2021

BIOCHEMISTRY — HONOURS

Paper : SEC-A-2

(Protein Purification Techniques)

Full Marks : 80

The figures in the margin indicate full marks.
Candidates are required to give their answers in their own words as far as practicable.

1. Answer ***any ten*** questions : 2×10

(a) Write down the separating principle of SDS-PAGE.

(b) What is mass spectrometry?

(c) What do you mean by isoelectric pH of a protein?

(d) Give example of an anion exchanger and a cation exchanger.

(e) Write two differences between mobile phase and stationary phase.

(f) What is 'salting in' of protein?

(g) What is dialysis?

(h) Why ammonium sulphate $\left[(NH_4)_2 SO_4\right]$ is most commonly used in salting out of proteins?

(i) What are the common buffers used in HPLC?

(j) Why is C_{18} column mostly used in HPLC?

(k) What is Svedberg unit?

(l) What is blank run on HPLC analysis?

2. Answer ***any four*** questions :

(a) (i) An enzyme of M.W. = 26,000 and pI = 5.5 is contaminated with a protein of similar M.W. but with pI = 7.2 and another protein of M.W. = 120,000 and pI = 5.4. Suggest a purification strategy.

(ii) What are the functions of APS and β-mercaptoethanol in SDS-PAGE? 3+2

(b) (i) What is R_f value in TLC? On which factors does R_f value depend?

(ii) Define specific activity of proteins with unit. Why does it increase during protein purification? (1+2)+(1+1)

(c) (i) What are the differences between adsorption chromatography and affinity chromatography?

(ii) Name two common matrix used in partition chromatography. 3+2

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(d) (i) Write down the principle of gel filtration chromatography and state one of its uses.

(ii) Tropomysin, a 70kd muscle protein, sediments more slowly than does haemoglobin (65kd). Their sedimentation coefficients are 2.6 S and 4.31 S. Which structural features of Tropomysin account for its slow sedimentation? (2+1)+2

(e) (i) The octapeptide Ala-Val-Gly-Trp-Arg-Val-Lys-Ser was digested with the enzyme trypsin. Would ion-exchange or gel filtration chromatography be most appropriate for separating the products? Explain.

(ii) Suppose that the peptide was digested with chymotrypsin. What would be the optimal separation technique? 3+2

3. Answer ***any four*** questions :

(a) (i) How would you purify integral and peripheral membrane proteins?

(ii) What is solvent fractionation?

(iii) Name two membranes used in dialysis.

(iv) When would you employ dialysis during protein purification?

(v) Differentiate between ultrafiltrations and dialysis. 2+2+2+2+2

(b) (i) What is isopycnic centrifugation?

(ii) What do you mean by gradient elution of ion exchange chromatography?

(iii) In what order would the following proteins emerge upon gel filtration of a mixture on sephadex G 200 : Myoglobin (M = 16,000), Catalase (M = 500,000), Cytochrome C (M = 12,000), Chymotrypsinogen (M = 26,000) and Serum albumin (M = 65,000)? Explain.

(iv) What are the working principles of horizontal electrophoresis using agarose gel?

(v) Write down the application of ultracentrifugation. 2+2+2+2+2

(c) (i) What is void volume?

(ii) In a chromatographic column, the stationary phase has an exclusion limit of 80,000 MW. If you tried to use this column material to separate alcohol dehydrogenase (M.W. = 150,000) from α-amylase (M.W. = 55,000), what would happen and why?

(iii) What is katal?

(iv) What is the difference between SDS-PAGE and NATIVE – PAGE?

(v) Why is extraction of proteins from a source done under ice-cold solution? 2+2+2+2+2

(d) (i) A solution containing egg albumin (pI = 4.6), β-lactoglobulin (pI = 5.2) and chymotrypsinogen (pI = 9.5) was loaded on to a column of DEAE allulose at pH = 5.4. The proteins are then eluted with buffer with pH = 5.5, with an increasing salt concentration. Predict the elution pattern.

(ii) Define the following : Partition coefficient, retention time, resolution, reverse phase chromatography. 2+(2×4)

(e) (i) Is ion exchange a chemisorption process?

(ii) What are the disadvantages of HPLC?

(iii) In what direction will the following proteins move in an electric field?

(A) β-lactoglobulin (pI = 5.2) at pH = 5.0 and pH = 7.0

(B) Ribonuclease (pI = 9.5) at pH = 4.5 and pH = 9.5

(iv) From a mixture of Glu (pI = 3.08), His (pI = 7.64) and Arg (pI = 10.76), how can you retrieve individual amino acids? 1+2+4+3
