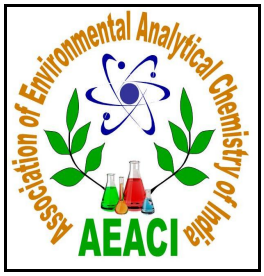
EXPERIMENTS IN

ANALYTICAL CHEMISTRY

A.V.R. Reddy
K.K. Swain
K. Venkatesh



ASSOCIATION OF ENVIRONMENTAL ANALYTICAL CHEMISTRY OF INDIA

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For copies and other information, please write to:

Secretary,
Association of Environmental Analytical Chemistry of India
C/o Analytical Chemistry Division
Bhabha Atomic Research Centre
Trombay, Mumbai 400 085
INDIA
FAX : +91 22 25505151

Association of Environmental Analytical Chemistry of India (AEACI)

Association of Environmental Analytical Chemistry of India (AEACI) was founded on 30th November, 2010 during a meeting held at Analytical Chemistry Division (ACD) in Bhabha Atomic Research Centre (BARC), Trombay, Mumbai to provide a common platform to all the Indian scientists and scholars working in the field of Analytical & Environmental Chemistry within the country from various Universities or Institutes or Industries. AEACI is a non-profit-making organization with its Head-Quarters at Analytical Chemistry Division, BARC, Mumbai. AEACI aims to promote the analytical environmental sciences and technology in India, to disseminate scientific and technological knowledge within the country and to advance both national and international cooperation (in particular south Asians countries) in the area of analytical and environmental Chemistry. The motivation to commence a national forum like AEACI came from the discussion in the School of Analytical Chemistry held at BARC, Mumbai during November 19th to 26th November in 2011. Since its inception, the Association has evolved magnificently to represent a truly National Organization and at present, it comprises about 155 life-members from different parts of India. The Executive committee of AEACI , which manages all the activities of AEACI, is being elected triennially by the members of AEACI. The scientific and technological fora provided by AEACI comprise the following.

- Symposia – that address environmental issues in a broad sense, but always include analytical environmental chemistry as a central topic
- Workshops – that are dedicated to specific topical areas of analytical environmental research
- Short courses – that are intended to familiarize scientists or engineers with new techniques applicable in carrying our environmentally relevant investigations.
- To provide partial financial assistance to Indian Scientist (AEACI, members) to South Asian Countries.
- AEACI plans to have biannual Bulletins with guest editors from different fields in the area of analytical environmental chemistry.

To encourage scientists & technologists AEACI will institute a few awards to recognize and honour valuable contributions of Indian Scientists or scholars in the area of analytical environmental Chemistry. Please visit the “Awards” section in the AEACI website (www.aeaci.org) to know details about these awards and to nominate for awards.

R.K. Singhal

General Secretary, AEACI

About School on Analytical Chemistry

Analytical chemistry an interdisciplinary science and often is called **central science** that contributes to every branch of science. Analytical chemistry finds application in all the branches of science like chemistry, physics, biology, geology, materials science, nuclear science and technology, medicine, environment and industry. Analytical measurements are aimed at obtaining qualitative and quantitative information about the composition and structure of various materials that have relevance to both fundamental understanding as well as applications towards improving the quality of life. Obtaining precise and reliable data is the prime requirement in the studies involving medical products, clinical evaluations, Environment impact and remediation, and high tech products. These data may be used in decision making. In view of this, a host of instrumental methods have been developed. The pace of development compares with that of electronics industry. It is also essential to evaluate essence of the problem and choose the right method that is fit for the purpose. Therefore analytical scientists need a good working knowledge of the available techniques, awareness about the advancements made in instrumentation and methodologies, and have to adapt "continued education" approach for keeping abreast with the advancements in theoretical and experimental knowledge associated with the state-of-the-art analytical methodologies. One of the major requirements is understanding the underlying principle of the chosen method for a particular analysis. During the last century with the advent of electronics, rapid improvements in computer technology and automation of instrumental methods of analysis, a large number of instrumental methods are made available to analytical chemists. Veracity of analytical data not only depends on choosing the correct instrumental method, materials, procedures and evaluating the measured results into concentrations of the analytes but more on understanding the phenomenon of signal generation and deciphering reliably signal from background.

It was thought that this could be achieved through organizing a 7 day School of Analytical Chemistry with an objective of providing a forum for revisiting fundamentals of analytical chemistry and exchanging with experts, the latest developments in analytical chemistry and its applications. In addition to regular lectures on various aspects of analytical chemistry, laboratory experiments were included in this School to provide hands on experience to the participants. It is expected that the participants will take advantage of the presence of experts and interact with them in a manner that facilitates collective growth in the field of analytical chemistry. While formulating the syllabus for the first school, some topics to impart the essence of work-culture, safe practices and good laboratory practices, besides theoretical and experimental aspects were included.

I am very happy that Analytical Chemistry Division has taken lead in organizing this School in a Works-shop mode. It was planned for 50-60 participants, mainly comprising of research scholars, young academics and young scientists from research institutions like BARC, with Chemistry and Physics background. An examination is included on the last day of the Second School and the performance of the participants was very good. Feedback from the first School encouraged us to make this School a regular feature and for the first time, the third School will be organized in a university (S.K. University, Ananatapur) during February 24 - March 01, 2012. It is heartening that a fledgling Association, AEACI has come forward to extend cooperation to organize this School and thanks are due to all the members of EC, AEACI, particularly Dr. T.

Mukherjee, President, AEACI and director, Chemistry Group for all the support and guidance provided. Dr. R. Sinha, Director, BARC has been a source of inspiration in all our endeavours and his patronage to this School is gratefully acknowledged. Dr. S. Banerjee, Chairman, Atomic Energy Commission and Secretary, Department of Atomic Energy for his continued guidance and encouragement.

Organising such a School is not possible without the generous funds from the Board of Research in Nuclear Sciences (BRNS). I take this opportunity to thank Prof. P. Rama Rao, Chairman, BRNS, Shri S.G. Markandeya, Scientific Secretary, BRNS, Members of the Board and Dr. S. Kailas, Chairman, Basic Sciences Committee, BRNS for their support. I thank the cooperation extended by all the members of Analytical Chemistry Division and administrative colleagues.

A.V.R. Reddy
Head, Analytical Chemistry Division

Presidential Message

Greetings to each one of you from AEACI and from all the members of Managing Committee of AEACI. Hope that all of you have also received the hard copy of the pamphlet for the School on Analytical Chemistry to be held at S.K. University, Anantapur, during February 24 - March 01, 2012. If not, please download the same from the website http://www.aeaci.org . This School consists of lectures in the morning sessions and laboratory experiments in the evening sessions. The target participants will be young academics, research scholars, industrial personnel and budding scientists from research centres. A total of 50 applicants will be selected as participants for this School on all India basis. I am glad that my colleagues made efforts to publish a book on 'Experiments in Analytical Chemistry' which will serve as a laboratory manual not only in SAC 2012 but also to M.Sc. students in Analytical Chemistry.

Delegates and colleagues from India and overseas working in the area of environmental and analytical chemistry are welcome to join hands with us, by becoming life-members of AEACI, to promote the growth of environmental and analytical chemistry world-wide. We propose to work synergistically with other Societies in India and abroad, working in the field of environmental and analytical chemistry, to make use of the multifaceted applications of environmental analytical chemistry for the benefit of mankind. Let us all work together to take the discipline of Environmental and Analytical Chemistry to newer heights and make India as one of the internationally known leaders in this branch of science.

My best wishes to each one of you in this challenging endeavour and look forward to discuss at length scientifically during the upcoming events.

You are welcome to suggest the names of reputed scientists and academicians from India and abroad in this field who can deliver lucid and inspiring lectures in the upcoming events as well as in the continuous educational programmes of ***AEACI****.*

Dr. T. Mukherjee
Distinguished Scientist
Director, Chemistry Group
Bhabha Atomic Research Centre
Trombay, Mumbai 400 085

Preface

Analytical Chemistry is useful in all the branches of science like chemistry, physics, biology, geology, materials science, nuclear science and technology, medicine, environment and industry. Analytical measurements are aimed at obtaining qualitative and quantitative information about the composition and structure of various materials that have relevance to both fundamental understanding as well as applications towards improving the quality of life. Analytical scientists need a good working knowledge of the available techniques, awareness about the advancements made in instrumentation and methodologies. In view of this Analytical Chemistry Division, BARC has initiated to organize a series of School on Analytical Chemistry for the benefit of young scientists, academics and research scholars with chemistry background and those who use chemical instrumentation of analysis in their work. In this School lectures are planned in the morning hours, laboratory experiments in the afternoon hours to provide hands on experience to the participants and specialized plenary lectures in the evening hours to provide an insight into the frontiers of Analytical Chemistry. Although many good text books in the subject analytical chemistry are available, there is a paucity of books on experiments in Analytical Chemistry. This book on experiments in Analytical Chemistry is an effort to provide a simple introduction to about 25 experiments covering various aspects of instrumental methods of analysis. A few of the experiments are chosen in each school and therefore this book is written as a manual with a provision to note the observations and perform calculations. In addition a brief introduction is provided on a few relevant topics to inculcate laboratory work-culture, safe practices in the laboratory and good laboratory practices, besides theoretical and experimental aspects. This book is modeled on an IANCAS publication of Experiments in Radiochemistry: Theory and practice.

