



Date:
Registration number:

ST. JOSEPH'S COLLEGE (AUTONOMOUS), BENGALURU -27
B.Sc. – V SEMESTER
SEMESTER EXAMINATION: OCTOBER 2022
(Examination conducted in December 2022)
BCH 5222 : ANALYTICAL TECHNIQUES IN BIOCHEMISTRY-2

Time: 2 ½ Hours

Max Marks: 70

This paper contains THREE printed pages and FOUR parts

PART-A

Answer any SIXTEEN questions

16 x 1 = 16

1. What is meant by cell fractionation?
2. Write any one principle of cell theory.
3. What is differential centrifugation?
4. What is the role of SDS in SDS-PAGE electrophoresis?
5. Name the buffer used for western blotting.
6. What is the advantage of cellulose acetate electrophoresis over paper electrophoresis?
7. What is the purpose of staining the gel?
8. What are cation-exchangers?
9. Arrange the following organelles in the increasing order of sedimentation (lightest to heaviest) after centrifugation. The organelles are: ribosomes, mitochondria, nuclei.
10. Complete the reaction : $^{12}_6C \rightarrow ? + ^0_{-1}e$
11. State radioactive decay law.
12. What are radioactive probes?
13. The antigen or hapten used for radioimmuno assay is labeled with ^{125}I instead of ^{14}C or 3H . Give reason.
14. What is a green fluorescent protein?
15. Name the chemical reagent, which is used to cleave the peptide bonds on the C-terminal side of methionine residue?
16. What are exopeptidases?
17. What is the advantage of mass spectroscopy in protein sequencing technique?
18. What is the role of 2-mercaptoethanol in protein sequencing?

PART-B

Answer any TEN questions

10 x 2 = 20

19. Write any two methods that can be used to achieve homogenization during cell fractionation.

20. What is blotting? Write any one difference between western and southern blotting.
21. What is the polarity (polar/non polar) of the following in reverse phase chromatography?
 - a) stationary phase and b) mobile phase
22. Write the working principle of cellulose acetate electrophoresis?
23. Write the principle involved in HPLC method of analysis.
24. Name two reagents which are used for staining carbohydrates.
25. Describe enzymatic method of C-terminal analysis.
26. Define DNA sequencing. What is the role of ddNTPs in DNA sequencing?
27. Write any two advantages of automated DNA sequencing over manual DNA sequencing method.
28. Name the technique/method used for
 - a) studying the dynamics of fluorescent molecules in living cells.
 - b) measuring the spatial distance between two or more fluorophores.
29. What is alpha emission? Give an example.
30. Write any two conclusions drawn from Hershey Chase experiment using ^{32}P and ^{35}S radiolabeling.

PART-C

Answer any EIGHT questions

8 x 3 = 24

31. Explain density gradient centrifugation.
32. What is the principle of gel filtration chromatography? Explain with a neat diagram.
33. Outline the steps involved in Western blotting.
34. Explain paper electrophoresis with a suitable example.
35. What is autoradiography? Explain in vitro mode of autoradiography?
36. Explain the principle of radioimmunoassay?
37. How does PET scan work? Write any one application of PET scan.
38. Explain N-terminal amino acid analysis using dansyl chloride.
39. What is protein sequencing? What are the initial steps involved in protein sequencing prior to the N-terminal and C-terminal analysis.
40. Briefly explain the automated DNA sequencing using dideoxynucleotide method.

PART-D

Answer any TWO questions

2 x 5 = 10

41. A group of scientists homogenized pancreatic tissue before carrying out the cell fractionation to isolate organelle G. Explain why the scientists

- i) homogenized the tissue?
 - ii) filtered the resulting suspension?
 - iii) kept the suspension in ice-cold during the process?
 - iv) used isotonic solution during the process?
 - v) used buffered solution during the process?
42. Mn-54 has a half life of 314 days. Calculate the
- i) decay constant in terms of days^{-1} and s^{-1} and
 - ii) percentage of initial radioactivity remaining in the sample after 80 days. (3+2)
43. Oxytocin, a hormone peptide of nine amino acids, is widely used in obstetrics to induce uterine contractions. There is an intramolecular disulfide bond which must be reduced before sequencing. The reduced oxytocin has the composition: Asn Cys Cys Gln Gly Ile Leu Pro Tyr
Partial hydrolysis of reduced oxytocin led to the following fragments:

Asn-Cys
Cys-Tyr
Tyr-Ile-Gln
Cys-Pro-Leu
Ile-Gln
Leu-Gly
Gln-Asn-Cys

Reaction of reduced oxytocin with carboxypeptidase showed glycine as the first liberated amino acid at the right end.

Deduce the sequence of oxytocin.
