2020

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) What is salting out of proteins?

(b) Why is EDTA used in protein extraction method?

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

(d) Why is extraction of proteins from a source done under ice cold solution?

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

(f) How are peripheral membrane proteins extracted?

(g) What are the factors that affect the mobility and sharpness of separation of bands in electrophoresis?

(h) Write down two applications of dialysis.

(i) Write the name of different types of ion-exchange chromatography with example.

(j) What is the basis of separation of proteins in SDS-PAGE?

(k) Which of the following is not a gel filtration media used in gel filtration?

(i) Agarose gel

(ii) Polyacrylamide gels

(iii) Sephadex

(iv) Silica gel.

(l) What is solvent fractionation?

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

(a) (i) The proteins ovalbumin (pI = 4.6), urease (pI = 5.0) and myoglobin (pI = 7.0) were applied to a column of DEAE-cellulose at pH 6.5. The column was eluted with a pH 6.5 buffer and then with the same buffer containing increasing concentration of sodium chloride. In what order will the proteins be eluted from the column?

(ii) What is R_f value in TLC? 3+2

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

(ii) Why salt gradient is used in ion-exchange chromatography? 3+2

(c) (i) What is salting in of proteins?

(ii) Write down the principle of Ion-Exchange chromatography with proper diagram. 2+3

(d) (i) In what direction, i.e towards anode, towards cathode or stationary, will the following proteins migrate in an electric field at the pH indicated and why?

(A) Egg albumin at pH 5.0

(B) β-lactoglobulin at pH 7.0

(C) Chymo trypsinogen at pH 5.0

Given, pI of Egg albumin = 4.8
pI of β-lactoglobulin = 5.2
pI of Chymotrypsinogen = 9.5

(ii) What are the factors that affect electrophoresis? 3+2

(e) (i) Explain the phenomenon 'isoelectric precipitation' with an example.

(ii) What are the differences between normal phase HPLC and reverse phase HPLC? 3+2

(f) (i) Write down the principle of gel filtration chromatography.

(ii) Differentiate between isoelectric precipitation and isoelectric focussing. 3+2

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

(a) (i) What is ultracentrifugation? State some applications of it.

(ii) An enzyme examined by means of gel filtration in aqueous buffer at pH 7.0 had an apparent M.W. of 160,000. When examined by gel electrophoresis in SDS solution, a single band of apparent M.W. 40,000 was formed. Explain these findings. (4+2)+4

(b) (i) Why does specific activity increase in protein purification?

(ii) What are common buffers used in HPLC?

(iii) From a mixture of Lys (pI = 9.47), Asp (pI = 2.98) and His (pI = 7.64), how can you retrieve individual amino acids? 4+3+3

(c) (i) Write down the application of ion-exchange chromatography.

(ii) In what direction (toward the anode toward the cathode or remian stationary) will the following proteins move in an electric field?

(A) Urease (pI = 5.0) at pH 3.0 and pH 9.0

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

(iii) What is void volume in gelfiltration chromatography? 4+4+2

(d) (i) Give an example of compounds which makes up the gel used in protein electrophoresis. Briefly describe the structural feature of that compound which makes it suitable for electrophoresis.

(ii) What are the functions of β-mercapto ethanol and TEMED in SDS-PAGE?

(iii) What is Svedberg unit? 4+(2+2)+2

(e) (i) Name two common matrix used in partition chromatography.

(ii) What is exclusion limit in gel filtration?

(iii) In what order do the following proteins emerge upon gel filtration through a column contaning a gel that excludes all proteins of M.W. 200,000 and higher. Cytochrome C (M.W. = 13,000), Tryptophan Synthetase (M.W. = 117,000, hexokinase (M.W. = 96,000) glucose oxidase (M.W. = 154,000) and xanthine oxidase (M.W. = 300,000).

(iv) What is role the of SDS in SDS-PAGE? 2+2+4+2

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