MAASAI MARA UNIVERSITY

UNIVERSITY EXAMINATIONS 2022/2023

POSTGRADUATE

MSc. FIRST YEAR SECOND SEMESTER EXAMINATION

FOR

THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

CHE 8209: Special Methods in Analytical Chemistry

DATE: /April/ 2023 **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 term:
i. isothermal and temperature programming in GC analysis, ***(2 Marks)***
ii. Antigen. ***[1 Mark]***

b i. List the essential requirements for radioimmunoassay. ***(3 Marks)***
ii. State the various separation technique used in radioimmunoassay. ***(2 Marks)***
iii. Explain the ssupercritical Properties of CO_2 which makes it amenable in supercritical chromatography. ***(4 Marks)***

c. i. Elucidate the significant issues that limit the applicability of chromatographic approaches in rapid automated radiochemical analysis on difficult sample matrixes. ***(4 Marks)***

ii. State the practical problems that arise due to the decrease in column diameter. ***(2 Marks)***

d. i. State two outstanding features of radioactivity on which Radiochemical analysis is based on. ***(2 Marks)***
ii. State the assumptions on which radiochemical analysis relies on: ***(3 Marks)***
iii. Give reasons why Measurements of ionizing radiation and radionuclides in foods and environmental samples are required. ***(3 Marks)***
iv State the parameters measured in radiochemical analysis. **(3 Marks)**

e. i. Explain the ssupercritical Properties of CO_2 which makes it amenable in supercritical chromatography. ***(4 Marks)***
ii. Dicarboxylic acids ($HO_2C(CH_2)_nCO_2H$) cannot be analyzed easily directly by GC. However, by converting them to diesters ($CH_3O_2C(CH_2)_nCO_2CH_3$), good GC separation and detection is possible. What is a major effect of this derivatization that leads to improvement? ***(2 Marks)***

Question TWO (15 Marks)

a. Define the terms:
i. Radioimmunoassay. ***(2 Marks)***
ii. A label. ***(2 Marks)***

b. State three principles which make radioimmunoassay the most specific and sensitive than other immune assays. ***(3 Marks)***

c. Elucidate the general procedure of radioimmunoassay. ***(8 Marks)***

Question THREE (15 Marks)

a. i. What system components do you need for an HPLC apparatus? ***(2 Marks)***
ii. List three detectors suitable for HPLC and the substances detected. ***(4 Marks)***

b i. State the meaning of GC-HRMS and GC-GC-HRTOFMS in gas chromatography analysis. ***(2 Marks)***

ii. Discuss the characteristic applications features of; GC-HRMS and GC-GC-HRTOFMS in improving GC sample analysis efficiencies. ***(7 Marks)***

Question FOUR (15 Marks)

a. List four characteristic features of a good laboratory measuring equipment used in radiochemical analytical procedures. ***(2 Marks)***

b. Explain on how Counting rates measured with Geiger–Müller, scintillation, or semiconductor detectors are used to obtain qualitative and quantitative data. ***(3 Marks)***

c. Discuss on the two column separation formats generally considered for automated separations in radiochemical analysis. ***(10 Marks)***

END

CHE 8209: Special Methods in Analytical Chemistry

MARKING SCHEME 60/60

Question ONE (30 Marks)

a. Define the following term:

i. isothermal and temperature programming in GC analysis, *(2 Marks)*

- ***the temperature of the column is held constant throughout*** ✓1***the entire separation.***
- ***temperature programming method, the column temperature is either increased continuously or in steps as the separation progresses.*** ✓1

ii. Antigen. ***[1 Mark]***

An antigen is a substance with the ability to induce an immunological response. ✓1

b **i. List the essential requirements for radioimmunoassay.** ***(3 Marks)***

1. ***micro titter plate/ test tube***✓1/2
2. ***pure antigen***✓1/2
3. ***radio labelled antigen***✓1/2
4. ***anti body***✓1/2
5. ***standards***✓1/2
6. ***centrifuge***✓1/2
7. ***radio active counter***✓1/2

ii. State the various separation technique used in radioimmunoassay. ***(2 Marks)***

- ***gel filtration,*** ✓1/2
- ***electrophoresis,*** ✓1/2
- ***solid phase adsorption of ag,ab &*** ✓1/2
- ***fractional precipitate.*** ✓1/2

iii. Explain the ssupercritical Properties of CO_2 which makes it amenable in supercritical chromatography. ***(4 Marks)***

- One of the biggest limitations to most mobile phases in SFC is getting them to reach the critical point.

- This means extremely high temperatures and pressures, ✓1/2***which is not easily attainable.***

- The best gases for this are ones that can achieve a critical point at relatively low temperatures and pressures. ✓1/2

- CO_2 has a critical temperature of approximately 31 °C and a critical pressure of around 73 atm. ✓1/2

- These are both relatively low numbers and are thus ideal for SFC.