We thank all the authors who contributed to various experiments. We also thank other colleagues who have gone through the book critically. Association of Environmental Analytical Chemistry of India (AEACI) has come forward to publish this book and we record our gratitude to the members of executive committee, AEACI led by Dr. T. Mukherjee, President, AEACI and Director, Chemistry Group. Dr. R. Sinha, Director, BARC has been a source of inspiration in all our endeavors and his patronage to this School is gratefully acknowledged. We record our gratitude to Dr. S. Banerjee, Chairman, Atomic Energy Commission and Secretary, Department of Atomic Energy for his continued guidance and encouragement. We thank Prof. P. Rama Rao, Chairman, Board of Research in Nuclear Sciences (BRNS) for his encouragement and support.

This book is composed meticulously by Shri Sharad Nalavade and Shri Vishal N. Koli and we thank them for their whole hearted efforts. Our office colleagues Smt Vaishali Wade and Smt S D. Shinde deserve appreciation for their cooperation and contribution.

A.V.R. Reddy
K.K Swain
K. Venkatesh
Analytical Chemistry Division

February 18, 2012

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Chapter 1

GENERAL ANALYTICAL CHEMISTRY

A.V.R. Reddy, K. Venkatesh, Sanjukta A. Kumar, K.K. Swain and R. Verma

INTRODUCTION

Analytical Chemistry is the science of measurement based on a set of ideas and methods employing state-of-the-art technology. Analytical chemistry is useful in all the branches of science like chemistry, physics, biology, geology, materials science, nuclear science and technology, medicine, environment and industry. Analytical measurements are aimed at obtaining qualitative and quantitative information about the composition and structure of various materials that have relevance to both fundamental understanding as well as applications towards improving the quality of life. Analytical chemistry is of interdisciplinary nature and often is called **central science** that contributes to every branch of science. Today, analytical chemistry has a range of powerful tools to obtain the needed information. Obtaining precise and reliable data is the prime requirement in any analytical method for obvious reasons. This aspect is pertinent to nuclear industry and contributes significantly in various stages of nuclear fuel cycle. It is also essential to evaluate essence of the problem and choose the right method that is fit for the purpose. Therefore analytical scientists need a good working knowledge of the available techniques, awareness about the advancements made in instrumentation and methodologies, and have to adapt “continued education and update” approach by which each analytical scientist could update oneself with the theoretical and experimental knowledge associated with the state-of-the-art analytical methodologies. Chemistry in general and Analytical Chemistry in particular, is an experimental science. Therefore to better understand Analytical Chemistry, it is essential to have an opportunity to experience various experimental aspects so that the observations made could be analysed in the theoretical frame besides examining whether the data obtained are useful for the intended purpose. Over the years, there is a paradigm shift in the analysis from classical chemistry to instrumental methods of analysis. As instrumental methods are ratio methods, there is a need to have standards / reference materials to develop calibration methods. Although instrumental methods of analysis help achieving reduced detection limits, classical methods are absolute methods for quantitative analysis and the principles in qualitative analysis are very important in various steps of instrumental analysis.

Thus, major requirements for effective analysis are understanding the underlying principle of the chosen method for a particular analysis and various steps that are needed like selecting the method for collecting the sample, preparation of experimental sample, dissolution method if required, signal generation, quantitative measurement of the signal, converting the signal to concentration, analysis of data and reporting the data with stated uncertainty. During the last century with the advent of electronics, improvements in computer technology and automation of instrumental methods of analysis, a large number of instrumental methods are available to analytical chemists. Veracity of analytical data not only depends on choosing the correct instrumental method, materials, procedures and evaluating the measured results into concentrations of the analytes but more on understanding the phenomenon of signal generation and deciphering the signal from background. Materials that are suitable to obtain information quantitatively / proportionally are good detector materials. Electronic devices are used to decipher the signal from noise, digitize and convert them into concentration of the analyte using a suitable programme.

It is essential to have clarity and use the right terminology, like grammar in a language. For example, there is confusion between **analytical technique** and **analytical method**. An analytical technique is a fundamental scientific phenomenon that is useful for providing information on composition of substances whereas a method is a specific application of the technique. ICP MS is a technique used to determine large number of elements in wide variety of matrices, and determination of impurities in ground water using ICP MS is a method. Similarly **procedure** and **protocol** are not well distinguished by many. A procedure is a set of written instructions for carrying out a method (essentially meant for those with some background). On the other hand, detailed specific description of the method is a protocol and the instructions have to be followed without any exception. Yet another pair of terms is **precision** and **accuracy**. A few commonly used terminologies are given in the Table 1.1.

Table 1.1 : Some commonly used definitions

Analysis	A process that provides chemical or physical information about the constituents in the sample.
Determination	An analysis of a sample to find the identity, concentration, or properties of the analyte
Measurement	An experimental determination of an analyte's chemical or physical properties.
Technique	A chemical or physical principle used to analyze a sample.
Method	Means for analyzing a sample for an analyte in a matrix
Procedure	Written directions outlining how to analyze a sample.
Protocol	A set of written guidelines for analyzing a sample by an agency
Signal	An experimental measurement that is proportional to the amount of analyte
Total analysis technique	A technique in which the signal is proportional to the absolute amount of analyte ("classical" techniques)
Concentration technique	A technique in which the signal is proportional to the analyte's concentration; also called "instrumental" techniques.
Precision	An indication of the reproducibility of a measurement.
Accuracy	A measure of the agreement between an experimental result and its expected value
Sensitivity	A method's ability to distinguish between two samples reported as the change in signal per unit change in the amount of analyte.
Detection limit	A statistical statement about the smallest amount of an analyte that can be determined with confidence
Method's selectivity	A measure of a method's freedom from interferences
Robust method	A method that can be applied to analytes in a wide variety of matrices
Rugged method	A method that is insensitive to changes in experimental conditions
Method blank	A sample that contains all components of the matrix except the analyte
Calibration	The process of ensuring that the signal measured by a piece of equipment or an instrument is correct.
Standardisation	The process of establishing the relationship between the amount of analyte and a method's signal

Validation	The process of verifying that a procedure yields acceptable results.
QA/QC	Those steps taken to ensure that the work conducted in an analytical lab is capable of producing acceptable results.
Mean	The average of a set of data
Median	Middle value(s) when a set of data is arranged in ascending or descending order
Range	Difference between the largest and smallest values in a set of data
Std. dev (s)	A statistical measure of average deviation from the mean of a set of data
Error	A measure of bias in a result
Variance	Square of standard deviation (s)
Sampling error	Error occurred during sampling process
Method error	Error due to limitations of the analytical method
Personal error	Error due to analyst & his/her approach
Measurement error	Error due to limitations in the equipment / instrument used
Determinate error	Error that can be traced to the source – above FOUR
Outlier	A datum which is far larger / smaller than the remaining data
Repeatability	The precision for analysis in which the only source of variability is the analysis of replicate samples
Reproducibility	Precision of results of several samples, several analysts or several methods.
Indeterminate errors	Sources are not known but affects the scatter around the central value (mean)
Uncertainty	The range of possible values for a measurement
Confidence intervals	Range of results around a mean value that can be explained by random error
Histogram	Profile of frequency as a function of the range of measured values
Normal distribution	Normalised frequency (probability) of measured values
Degrees of freedom	Number of independent values on which the result is based
Significance test	A statistical test to determine whether the difference between two values is significant or not.
Null hypothesis	A statement that the difference between two values can be explained by indeterminate error
SRM / CRM	Material certified with known concentrations of the analytes
Type 1 error	The risk of falsely rejecting null hypothesis
Type 2 error	The risk of falsely retaining the null hypothesis
t- test	For comparing two mean values
F - test	For comparing two variances

It is important to have documented haromonised procedures. It is crucial to document procedures and observations as it is essential for further calculations as well as for archiving.

