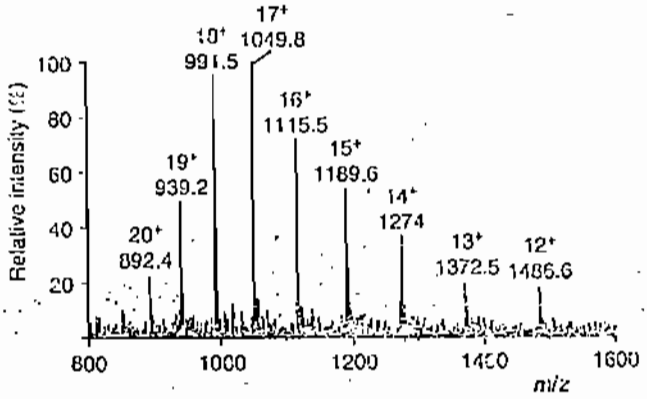
BEL420 Analytical Methods in Biotechnology
Major Exam 2nd semester 2006-07.

Max. Marks:40 Time: 2 hours

Part A (25 Marks)

Q.1 a. Examine the electrospray spectrum of phage lysozyme. Determine the mol wt of this protein. 2



b. Distinguish between FAB and MALDI as sources of ionization of biomolecules. Highlight the advantages/disadvantages of these techniques. 2 + 2 = 4

Q.2 a. Define one Curie (Ci). How is it related to disintegration per minute? 2

b. Why are fluors added to the counting liquids in liquid scintillation counting? What is the difference between a primary and a secondary fluor? 4

c. Good way to decontaminate ^{32}P contaminated glassware is to store it in lead containers. How long should glassware contaminated with 2×10^6 cpm be stored so that radioactivity is no more than 100 cpm? Could storage be used to decontaminate glassware containing ^{3}H (Half-life, ^{32}P=14.2 days; ^{3}H=12.3 years) 3

d. A mutant of *E. coli* has the property of dividing asymmetrically, and producing a normal cell and one having approx $1/10^{th}$ volume at a high frequency. The small cells never divide again. How could you determine whether the small cells have the normal amount of DNA? 3

Q.3 A stretch of DNA is being sequenced as shown below by Sanger's method. Show the length of the reaction products on the 4-lane (A,C,G,T) gel.

5'-CCGATCTATACCAT-3'
GGTA-5' 2

Q.4 a. What is the difference between sedimentation-velocity and sedimentation equilibrium method? Write a practical application for each. 3

b. 2-D liquid chromatography is being used to achieve good resolution of proteins. What principles, in your opinion, can be employed in this methodology? 2

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Part B (15 Marks)

1. (a). It was observed that the purification of a recombinant protein is difficult using size exclusion chromatography, ion exchange chromatography and hydrophobic interaction chromatography. What ean be done at any level like chromatography, genetie manipulation etc or both to get sufficient amount of that purifiecl recombinant protein? Explain with merits and demerits of your proposition. (5)

   (b). What kind of considerations need to be taken for the preparation and purifieation of recombinant proteins to be used in different purposes like human health care, industrial purposes, structural studies ete. (5)

   (c). A protein preparation is exhibiting substantial secondary structure from Far-UV CD speetroscopy. However, the near-UV CD spectroscopy is not showing the existence of tertiary structure. Although the X-ray diffraction data confirms the presence of tertiary contacts. What could be the reason for this and how are you going to trouble shoot the problem. (5)