(b) Discuss the principle of ion exchange chromatography and explain how can a sample be eluted-out from the ion exchange column.

(c) Sephadex G-200 has a fractionation range of 5-600 kDa. Draw and explain the Elution profile obtained on separation of Myosin (212 kDa), Ovalbumin (45kDa) and BSA (66.2kDa). (5,5,5)

5. (a) Define sedimentation coefficient and write about the factors that affect sedimentation coefficient.

(b) If a solution has absorbance of 1.0 and is diluted 1:1, what will be absorbance change? Justify your answer.

(c) Write a short note on the staining procedures for protein sample in electrophoresis. (5,5,5)

6. Write the principle and any **two** applications of the following :

(a) Agarose gel electrophoresis

(b) Affinity chromatography

(c) Ultrasonication

(d) Molecular sieve chromatography

(e) Thin-layer chromatography (5×3=15)

[This question paper contains 4 printed pages.]

Your Roll No.....

Sr. No. of Question Paper : 6031 **H**

Unique Paper Code : 2494001203

Name of the Paper : Techniques in Biochemistry

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

Semester : II

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 **six** questions.
3. Attempt any **four** questions.
4. **All** questions carry equal marks.
5. Question no. **1** is compulsory.

1. (a) Mention the role of the following chemicals used in electrophoresis :

(i) Ammonium persulphate

(ii) TEMED

(iii) Glycerol

(iv) Ethidium bromide

(v) Bromophenol blue

(vi) N, N'- Methylene bis (Acrylamide)

(b) Write reasons for the following :

(i) Quartz or fused silica cuvettes are used when working with a UV- Spectrophotometer.

(ii) Swinging bucket rotors allow better separation during centrifugation.

(iii) Electrophoretic migration of molecules depends on their charges and mass.

(iv) Absorbance is a unitless parameter.

(v) Gel filtration chromatography can be used for the determination of molecular mass of a protein.

(vi) Dialysis can be used for the purpose of the de-salting. (3,12)

2. Differentiate between :

(a) Isopycnic and rate zonal gradient centrifugation

(b) SDS-PAGE and Native PAGE

(c) Salting-in and salting-out

(d) Single beam and double beam spectrophotometer

(e) Cation and anion exchange chromatography (3×5=15)

3. (a) Write a short note on types of rotors used in centrifugation.

(b) Derive Beer-Lambert's law and discuss its limitations.

(c) Discuss the role of the following :

(i) Sucrose in centrifugation

(ii) Ethidium bromide in electrophoresis of DNA (6,5,4)

4. (a) Write the principle of paper chromatography and mention its limitations.