- a downside. In this case, CO_2 lacks polarity, which makes it difficult to use its mobile phase properties to elute polar samples. ✓1/2

c. i. Elucidate the significant issues that limit the applicability of chromatographic approaches in rapid automated radiochemical analysis on difficult sample matrixes. ***(4 Marks)***

- use of high-performance ion chromatography (HPIC) with online radiometric detection for qualitative analysis of Hanford waste samples was tried. ✓1
- Long separation times (40 minutes to several hours), ✓1***matrix effects on the***✓1/2
reproducibility and reliability of the separation procedure, ✓1/2 ***limited capacity of the separation material***✓1/2***, and speciation problems.*** ✓1/2

ii. State the practical problems that arise due to the decrease in column diameter. *(2 Marks)*

The practical problems that arise due to a decrease in column diameter are the requirement of small particle size ✓1/2***and high pressure drop.*** ✓1/2 ***But it increases column efficiency***✓1**.**

d. i. State two outstanding features of radioactivity on which Radiochemical analysis is based on: ***(2 Marks)***
- the high sensitivity and ease of measurement of radioactive radiation✓1***; and***
- the possibility of labelling chemical compounds with radioactive tracers. ✓1

ii. State the assumptions on which radiochemical analysis relies on: ***(3 Marks)***

- those different isotopes of the same element exhibit the same properties in any macroscopic physical ✓1***or chemical process,*** ✓1 ***and***

- that radioactive labeling does not influence the other properties of a chemical species✓1**.**

iii. Give reasons why Measurements of ionizing radiation and radionuclides in foods and environmental samples are required. ***(3 Marks)***
- for the assessment of exposure to both natural and artificial radiation sources, ✓1
- determination of compliance with government regulations, ✓1 ***and***
- studies of the movement and retention of artificial radionuclides in food and environmental media and of the composition of the natural radiation environment. ✓1

iv State the parameters measured in radiochemical analysis: **(3 Marks)**
- monitor the presence and ecological behaviour of radionuclides that are present in food and environmental samples✓1***;***
- identify the pathways by which human exposure results✓1***; and***
- estimate the dose to humans. ✓1

e. i. Explain the ssupercritical Properties of CO_2 which makes it amenable in supercritical chromatography. (4 Marks)
- One of the biggest limitations to most mobile phases in SFC is getting them to reach the critical point. ✓1
- This means extremely high temperatures and pressures, which is not easily attainable. ✓1
- The best gases for this are ones that can achieve a critical point at relatively low temperatures and pressures. ✓1/2

- CO_2 has a critical temperature of approximately 31 °C and a critical pressure of around 73 atm. ✓1/2

- These are both relatively low numbers and are thus ideal for SFC. ✓1/2

- a downside. In this case, CO_2 lacks polarity, which makes it difficult to use its mobile phase properties to elute polar samples. ✓1/2

ii. Dicarboxylic acids ($HO_2C(CH_2)_nCO_2H$) cannot be analyzed easily directly by GC. However, by converting them to diesters ($CH_3O_2C(CH_2)_nCO_2CH_3$), good GC separation and detection is possible. What is a major effect of this derivatization that leads to improvement? ***(2 Marks)***

1) ***increased volatility***✓1, ***2) decreased polarity***✓1

Question TWO (15 Marks)

a. Define the terms:

i. Radioimmunoassay. (2 Marks)

- This is a technique used to determine concentration of antigen in given sample. ✓1***This technique is very sensitivity it can detected 0.001 µg/ml.*** ✓1

ii. A label. (2 Marks)

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***This helps to measure the amount of antigen or anti body present.*** ✓1

b. State three principles which make radioimmunoassay the most specific and sensitive than other immune assays. (3 Marks)

- ***An immune reaction i.e. antigen, antibody binding.*** ✓1
- ***A competitive binding or competitive displacement reaction***✓1
- ***Measurement of radio emission (it gives sensitivity)*** ✓1

c. Elucidate the general procedure of radioimmunoassay. **(8 Marks)**

- ***A known quantity of an antigen is made radio active frequently by labelling it with gamma radioactive isotopes of iodine attached to tyrosine.*** ✓1
- ***This radio labelled antigen is then mixed with a known amount of antibody for that antigen and as a result the two chemically bind to one another.*** ✓1
- ***Then a sample of serum from a patient containing an unknown quantity of that same antigen is added.*** ✓1
- ***This causes the unlabelled anti gen from the serum to complete with the radio labelled antigen for antibody binding site.*** ✓1
- ***The concentration of cold antigen is increased more of it binds to the antibody displacing the radiolabelled variant and reducing the ratio of antibody – bound radio labelled antigen to free radio labelled antigen.*** ✓1

