5. Differentiate between the following :

   (i) Differential and Density gradient centrifugation

   (ii) Paper chromatography and Thin layer chromatography

   (iii) Continuous SDS-PAGE and Discontinuous SDS-PAGE

   (iv) Single and Double beam spectrophotometer

   (v) Absorbance and Transmittance (3×5=15)

6. Write short notes on the following **(Any three)** :

   (a) Gel filtration chromatography

   (b) Methods of elution in ion-exchange chromatography

   (c) Staining procedures for proteins

   (d) Methods of cell lysis (5×3=15)

[This question paper contains 4 printed pages.]

Your Roll No.....

Sr. No. of Question Paper : 3499 **I**

Unique Paper Code : 2494001003

Name of the Paper : Techniques in Biochemistry (UGCF-NEP)

Name of the Course : **B.Sc. (Hons.) Biochemistry**

Semester : I

Duration : 2 Hours Maximum Marks : 60

Instructions for Candidates

1. Write your Roll No. on the top immediately on receipt of this question paper.
2. There are **6** questions.
3. Attempt any **4** questions.
4. **All** questions carry equal marks.
5. Question no. **1** is compulsory.

1. (a) Define the following **(Any five)** :

   (i) Salting out.

   (ii) Lambda max

   (iii) Retention factor

   (iv) Native PAGE

   (v) Hyperchromic shift

   (vi) Molar extinction coefficient

   (b) Why is ammonium sulphate preferred over other salts for salt fractionation of proteins?

   (c) What is the principle of SDS-PAGE? How is it different from agarose gel electrophoresis? (5,4,6)

2. (a) Write down the principle and applications of ion-exchange chromatography.

   (b) Three proteins A, B and C having pl values 2, 5 and 6 respectively were separated using DEAE cellulose column at pH 7.0 using salt gradient elution. What will be the order of elution of proteins? Explain.

   (c) Name any two cation exchangers and two anion exchangers. (6,5,4)

3. (a) What is Beer-Lambert law and what are its limitations?

   (b) A solution of protein, in a 1-cm quartz cuvette, has an absorbance value of 0.5 at 280 nm. What is the concentration of protein solution in mg/ml? Assume that the molar extinction coefficient of protein at 280 nm, ε_{280}, is 20,000 M^{-1} cm^{-1} and molecular weight of protein is 20,000 Da.

   (c) Why do we prefer plotting absorbance v/s concentration graph for determination of concentration of the sample rather than transmittance v/s concentration? (6,5,4)

4. (a) Define sedimentation coefficient and write down Svedberg equation that relates sedimentation coefficient with molecular weight of the particle.

   (b) If you wish to centrifuge a sample at RCF of 10,000 g, then at what rpm value will you set the centrifuge if r is 10 cm?

   (c) Write down the criteria for selection of matrix and buffer for ion-exchange chromatography.

   (d) Comment on the functions of TEMED and Bis-acrylamide in SDS-PAGE. (3,5,5,2)