Name : .....

Roll No. : .....

Invigilator's Signature : .....

CS/M.Tech/(INT PhD)Mol.Bio./Micro.Bio/SEM-1/PHMB/PHMC-102/2011-12

2011

LABORATORY TECHNIQUES

Time Allotted : 3 Hours *Full Marks* : 70

The figures in the margin indicate full marks.

Candidates are required to give their answers in their own words as far as practicable.

1. Answer any *five* questions. 5 × 2 = 10

i) a) What type of material is generally used to prepare solid stationary phase for Gas Liquid Chromatography (GLC) ?

b) What is basic principle to separate vaporized analyte by GLC ? 1 + 1

ii) a) Define "Electrophoresis".

b) Who discovered this technique for the first time ?

1 + 1

iii) "One of the vitamins can be used to polymerize acryl amide." Explain. 2

iv) a) Why base hydrolysis is needed to conduct amino acid analysis of a protein ?

b) What are the conditions to be applied for acid hydrolysis of a protein to determine its amino acid composition ? 1 + 1

v) Fill in the blank :

The electron microscope uses ..... and ..... lenses. 1 + 1

vi) a) Why a solution of proteins after adding sodium dodecyl sulphate (SDS) has to be heated to conduct Polyacryl amide Gel Electrophoresis (PAGE) ?

b) Why potassium dodecyl sulphate (KDS) cannot be used in PAGE ? 1 + 1

vii) a) What is the life time fluorescence emission ?

b) Phosphorescence occurs from which excited states in terms of spin ? 1 + 1

viii) Differentiate between intersystem crossing and internal conversion. 2

2. Write short notes on any *three* of the following : 3 × 5 = 15

i) Electromagnetic radiation

ii) Preparative ultracentrifuge

iii) Scintillation counter

iv) Thermal conductivity detector

v) Chemical shift.

3. Answer any *three* questions : 3 × 10 = 30

i) a) Define "Salting Out".

b) After dialyzing a mixture of protein and sodium chloride solution, how will you prove that the dialyzed protein solution has become free of salt ?

c) Draw the steps to explain Northern Blot providing proper diagrammatic illustration for electroblotting.

d) How does Western Blot differ with Eastern Blot ?

2 + 2 + 5 + 1

ii) a) Which peptide bonds of a protein are specifically cleaved by *o*-iodosobenzoate and 2-nitro-5-thiocyanobenzoate ?

b) Draw a flowsheet to explain how N-terminal amino acid end of a protein is determined using Edman reagent.

c) Reduction of the original peptide with mercaptoethanol followed by alkylation of cysteine residue with iodoacetate yielded two smaller peptides (*A* and *B*). Suggest the likely structure of the original peptide from the following data.

Peptide (A)

i) Contained ala – gly – cys – glu = arg – ile

ii) Carboxy peptidase *A* liberated isoleucine

iii) Treatment of fluorodinitrobenzene (FDNB) yielded derivative of glycine

iv) Treatment with trypsin yielded two peptides – one contained glu-isoleu, the other contained gly – ala – cys – arg .

Peptide (B)

i) Contained thr – val – cys – phe

ii) Carboxy peptidase *A* liberated valine

iii) Chymotrypsin liberated valine and a tripeptide containing cys – thr – phe

iv) Treatment of fluorodinitrobenzene (FDNB) yielded derivative of threonine. 2 + 3 + 5

iii) a) Describe how Scanning Electron Microscope works.

b) Outline basic principles involved in Phase Contrast Microscopy to examine biological samples.

c) Provide a diagram to explain confocal microscopy.

3 + 3 + 4

iv) a) Describe Stokes equation.

b) What are the reactions that permit current passage from cathode to anode in electrophoresis ?

c) What should be pH of buffer to be used for running and stacking gels in 'Disc' electrophoresis ?

d) "Glycine is used to adjust pH of the buffer of upper reservoir and HCl to adjust pHs of stacking and running gel buffers in SDS-PAGE". Explain critically. 2 + 2 + 2 + 4

4. Answer any *one* question : 1 × 15 = 15

i) a) What is the basic difference between Normal Phase and Reverse Phase HPLC ?

b) "Reverse Phase columns should never be used with aqueous bases." Why ?

c) "Structural properties of the analyte molecule play an important role in its retention characteristics when Reverse Phase HPLC is conducted." Explain in detail.

d) Why retention time of the separable components is increased when more water is added to the mobile phase of Reverse Phase HPLC ?

e) Why pH should be controlled in Reverse Phase HPLC ?

f) Why higher product concentrations and purities may be obtained by Displacement Chromatography compared to other modes of chromatography ?

2 + 2 + 4 + 2 + 2 + 3

ii) a) Explain cortically how a pH meter works.

b) Describe the preparation of 3 litres of a 0.2 M acetate buffer, pH 5.00, starting from solid sodium acetate anhydride (MW 136) and a 1 M solution of acetic acid. 8 + 7

iii) The fundamental vibrations in H_2O have the frequencies ν_1 (symmetric stretching) 3652 cm^{-1}, ν_2 (bending) 1595 cm^{-1}, ν_3 (antisymmetric stretching) 3756 cm^{-1}. Besides these the following transitions at 3151.4 cm^{-1}, 5332.0 cm^{-1} and 6874 cm^{-1} are also observed. These are either weak or medium. Assign these transitions and also calculate the energy in joules for each transition.