



**RAJARATA UNIVERSITY OF SRI LANKA
FACULTY OF APPLIED SCIENCES, MIHINTALE**

B.Sc. (Special) Degree in Applied Biology

Fourth Year Semester I Examination – April/ May 2015

MIB 4205 – ANALYTICAL TECHNIQUES IN BIOLOGY

Time: TWO (02) hours

Answer ALL questions.

1. a) Illustrate the important components of a spectrophotometer by using a schematic diagram only. **(25 Marks)**
b) Mention the functions of those components. **(15 Marks)**
c) Draw a generalized graph for absorption of a compound at increasing concentrations to illustrate the shape of the graph. **(10 Marks)**
d) Mention the reasons for this shape. **(20 Marks)**
e) Molar Extinction Coefficients of common biomolecules are known. Therefore the concentration of such a molecule can be calculated by using Beer's Law ($A = \epsilon cl$), when 'A' has been determined. However, in practice, the concentration is determined based on a standard curve/ graph.
Explain the reasons for not using ' ϵ ' for calculating the concentration. **(30 Marks)**
2. a) List the variables that influence the settling of a particle in a centrifuge. **(20 Marks)**
b) Give a comparative account on analytical and preparative ultracentrifugation. **(50 Marks)**
c) The condition of centrifugation used to separate particles can be expressed in 'rpm' or 'g'. Briefly explain why 'g' is preferred over 'rpm'. Mention the other information that you need to give if you use rpm in your reporting. **(30 Marks)**
3. a) State different steps (in correct order) and the corresponding analytical methods that you would follow to characterize individual proteins in a plant tissue (details of methods are not required). **(50 Marks)**
b) There are five proteins in a mixture, with different pI values as 2, 3, 6, 10 and 13.
Write down the expected order of elution when a cation exchange column equilibrated at pH 8.0 was used. Explain your answer. **(50 Marks)**

4. a) "Mesh size of the stationary phase influences the flow rate and resolution in column chromatography." Explain the above statement. **(30 Marks)**

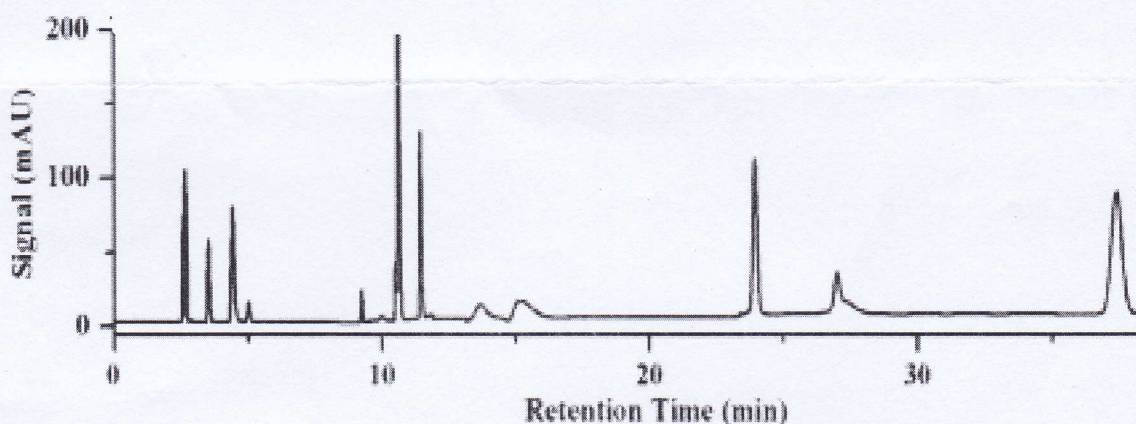
b) A student analyzing a plant extract (prepared in 80% aqueous MeOH) using HPLC, obtained the following graph. Program details are as follows.

Running time: 40 minutes

column dimensions: 250 x 2.00 mm

Stationary phase: Taxil PFD, 3 μ m

Mobile phase: MeOH - gradually changes from 100% - 50% aqueous in running time



i) Explain the above graph. **(30 Marks)**

ii) State any four ways to remedy the occurrence of overlapping peaks. **(20 Marks)**

iii) Discuss why HPLC analysis of samples is usually programmed to carryout simultaneously in several wave lengths of UV radiation. **(10 Marks)**

iv) Give one advantage of HPLC over GCMS in analyzing samples. **(10 Marks)**