Analytical chemistry has become a multi disciplinary subject in which chemical instrumentation has made great inroads. It is essential that efforts be made to identify the appropriate methods and methodologies for obtaining quality analytical data that are precise, accurate and reliable, which would stand to the scrutiny by regulators in various branches of science and technology. An attempt is made to introduce briefly some aspects on topics like classical methods, instrumental methods, signal generation and quality assurance & quality control in analytical chemistry in this part. In the second part, a few experiments are included covering most of the areas that an analyst would like to be exposed in the induction period into the subject.

Basic Tools in Analytical Chemistry

As Analytical Chemistry is a quantitative science, it deals with numerical and experimental tools. Measurements and numerical calculations are integral part of every determination e.g. concentration of a species in a solution, evaluating equilibrium constant, reaction rates, and so on so forth.

Units

Measurement data consists of a number and a unit to express the quantity e.g. mass of a sample is 5.2mg. Unfortunately a few different units like oz are also used for the same quantity. In order to have to use the same units, a common set of fundamental units, called SI units are defined which are given in Table 1.2. Some more derived units and their equivalent SI units are given in Table 1.3. It is advisable to practice to use only these units so that the basic tool of numbers will be on same scale and international comparison of results is easy.

Table 1.2 Fundamental SI units

Measurement	Unit	Symbol
mass	kilogram	kg
volume	liter	L
distance	meter	M
temperature	Kelvin	K
time	second	s
current	ampere	A
amount of substance	mole	mol

Table 1.3 : Other SI and Non-SI Units

Measurement	Unit	Symbol	Equivalent SI Unit
length	angstrom	A	1 Å = 1× 10^{-10} m
force	newton	N	1 N = 1 m · kg/s^2
pressure	pascal	Pa	1 Pa = 1 N/m^2=1 $kg/(m{\cdot}s^2)$
	atmosphere	atm	1 atm = 101, 325 Pa
energy, work, heat	joule	J	1 J = 1 N · m = 1 m^2 · kg/s^2
power	watt	W	1 W = 1 J/s= 1 m^2 · kg/s^3
charge	coulomb	C	1 C = 1 A · s
potential	volt	V	1 V = 1 W/A= 1 m^2 · $kg/(s^3{\cdot}$ A)
temperature	degree Celsius	^oC	^oC = K – 273.15
	degree Fahrenheit	^oF	^oF = 1.8(K-273.15) + 32

Significant Figures

The analytical data of a measurement are expected to provide magnitude and uncertainty. If a sample is weighed in a balance and its mass is 2.4769 g the value implies that uncertainty is in the last digit which could be ±0.0001 g and corresponding relative uncertainty is 0.0001 / 2.4769 x 100 = 0.0430730 %, however it has to be rounded to 4^{th} digit as it is the position of the last digit. Thus the value will be 0.0431%. Significant figure of a measured quantity includes all the digits of the datum that is exactly known and also the last digit which contains a degree of uncertainty. In combining many results through a formula it often becomes ambiguous to fix the significant digit. Final result shall have the least significant digit as one of the parameters (data) that has lowest significant digit, e.g. in a sum of three data like

X = 147.2341 + 152.15 + 99.723
= 399.1071 (to be rounded to 399.11)

as the lowest significant digit is in 2^{nd} decimal place in the second datum. Important point is that rounding or truncating shall not be done till the final result. Absolute uncertainty in addition and subtraction, and relative uncertainty in multiplication and division are conserved, not the significant digits.

To avoid confusion, scientific notation is followed by fixing the significant digits e.g. $1x10^3$ has one significant digit, whereas $1.000\ x10^3$ has three significant digits. It is always better to use the scientific notation. In the case of log values like pH, number of decimals are significant digits e.g. pH=2.7 has one significant digit whereas pH 2.70 has two significant digits, as 2 is equal to 10^2. In analytical measurements, a large number of data are obtained and calculations are performed to obtain values for quantities like weight, concentration, molarity, rate constant and many more. Therefore in these calculations, one has to use an acceptable method to report the results with uncertainty. These data form the basis for understanding various phenomena based on often small differences in the results. Thus it becomes crucial to report results in an appropriate manner.

Basic Equipment and Instrumentation

Balance is used to measure the mass and is one of the essential equipment in a chemistry lab. One should be very careful while using a balance. Standard Operation Procedure (SOP) has to be read, assemble the required accessories and notebook and then carry out weighing and transfers. It is part of Good Laboratory Practices (GLP) that the data be noted in the logbook as well as to enter date, time, analyst's name and the material weighed in the instrument's logbook. Balance has to be calibrated periodically using certified weights.

Glassware like pipette, standard flask, cylinders and beakers are used to measure volume of a liquid sample. Glassware should be cleaned with dilute chromic acid followed by water, and have to be dried in an oven and cooled before using them. Propipette has to be used to suck the liquid sample to a pipette. After transfer of liquid, tip should not be blown out. Glassware that have to be used in critical experiments, have to be stored in a desiccator.

Laboratory Note Book

Lab note book often known as logbook, is used to note the data, sequence of operations, observations etc. It is an essential tool for achieving the data, verifying data and storing data. In addition, log books are used to record observations associated with operation of

the instruments and hence it will become a 'track record'. Every person and equipment in the lab shall have a log book with index. Each instrument's log book shall have SOP.

Safety Practices in a Chemical Laboratory

It is important and necessary that all laboratory staff are qualified, trained and well versed with the requirements in the laboratory, available equipment, chemicals and develop an attitude to follow safe practices. They as well shall ensure that the newcomers are provided with training and information for safe practices. Laboratory in-charge is responsible for proper functioning of all the safety equipment, personal protective wares and shall act in a manner that good laboratory practices are implemented by all the employees as well as trainees and students. A list of **Do's** and **Dont's** is displayed in the laboratory at the entrance as well as a written copy is given to all concerned. It is the responsibility of every member of the laboratory to ensure that safety devices and precaution manuals are located in a place that is easy to find. It is essential to display the general safety procedures as posters at the entrance of the laboratory. In the case of equipment, all standard operation procedures (SOP) and safety instructions concerned with the equipments are displayed by the side of the equipment. Laboratory staff should undergo an annual medical examination. It is the responsibility of the laboratory in charge to designate two or more staff members for emergency contact and they shall be given training handling emergencies. It is important to follow the safety do's and don'ts of the laboratory, not only for one's own safety but also for others' safety. The following guidelines shall be followed in a chemical laboratory.

1. Read "know your laboratory" manual before working in the lab. If it is not available, learn from the lab in-charge or the deputy. Note down precautions before using electrical items and gas cylinders.

2. Always wear safety shoes, laboratory coats and safety glasses

3. Don't work alone in the laboratory

4. Eating, drinking and smoking should be prohibited in the laboratory

5. Keep hot plate and hot glassware in a designated place that has a display so that other members will not touch them.

6. Do not keep more chemicals in the laboratory than necessary for the ongoing work. Store the rest of the chemicals in a safe place.

7. Used liquids/chemicals should be disposed off in a proper way as per the procedure precisely.

8. Always have a first-aid kit ready in the laboratory.

9. Take care of fire extinguishers, fume hoods, chemical spill kit, eye washes and other Safety devices.

10. Check analytical procedures thoroughly before starting the work with organic liquids which may be dangerous.

11. Be careful with power supply, gas cylinders and heating equipments.

12. Work as much as possible in a fume hood and always add acid/base to water. Slowly add strong acids and bases to water to avoid sputtering.

13. If there is an accidental skin contact, thoroughly flush the contaminated area with water and seek medical attention.

14. Remove and replace broken glassware.

15. Instruct new laboratory persons in detail.

16. Avoid contact with chemicals which may cause external or internal injuries. External injuries are caused by skin exposure to caustic/corrosive chemicals (acid/base/reactive salts).

17. Prevent as far as possible inadvertent spills and splashes and equipment corrosion.

18. Internal injuries may result from toxic or corrosive effects of chemicals accidentally ingested and absorbed by body.

19. Use auto pipettes and avoid mouth pipetting.

20. Never use glassware for hydrofluoric acid treatment.

21. Avoid exposure to fumes of inorganic acids and bases as these irritates or damages eye, skin and create respiratory problems. Hot acids quickly react with the skin.

22. Store acids and bases separately, in well-ventilated areas and away from volatile organic and oxidisable substances.

23. Don’t use perchloric acid together with organic reagents, particularly volatile solvents in one fume hood as it reacts violently on contact with organic materials.

24. Certain chemicals like NaOH produce considerable heat on dissolution, which may cause burns. Care should be taken in these operations.

25. Label the chemicals that are highly toxic and may also be carcinogenic. Avoid inhalation, ingestion and skin contact.

