END

Mid-semester examination

BT 501: Biotechniques (July-Nov, 2022) **November 26, 2022**

Maximum marks: 60 (to be normalized to 50) **Time: 3 hours (9 A.M. - 12 Noon)**

==========

Instructions:

1. **This question paper contains 6 pages, and is divided into two sections: Section A and Section B.**
2. **Section A contains 6 questions (5 marks each).**
3. **Section B contains 4 questions (marks indicated against each question).**
4. **Attempt all the questions.**
5. **Do not mix the questions of one section with another.**

==========

Section – A

Q1. Consider the following hypothetical situations:

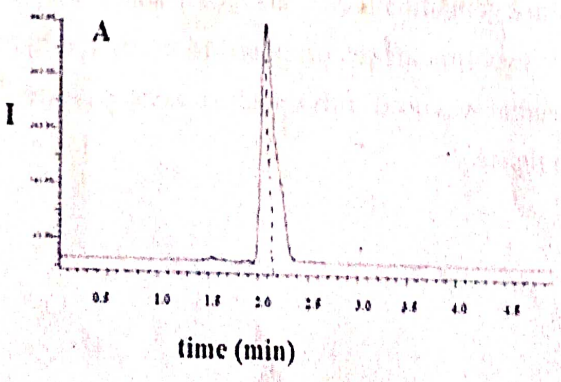
1. What happens if all analytes in a chromatographic separation spend the same amount of time in the mobile phase.

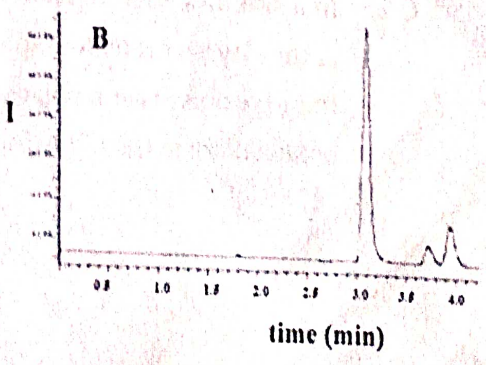
2. What happens to the retention time of the analytes, if we double the length of the column.

3. If we double the length of the column; will it double the chromatographic resolution?

Rationalize your answer.

Q2. The two chromatograms below were obtained using a **Reverse phase HPLC** stationary phase (**C-18 column**) and an acetonitrile/water mobile phase for the separation of three compounds in a mixture. One of the chromatograms was obtained using 12% acetonitrile in water and the other was obtained using a 6% acetonitrile in water mobile phase. (t_M = 0.7 for both A and B).



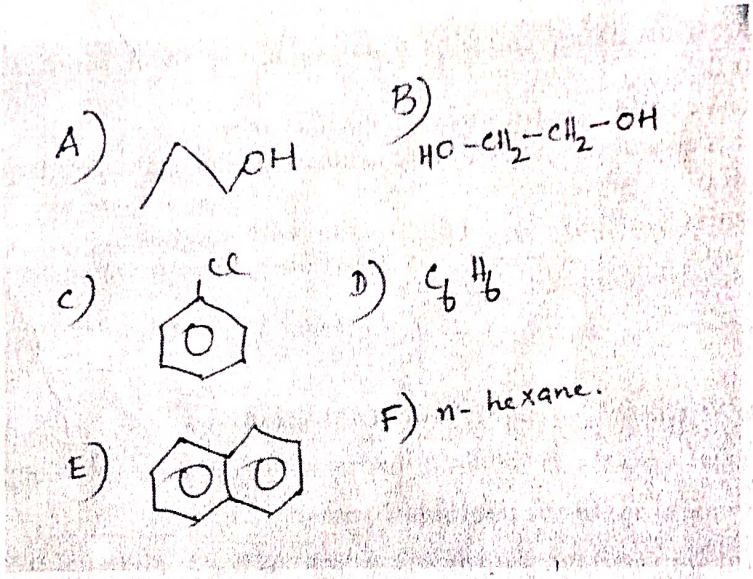


A) Calculate number of plates and retention factor.

B) Which chromatogram (A or B) has better resolution? What is the reason for better resolution of one chromatogram over the other?

C) Which mobile phase (12 % acetonitrile or 6 % acetonitrile) was used to obtain chromatogram B?

Q3. Predict the elution order of the following solutes in reversed phase HPLC.



