



B.TECH DEGREE EXAMINATIONS: APRIL /MAY 2024

(Regulation 2018)

Fourth Semester

BIOTECHNOLOGY

U18BTI4203: Instrumental Methods of Analysis

COURSE OUTCOMES

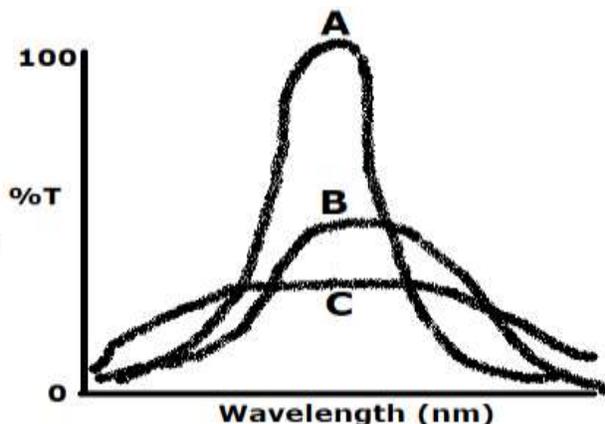
- CO1: Understand and apply the statistical principles to solve biological issues, and apply appropriate extraction methodologies to process biological samples
- CO2: Compare, apply and interpret the data of biological solutions acquired from different spectroscopy techniques
- CO3: Describe, apply and evaluate the data originated by chromatographic techniques to solve biological problems
- CO4: Explain, apply and evaluate the data obtained from different electrophoretic techniques
- CO5: Describe and apply mass spectrometry, x-ray diffraction and NMR techniques in the broad field of biotechnology
- CO6: Discuss the fundamentals of centrifugation techniques

Time: Three Hours

Maximum Marks: 100

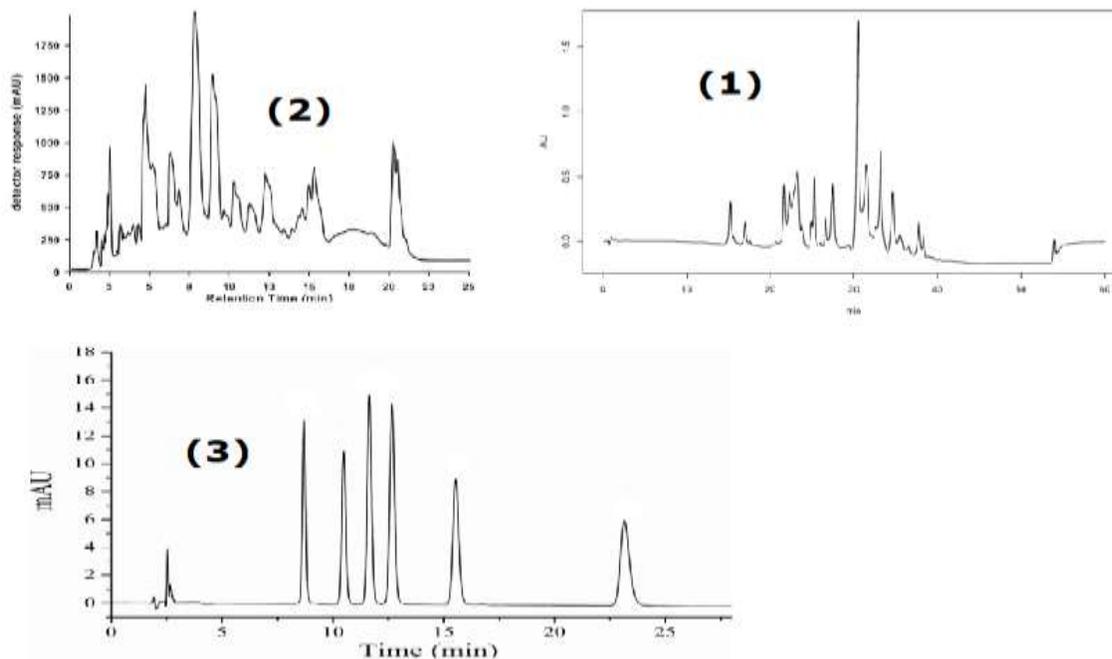
Answer all the Questions:-
PART A (10 x 2 = 20 Marks)
(Answer not more than 40 words)

1. Calculate the limits of quantification (LoQ) of an experimental assay for the furnished values. CO1 [K₃]
STEYX: 0.033; Slope: 0.007
2. What is a standard operating procedure (SOP)? CO1 [K₂]
3. Write the principle of nephelometry CO2 [K₂]
4. Observe the below depicted image related to the bandwidth of three different filters. CO2 [K₅]
Which labelled filter(s) is/ are to be considered as highly efficient for the generation of monochromatic light?



