INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

MID SEMESTER EXAMINATION - BT 501 Biotechniques

September 2023. Time: 2 hours. QP has 3 pages, 7 Questions

Questions 1-6: 10 marks each. Q7: 20 marks. Total: 80 Marks

Q1. Answer the following questions from the choices given **(2 marks each)**

1. 1: What does the retention factor, k', describe?

a) The distribution of an analyte between the stationary and the mobile phase

b) The migration rate of an analyte through a column

c) The velocity of the mobile phase

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1. 2. What does the selectivity factor, describe?

a) The proportional difference in widths of two chromatographic peaks

b) The maximum number of different species which a column can separate simultaneously

c) The relative separation achieved between two species

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1. 3. What useful information can be found from a Van Deemter plot?

a) The selectivity factor

b) Optimum mobile phase flow rate

c) Optimum column temperature

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1. 4. Resolution is proportional to the square root of the number of theoretical plates in a column. For example, doubling the column's length increases resolution by a factor of 2

True or false?

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1. 5. In reverse phase chromatography, the stationary phase is made of a _____

A) Polar substance B) Non Polar Substance C) Neutral molecule D) Metals

Q2. Explain the principle and working of a flow cytometer **(10 marks)**

Q3. HPLC chromatogram of a hypothetical mixture of four different protein/peptide segments USING REVERSE PHASE (C-8) column is shown below:

Protein - Sequence 1: ACDEFGHI; Sequence 2: TVWILFHI;
Sequence 3: DEGKMNRK and Sequence 4: KLMNPRST

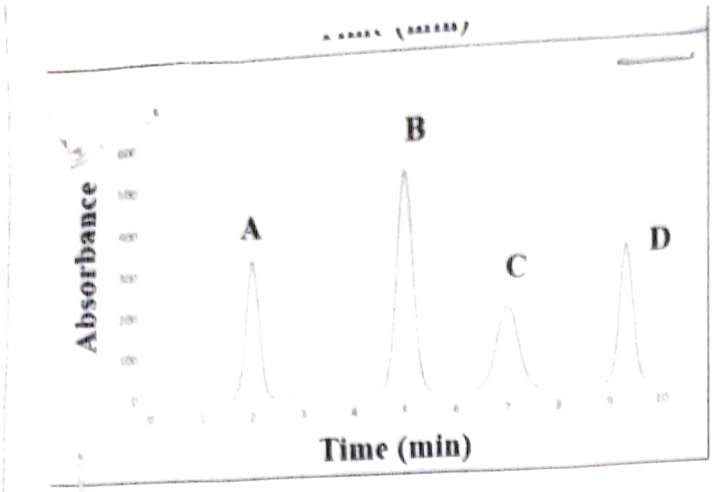


Figure 1. HPLC Chromatogram

*Hydrophobicity Data from Cambridge University press

Match Sequence 1, 2, 3 and 4 against A, B, C and D. **(4 marks)**

What changes (qualitatively) in the sequence of elution do you expect if you use

(i) C-18 column **(3 marks)**

(ii) Normal Phase column **(3 marks)**

Q4. The data from a typical chromatogram given below (Table 1). This data was obtained using HPLC stationary phase (C-18 column) and an acetonitrile/water mobile phase, for the separation of two compounds in a mixture. Both compounds are peptides with the following sequence

Peptide 1: ADEFGDEVDEAA; Peptide 2: AVIFGLEVDAAA

Table 1

	Component A	Component B
t_M (min)	1.53	1.53
t_R (min)	8.36	9.18
$w_{½}$ (min)	0.62	0.68

Table 2

compound	pentane	heptane	toluene
b. p	35 °C	98 °C	110 °C

A) Match peptides (1 and 2) against their peaks (component A or component B) **(5 marks)**

B) Are the two peaks corresponding to component A and component B, well resolved OR do you expect an overlap? Explain Why? / Why not? **(5 marks)**

Q5. A) The vibration frequency of $^{1}H\ ^{35}Cl$ is 2990.6 cm-1. Without calculating the bond force constant, estimate the frequency for $^{1}H\ ^{37}Cl$, and $^{2}D\ ^{35}Cl$. **(6 marks)**