Rationalize your answers

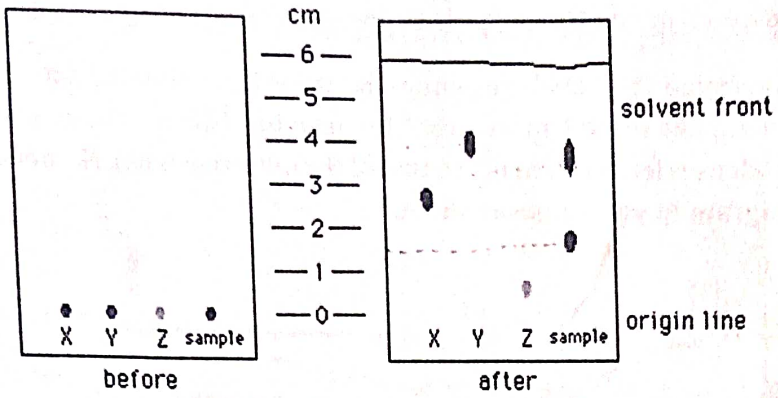
Q4. How are the height equivalent to a theoretical plate of a chromatographic column related to the various flow and kinetic parameters which cause peak broadening?

Describe how the "A" term of the van Deemter equation contributes to band broadening.

Describe how the "B/u" term of the van Deemter equation contributes to band broadening. Why is it inversely proportional to mobile phase flow rate?

Describe how the "Cu" term of the van Deemter equation contributes to band broadening. Why is it directly proportional to the mobile phase flow rate?

Q5. In a scientific investigation three constituents X, Y and Z are suspected in a mixture. A thin layer chromatographic experiment was proposed to verify the hypothesis. In this experiment a nonpolar solvent was used with a polar stationary phase. The results obtained are in the following figure.



Answer the following questions:

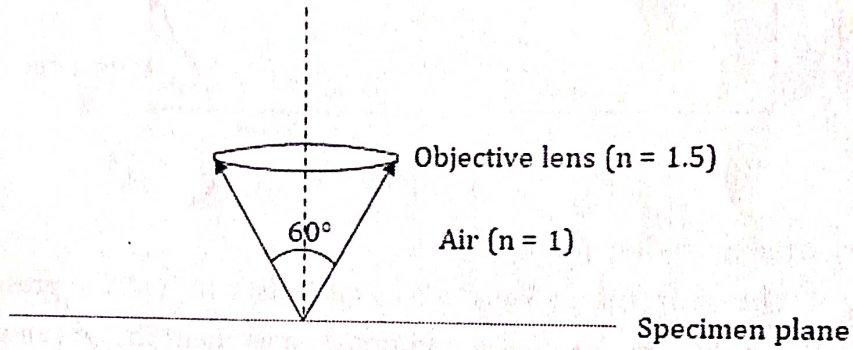
1. Out of the three suspected constituents, which all are present in the sample?
2. Could you detect any additional constituent in the sample other than the suspected ones?
3. What is the Rf value of the unsuspected constituent (if any)?
4. Assign the polarity of the identified constituents.

Q6. Can we determine the Molecular weight of a protein from the values of relative migration R_f in an SDS PAGE; If yes How?

Section B

Q.7 Short answer questions. Part (A) and (B) are numerical problems. Parts (C) – (L) should be answered in one-three words or one sentence. **{1 × 12 = 12 marks}**

A. Determine the Abbe's resolution limit for the following set-up of the objective lens when light of 400 nm is used for imaging (Given: the numerical aperture of the condenser lens is higher than that of the objective lens). **No need of reproducing the diagram in your answer sheet.**



B. What will be the wavelength of the electrons in an electron microscope operating at 100 kV. (Given charge on electron = 1.6×10^{-19} C, mass of electron = 9.1×10^{-31} kg, Planck's constant = 6.626×10^{-34} m^2 kg s^{-1})

C. Resolution of linear mode time-of-flight (TOF) mass analyser is poor. Name any one method that is used to improve the resolution of TOF.

D. In scanning electron microscopy, the interaction of electron beam with the specimen results in various types of electrons. Which electrons are generally used for the SEM imaging?

E. The electron gun used in electron microscope wherein the electrons are pulled out of the tip using an electric field giving highly coherent electron beam is called _____.

F. The resolution in AFM is determined by
(a) The wavelength of the laser that reflects back from the back of the tip
(b) The size and shape of the tip
(Write the correct option in your answer booklet.)

