234106011

End-semester examination

BT 501: Biotechniques (July-Nov, 2023) **November 21, 2023**

Maximum marks: 40 **Time: 3 hours (9 A.M. - 12 Noon)**

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Instructions:

1. **This question paper contains 3 pages.**
2. **Attempt all the questions.**
3. **If possible, don't answer more than one question on one page. Start a new question on a new page.**
4. **An appendix is provided with isotopic abundances of selected elements.**
5. **A graph Sheet will be provided to you. If you need more than one, please ask the TAs.**

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Q.1 Answer the following questions. **{1 × 10 = 10 marks}**

(a) The resolution of an atomic force microscope is limited by the _____.

(b) What is the role of tube lens in infinite conjugate imaging?

(c) How is circularly polarized light generated from plane polarized light?

(d) Name any one fluorescence microscopic method that rejects out of focus light without the need of a pinhole.

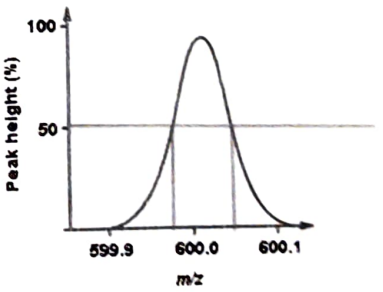
(e) Name two methods that are used to improve the resolution of time-of-flight mass analyser.

(f) Name any two lens aberrations.

(g) What does phase plate do in phase contrast microscopy?

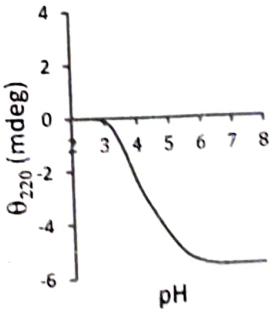
(h) Two point objects are separated by 320 nm. Calculate the longest wavelength of light that can theoretically resolve (Abbe's resolution criterion) these two objects (Assume numerical aperture to be 0.75).

(i) Determine the resolution of the mass spectrum peak shown here.



(j) Why are protein folding/unfolding studies using circular dichroism spectroscopy usually carried out at 220 nm?

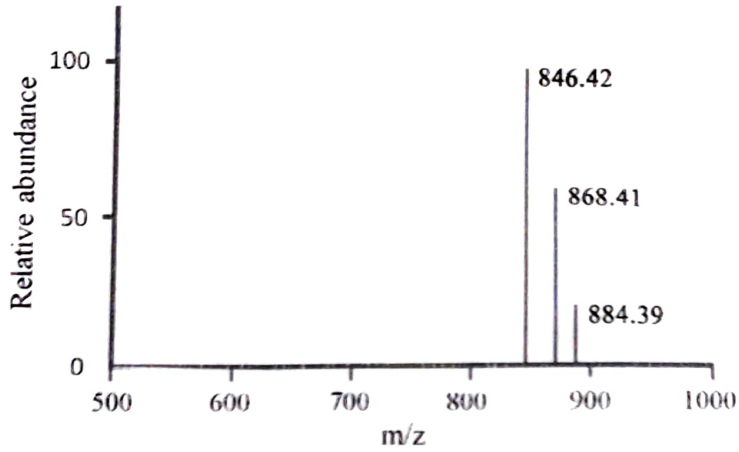
Q.2 The circular dichroism data of a protein at 220 nm and different pH values is shown here. Interpret this data. **{3 marks}**



Q.3 How are type II secondary electron (SE2) generated in a scanning electron microscope? How do they adversely affect resolution? **{3 marks}**

Q.4 A chemist synthesized the peptide, AQLRGNST (expected monoisotopic mass: 845.425 Da). The peptide turned out to more than 98% pure in high performance liquid chromatography. The MALDI mass spectrometry of the peptide is shown below:

{3 marks}



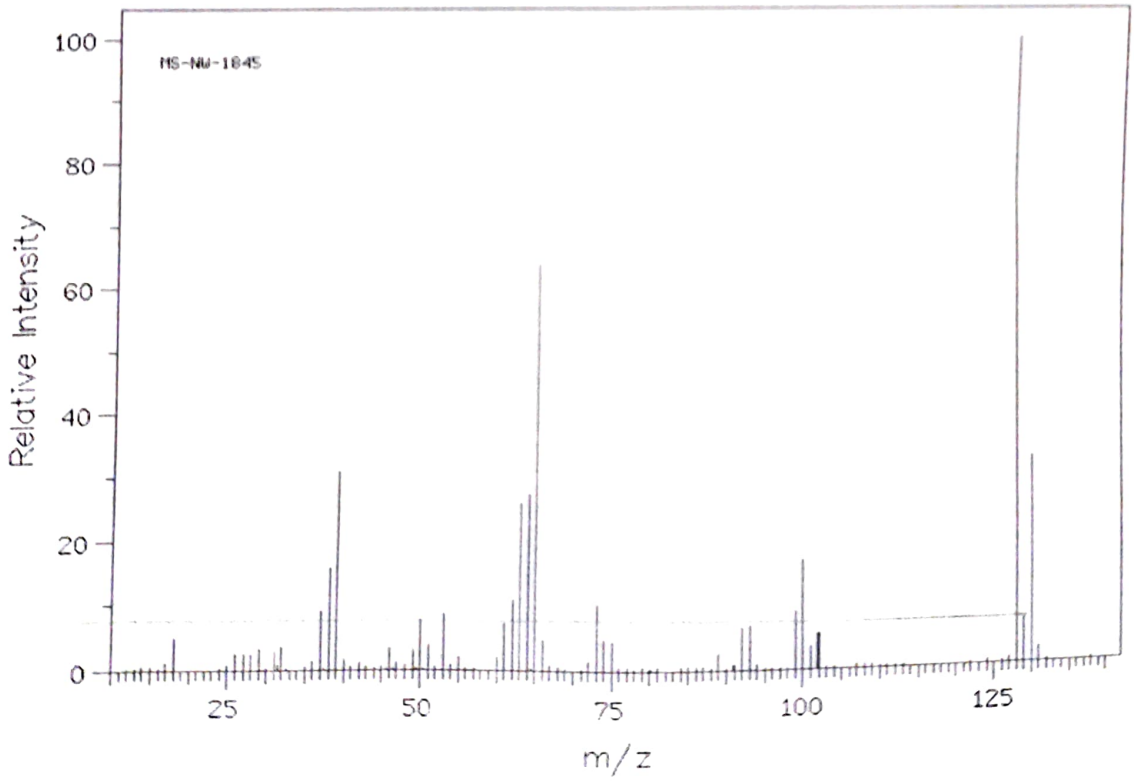
Explain the three peaks observed in the mass spectrum.

