MAASAI MARA UNIVERSITY

UNIVERSITY EXAMINATIONS 2023/2024

POSTGRADUATE

MSc. FIRST YEAR SECOND SEMESTER EXAMINATION

FOR

THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

CHE 8209: Special Methods in Analytical Chemistry

DATE: /May/ 2024 **TIME:**

Duration: 3 Hours

INSTRUCTIONS

1. This paper contains **FOUR** (4) questions.
2. Answer question **ONE (1)** and any other **Two** (2) questions.
3. Do not forget to write your Registration Number.

Question ONE (30 Marks)

a. Define the following terms:
i. Unstable Isotopes ***[1 Mark]***
ii. Stable Isotopes ***[1 Mark]***
iii. GCXGC chromatography system. ***[2 Marks]***
iv. Antigen. ***[1 Mark]***

b.i. List the main Applications of GC-TOF-MS. ***[2 Marks]***
ii. Highlight on the advantages of a GC x GC-MS. ***[3 Marks]***

c. i. List the desirable features of the ovens used in gas chromatography. ***3 Marks]***
ii.. Elucidate on the temperature requirements of a GC sample injection port. ***[2 Marks]***
iii. State and explain on the commonly used support material for the packed column in gas chromatography. ***[1 Mark]***

d. i. State the kind of treatment given to solid samples for analysis with the Gas Chromatography. ***[1 Mark]***
ii. Give reasons why Slow injection of large samples leads to band broadening and loss of resolution. ***[1 Mark]***
iii.. Elucidate on the principal features of a GC-TOF-MS analytical technique. ***[3 Marks]***

e. i. List the requirements for RIA ***[3 Marks]***
ii.. State the properties of Radioisotopes which make them important analytical tools. ***[2 Marks]***
iii. Enumerate some of the applications of GC-MS Systems. ***[4 Marks]***

Question TWO (20 Marks)

a. Describe a stepwise procedure for Radiochemical analyses. ***[5 Marks]***

b.i. Radiochemical analysis is based on two outstanding features of radioactivity: ***[2 Marks]***
ii.. State the properties of Radioisotopes which make them important analytical tools. ***[2 Marks]***

iii. State the three working stages in radiochemical analysis. ***[3 Marks]***
iv. Explain on the Sample preparation of radioisotopes for analysis. ***[2 Marks]***

c. Discuss on how Chemical Separation of radionuclides is carried out. ***[6 Marks]***

Question THREE (20 Marks)

a. Define the term Immunoassay. ***[2 Marks]***
b. Explain on why proteins are good in Immunoassaying. ***[2 Marks]***
c. Explain on the use of labels in IA technique. ***[4 Marks]***
d. Describe the principles of Radio immunoassay technique. ***[5 Marks]***
e. Explain the assay procedure in RIA. ***[4 Marks]***

f. List the functions of a modulator in GC-GC-MS. ***[3 Marks]***

Question FOUR (20 Marks)

a. i. Give one disadvantage of nitrogen, when used as a carrier gas in gas chromatography. ***[2 Mark]***

ii. State the advantages of Capillary gas Chromatography over HPLC. ***[2 Mark]***

iii. Elucidate the differences between 2-Dimensional GC X GC-MS and GC. ***[5 Marks]***

b. i. The relative peak areas of a mixture of four steroids separated by HPLC, along with relative response factors is given in the table below. Based on this information, what percentage of the total steroid content is comprised of estradiol? ***[6 Marks]***

Compound	Relative Peak area	Relative detector Response
Estradiol	32.4	0.72
Estrone	47.1	0.68
Testosterone	40.6	1.00
Estriol	27.3	0.95

ii. Name the main effect which results from slow injection of a large sample volume in Gas Chromatography. ***[1 Mark]***

c. Elucidate on Immunoassay industrial applications in friction and wear out. ***[4 Marks]***

END

CHE 8209: Special Methods in Analytical Chemistry

MARKING SCHEME 70/70

Question ONE (30 Marks)

a. Define the following term:

i. Unstable Isotopes *[1 Mark]*
–isotopes that continuously and spontaneously breakdown/decay into other lower atomic weight isotopes. ✓1
ii. Stable Isotopes *[1 Mark]*
–isotopes that do not naturally decay but can exist in natural materials in differing proportions✓1
iii. GCXGC chromatography system. *[2 Marks]*