26. Nearly all organic solvents are hazardous and should be treated with extra caution.

27. Carefully read the laboratory experiment protocol before hand, discuss with instructor before performing the experiment.

28. Make a list of questions regarding the experiment and get it clarified before commencing.

29. Make a brief outline of the experiment in your logbook with required reagents and solutions before the start of the experiment.

30. Prepare data recording format before start of the experiment

31. All data shall be recorded in a log book. Do not use loose sheets.

32. Laboratory procedures shall be followed exactly as they are given by the instructor.

33. Note down all the observations in logbook, such as color changes, endothermic or exothermic changes, changes in physical state, boiling point, melting point and freezing point.

34. Review your observations and results, and decide whether experiment has to be repeated or not.

35. When in doubt, repeat a portion of the experiment. If you are unsure, discuss with the in-charge.

36. Clean your glassware at the end of the experiment.

37. Report any dangerous observation made in the lab.

In addition to the safety measures taken in normal chemical laboratory, following additional precautions are to be taken in a Radioactive Laboratory.

1. Persons entering the radioactive area (*Amber and Red zone) should wear the protective clothing like shoe covers and lab coats.

2. A film badge or a TLD for personnel monitoring of radiation exposure must be worn by the person while working in the radioactive laboratory.

3. All operations with exposed sources of radioactivity must be carried out in fume hood / glove box with proper ventilation.

4. Personnel should wear surgical gloves during these operations to avoid direct physical contact with the radioactive substances.

5. No work with radioactive material should be carried out by anybody having an open cut, skin lesion or injury.

6. Any spillage of radioactive solution, contamination of personnel or work area by any accident must be reported to the laboratory-in-charge.

7. All wastes of liquids and solids must be separated and stored in containers.

8. All radioactive materials must be stored, sealed and **labelled** properly, with date and **person's** name written on it.

9. Active samples should not be removed from the laboratory without the permission of laboratory in-charge.

10. After removing the gloves, hands should be washed with detergent solution and water.

11. Hands should be monitored using hand monitors provided in the green zone of the radioactive laboratory. Only when the hands are free from contamination one should leave the laboratory.

12. Report cases of high count rate to laboratory health physicist immediately and follow proper decontamination methods.

13. Dose records of all the occupational workers should be maintained by management of facility.

14. Radioactive symbol should be displayed at the entrance as well as active areas of the laboratory.

NOTE: * Amber Zone stands for low radioactive area and Red zone indicates high radioactive area

Sampling and Sample Preparation

The term "sample" in analytical chemistry is applied to a portion of material selected in an appropriate manner, to represent a larger body of material. The result obtained from the samples is merely an estimate of the quantity or concentration of a constituent or property of the bulk material from which this sample is taken. The parent material may be homogeneous or heterogeneous. The use of a sample is always likely to introduce an uncertainty, arising from heterogeneity of the parent material and in extrapolating from the smaller portion to the larger portion called the "sampling error."

Sampling consists of two important steps- the sample collection step (sampling) and sample preparation step. The overall uncertainty is more often limited by problems with sampling or sample preparation than by the analysis of the prepared sample. If samples collected are the wrong size or type, even the best lab analysis of those samples may not be able to correctly represent the analysis of bulk material and thus may not serve the intended purpose.

The overall uncertainty (represented by σ^2 total) is made up of additive contributions from sampling, sample preparation, and the analytical measurement.

$$\sigma^2_{total} = \sigma^2_{sampling} + \sigma^2_{preparation} + \sigma^2_{measurement}$$

All three contributions are potentially important. Analyses are typically designed to minimize each. Heterogeneous samples provide the greatest sampling challenges.

Sampling Objectives:

1. Obtaining a representative sample
2. Obtaining a homogeneous sample
3. Sampling should include random sampling
4. Sampling and sample handling should not change analyte concentrations

Experience and common sense are always part of a successful analytical sampling.

Sampling Techniques:

Mixing

Mixing is combining of components, particles, or layers into a more homogeneous state. The mixing may be achieved manually or mechanically by shifting the material with stirrers or pumps or by revolving or shaking the container. The process must not permit segregation of particles of different size or properties. Homogeneity may be considered to have been achieved in a practical sense when the sampling error of the processed portion is negligible compared to the total error of the measurement system.

Reduction of size

Decreasing the size of the laboratory sample or individual particles, or both is achieved by the division of the bulk material. Division of the size of the laboratory sample is generally accomplished manually by coning and quartering or by riffling or mechanically by rotary dividers. Reduction of particle size may be accomplished by milling or grinding**.** Simultaneous division and reduction may also be achieved with mills having stream diverters.

Coning and quartering

Coning and quartering is one of the methods for preparing representative sample from the powdered sample by forming a conical heap. The heap is spread out into a circular, flat cake. The cake is divided radially into quarters and two opposite quarters are combined. The other two quarters are discarded. The process is repeated as many times as necessary to obtain the quantity desired for the final use.

Riffling

The separation of a free-flowing sample into (usually) equal parts by means of a mechanical device composed of diverter chutes.

Milling/grinding

In milling and grinding mechanical reduction of the particle size of a sample is achieved by attrition (friction). The required particle size of a sample is related to the size of the test portion and the number of particles required to ensuring homogeneity among test portions. The reduction in particle size may sometimes result in particles of different hardness and density, which produces in-homogeneity during the preparation of the test sample or during the withdrawal of the test portion.

SAMPLE TYPES

Random sample:

The sample **so** selected that any portion of the population has an equal chance of being chosen.

Representative sample

A sampling plan that adequately reflects the characteristics of the population. The degree of representativeness of the sample may be limited by cost or convenience.

Selective sample:

A sample that is deliberately chosen by using a sampling plan that screens out materials with certain characteristics and/or selects only material with other relevant characteristics.

Stratified sample:

A sample consisting of portions obtained from identified subparts (strata) of the parent population. Within each stratum, the samples are taken randomly. The objective of taking stratified samples is to obtain a more representative sample than that which might otherwise be obtained by random sampling.

Convenience sample:

A sample chosen on the basis of accessibility, expediency, cost, efficiency, or other reason not directly concerned with sampling parameters.

Replicate (duplicate) sample:

Multiple (or two) samples taken under comparable conditions. A duplicate sample is a replicate sample consisting of two portions. The umpire sample is often used to settle a dispute and the replicate to estimate sample variability.

Umpire sample/referee sample/reserve sample:

A sample taken, prepared, and stored in an agreed manner for the purpose of settling a dispute, if arises.

Sequential sample:

Units, increments, or samples taken one at a time or in successive predetermined groups, until the cumulative result of their measurements (typically applied to attributes), as assessed against predetermined limits, permits a decision to accept or reject the population or to continue sampling.

Multistage sampling:

Samples taken in a series of steps with the sampling portions constituting the sample (units or increments) at each step being selected from a larger or greater number of portions of the previous step, or from a primary or composite sample .

Sample Preparation, storage and handling:

In analytical chemistry, sample preparation refers to the ways in which a sample is treated prior to its analysis. Preparation is a crucial step in most of the analytical techniques, because the techniques are often not responsive to the analyte in its in-situ form, or the results are distorted by interfering species. Sample preparation may involve dissolution, reaction with some chemical species, pulverizing, treatment with a chelating agent (e.g. EDTA), masking, filtering, dilution, sub-sampling or many other techniques. Sample preservation, storage, and handling must be established in the work plan prior to sample collection.

Typical Sampling Protocol for elemental boron:

Pure boron is highly reactive even at room temperature and forms oxides of boron. Due care should be taken to ensure that material is sampled complying with the following:

1. The representative sample of 25g shall be taken from each package from the batch/lot.
2. Samples shall be drawn once through in Air Conditioned or dust free room.
3. The contents of each container selected for sampling shall be mixed thoroughly before drawing the sample.
4. The samples shall be placed in clean, dry and airtight polypropylene containers.

5. The sample container shall be of such size that it is almost completely filled by the sample. Each container shall be sealed airtight and stored at below or below 27^0C.

Sample preparation, storage and handling should consider the following:

Sample volume: Samples should be collected using equipment and procedures appropriate to the matrix, the parameters to be analyzed, and the sampling objective. The volume of the sample collected must be sufficient to perform the analysis requested, as well as the quality assurance/quality control requirements

Matrix spike/matrix spike duplicate (MS/MSD) sample: A Matrix Spike and Spike Duplicate (MS/MSD) are representative but randomly chosen samples that have known concentrations of analytes of interest added to the samples prior to sample preparation and analysis. They are processed along with the same un-spiked sample. The purpose of the MS/MSD is to document the accuracy and precision of the method for that specific sample.

