UNIVERSITY OF JOHANNESBURG

DEPARTMENT OF BIOCHEMISTRY

BIC0088

Advanced Analytical Techniques

EXAMINATION

JUNE 2016 (SUPPLEMENTARY)

LECTURERS:

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MODERATOR:

PROF Z APOSTOLIDES (UP)

TIME: 3 HOURS

Answer all questions. Use relevant equations to add to your answers.

QUESTION 1 **[10]**

1.1 Detectors are often compared with regards to their analytical ranges. Supply a definition of the 'analytical range' as a performance parameters of a method and explain why an analyst should be aware of it. (2)

1.2 You have to evaluate two analytical methods for drug quantification from the literature. The following concentrations were determined by two different analysts using two different methods:

Method [1]: 2.3, 2.5, 2.2, 2.6, and 2.5 nM

Method [2] : 310, 350, 320, 340, 330 pg/ml

How would you compare the two methods with regard to their precision? (6)

(The following equation can be used: $s = \sqrt{\sum (x_i-x)^2 /n-1}$)

1.3 What type of information can be deduced from the 'standard deviation' vs. the 'standard error of the mean'? (2)

QUESTION 2 **[5]**

'The strength of electrophoresis as a separation technique lies in its ability to separate charged biomolecules with different electrophoretic mobilities'. Discuss this statement by referring to (and explaining) the forces acting upon a charged molecule within a gel matrix when an electric field is applied.

QUESTION 3 **[10]**

NB! : Define all abbreviations used.

2D electrophoresis is the corner stone of gel-based proteomics.

3.1 Briefly (pointwise) describe the principles / techniques involved (4)

3.2 Draw a sketch of a typical 2D gel image. Indicate (i) the positions of the electrodes, (ii) the low pH and high pH regions, (iii) the low Mr and high Mr regions and (iv) where acidic vs. basic proteins will be found. (4)

3.3 If an initial result with a 3-10 pH gradient indicated that most of the proteins are neutral and not well separated, how will you change experimental conditions to obtain improved resolution? (1)

3.4 Which MS technique would you use in conjunction with 2D gels? (1)

QUESTION 4 **[5]**

What are the advantages of using derivitization in (i) LC and (ii) GC?

QUESTION 5 **[10]**

Describe how mass spectrometry is used in order to:

(i) determine the accurate mass of a protein or peptide

(ii) obtain sequence information of a peptide

(Make use of sketches to aid your answers)

QUESTION 6 **[10]**

6.1 Name the types of radioactive decay and a biomedical application. (5)

6.2 A scientist starts an experiment with 120 g of Radium with a half-life of 11 days. How much will be left after 44 days and what percentage of the original Radium is left? (2)

6.3 The half-life of ^{33}P is 25.4 days. Calculate the age of the sample at which 22% of the radioactive nuclei originally present has decayed. (3)

QUESTION 7 **[10]**

7.1 a) Define biological centrifugation (2)

b) When designing a centrifugation protocol, what should one **remember** to take into account regarding the centrifugal field? (5)

7.2 List the uses of analytical ultra-centrifugation (2)

7.3 A fixed-angle rotor exhibits a minimum radius, rmin, at the top of the centrifuge tube of 3.5 cm, and a maximum radius, rmax, at the bottom of the tube of 7.0 cm. If the rotor is operated at a speed of 20 000 r.p.m., what is the relative centrifugal field, RCF, at the top and bottom of the centrifuge tube? (1)