Chromatography system which employs a pair of columns✓1 – non-polar and polar columns in series connected by a modulator. ✓1

iv. Antigen. *[1 Mark]*
An antigen is a substance with the ability to induce an immunological response. ✓1

b.i. List the main Applications of GC-TOF-MS. *[2 Marks]*
GC-TOF-MS has been proven very useful for target screening ✓1***of organic pollutants in water, pesticide residues in food, anabolic steroids in human urine and xenoestrogensin human-breast tissues as well as non-target screening.*** ✓1
ii. Highlight on the advantages of a GC x GC-MS. *[3 Marks]*
•Unprecedented selectivity (three separation dimensions with regard to volatility, polarity, and mass). ✓1
•High sensitivity (through band compression). ✓1
•Enhanced separation power✓1
•Increased speed of separation (comparable to ultra-fast GC experiments, if the number of peaks resolved per unit of time is considered) ✓1

c. i. List the desirable desirable features of the ovens used in gas chromatography. *[3 Marks]*

a) It must have a fast rate of heating✓1
b) Power consumption should be kept low✓1
c) It should have proper insulation✓1

ii.. Elucidate on the temperature requirements of a GC sample injection port. *[2 Marks]*

-Sample injection port must be maintained at a temperature at which rapid vapourization occurs✓1 ***but thermal degradation does not occur. The column is maintained at a different temperature.*** ✓1

iii. State and explain on the the commonly used support material for the packed column in gas chromatography. ***[1 Mark]***

- Diatomaceous earth is the commonly used support material for the packed column in gas chromatography. The columns could be made of glass or metal. ✓1

d. i. State the kind of treatment given to solid samples for analysis with the Gas Chromatography. ***[1 Mark]***

Solid samples must be dissolved in volatile liquids for introducing it into the column. They can be introduced directly using solid injection syringes. ✓1

ii. Give reasons why Slow injection of large samples leads to band broadening and loss of resolution. ***[1 Mark]***

-Slow injection of large samples leads to band broadening and loss of resolution. Hence, for desired column efficiency, samples should not be too large. ✓1

iii.. Elucidate on the principal features of a GC-TOF-MS analytical technique. ***[3 Marks]***

•High resolution detection in GC-TOF-MS offers not only the high mass accuracy of molecular and fragment ions ✓1***but also the accurate isotopic distribution with regard to isotope intensities and isotope-resolved information for element assignments.*** ✓1

•It is extremely helpful for unknown compounds for which no Library spectrum is available for database searching. ✓1

e. i. List the requirements for RIA ***[3 Marks]***

-Preparation & characterization of the Antigen [Ligand to be analyzed] ✓1
Radiolabeling of the Antigen
-Preparation of the Specific Antibody ✓1
Development of Assay System✓1

ii.. State the properties of Radioisotopes which make them important analytical tools. ***[2 Marks]***

- ***They behave chemically the same as the isotopes of the elements***✓1***, And***
- ***Their radiation indicates their position and quantity N***✓1

iii. Enumerate some of the applications of GC-MS Systems. ***[4 Marks]***

•Quantization of pollutants in drinking and waste water✓1
•Quantization of drug in metabolites and urine is done for the pharmacological and forensic use. ✓1
•Identification of unknown organic compounds in hazardous waste dumps and reaction products by synthetic organic chemistry. ✓1
•Used for drug analysis, pesticide and herbicide detection. ✓1

Question TWO (20 Marks)

a. Describe a stepwise procedure for Radiochemical analyses. ***[5 Marks]***

• Radiochemical methods/analyses involve monitoring spontaneous emissions of particles or electromagnetic radiation from unstable atomic nuclei✓1

• The intensity of the emitted particles or electromagnetic radiation is used for quantitative analysis, ✓1 and the energy of the emissions is used for qualitative analysis✓1

• Emissions of alpha particles, electrons, positrons, neutrons, protons, and gamma rays can be useful✓1

• Gamma rays are energetically identical to X rays however; they are emitted as a result of nuclear transformations rather than electron orbital transitions. ✓1

b.i. Radiochemical analysis is based on two outstanding features of radioactivity: ***[2 Marks]***

1)The high sensitivity and ease of measurement of radioactive radiation✓1
2)The possibility of labelling chemical compounds with radioactive tracers. ✓1

ii.. State the properties of Radioisotopes which make them important analytical tools. ***[2 Marks]***

1. They behave chemically the same as the isotopes of the elements, ✓1
and
2. Their radiation indicates their position and quantity N✓1

iii. State the three working stages in radiochemical analysis. ***[3 Marks]***

•sample preparation, ✓1
•chemical separation, ✓1 and
•radioactivity measurement. ✓1

iv. Explain on the Sample preparation of radioisotopes for analysis. ***[2 Marks]***

•Sample preparation, the element whose radioisotope (or labelled compounds) is to be determined is separated from the sample. ✓1
•The final measurement of the activity of the element (or labelled compounds) is made on the isolate. ✓1

c. Discuss on how Chemical Separation of radionuclides is carried out. ***[6 Marks]***

Chemical Separation of radionuclides is performed with the aid of a suitable Carrier. ✓1
• Generally, the carrier is a stable isotope (or a suitable compound) that is added to the radioactive compound in a small but detectable amount and has identical chemical properties✓1
• Both the radioactive isotope and the carrier must be in the same chemical form✓1
• The isotopic carrier is irreversibly mixed with the radioactive compound and cannot be separated from it again by chemical means Such a carrier can therefore be used only when a lower specific activity is sufficient for the subsequent operations✓1

