This question paper contains 5 printed pages.]

Your Roll No. ....

1361   **A**

B.Sc. (Hons.)/Bio-Chemistry/II Sem.
Paper—BCHT–203–BIOCHEMICAL AND
BIO-PHYSICAL TECHNIQUES

Time : 3 Hours   *Maximum Marks : 75*

(Write your Roll No. on the top immediately

on receipt of this question paper.)

Attempt six questions in all.

Question No. 1 is compulsory.

Use of scientific calculator/log tables may be allowed.

1.   (A) State True or False. Reason out your answer.

(a)   All fluorescent compounds have cyclic structures.

(b)   DNA exhibits hypochromacity on heating.

(c)   TLC is more sensitive than paper chromatography.

(d)   Affinity matrix can be prepared by attaching a legand
with PEG.

(e)   Native gel electrophoresis can be used for determining
the molecular weight of proteins. $(2 \times 5 = 10)$

(B) Give the importance of the following in the sand instrument/
process :

[P.T.O.

(a) Salt gradient in ion exchange chromatography

(b) Peristaltic pump in HPLC

(c) Vacuum pump in ultracentrifuge

(d) Stationary phase in paper chromatography

(e) Supporting material in GLC

(f) Nitrocellulose membrane in ultra-filtration

(g) Organic solvents in protein precipitation

(h) Tracking Dye in gel electrophoresis

(i) Quartz cuvettes in UV spectrophotometry $(1 \times 9 = 9)$

2. (a) Why is ammonium sulphate a salt of choice for salting out ? 3

(b) Explain the concept of theoretical plates in chromatography. 3

(c) The relative molecular mass of a protein was investigated by exclusion chromatography using Sephacryl S300 column and using aldolase, catalase, ferritin, thyroglobulin and blue dextran as standard. The following elution were obtained :

Protein	Molecular Mass	Retention Volume (cm)
Aldolase	153000	22.5
Catalase	210000	21.4
Ferritin	444000	18.2
Thyroglobulin	669000	16.4
Blue Dextran	200000	13.6
Unknown	?	19.5

What is approx. Molecular mass of the unknown protein   4

(d) Why is affinity chromatography considered more effective than other chromatographic methods ? Describe the use of any one affinity chromatography matrix in purification of a macromolecules.   4

3. (a) Explain Lambert Beer's Law. Give its limitations.   3

(b) What are intrinsic and extrinsic fluors ? Give an example of each and their application in biology.   4

(c) A solution at a concentration of 32 mg/ml of a substance having a molecular weight of 423 has an absorbance of 0.27 at 540 nm measured in a cuvette with a 1 cm. path length. What is the molar absorption coefficient at 540 nm ? Assume that Beer's law is obeyed.   3

(d) What are monochromatons? Explain the different types of monochromatons used in spectrophotometry.   4

4. (a) Define and explain the significance of Svedberg constant. 2

(b) Explain with one example the difference between density gradient and differential centrifugation.   5

(c) For the pelleting of a microsomal fraction from a liver homogenate, an ultra centrifuge is operated at a speed of 40,000 rpm. Calculate the angular velocity (w) in radians per second.   2

(d) Describe the different types of rotors used in centrifugation.   5

5. (a) Why is electrophoresis done in solutions having low salt concentrations ? 2

(b) What is the principle of separation of biomolecules by agarase gel electrophoresis ? What factors affect the separation ? 3

(c) Will two molecules having the same molecular weight and charge have the same mobility ? Explain. 2

(d) Give the principle and application(s) of iso electric focussing. 4

(e) An enzyme examined by means of gel filtration in aqueous buffer at pH 7 had an approximate molecular weight of 160 kd. When run on SDS-PAGE, the same protein gave a single band of molecular weight corresponding to 40 kd. Explain and interpret the results. 3

6. (a) Define the following terms : $1 \times 4 = 4$

(i) Void volume

(ii) R_f value

(iii) Retention time

(iv) Exclusion limit

(b) Guard columns are used in HPLC. Comment. 2

(c) Modified matrix improve the resolution of paper chromatography. Comment. 2

(d) Explain the types of detectors used in GLC. 6

7. Differentiate between the following :

    (a) Rational and isogenic centrifugation

    (b) Western and Southern Blotting

    (c) Equilibrium and reverse dialysis

    (d) Resolving and stacking gels. $3.5 \times 4 = 14$

8. Write short notes on the following :

    (a) Lyophilization

    (b) Ligands in affinity chromatography

    (c) Paper electrophoresis

    (d) Reverse phase chromatography

    (e) Nucleic acid and protein staining   2, 3, 3, 3, 3