- ***The radioactivity falls because unlabelled antigen dilutes it.*** ✓1
- ***The count obtained from the radioactivity are used to determine the hapten concentration in the sample the interpretation being done on the standard curve.*** ✓1

Test tube-1 blank	***Ab +Ag***
Test tube-2 calibrator	***Ab+pure Ag + Ag***
Test tube-3 sample	***Ab + sample A g + Ag*** ✓1

Question THREE (15 Marks)

a. i. What system components do you need for an HPLC apparatus? (2 Marks)

Solvent pump with solvent tank, injection valve for the sample, possibly a guard column, then the actual chromatographic column and then the detector recorder system. ✓1***In case of a preparative process also valves to guide the flow and product collecting tanks are needed***✓1

ii. List three detectors suitable for HPLC and the substances detected. (4 Marks)

- ***UV-VIS detector:*** *able to detect UV-active compounds*✓1
- ***FT-IR*** *which is able to detect IR active compounds*✓1
- ***Light scattering detector*** *which detects the light scattering of the whole mixture*✓1
- ***Fluorescence detector:*** *which is able to detect fluorescent*✓1

b i. State the meaning of GC-HRMS and GC-GC-HRTOFMS in gas chromatography analysis. (2 Marks)

GC-HRMS - gas chromatography coupled to high-resolution mass spectrometry. ✓1

GC-GC-HRTOFMS- Double gas chromatography coupled to High-resolution time-of-flight mass spectrometry. ✓1

ii. Discuss the characteristic applications features of; GC-HRMS and GC-GC-HRTOFMS in improving GC sample analysis efficiencies. (7 Marks)

GC-HRMS

- analytical method that combines excellent sample separation power of gas chromatography with improved identification based on an accurate mass measurement. ✓1

- identification and structure elucidation of unknown volatile and semi-volatile organic compounds. ✓1

-It offers high resolution, exceeding 60 000 with five orders of magnitude dynamic range. ✓1

-It is capable of working in both modes, either full-scan mass analysis or selected ion monitoring (SIM). ✓1/2

GC-GC-HRTOFMS-

- fast GC × GC measurements requires high acquisition speed spectra per second thus, HRMS (maximum 20) cannot be amenable with it. ✓1

- it uses HRTOFMS with the possibility to record up to 500 spectra per second.

-High separation efficiency✓1

- HRTOF allows recording of an accurate full-scan analysis even at low concentrations. ✓1

-allows post target screening of unknown compounds. ✓1/2

Question FOUR (15 Marks)

a. List four characteristic features of a good laboratory measuring equipment used in radiochemical analytical procedures. **(2 Marks)**

It should show:

- a high radiation sensitivity, ✓1/2

- high detection efficiency, ✓1/2

- low background, ✓1/2***and***

- high stability. ✓1/2

b. Explain on how Counting rates measured with Geiger–Müller, scintillation, or semiconductor detectors are used to obtain qualitative and quantitative data. **(3 Marks)**

- Counting rates are of detectors are usually compared with suitable standards to obtain quantitative data. ✓1

- Pulse analysers (either single or multichannel), when coupled to a suitable radiation✓1 ***detector, can yield qualitative as well as quantitative data about individual components of the sample.*** ✓1

c. Discuss on the two column separation formats generally considered for automated separations in radiochemical analysis. **(10 Marks)**

-The first involves high-performance chromatographic techniques coupled with online flow-through scintillation ✓1***detection for intermittent sampling and quantification of actinides and fission products in water:***

- description of the use of high-performance ion✓1 ***chromatography (HPIC) with online radiometric detection for qualitative analysis of Hanford waste samples has been done. Long separation times (40 minutes to several hours), matrix effects on the reproducibility and reliability of the separation procedure,*** ✓1***limited capacity of the separation material, and speciation problems are significant issues with these chromatographic approaches that limit their applicability for rapid automated radiochemical analysis on difficult sample matrixes.*** ✓1

- The second approach uses selective separation chemistries in extraction chromatographic or solid-phase extraction formats to rapidly and selectively isolate species of interest from stable matrix and radiological interferences: ✓1

-The species of interest are strongly and preferentially retained by the column material under the solution conditions selected for the separation. ✓1 ***Following a wash step (or sequence***

of wash steps) to remove unretained or slightly retained sample components✓1, the species of interest are abruptly released by creating a large drop ✓1in the affinity factors via changes in the mobile phase composition or even by doing reaction chemistry on the retained species. Thus, this separation format relies on selective ✓1uptake and release properties rather than on the chromatographic efficiency of a long separation column, where analytes separate by migrating at different rates down a column ✓1due to (often small) differences in distribution ratios.

END