QUESTION 8 **[2]**

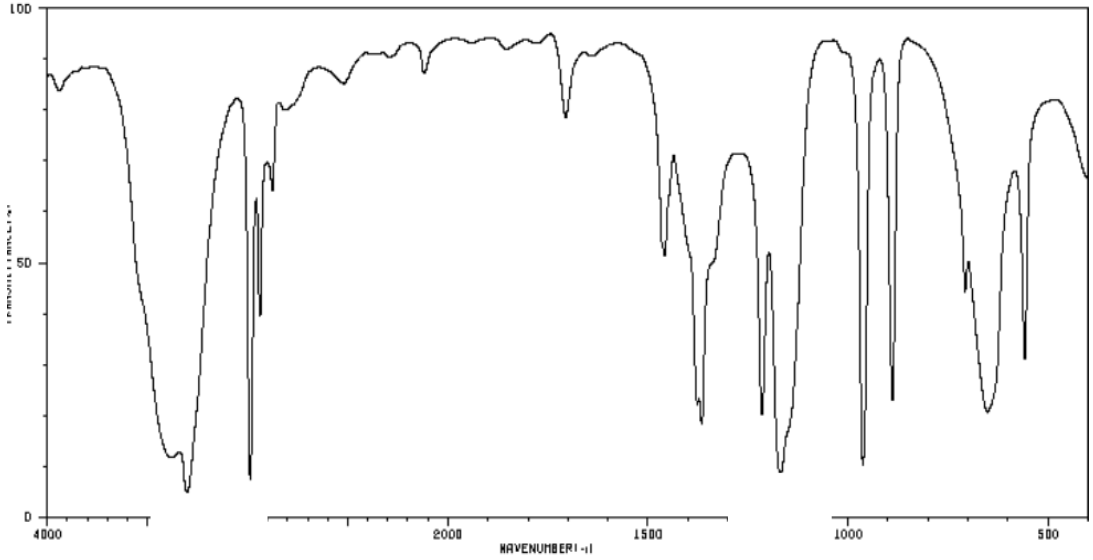
8.1 Draw the electron configuration of Sulphur (S) and identify the spin direction of the outermost electron. (1)

8.2 Draw the electron configuration of Gallium (Ga) and identify the spin direction of the outermost electron. (1)

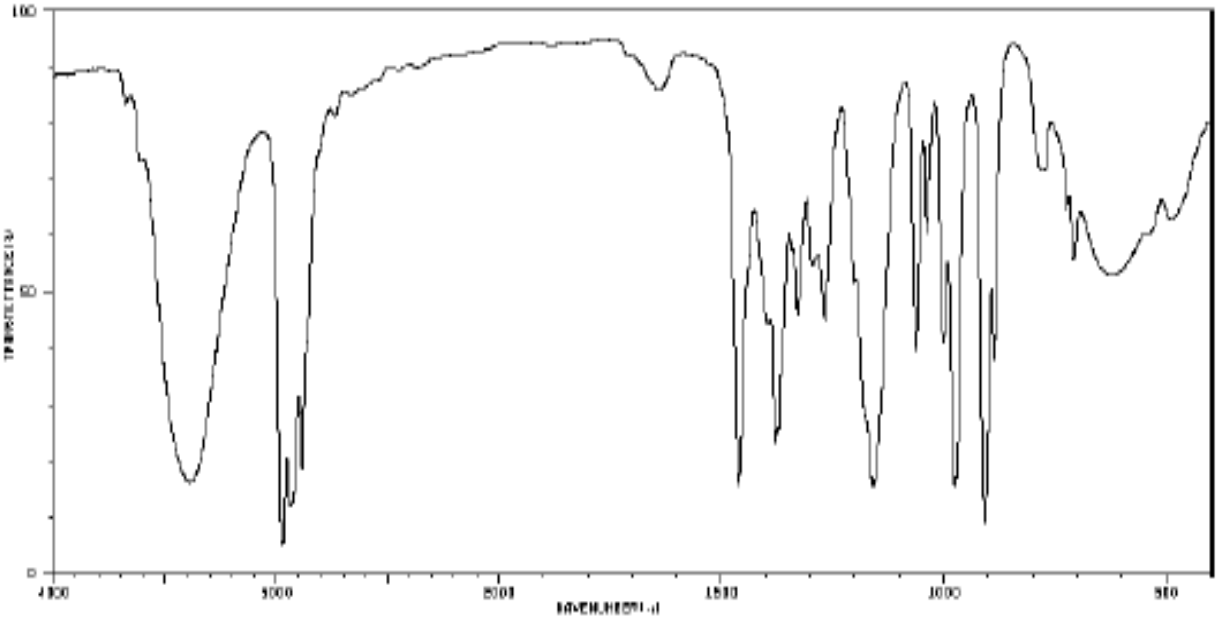
QUESTION 9 **[6]**

Label at least 3 (three) peaks in the following IR spectra that are consitent with the given molecular formula (6)

