V(3rd Sm.)-Biochemistry-H/SEC-A-1/CBCS

2021

BIOCHEMISTRY — HONOURS

Paper : SEC-A-1

(Tools and Techniques in Biochemistry)

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 the usual range of pH scale at 25°C? What is its significance?
- (b) Can pH of a solution be negative? How does pH of a solution vary with temperature?
- (c) State Lambert Beer's Law. Mention its limitations.
- (d) What are chromophores and auxochromes?
- (e) What is λ max? What is its significance in UV-visible spectrum?
- (f) What is the significance of the "blank spectrum" in UV-visible absorption spectroscopy?
- (g) If the fasting blood glucose in man is 5 mmol/litre, what is the concentration expressed as mg per 100 ml of the blood? (M.W of glucose = 180)
- (h) What conditions must be maintained for storing biological samples?
- (i) How would you make quantitative transfer of a liquid? Why this transfer is important?
- (j) Mention the three main buffer systems found in living material. Why they are important?
- (k) Explain the mechanism of buffer action of an acidic buffer.
- (1) Name one intrinsic & one extrinsic fluors.
- (m) A sample of plasma 0.2 ml was mixed with 0.8 ml of distilled water and 2 ml of biuret reagent. The absorptivity of the mixture after 10 minutes was the same as that obtained from mixing 1 ml of albumin (1.3 mg/ml) and 2 ml of biuret reagent. Calculate the concentration of protein in the plasma.

Please Turn Over

2. Answer any four questions:

(a) Discuss the principle for determining the concentration of unknown protein by BCA method. Compare BCA & Folin-Lowry method.
 3+2

(2)

- (b) Why does fluorescence occur at a lower frequency than that of incident radiation? What do you mean by the term "fluorescence quenching"? On what factors does it depend? 2+1+2
- (c) Mention the two electrodes used in a pH meter and write down the cell diagram of the electrochemical cell formed between the two electrodes. Can't we measure the pH of a pH-meter using only one electrode? Explain in short.
 1+2+2
- (d) The quantum yield of a photochemical reaction is 0.66. Calculate the no. of moles of reactants converted to product if the radiation was done by 3×10^{18} quanta per second for 5 minutes. 5
- (e) You are supplied with 100 ml 0.1 (N) NaOH & 100 ml 0.1 (N) acetic acid. How will you use these to prepare 100 ml of a buffer solution of pH 5.0 at 25°C? [Given K_a of acetic acid = 1.75×10^{-5} at 25°C] 5
- 3. Answer any four questions:
 - (a) (i) Discuss about the radiation hazards one may face when working in such laboratories. What safety practices one should maintain to avoid particularly this type of risk?
 - (ii) Mention amino acids responsible for protein fluorescence. Define FRET with an example.

(3+3)+(1+3)

- (b) (i) Draw a simple schematic block diagram of the instrumentation used in single beam UV-vis. spectrophotometer and also in UV-vis. spectroflurimeter.
 - (ii) Mention the differences in making the design of these two instruments and also explain why such differences are made in the design while both working on in the UV-visible spectroscopy.

(3+3)+(2+2)

- (c) (i) Why does a protein solution exhibit UV-visible spectra? The UV spectrum of 1 mM tyrosine shows a red shift in λ max on increasing the pH of the medium. Explain it with proper reasons and diagram.
 - (ii) How does solvent affect the UV-vis. spectrum determined for chromophores? What choices should we take for the use of solvents in UV-vis. spectroscopy? (2+3)+(2+3)
- (d) (i) Define absorbance and transmittance of a solution. Which among them is additive and why? A spectrophotometer cell when filled with liquid A transmits 50% and when filled with liquid B transmits only 25% of the incident light of a certain wavelength. What would be the optical density at this wavelength when the same cell is filled with a mixture of equal volumes of two liquids?

- (ii) An aqueous solution of a triphosphate derivative (molar mass 602 g mol⁻¹) was prepared by dissolving 15.1 mg in 500 ml of water. When the solution was taken in a cell of pathlength 1.00 cm, the measured absorbance was 0.505 at its λ max? Calculate absorptivity and % transmittance for a solution with twice the concentration. (3+3)+4
- (e) (i) A 10 ml solution of 0.1 (M) acetic acid was titrated against 0.5 (M) NaOH solution pH metrically. Draw the pH-metric titration curve for this mentioning the axis properly and also indicate the equivalence point on the graph. What would be the pH when just 1 ml 0.5 (M) NaOH was added to the 10 ml 0.1 (M) of such acetic acid solution? ($K_a = 1.75 \times 10^{-5}$ at that temp)
 - (ii) What is buffer capacity? Why is buffer range pKa \pm 1? (3+3)+(2+2)