• For example, barium or lead can serve as carriers when separating radium iron or yttrium may be applied in separations of rare earth elements, etc✓1
• In certain cases, when no stable isotope is available, for example, as in plutonium separation, a separation of carrier free radionuclide is also possible✓1

Question THREE (20 Marks)

a. Define the term Immunoassay. ***[2 Marks]***
An immunoassay is a test that uses antibody and antigen complexes as a means of generating a measurable result✓1. An antibody: antigen complex is also known as an immune-complex. ✓1
b. Explain on why proteins are good in Immunoassaying. ***[2 Marks]***
Proteins or glycoproteins make the best antigens because they are the best at stimulating antigen recognition molecules. ✓2

c. Explain on the use of labels in IA technique. ***[4 Marks]***
All immunoassays require the use of labeled material in order to measure the amount of antigen or antibody present. ✓1
A label is a molecule that will react as part of the assay, so a change in signal can be measured in the blood: reagent solution. ✓1
Examples of a label include
a radioactive compound, ✓1
an enzyme that causes a change of color in a solution, a substance that produces light. ✓1

d. Describe the principles of Radio immunoassay technique. ***[5 Marks]***
The principle of RIA involves competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody. ✓1
The labeled antigen is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody. ✓1
Then test samples of unlabeled antigen of unknown concentration are added in progressively larger amounts. ✓½
The antibody does not distinguish labeled from unlabeled antigen, so the two kinds of antigen compete for available binding sites on the antibody. ✓1
As the concentration of unlabeled antigen increases, more labeled antigen will be displaced from the binding sites. ✓½
The decrease in the amount of radiolabeled antigen bound to specific antibody in the presence of the test sample is measured in order to determine the amount of antigen present in the test sample. ✓1

e. Explain the assay procedure in RIA. ***[4 Marks]***
Add known amounts of the test sample + labelled antigen into the microtitre wells Incubate- allow the reaction to reach ✓1***completion Decant & wash contents of the well - removes all unbound antigens***✓1
Radioactivity remaining in the Microtitre wells measured by a Counter [GM counter, Scintillation counter etc] ✓1
- Intensity of radioactivity is inversely correlated with the conc of antigens in the test sample✓1

f. List the functions of a modulator in GC-GC-MS. ***[3Marks]***

i. ***It connects the first and second columns***✓1
ii. ***Traps eluate from the first column for a fixed period of time.*** ✓1
iii. ***It subsequently remobilizes, focuses and inject the eluate into the second column***✓1

Question FOUR (20 Marks)

a. i. Give one disadvantage of nitrogen, when used as a carrier gas in gas chromatography. ***[2 Mark]***

- Nitrogen has reduced sensitivity. ✓1 ***It is still one of the commonly used carrier gas in gas chromatography.*** ✓1

ii. State the advantages of Capillary gas Chromatography over HPLC. ***[2 Mark]***

GC has larger H values✓1 ***and higher efficiencies than HPLC***✓1

iii. Elucidate the differences between 2-Dimensional GC X GC-MS and GC. ***[5 Marks]***
•In this technique two dimensional GC is coupled with mass spectroscopy. ✓1***This coupling will lead to the better resolution of the peaks in GC.*** ✓1

•Sometimes in one dimensional GC the analyte is unable to be distributed along the whole retention time. ✓1

•The two-dimensional GC leads to the better resolution. ✓1 ***with the help of two-dimensional GC components of the analytes are properly separated due to which in the MS even trace amount components will be identified.*** ✓1

b. i. The relative peak areas of a mixture of four steroids separated by HPLC, along with relative response factors is given in the table below. Based on this information, what percentage of the total steroid content is comprised of estradiol? ***[6 Marks]***

Compound	Relative Peak area	Relative detector Response
Estradiol	32.4	0.72
Estrone	47.1	0.68
Testosterone	40.6	1.00
Estriol	27.3	0.95

$\frac{32.4}{.72} = 45$ $\quad \frac{45}{183.64} = .245$

$\frac{47.1}{.68} = 69.3$ $\quad .245 \times 100 = 24.5\%$

$\frac{40.6}{1} = 40.6$

$\frac{27.3}{.95} = 28.7$

183.64

✓1X6 steps

ii. Name the main effect which results from slow injection of a large sample volume in Gas Chromatography. ***[1 Mark]***

Decreased resolution✓1

c. Elucidate on Immunoassay industrial applications in friction and wear out. ***[4 Marks]***

When the 2 surfaces are rubbed, ✓1one is neutron activated so that it becomes✓1 radioactive, without a lubricant the other surface is auto-radio graphed. ✓1

This indicates the amount of matter transferred (wear out of piston rings). ✓1

End