C5H8O

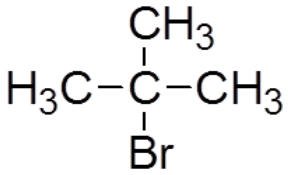


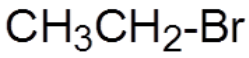
C6H10O



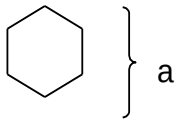
QUESTION 10 **[10]**

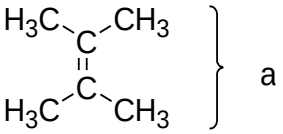
10.1 How many NMR signals would you expect to see for the following molecules? (2)

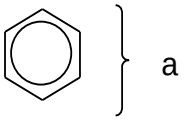


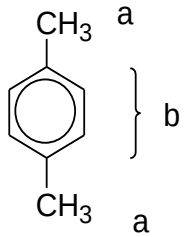


10.2 At which ppm position would you expect to see the following molecules? (8)









QUESTION 11 **[2]**

What are the differences and similarities in Raman and IR spectroscopies?

QUESTION 12 **[8]**

Pyruvate kinase is an enzyme that converts PEP into pyruvate via K^+ and Mg^{2+} ion cofactors and dephosphorylation generating ATP.

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

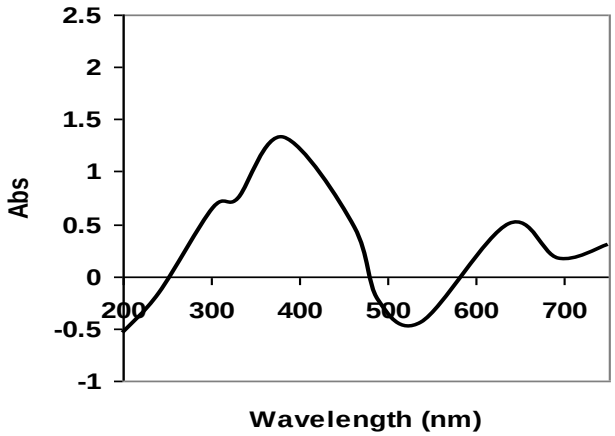
12.2 In a controlled system, how would you determine the threshold concentration of Mg^{2+} ions required for efficient enzyme reaction rates? (2)

12.3 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? (3)

QUESTION 13 **[8]**

The relationship between two proteins: Protein D and Protein M need to be studied. You begin by comparing their absorbance spectra and obtain the following profile:



13.1 What kind of spectrum is this? (1)

13.2 What does it tell you about the relationship between proteins D and M? (3)

13.3 How would you determine their secondary structure for relation studies? (1)

13.4 You suspect from the secondary structure that proteins D and M might form a complex. Using a **fluorescent** application, describe how you would go about confirming this. (3)

QUESTION 14 **[4]**

Nucleic acids contain chromophores in the form of pyrimidines which can be exploited for spectrophotometric studies.

14.1 After subjecting 10 µg dsDNA in water to high temperatures (>80˚C) the absorbance observed rises from 0.234 to 0.656 A.U. at 260 nm. If using the standard equation to calculate concentration, an inaccurate value of around 30 µg would be obtained based on the new A.U. What could be the cause of this higher A.U. value? (1)

14.2 You wish to study the binding relationship between a plasmid and protein suspected of having polymerase activity. How would you make use of **absorbance** spectroscopy to determine if this is the case? (2)

14.3 You have an unknown concentration of cDNA that yields an absorbance of 0.873. Name a **spectrophotometric** application you could use to determine the concentration of this sample? (1)

Total [100]