Sample Container: Containers must be compatible with the sample matrix, clean and labeled appropriately. The exterior of the sample containers must be wiped clean and dry prior to sample packaging. To prevent leakage of aqueous samples during shipping, sample containers should be no more than 90 percent full. If air space would affect sample integrity, fill the sample container completely and place the container in a second container to meet the 90 percent requirement.

Sample Preservation: When a preservative other than cooling is used, the preservative is generally added after the sample is collected. If necessary, the pH must be adjusted to the appropriate level and checked with pH paper in a manner, which will not contaminate the sample. The laboratory performing the analysis should be contacted to confirm the requirements for sample volumes, container types, and preservation techniques.

Analysis Methods

Analytical Chemistry deals with methods for determining the chemical composition of samples of matter. A qualitative method yields information about the identity of atomic or molecular species or the functional groups in the sample; a quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components. Analytical methods are classified broadly as classical and instrumental methods of analysis. This classification is largely historical with classical methods, sometimes called wet-chemical methods, preceding instrumental methods by a century or more.

Classical Methods

In the early years of chemistry, most analyses were carried out by separating components of interest in a sample by precipitation, extraction, or distillation. For quantitative analyses, the separated components are treated with reagents that yield product. The products could be recognized by their colour and odour, boiling points or melting points, their solubility in a series of solvents, their optical activities, or their refractive indexes. For quantitative analyses, the amount of the analyte was determined either by gravimetric or titrimetric measurement.

Gravimetric analysis

Gravimetric analysis involves determining the amount of material present by weighing the sample before and/or after some transformation. A common example is the determination of the amount of water in a hydrate by heating the sample to remove the water such that the difference in weight is due to the loss of water.

Volumetric Titrations

Titration involves the addition of a reactant to a solution being analyzed until the equivalence point is reached. Often the amount of material in the solution being analyzed may be determined. Most familiar example is the acid-base titration using an indicator that changes its color after the end point. There are many other types of titrations, for example potentiometric titrations. These titrations may use different types of indicators to reach the equivalence point.

Instrumental Methods of Analysis

Instrumental methods of chemical analysis have become the principal means of obtaining information in diverse areas of science and technology. These methods of analyses are based on the measurements of a physical property of the analyte such as conductivity, electrode potential, light absorption or emission, mass-to-charge ratio, fluorescence and radioactivity. A variety of instrumental techniques have been developed for the application to chemical analysis. They include mass spectrometric, optical spectroscopic, nuclear, thermal, surface, electrochemical and separation methods. They are commonly used individually. Two or more instrumental techniques are often used in combination to gain advantages that neither can provide singly. Such combinations of the techniques produce *"hybrid" or "hyphenated" techniques*. In general, all of these instrumental techniques function by converting information in the non-electrical domain into the electrical domain, which can be measured reliably. For Example, in spectrometry when an analyte is probed with a monochromatic light, the analyte absorbs the light if matches one of its energy states resulting a decrease in intensity of the probing light. The difference in intensity is proportional so concentration of the analyte. Intensity of light is measured as an electric pulse (current / potential) and difference is compared.

Classification

Classification of techniques is mainly based on the physical property of the analyte used by the instrument for its quantification.

Spectroscopic techniques involve the interaction of electromagnetic radiation with atoms or molecules. General subcategories of spectroscopic techniques are those in which matter absorbs, emits, or scatters electromagnetic radiation. In addition to quantitative data, qualitative information on the identity of atoms, molecular functional groups, molecules, and changes in bonding environments can be obtained by optical spectroscopy. Spectroscopic techniques consist of different categories such as atomic absorption spectroscopy, atomic emission spectroscopy, ultraviolet-visible spectroscopy, x-ray fluorescence spectroscopy, infrared spectroscopy, Raman spectroscopy, nuclear magnetic resonance spectroscopy, photoemission spectroscopy and Mössbauer spectroscopy.

Mass spectrometric (MS) techniques involve the generation of ions of analytes present in the sample, their separation according to mass-to-charge ratio, and subsequent detection.

MS is a powerful technique, providing qualitative and quantitative information on the atomic or molecular composition of inorganic or organic materials. Advances in the creation of gas phase ions from macromolecules have resulted in a variety of applications of MS to problems in biochemistry and molecular biology, in addition to general chemistry and material science. There are several ionization methods to generate ions from sample e.g. electron ionization, chemical ionization, electrospray, fast atom bombardment and matrix-assisted laser desorption/ionization which are useful for mass spectometric techniques. Also, mass spectrometric techniques may be categorized by approaches of mass analyzers such as magnetic-sector, quadrupole mass analyzer, quadrupole ion trap and time-of-flight.

Nuclear analytical techniques are based on detection and measurement of natural or induced radioactivity and use of radioactive tracers. These techniques involve the emission of particles or electromagnetic radiation from the nucleus of an element, rather than electronic phenomena common in the more traditional optical spectroscopic techniques. Nuclear analytical techniques, such as neutron activation analysis (NAA), which are generally considered as reference methods for many analytical problems, are useful in almost all fields of science and technology. Various nuclear analytical techniques include, Activation analysis (including NAA, Charge Particle Activation Analysis, Photon Activation Analysis), Isotope Dilution Technique, Ion Beam Analysis (including Particle Induced X-ray Emission, Particle Induced Gamma-ray Emission, Nuclear Reaction Analysis, Rutherford Back Scattering) and X-ray Fluorescence. Even though XRF is an optical spectroscopic technique, it is included in nuclear analytical techniques as the instruments used are similar to other nuclear techniques.

Surface analytical techniques provide the information on the surface materials of the samples analyzed. The analysis of surface starts with defining what a surface is. Generally, it is considered that the top 4 – 5 layers thick or top 10 Á depth is considered to be the surface of the sample. Some surface science techniques also are spectroscopic in nature, but differ in sampling considerations and the portion of the sample analyzed. A number of surface analytical approaches, such as atomic force microscopy (AFM) and scanning tunneling microscopy (STM), do not involve electromagnetic radiation.

There are generally four particle beams, namely electrons, ions, neutrons and photons and there are four other fields, namely thermal, electric, magnetic and surface sonic waves that can be used as input probes in surface analytical techniques. When one employs any one of these eight input probes, they give rise to emission or transmission or scattering of the four particle beams (except the magnetic field) namely electrons, ions, neutrons and photons. These particles carry information of the surface to a suitable detector. The detector assembly can be tuned to count the number of particles emitted (intensity), or it can identify the chemical nature of the species emitted in the case of ions and neutrons or can be made to analyse the energy or angular distribution of the particles emitted. Any or all of these four types of information on the emitted particle are used to develop better understanding of the surface under study.

Thermal techniques involve the study of materials properties as they change with temperature. *Thermal Analysis* is also often used as a term for the study of Heat transfer through structures. Many of the basic engineering data for modelling such systems come from measurements of heat capacity and Thermal conductivity. Several methods are commonly used - these are distinguished from one another by the property which is measured. Some of them are listed here.

- Thermogravimetric analysis (TGA): mass
- Differential thermal analysis (DTA): temperature difference
- Differential scanning calorimetry (DSC): heat difference

- Thermomechanical analysis (TMA): dimension
- Evolved gas analysis (EGA) : gaseous decomposition products

Electroanalytical techniques comprise another broad, general classification. These methods generally depend upon some approach to monitor the process of electron transfer to or from an analyte. Generally electrochemical techniques measure the electric potential in volts and/or the electric current in amps in an electrochemical cell containing the analyte. These methods can be categorized according to which aspects of the cell are controlled and which are measured. The three main categories are potentiometry (the difference in electrode potentials is measured), coulometry (the cell's current is measured over time), and voltammetry (the cell's current is measured while actively altering the cell's potential).

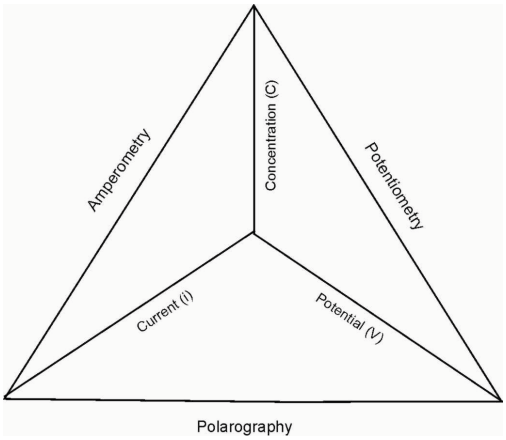


Fig. 1.1 : Electrochemistry Triangle

1 Polarography (Concentration (C)-constant)
2 Potentiometry (Current (i)-constant)
3 Amperometry (Potential (V)-constant)

Electrochemical techniques are used to derive both qualitative and quantitative information about elements, ions, and compounds in a wide variety of matrices.