B) Write the increasing order of Carbon-Carbon stretching frequency in ethane, ethene and ethyne. **(2 marks)**

C) In an IR spectrum, the wavelength corresponding to the peak at 1720 cm^{-1} is _____ **(2 marks)**

Q6. A) Absorption and emission spectra of three aromatic amino acids, F, Y and W are shown in the figure 5 A. Draw an approximate spectrum of the molecule in figure 5B, with x axis and y axis properly labeled. Explain how it is similar or different from the spectrum of F, Y and W **(4 marks)**

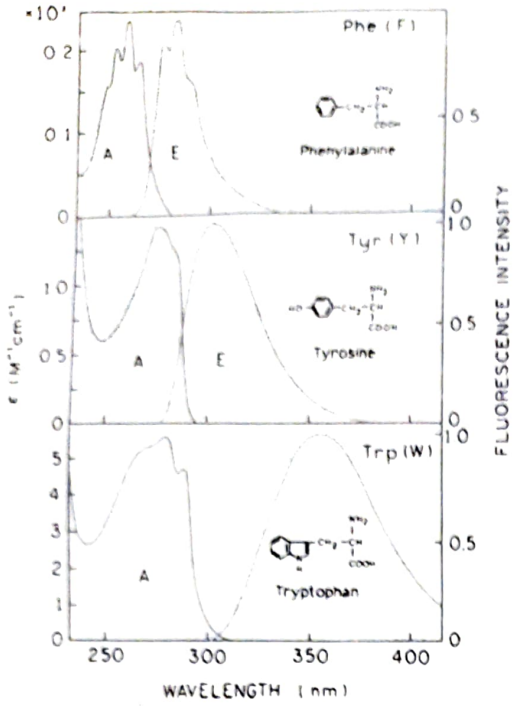


Figure 5 A

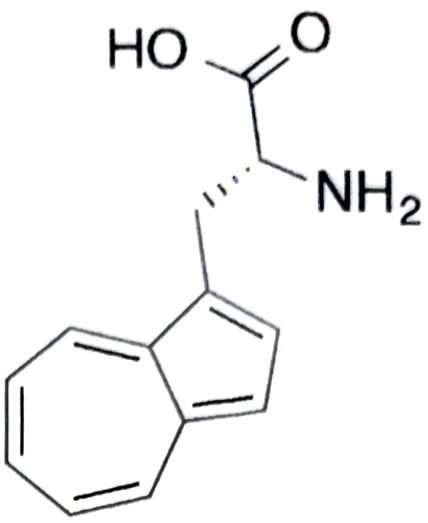
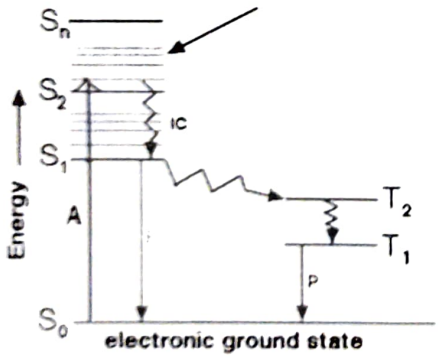


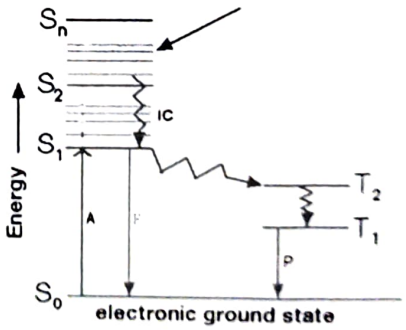
Figure 5 B

B) In the Jablonski Diagram shown below, if the absorption **A** and fluorescence **F** is strictly happening as shown in Case A and Case B, what would be its effect in

i) Fluorescence intensity ii) Quantum yield and iii) wavelength **(6 marks)**



Case A



Case B

Q7. Write Short notes on

i) Gradient elution
ii) Partition coefficient
iii) Gel filtration chromatography
iv) 2D Gel Electrophoresis

(5 marks each)

End of Questions