Q.5 The refractive index of glass is 1.5. **{4 marks}**

(a) Calculate the optical path length if the thickness of the material is 10 μm.

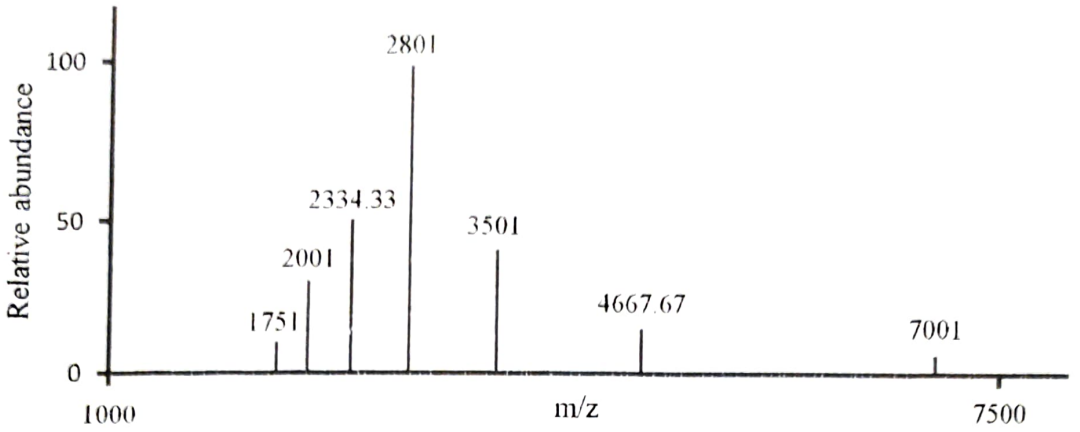
(b) What will be the phase shift of the 600 nm light (with respect to air) when the light comes out of the slab?

Q.6 The electron impact ionization spectrum of an organic compound is shown below. Determine the molecular formula of the molecule. **{5 marks}**

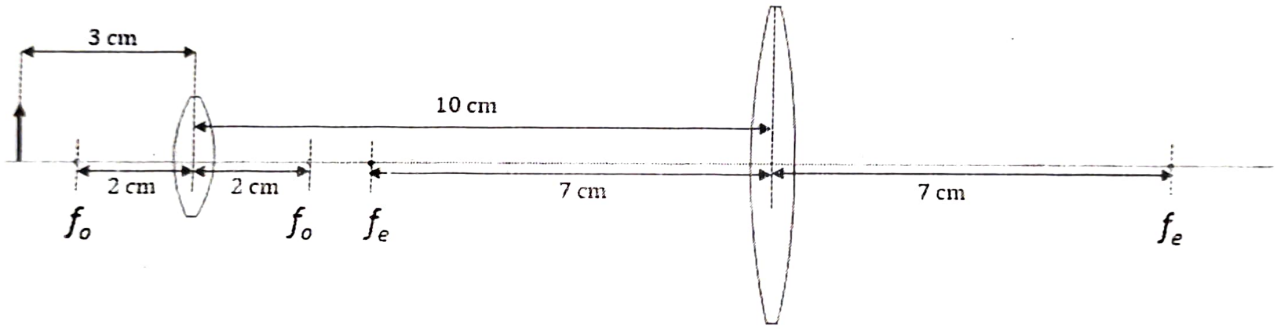


Q. 7 Consider that the mass spectrum shown below is obtained for a protein through the positive ion mode ESI-MS. Calculate the mass of the protein from the data given.

{6 marks}



Q. 8 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}**

Appendix

Isotopic composition of some common elements						
Element	M+		M+1		M+2	
Hydrogen	^{1}H	99.98%	^{2}H	0.02%		
Carbon	^{12}C	98.9%	^{13}C	1.1%		
Nitrogen	^{14}N	99.6%	^{15}N	0.4%		
Oxygen	^{16}O	99.8%			^{18}O	0.2%
Sulfur	^{32}S	95.0%	^{33}S	0.8%	^{34}S	4.2%
Chlorine	^{35}Cl	75.8%			^{37}Cl	24.2%
Bromine	^{79}Br	50.7%			^{81}Br	49.3%
Iodine	^{127}I	100.0%				