Separations represent an important category of analytical chemistry. Separation processes are used to decrease the complexity of material mixtures. Most samples are complex mixtures of atoms, ions and molecules. Separation of the analytes or removal of matrix enhances the signal. Thus, the individual sample components might be separated prior to measurement. The two most common separation techniques are chromatography and electrophoresis. *Chromatographic techniques* involve partitioning of components in a sample between a flowing “mobile” phase and an immobile “stationary” phase. Separation is achieved exploiting different intermolecular interactions of the sample components in the two phases which results in different velocities of components through a column containing the stationary phase. Chromatographic techniques include Ion Chromatography (IC), Gas Chromatography (GC) and Liquid Chromatography / High Performance Liquid Chromatography (HPLC). *Electrophoretic techniques* involve separation of charged molecules by their migration in an electrical field. Electrophoretic techniques are traditionally associated with separations in biochemical systems. Instruments used in separations require the integration of a detection technique to measure the separated components. The choice of an appropriate chromatographic detector is governed by the specific requirements of the analysis. Numerous detection methods are available, often employing common instrumental techniques, such as mass spectrometry or optical spectrometry.

Gas chromatography hyphenated with a mass spectrometer known as GC-MS. GC-MS allows separation of volatile compounds from a complex mixture followed immediately by sequential mass specific detection (and structural elucidation) of each of the separated compounds. Hyphenated techniques are unlimited depending on the application and effective benefit of hyphenation. Some of the examples of hyphenated techniques:

- Chromatography-mass spectrometry (LC-MS or HPLC-MS)
- Capillary electrophoresis-mass spectrometry (CE-MS)
- Gas chromatography-mass spectrometry (GC-MS)
- Liquid chromatography-infrared spectroscopy (LC-IR)

Basic Function of Instrumentation

The role of a chemical instrument is to obtain information about a sample. This process involves converting the information contained in the chemical or physical properties of analytes, into meaningful data. Several transformations may be necessary to accomplish the measurement; the number needed depends upon a variety of factors including the instrument and the quality of data needed and the quantity of data required. The flow of information in an instrumental measurement may be divided into different steps, as illustrated in Fig. 1.2. The measurement begins with a signal generator, the portion of the instrument that creates a signal as a result of direct interaction of energy with the analyte. The energy involved is often electromagnetic radiation, thermal heating, or electricity. The resulting signal is directed to an input transducer, a device that transforms the signal from the non-electrical domain (the desired physical or chemical characteristic: chemical composition, light intensity, pressure, chemical activity) into the electrical domain. The electrical signal is then transformed into a more usable form by signal modifiers. This involves operations such as amplification, attenuation or filtering. Finally, the modified electrical signal is converted by an output transducer to information in a format which can be recorded and interpreted by the analyst.

Signal Generation, Blank Corrections and Calibration

Scientists began to exploit the physical properties like conductivity, electrode potential, absorption / emission of light and mass to charge ratio for quantitative analysis. In the process many ‘instrument’ based methods have emerged. These methods are relative or ratio methods, and do not give absolute values. However, to use a physical property, most important pre-requisite is to understand the measureable signal that can be induced by this physical property. Besides, the optimization of conditions play a crucial role in obtaining a signal that is much larger than the background. While using an instrumental method, it is essential to know the phenomenon that is exploited, the process of signal generation and its proportionality to quantity of the analyte, it’s specificity to that analyte, reproducibility, repeatability and above an all the stability of signal. Some of the characteristic properties used alongwith this application in various instrumental methods are given in Table 1.4.

An analytical instrument can be viewed as a communication tool between the analyst and the system under study. Generally, a stimulus like electromagnetic radiation, electrical, mechnical or nuclear energy is used to retrieve the desired information from the analyte. The information is contained in the phenomenon that results from the interaction of the stimulus with the analyte. For example when a monochromatic electromagnetic radiation is passed through a sample, some of the light is absorbed by the analyte and consequently intensity is decreased which is directly related to concentration of the analyte. Intensity of light is measured before and after is interaction with the sample to obtain a measure of the analyte concentration.

Table 1.4: Chemical and Physical properties used in Instrumental Methods

Characteristic properties	Instrumental Methods
Emission of radiation	Emission Spectroscopy (X-ray, UV, Visible, electron, Auger); fluorescence, phosphorescence, and luminescence (X-ray, UV, and visible)
Absorption of radiation	Spectrophotometry and photometry (X-ray, UV, visible, IR); photoacoustic spectroscopy; nuclear magnetic and electron spin resonance spectroscopy
Scattering of radiation	Turbidimetry; Nephelometry; Raman spectroscopy
Refraction of radiation	Refractometry; Interferometry
Diffraction of radiation	X-ray and electron diffraction methods
Rotation of radiation	Potentiometry; Chronopotentiometry
Electrical charge	Coulometry
Electrical current	Amperometry; polarography
Electrical resistance	Conductometry
Mass	Gravimetry (quartz crystal microbalance)
Mass - to - Charge ratio	Mass spectrometry
Rate of reaction	Kinetic methods
Thermal characteristics	Thermal gravimetry and titrimetry; differential scanning calorimetry; differential thermal analyses; thermal conductometric methods
Radioactivity	Activation and isotope dilution methods

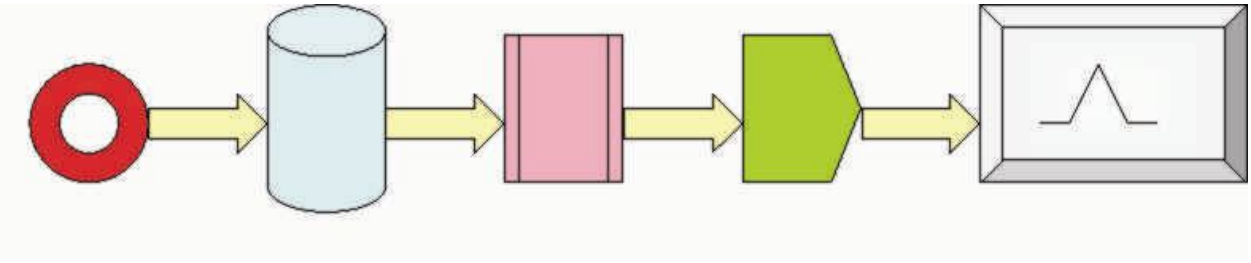
Table 1.5 : Some examples of Instrument Components

Instrument	Energy source (stimulus)	Analytical information	Information sorter	Input transducer	Data of Domain of transduced information	Signal processor readout
Photometer	Tungsten lamp	Attenuated light beam	Filter	Photodiode	Electrical current	Amplifier digitizer LED display
Atomic emission spectrometer	Inductively coupled plasma	UV or visible radiation	Monochromator	Photomultiplier tube	Electrical current	Amplifier digitizer digital display
Coulometer	Direct current source	Charge required to reduce oxidize analyte	Cell potential	Electrodes	Time	Amplifier digital timer
pH meter	Sample / glass electrode	Hydrogen ion activity	Glass electrode	Glass – calomel electrodes	Electrical voltage	Amplifier digitizer digital display
Mass spectrometer	Ion source	Mass-to-charge ratio	Mass analyzer	Electron multiplier	Electrical current	Amplifier digitizer computer display
Gas chromatograph with flame ionization	Flame	Ion concentration vs time	Chromatographic column	Biased electrodes	Electrical current	Electrometer digitizer computer system

Tables 1.4 and 1.5 are from Instrumental Analysis by Skoog, Holler and Crouch, Brooks / Cole, 2007.

A wide variety of devices are used to convert information from one form to another. Representation of the information is called encoding and nodes of encoding

information are called data domains. Data domain are broadly classified as non-electrical domains (length, density, pressure, intensity of light etc.) and electrical domains (current voltage charge frequency etc.). Any measurement is considered as a series of interdomain conversions. For example to measure fluorescence of quinine in water, the block diagram represents various steps in the final signal generation.