5. Observe the furnished diagram below of different liquid chromatograms. Which labelled chromatogram reveals the complete purification of the analytes? Justify the same

CO3 [K₅]

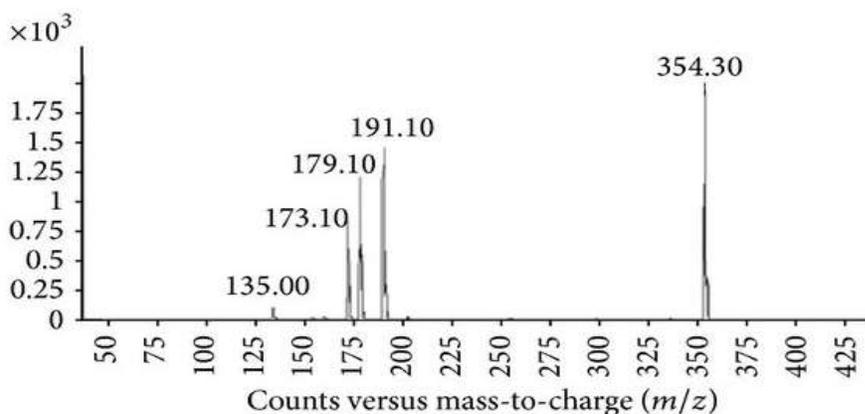


6. Assumed that you have procured the below shown adsorbent for the planar chromatography analysis. CO3 [K₄]

- i) What does the letter “G” denote?
- ii) Can you view the separated analytes under short UV (254 nm) if the adsorbent is used as a stationary phase?



7. Define electrophoresis CO4 [K₂]
8. Write the principle of isoelectric focusing CO4 [K₂]
9. Observe the shown mass spectrum (MS) and identify the parent and abundant daughter ion CO5 [K₄]

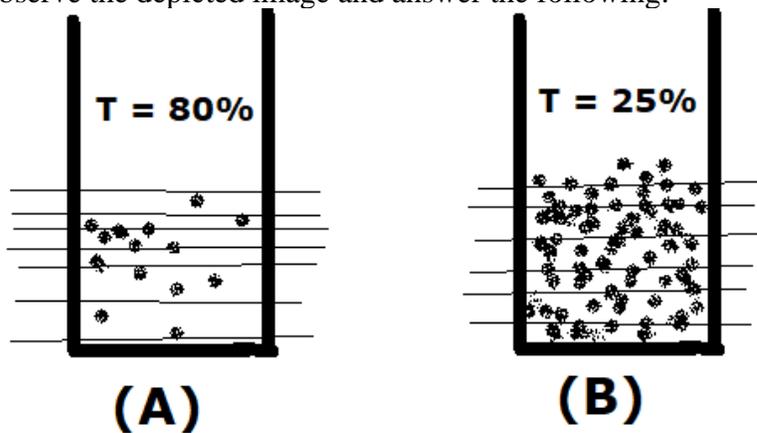


10. Write the name of the unit that is used to express the sedimentation coefficient

CO6 [K₁]

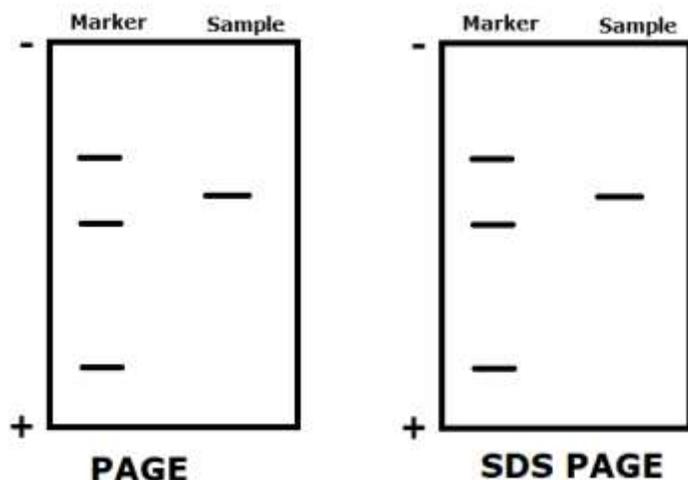
**Answer any FIVE Questions:-
PART B (5 x 16 = 80 Marks)
(Answer not more than 400 words)**