G. Name any one method that can be used to study the binding of a non-fluorescent small molecule to a macromolecule.

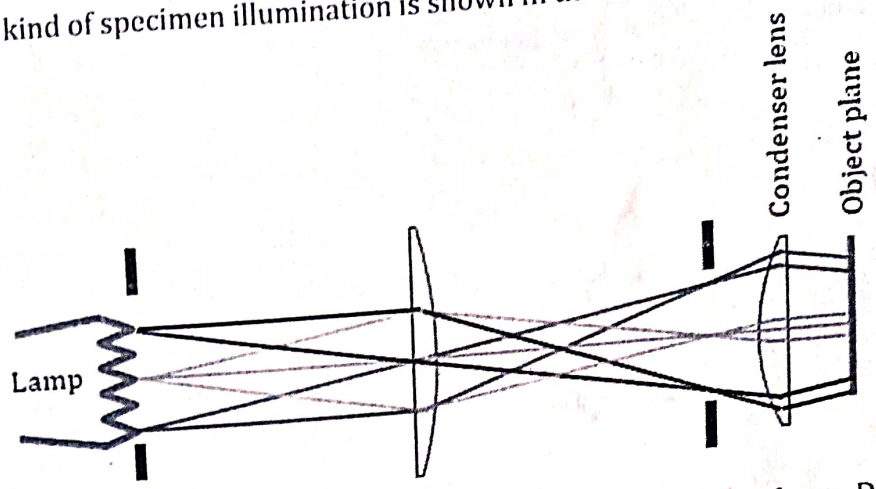
H. Which of the following techniques does not/do not need a pinhole to reject the out of focus light?
(a) Laser scanning confocal microscopy
(b) Multiphoton fluorescence microscopy
(c) Total internal reflection fluorescence microscopy
(Write down the correct option(s) in your answer booklet.)

I. Which light microscopy contrast method needs polarized light to achieve contrast?

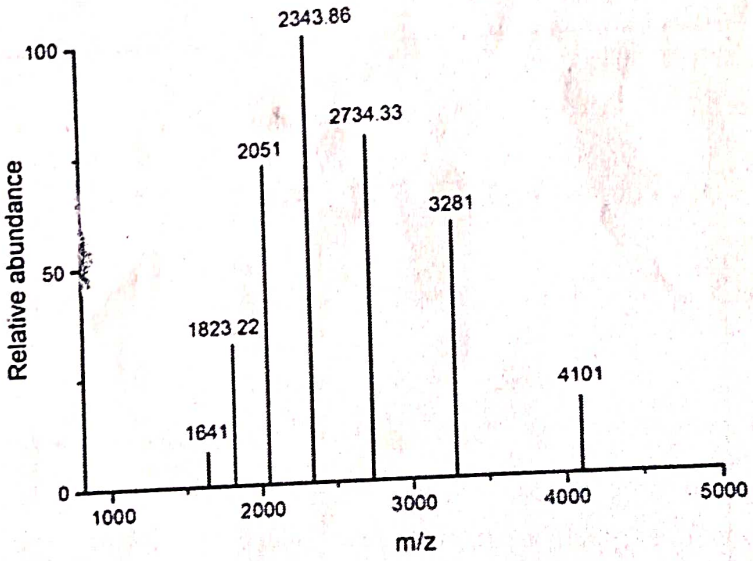
J. Name three important elements present in a filter cube that is used in epifluorescence microscopy.

K. What is the role of tube lens in the infinity conjugate imaging?

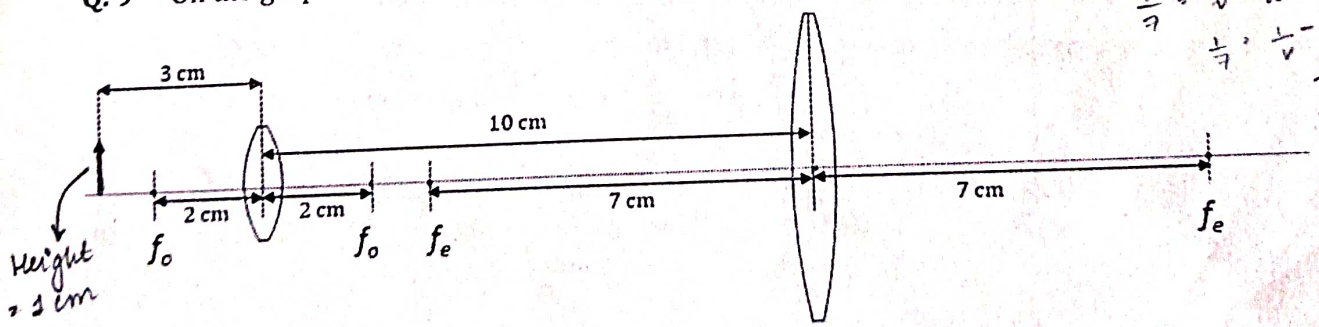
L. What kind of specimen illumination is shown in the diagram below?



Q. 8 Consider the positive-ion mode ESI-MS data of a protein shown here. Determine the molecular weight of the protein. **{6 marks}**



Q. 9 On the graph-sheet provided, draw the following diagram with the correct dimensions.



(a) Draw a neat ray diagram to show the final image formation. **{4 marks}**
(b) What kind of final image is formed? **{1 mark}**
(c) Using the ray diagram, determine the final magnification achieved? **{1 mark}**

Q. 10 The infrared spectrum and the electron impact mass spectrum of an organic compound are shown below. Determine the molecular formula of the compound from this data.

{6 marks}