Stimulus **Sample** **Detector** **Signal** **Readout**

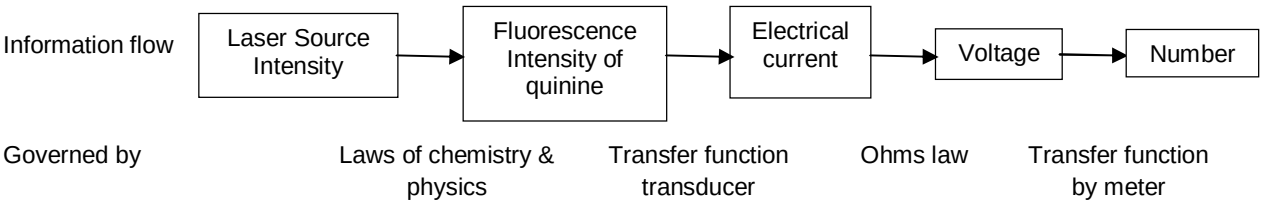


Fig. 1.2 : Block diagram of an analytical instrument

Measurement of intensity of fluorescence is most important as it is proportional to the concentration of quinine in water while water does not contribute to the signal. Laser stimulates the analyte by providing electromagnetic energy. This radiation interact which quinine to produce fluorescence, characteristic of quinine. Radiation unrelated to quinine is removed from the beam of the light using an optical filter. The intensity of fluorescence, a non electrical information, is encoded into an electrical signal by the device input transducer (phototransducer) which converts input radiant power impinging on it into electrical output (current). The current from the phototransducer is passed through the resistor to convert it into voltage. This voltage is proportional to current which in turn is proportional to fluorescence and thus to the concentration of quinine. Voltage is passed through a meter that converts it into digital number for direct reading.

Calibration

Since the signal obtained in the instrumental method is proportional to the concentration, it has to be converted to absolute concentration. This can be achieved by repeating the measurement with a standard samples having a know amount of the analyte and comparing signal from samples with that of the standard. Since the determination of the analytes depends on the signals that is generated and manipulated through a series of steps, it is possible that there could be a signal without the analyte (blank). It is absolutely essential to analyse blank samples in an identical manner like the analysis of samples and standards. Care should be taken that blank signal, if any, has to be subtracted from the signal of the sample. More appropriately this can be achieved by measuring the signals for a series of standards that covers the dynamic range of the instrumental method. Calibration involves plotting the signal from the standards as a function of the mass of the analyte on the premise that the range of the mass gives a linear relations with the signal. From the slope and intercept, concentration of the

analyte in the sample can be calculated. Another method is to use an internal standard in which constant quantity of the analyte is added to all the samples and blanks. From the signals and using appropriate manipulations analyte concentration can be obtained.

Treatment of Analytical Data

It is difficult and often impossible to have a total control on experimental variables and thus analytical measurements yield a range of results. Sampling methods, analytical techniques and instrument signals are potential sources of error. A measurement, repeated under identical conditions, yields a range of results. For example, in titration the titre value of triplicate / replicate titration is not expected to be same and thus the titre value flucations in a small range of a central value. The results have to be objectively evaluated to arrive at a reasonable value with its range by means of statistical methods of treatment of data. Often the range of values (uncertainty) is quoted as error. The sources for fluctuations can be due to random (indeterminate) and systematic (determinate) errors. A third type of error, which is encountered occasionally, is gross error, which arises in most instances from carelessness of the experimenter. Terms accuracy and precision are commonly used to define systematic error and random error respectively. Accuracy describes the correctness of an experimental result, essentially nearness to true value. The only type of measurement that can be completely accurate is one that involves counting of objects or head counting in a class room. All other measurements contain errors and give only an approximation of the true value. In chemical measurements, efforts are made to obtain value that is as close to true value as possible. In reality 'true value' is not known. Therefore, average value obtained from replicate measurements is taken as a starting point in statistical treatment of data.

Both absolute and relative errors bear a sign, a positive sign indicating that the measured result is greater than its true value and a negative sign the reverse.

Systematic errors have a definite value, have an assignable cause, and are of same sign and magnitude for replicate measurements made in exactly the same way. Thus systematic errors results in a bias in the measurement technique

Systematic errors are of three types: instrumental, personal and method errors. Systematic instrumental errors are commonly detected and corrected by calibration using suitable standards. Personal errors are those introduced into a measurement by judgment of experimenter and can be minimized by care and training. Method based errors are often introduced from non-ideal chemical and physical behavior of the reagents and reactions upon which an analysis is based. Possible sources include slowness or incompleteness of chemical reactions, losses by volatility, adsorption of analytes on solids, instability of reagents, contaminants, and chemical interferences. Systematic method errors are usually more difficult to detect and correct, than the instrumental and personal errors. The best and surest way involves validation of the method using it for the analysis of standard materials that resemble the samples to be analysed both in physical state and in chemical composition, if the analyte concentrations of these standards are known with a high degree of certainty.

Precision describes the reproducibility of results; for two or more replicate measurements, or measurements that have been made in exactly the same way. Three terms are used to describe precision of a set of replicate data: *standard deviation, variance, and coefficient of variation.* A careful distinction must be made between reproducibility and repeatability. Repeatability is within-run precision, measurements made in rapid succession whereas reproducibility is between-run precision; measurements made on different occasions.

Standard Deviation

In the absence of knowledge of true value, average value $\bar{x}$ is taken as the central tendency value and deviations of each measurements (x_i) from $\bar{x}$ are computed. Root mean square deviation is taken as a convention, as standard deviation. The standard deviation (s) for a set of data that is of limited size (n= 20) is given by equation

$$s = [\Sigma (x_i - \bar{x})^2/(n-1)]^{1/2}$$

Root mean square of the deviations represents the spread and thus (s) is a measure of precision. As already one degree of freedom is used to calculate $\bar{x}$ assuming that it represents central value, the number of degrees of freedom is reduced by one, else s will be underestimated (smaller value).

Variance is the square of standard deviation (s^2). Precision of measurements in terms of standard deviation rather than variance is preferred because the standard deviation carries the same units as the measurement itself. Relative standard deviation of a data sample is given by

$$RSD = s / \bar{x}$$

The relative standard deviation expressed as a percent is also known as the coefficient of variation (cv) for the data.

$$CV = \% RSD = (s / \bar{x}) 100$$

The mean and standard deviation for a set of data, called *statistics*, are of primary importance. The mean provides the best estimate of variable being measured and standard deviation of mean provides information about the random error associated with the measurement. A systematic error will not be revealed by statistical analysis; it must be sought by a careful study of the basic processes and methods employed. The statistical analysis of random errors can lead to certain conclusions with regard to the reproducibility of the measurements.

Distribution of errors

When analytical measurements are repeated on the same samples, the data of replicate measurements are scattered due to random errors. Random errors reflect in the imprecise data. This set of data is generally organized in equal sized data groups and corresponding frequencies are computed. The variation of frequency as a function of ascending value data groups results in a histogram. If the data set is very large tending to infinity then this histogram will become a Gaussian curve. A Gaussian distribution is characterized by the mean and width of the distribution (σ). The Gaussian curve has the following characteristics:

1. The most frequently observed result is the mean (μ) of the entire data set.
2. The data cluster symmetrically around the mean value.
3. Small deviations from μ are more frequent than large deviations.

4. In the absence of systematic errors, μ approaches true value, although in practice always there would be some systematic error.

The mean of a finite set of data rapidly approaches the true mean when the number of value increases beyond 20. However, in practice 20 replicates are difficult to take. Narrow distribution indicates that the data set has a better precision.

From the Gaussian curve it can be calculated that 68.3% of the data lie within $\pm 1\sigma$. Similarly 95.4% and 99.7% values lie within $\pm 2\sigma$ and $\pm 3\sigma$ respectively.

Student's t Test

(If calculated t is greater than value shown, reject the null hypothesis.)

SIGNIFICANCE LEVEL FOR ONE-DIRECTION TEST			
df	.10	.05	.01
1	3.078	6.314	31.821
2	1.886	2.920	6.965
3	1.638	2.353	4.541
4	1.533	2.132	3.747
5	1.476	2.015	3.365
6	1.440	1.943	3.143
7	1.415	1.895	2.998
8	1.397	1.860	2.896
9	1.383	1.833	2.821
10	1.372	1.812	2.764
11	1.363	1.796	2.718
12	1.356	1.782	2.681
13	1.350	1.771	2.650
14	1.345	1.761	2.624
15	1.341	1.753	2.602
16	1.337	1.746	2.583
17	1.333	1.740	2.567
18	1.330	1.734	2.552
19	1.328	1.729	2.539
20	1.325	1.725	2.528
21	1.323	1.721	2.518
22	1.321	1.717	2.508
23	1.319	1.714	2.500
24	1.318	1.711	2.492
25	1.316	2.060	2.787
26	1.315	2.056	2.779
27	1.314	2.052	2.771
28	1.313	2.048	2.763
29	1.311	2.045	2.756
30	1.310	2.042	2.750
120	1.289	1.980	2.617

Confidence Intervals

In a chemical analysis the true value of the mean cannot be determined as it needs large number of replicate measurements. Statistics are helpful in establishing intervals surrounding an experimentally determined mean (x) within which the population mean μ is expected to lie with a certain degree of probability. This interval is known as Confidence Interval (CI). In a measurement if the measured value is 6.10 ± 0.11 with a probability of 99%, then it means that the measured value lies in the interval of 5.99 to 6.21 with 99% probability.

