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]**

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

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

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

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

1.4. 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)

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

QUESTION 2 **[10]**

In protein research, chromatography is often used in conjunction with electrophoresis to characterize the physico-chemical properties of an isolated/purified protein. Assume that your starting point is *E. coli* cells in which the target protein is fused to an affinity tag and has been over-expressed.

2.1. **Name** the chromatographic techniques you will use to purify the protein; and the order in which you would use them. Explain. (2)

2.2. **Explain** in detail the principles of the chromatographic methods mentioned in (**2.1**.) (6)

2.3. Which chromatographic technique would you use to estimate the mass of a protein? Elaborate on the principle of this technique. (2)

QUESTION 3 **[10]**

Gas chromatography is widely used for the qualitative and quantitative analysis of a large number of low-polarity compounds because it has high sensitivity, reproducibility and speed of resolution. The areas of gas chromatography applications are ever-increasing and includes pharmaceutical industry, research, food industry, medical and forensic fields, and environmental monitoring.

3.1. List the major components of a GC system (5)

3.2. What are the operating temperature modes (*i.e.*, adjustment of the column oven) for chromatographic separation in a GC system? Explain each mode. (2)

3.3. Name three types of detectors that are in common use in conjunction with 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.

4.1. Explain (briefly) how the MALDI technique functions (3)

4.2. Explain how the electrospray ionization (ESI) source functions (3)

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

QUESTION 5 **[10]**

The Fourth Industrial Revolution (**4IR**) era entails the integration of advanced technologies in the physical, digital, and biological domains. This includes the confluence and convergence of emerging technologies such as artificial intelligence (AI), the Internet of Things (IoT), big data analytics, cloud computing, robotics, wireless telecommunications, and a fusion of real- and virtual reality. In the life sciences, the 4IR technologies are inevitably revolutionizing fundamental and translational research and applications (Tugizimana *et al*., 2020; Tinte *et al*., 2021).

5.1. Describe the advantages of AI (in the form of analytical intelligence) and IoT-enabled chromatographic systems in terms of **(i)** system suitability test, **(ii)** mobile phase monitoring and blending, and **(iii)** gradient optimization. (4)

5.2. In mass spectrometry, tandem mass spectrometry (i.e., ion fragmentation) produces distinct patterns of fragment peaks at different mass-to-charge ratios (*m/z*) that aid in the annotation of chemical structures. **Name** four fragmentation strategies that can be used to perform fragmentation (4)

5.3. Virtual metabolomics mass spectrometer (ViMMS), an *in-silico* framework, can improve one of the fragmentation strategies in **5.2**. **Name** the strategy (in consideration) and **explain** how this is improved by ViMMS (2)

Total marks for Part 1: [50]

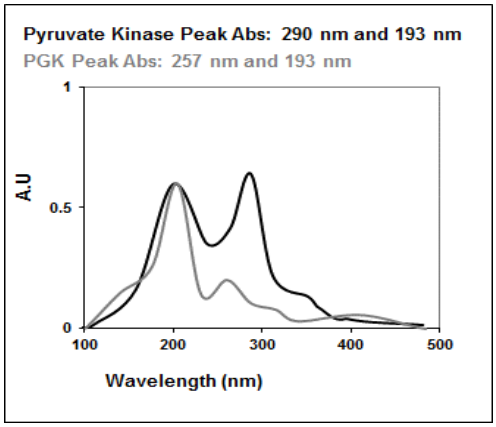
PART 2

SECTION A (UV/Vis spectroscopy)

[10]

QUESTION **[10]**

Pyruvate kinase is an enzyme that converts PEP into pyruvate via K^+ and Mg^{2+} ion cofactors and dephosphorylation generating ATP. You isolate some pyruvate kinase in order to perform studies on it. When you compare its absorption spectra in water to the enzyme phosphoglycerate kinase (PGK) you observe a different profile despite the two enzymes having similar functions:



a) What does this comparison tell you about the composition of pyruvate kinase versus other similar kinase enzymes? (2)

b) The spectra above are an example of an absolute spectra comparison. What spectrophotometric method could you use to determine the similarity between these two enzymes (Hint: isosbestic points) (1)

c) What is the corresponding molar extinction coefficient of pyruvate kinase if you obtained this spectrum (A_{290nm} = 0.589) using a concentration of 165 mol / dm^3? (Assume a standard light path length) (2)

d) You would like to study the binding relationship between pyruvate kinase and PEP so you couple a fluorophore to PEP which quenches its signal upon binding to pyruvate kinase. However, low PEP fluorescence readings are observed before binding with pyruvate kinase. What could be the possible **technical** causes of this? (2)

e) Describe how you could use luminescence to study pyruvate kinase reaction efficiency rates (3)

SECTION B (Spectroscopic techniques II)

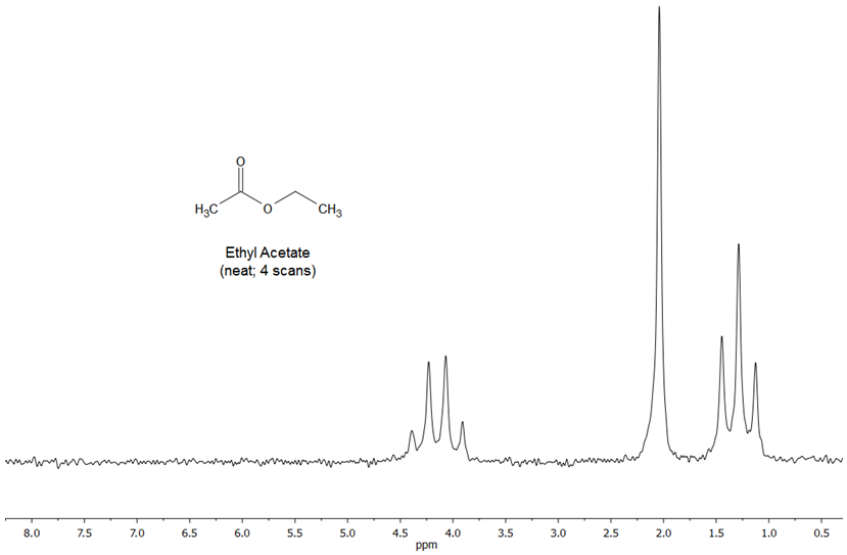
[20]

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. 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 / interpret** the (above) 1D proton-NMR spectrum of ethyl acetate – describing each chemical shift. (3)

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]**

What are the applications of analytical ultracentrifugation?

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. How can you ensure that the membrane fractions are not contaminated with myosin? (1)

QUESTION 3 **[10]**

3.1. What is electroendosmosis and how can this phenomenon be resolved? (4 × ½ = 2)

3.2. You obtain two kidney specimens. One is from a healthy patient and the other from a patient that has chronic kidney failure. A proteomic approach is required to compare the two states (healthy vs disease) with each other.

3.2.1. What electrophoretic technique will you use to identify differentially expressed proteins in the damaged kidney? (1)

3.2.2. Explain the principle of the technique mentioned in (3.2.1). (10×½=5)

3.2.3. You identify two proteins (using the method in (3.2.1)) that are absent in the healthy kidney extract but expressed in the damaged kidney extract. How will you isolate the proteins and with what other technique will you be able to identify them? (4 × ½ = 2)

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