DEPARTMENT OF BIOCHEMISTRY

BIC 8X01

Advanced Analytical Techniques

EXAM

04 JUNE 2021

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MODERATOR:

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TIME: 3 HOURS

TOTAL MARKS: 100

This paper consists of two main parts, 1 and 2. Answer all questions. Use relevant equations and diagrams to add to your answers where applicable.

PART 1

QUESTION 1 [10]

If you have to assess and compare the performance of two analytical methods, which performance indicators would you evaluate? List the **5 most important parameters** with a short **description** of each.

QUESTION 2 [10]

The basis of all forms of chromatography is the distribution or partition coefficient.

2.1 Define this coefficient and explain how it relates to the retention factor (2)

2.2 How can the retention factor be determined by analyzing a chromatogram? (2)

2.3 What is the selectivity factor of a chromatographic system and what influences this factor? (2)

2.4 Chromatographic columns are often compared with regards to the number of 'theoretical plates'. Explain what this is and why two well-separated analytes will have different values for the number of theoretical plates. (2)

2.5 Why are HPLC/UHPLC chromatographic columns housed in a column oven? (2)
(explain your answer by referring to chromatographic concepts)

QUESTION 3 [10]

3.1. In protein research, chromatography is often used in conjunction with electrophoresis to characterize the physico-chemical properties of an isolated/purified protein. With the focus on chromatography, mention **three** different techniques that can be used and indicate the **properties** of the protein that can be determined. (3)

3.2. Reverse phase liquid chromatography is one of the most widely used separation/ analytical techniques. Describe the composition of such as system and mention the order of elution of polar / mid-polar / non-polar analytes (4)

3.3. You work in a laboratory that performs analyses of environmental samples and must write a motivation for the use of gas chromatography (GC) as an analytical technique for the analysis of chlorinated pesticides. As part of your motivation, **explain** how chromatographic principles are applied in the case of GC and **which type** of analytes are suitable for analysis by GC (3)

QUESTION 4 **[10]**

Mass spectrometry, often regarded as the science of ions, has wide range of applications in life sciences. Please answer the following questions.

    a) **Explain** (briefly) how the MALDI technique functions (3)

    b) **Explain** how the electrospray ionization (ESI) source functions (3)

    c) What **types of ions** that can be formed during the ESI process of an analyte **M** (4)

QUESTION 5 **[10]**

Mass spectrometry is a very powerful analytical technique with wide applications in the biological sciences.

5.1. **Explain in detail** the events that happen during electron impact ionization of analytes that lead to the generation of a mass spectrum of an analyte. (7)

5.2. To obtain structural information on biomolecules and sequence information (in the case of proteins and peptides), tandem MS experiments are performed. **Explain** how quadrupole technology has been applied to make tandem MS possible (3)

Total marks for Part 1: [50]

PART 2

SECTION A (UV/Vis spectroscopy)
[10]

QUESTION 1 [6]

1.1. Ultraviolet-visible (UV/Vis) light spectroscopy is one of the analytical techniques used in analytical chemistry and biochemistry for routine analyses and research work. This technique, like any other spectroscopic techniques, is fundamentally based on the interaction of light (electromagnetic radiation) with matter, a quantum phenomenon. Describe an **electromagnetic radiation**, and make a reference to UV/Vis spectroscopy (6 lines max). (3)

1.2. In UV/Vis spectroscopy, structural components of molecules that are responsible for interaction with electromagnetic radiation are known as chromophores. What are the types of chromophores found / explored in proteins? (3)

QUESTION 2 [4]

2.1. The A solution with a concentration of 0.10M is measured to have an absorbance of 0.42. Another solution of the same biochemical analyte is measured under the same conditions and has absorbance of 0.32. What is its concentration? (2)

2.2. A solution of protein B shows a transmittance of 20%, when taken in a cell of 2.5 cm thickness. What is the molar concentration of this solution, if the molar absorption coefficient is 12000 $dm^3\,mol^{-1}\,cm^{-1}$. (2)

SECTION B (Spectroscopic techniques II)
[20]

QUESTION 1 [10]

1.1. In a table format, compare Raman and infrared (IR) spectroscopy techniques, specifically on the following aspects: (i) type / mode; (ii) what is measured; (iii) the criterion for a band/peak to appear in the spectrum; (iv) molecular structural information recorded (8)

1.2. Write briefly about bending vibrations in IR spectroscopy (5 lines max) (2)

QUESTION 2 [5]

Signals recorded on a 1D ^{1}H NMR spectrum are chemical shifts representing frequencies from all NMR-visible nuclei (protons, H's) in a sample.

2.1. Explain the use of deuterium oxide and TSP in the sample preparation step, in a proton-NMR experiment (2)

2.2. Write down the expression showing the dependence of the S/N ratio on the number of scan. (1)

2.3. The fundamental rule governing (first order) multiplet intensities for spin 1/2 nuclei with all couplings identical is Pascal's triangle (n = number of equivalent couplings). Explain (4 lines max) a triplet in a 1D proton-NMR spectrum. (2)

QUESTION 3 [5]

Signals recorded on a 1D ^{1}H NMR spectrum are chemical shifts representing frequencies from all NMR-visible nuclei in a sample. Thus, in 1D ^{1}H NMR metabolite profiling of plant extract samples, the generated spectrum is the result of the superposition of the NMR spectra of all NMR-visible single compounds present in the extracts.

3.1. To extract information from such analyses, data processing is required prior to statistical analyses. Explain (very briefly, 5 lines max) spectral 'bucketing or binning'. (3)

3.2. Due to the complexity of such samples, multidimensional NMR methods are often employed. Give two examples (full name) of such methods. (2)

SECTION C (Centrifugation & Electrophoresis)
[20]

QUESTION 1 [5]

1.1. A centrifuge rotor is spinning at 25000 rpm. The 'top' of the cell is 5.5 cm from the rotor's central axis, and the 'bottom' of the cell is 9.5 cm from the central axis. What are the *g*-forces on a particle found at the top and at the bottom of the tube? (2)

1.2. A protein-RNA complex (partial volume, v = 0.71 cm^3 g^{-1}) gives a sedimentation coefficient of 12.7 S in 10% sucrose, 50 mM Tris buffer, pH 7.4 at 4°C. What will be the velocity of

sedimentation of the complex under these conditions when the complex is found 5.5 cm from the central axis of a rotor spinning at 4.0×10^4 rpm? (3)

QUESTION 2 **[5]**

Primary skeletal muscle tissues (from mouse) is sent to your lab for analysis. The client wants you to isolate the proteins and organelles of the tissues for further analysis.

2.1. What technique will you use to ensure successful separation? Mention the major steps in the correct order. (4)

2.2. To ensure that the membrane fractions are not contaminated with myosin you include an extra step. Mention this step? (1)

QUESTION 3 **[10]**

You are given two liver extracts of which the one was obtained from a healthy patient and the other from a patient diagnosed with hepatocellular carcinoma (a form of liver cancer). A proteomic approach is required to compare the two states (healthy vs cancer) with each other.

3.1. What electrophoretic technique will you use to identify differentially expressed proteins in the cancerous extract? (1)

3.2. Explain the principle of the technique mentioned in (3.1). (7)

3.3. You identify two proteins (using the method in (3.1)) that are absent in the healthy tissue extract but expressed in the cancer tissue extract. How will you isolate the proteins and with what other technique will you be able to identify them? (2)

Total marks for Part 2 (sections A, B, and C): [50]