11. a) Describe the stepwise protocol of supercritical fluid extraction (SFC) process with a simple block diagram 12 CO1 [K₂]
 b) Write a short note on process validation with a suitable example 4 CO1 [K₂]
12. a) Elaborate in detail the principle and instrumentation of double beam UV spectrometry with a neat block diagram 12 CO2 [K₂]
 b) Observe the depicted image and answer the following: 4 CO2 [K₄]

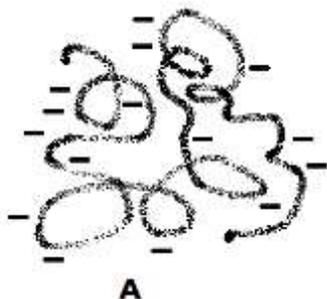


- i) State the most appropriate law that relates the above cited diagram
 ii) Calculate the absorbance (%) for both the conditions
13. a) Describe the principle and instrumentation of HPLC with a neat block diagram 12 CO3 [K₂]
 b) Assume that you are assigned to prepare a mobile phase (100 ml) with the following composition to separate the polar analytes. [Hint: Polarity index table is furnished at the end of the question paper] 4 CO3 [K₅]
 Ethanol: ethyl acetate: acetonitrile: water (4: 3: 2: 1)
 i) Calculate the polarity index (P') of the mobile phase.
 ii) Whether the prepared mobile phase solvent can be used for the separation process? Yes/ No, justify the same
14. a) Give a detailed account on the principle and instrumentation of agarose gel electrophoresis (AGE) 12 CO4 [K₂]

- b) What information you can infer from the below depicted electrophoretic gel images of protein sample? 4 CO4 [K₅]



15. a) Discuss in brief the principle (**ESI is sufficient**) and instrumentation of mass spectrometry 12 CO5 [K₂]
 b) Illustrate the principle of centrifugation process 4 CO6 [K₂]
 16. a) Discuss the principle and working mechanism of gel filtration with appropriate steps 12 CO3 [K₂]
 b) Assume that the below depicted charged protein “A” should be purified from a heterogeneous mixture using a chromatography technique. Which technique will you prefer? Why? 4 CO3 [K₄]



Polarity index table

Properties of Common Chromatographic Mobile Phases

Solvent	Refractive Index ^a	Viscosity, cP ^b	Boiling Point, °C	Polarity Index, P'	Eluent Strength, ε ^d
Fluoroalkanes ^d	1.27–1.29	0.4–2.6	50–174	<-2	-0.25
Cyclohexane	1.423	0.90	81	0.04	-0.2
n-Hexane	1.372	0.30	69	0.1	0.01
1-Chlorobutane	1.400	0.42	78	1.0	0.26
Carbon tetrachloride	1.457	0.90	77	1.6	0.18
i-Propyl ether	1.365	0.38	68	2.4	0.28
Toluene	1.494	0.55	110	2.4	0.29
Diethyl ether	1.350	0.24	35	2.8	0.38
Tetrahydrofuran	1.405	0.46	66	4.0	0.57
Chloroform	1.443	0.53	61	4.1	0.40
Ethanol	1.359	1.08	78	4.3	0.88
Ethyl acetate	1.370	0.43	77	4.4	0.58
Dioxane	1.420	1.2	101	4.8	0.56
Methanol	1.326	0.54	65	5.1	0.95
Acetonitrile	1.341	0.34	82	5.8	0.65
Nitromethane	1.380	0.61	101	6.0	0.64
Ethylene glycol	1.431	16.5	182	6.9	1.11
Water	1.333	0.89	100	10.2	Large

^aOn Al₂O₃. Multiplication by 0.8 gives ε^d on SiO₂.