the most suitable technique for effective separation of individual components elucidating the principle and protocol to be followed.	CO2	PO 1,5	8
		c)	PFGE is a powerful tool for characterizing various strains at the DNA level, obtaining relevant information on genome size and constructing the physical and genetic map of the chromosome of bacteria. List the types of PFGE and illustrate their working.	CO2	PO 1,5	8
			OR			

4		a)	The relative electrophoretic motilies of a 30 kDa protein and a 92 kDa protein used as standards on a SDS-PAGE are 0.80 and 0.41respectively.
			  i) Calculate the apparent mass of a protein having a mobility of 0.62 on this gel. (Mobility is inversely proportional to log. Mass).
			  ii) List and elucidate the factors that affect an electrophoresis run
				CO2	PO 1,5	10

		b)	Identify the electrophoretic techniques that can be used for the following cases. Justify your answers
			  (i) In vitro detection of protein–DNA binding
			  (ii) Resolving DNA
			  (iii) Zymogram analysis
			  (iv) Determine disulphide linkages in a protein
			  (v) single-strand conformation polymorphism (SSCP) analysis
				CO2	PO 1,5	10

			UNIT - III			
5		a)	Identify the technique which is based upon the differences in the sedimentation rate of biological particles of different size and density. Explain its principle and working with a schematic.	CO3	PO 1,5,12	7
		b)	DSC can elucidate the factors that contribute to the folding and stability of native biomolecules, including hydrophobic interactions, hydrogen bonding, conformational entropy, and the physical environment. Justify the statement	CO3	PO 1,5,12	6

		c)	The mass spectra of two compounds are shown below. One is propanone (CH₃COCH₃) and the other is propanal (CH₃CH₂CHO). (i) Identify the ions in the major peaks (ii). Identify the compound in each case 	CO3	PO 1,5,12	**7**
			OR			
	6	a)	A biochemist is interested in studying the topological images of a protein –DNA interactions. i) Identify a suitable technique that can be used ii) Explain the principle and working of this technique	CO3	PO 1,5,12	**6**
		b)	A plant extract was run through GC and needs to be further analyzed by MS to get information on it structural components. i) Select the ionization technique and the analyzer that is most suited for running the GC MS of this sample. ii) Illustrate the principle and working of the selected technique.	CO3	PO 1,5,12	**8**
		c)	Differentiate between fluorescence and phosphorescence.	CO3	PO 1,5,12	**6**
			UNIT - IV			
	7	a)	From the NMR spectrum given., identify the following with reasons : i) how many different kinds of protons are present. ii) how shielded or deshielded the proton is. iii) the number of protons of that type. **iv**) the number of protons on adjacent atoms.	CO4	PO 1,5,12	**4**

		b)	This graph shows a typical x-ray absorption spectrum, with absorption plotted vs. the energy of the incoming photon. Identify the following regions of the spectra: i) Edge, ii) XANES, iii)NEXAFS, iv) EXAFS v)White line and vi) E_o 	CO4	PO 1,5,12	**6**
		c)	An aliquot of a solution containing a light absorbing substance at a concentration of 5g/ml was placed in a 2cm light path cuvette. The cuvette was placed in a spectrophotometer and a beam of light of wavelength λ was passed through the cuvette containing the solution. A transmission value of 80% was recorded. i) Find the absorbance of the solution. ii) The molar extinction co-efficient if the molecular mass of the substance is known to be 410.	CO4	PO 1,5,12	**5**
		d)	A researcher has purified a recombinant protein and wants to check whether it has adopted a folded structure or not optically? Identify the technique that he should follow. Justify your choice.	CO4	PO 1,5,12	**5**
			OR			
	8	a)	Differentiate between ENDOR and ELDOR.	CO4	PO 1,5,12	**4**
		b)	Differentiate between Infrared spectroscopy and Raman spectroscopy.	CO4	PO 1,5,12	**6**
		c)	What is chemical shift? How is it measured? What are the factors that affect the chemical shift?	CO4	PO 1,5,12	**10**
			UNIT - V			
	9	a)	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.?	CO3	PO 1,5,12	**4**
		b)	What is Scintillation proximity assay? Illustrate its principle and working. Add a note on its advantages.	CO4	PO 1,5,12	**10**
		c)	List any six rules that should be followed while handling radioisotopes.	CO3	PO 1,5,12	**6**
			OR			
	10	a)	What types of radiation can be detected by an ionization counter? Justify.	CO3	PO 1,5,12	**4**
		b)	Differentiate between solid and liquid scintillation counting.	CO4	PO 1,5,12	**6**
		c)	List the merits and demerits of any three commonly used radioisotopes.	CO 3	PO 1,5,12	**6**
		d)	A 1 mCi source of ^{125}I gives a dose of 10 mSv h^{-1} at 1 cm. What will be the dose rate at 5 cm?	CO 3	PO 1,5,12	**4**

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