Confidence Intervals can be used when sigma is unknown using a comparison test known as t test. t is calculated as $t=(\bar{x}-\mu)/s$. For the mean of n measurements, t can be calculated as $t=(\bar{x}-\mu)/(s/\sqrt{n})$, where s is a good estimate of σ. The calculated t value is compared with that of the computed value given in Table 1.5. If the calculated t value is larger than the computed value then the two values differ significantly (Null hypothesis is rejected.

Some Aspects of Quality in Analytical Chemistry

Analytical Method Validation

Analytical chemists endeavor to develop new analytical methods that are recognized or accepted as standard methods. For any new method developed, one has to finalize the optimum experimental conditions. It has to be tested for providing results with acceptable precision and accuracy. For optimizing, influences of various parameters are assessed both by experiments and mathematical models, if possible. An optimized method would be adopted if it is demonstrated that acceptable results could be obtained by this method. Similarly a method is verified for single operator characteristic, method, precision, accuracy, detection limit etc. The ultimate step is to establish that the method is transferable to other laboratories. An important step towards this is collaborative testing of the method. Thus analytical method validation is a process of performing several tests designed to verify that an analytical test system is suitable for its intended purpose and is capable of providing useful and valid analytical data. A validation study involves testing multiple attributes of a method to determine that it can provide useful and valid data when used routinely. First critical step for establishing the requirements of the analysis using this method is “study design”. The purpose of the method must be understood, and the accuracy limits must be set. These may include analysis of reference standards, blanks and samples with known concentrations.

The method validation process includes the following steps:

1. Establishment of the intended use of the method and its performance requirements.
2. Definition of the analytical method to be validated (this may involve preliminary method development activities).
3. Development of a specific validation protocol, including descriptions of the parameters to be assessed, test procedures and criteria for results.
4. Approval of the validation study protocol.
5. Performance of the study as described in the protocol, and verification that the results for all tests meet acceptance criteria.
6. Quality control (QC) of all data.

Because the design of a validation study is dependent on the method type, its intended use and the specific method requirements, protocol for each validation study has to be finalized. It shall cover accuracy, precision, specificity, linearity, range, robustness, lower limit of quanititation and intermediate precision. The degree of precision obtained when the method is performed over multiple test runs, on different days, by different analysts, using different equipment is known as intermediate precision.

Collaborative test

The goal of the collaborative test is to determine the expected magnitude of all three sources of error when a method is placed into general practice. When several analysts analyze the same sample one time, the variation in their collective results includes contributions from random errors and the systematic errors (biases) unique to the analysts. The position of the distribution of all the data can be used to detect the presence of a systematic error in the method. Collaborative testing provides a means of estimating the reproducibility. Two sample collaborative tests is also used for method validation, details are not included here. Analyzing reference material, blanks and blind samples, at times, is adequate for method validation though it is not complete.

Introduction to GLP

The term ***good laboratory practice*** (**GLP)** refers to a quality system for research and testing laboratories to try to ensure the uniformity, consistency, reliability, reproducibility, quality, and integrity of analytical data. GLP is concerned with the organizational processes and conditions under which test procedures are planned, performed, monitored, recorded, archived and reported. The primary objective of Good Laboratory Practice (GLP) is to ensure the generation of high quality and reliable test data.

Need for GLP

- Development of quality test data
- Mutual acceptance of data
- Avoid duplication of data
- Avoid technical barriers to trade
- Protection of human health and the environment

Scope of GLP:

GLP is indispensible in testing items contained in:

- Pharmaceutical products
- Pesticide products
- Cosmetic products
- Veterinary drugs
- Food and feed additives
- Industrial chemicals
- Nuclear industry

GLP principles include:

1. Test facility organization and personnel:

It is important to provide a sufficient number of qualified personnel, appropriate facilities, equipment and materials to carry out the analytical activities. Records of qualifications, job descriptions, training and experience of personnel are to be maintained. Laboratory personnel should understand their roles and responsibilities associated with their job.

2. Quality Assurance Program(QAP):

A well documented Quality Assurance Program (QAP) must be in place and individuals need to be designated as members of the QA team directly responsible to the management. It must be ensured that QA members not involved in the conduct of the study being assured. Periodic inspections should be carried out to determine compliance with GLP principles. Three types of inspection are routinely carried out:

- Study-based inspections
- Facility-based inspections
- Process-based inspections

3. Facilities:

Facility in which the work is carried out should be suitable with regards to size, construction and location. Adequate degree of separation of the different activities must be ensured. Suitable storage rooms for supplies and equipment must be provided. Archive facilities need to be envisaged for easy retrieval of study plans, raw data, final reports, samples of test items and specimen. Handling and disposal of chemicals must be carried out without affecting the integrity of the study or the environment.

4. Apparatus materials and reagents:

Apparatus should be of appropriate design and adequate capacity. Records of inspection, cleaning, maintenance and calibration of apparatus should be documented. Apparatus and equipment need to be calibrated to be traceable to national or international standards. Chemicals, reagent and solutions should be labeled to indicate identity, expiry and specific storage instructions.

5. Test systems:

Apparatus used for the generation of data must be appropriate design and of adequate capacity.

6. Test and reference items:

Receipt, handling, sub-sampling and storage of the samples must be done in a proper manner to ensure homogeneity and stability and avoid contamination or mix-up.

7. Standard Operating Procedures (SOPs):

Approved SOPs must be strictly followed to ensure the quality and integrity of the laboratory data. Any deviations from SOPs have to be ratified by the appropriate authority.

8. Performance of the study:

Title, nature and purpose of the study, test item identity and reference item used must be recorded along with information concerning the customer and facility.

9. Reporting of study results:

Final report generated for each study has to be signed sign with date of reporting. The report has to be approved by the competent authority. Corrections, additions or amendments should be signed and dated by the competent authority.

10. Storage and retention of records and materials:

The data on the following items need to be stored and archived:

1. The study plan, raw data and samples
2. Inspection data and master schedules
3. Qualification, training experience and job description
4. Maintenance and calibration data
5. Validation data
6. SOPs
7. Environmental, health & safety monitoring records

The essence of GLP can be summarized in a few short, general phrases:

- Know your experiment.
- Ask questions if you are unsure.
- Plan and prepare thoroughly well ahead of time.
- Clean your work area frequently.
- The work area shall be well kept.
- Pay attention and stay alert to what you are doing at all times.
- Safety aspects must be considered at all times.

Introduction to QA / QC

It is the endeavour of the analytical chemists to provide data of high quality. Analytical data are considered to be of high ***quality if they are fit for their intended purpose like use in operations, decision making and planning***. Six important characteristics of a high quality data are ***Accuracy, Validity, Reliability, Timeliness, Relevance and Completeness***. The concept of Quality Assurance and Quality Control assumes significance in achieving these six quality characteristics.

Quality Assurance comprises of all the planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy defined requirements of quality. Two principles included in QA are: "Fit for purpose", the product should be suitable for

the intended purpose; and "Right, the first time" and therefore mistakes should be minimized if they cannot be eliminated. QA includes management of the quality of raw materials, assemblies, products and components, services related to production, and management, production and inspection processes for which reliable analytical data play pivotal role.

Quality Control in Analytical Chemistry refers to all those processes and procedures designed to ensure that the results of a laboratory analysis are consistent, accurate, within specified limits of precision and comparable with the best international laboratories in the field. QC processes are of particular importance in Laboratories analysing critical samples where the concentration of chemical species present may be extremely low and close to the detection limit of the analytical method and also situations where decision making depends on measured data for example forensic cases, clinical support and high-tech materials.

The difference is that QA is process oriented and QC is product oriented. Quality Assurance makes sure one is doing the right things in the right way. Quality Control ensures the results of expected quality.

The three important terminologies in Quality can be summarized as:

- Quality Assurance: A set of activities designed to ensure that the analytical process is adequate to ensure that it will meet its objectives.
- Quality Control: A set of activities designed to evaluate an analytical work product. Input for Quality Assurance is data generated by Quality control.
- Testing: The process of executing a system with the intent of finding defects

LABORATORY EXPERIMENTS

Chapter 2

ANALYTICAL SPECTROSCOPY

Sanjukta A. Kumar, K.K. Swain, A.C. Sahayam, C. Venkateswarlu*, S. Thangavelu*, G. Kiran Kumar, R.K. Singhal, MKT. Bassan, Manisha V., T.S. Reddy [#] and A.V.R. Reddy*

** NCCCM, Hyderabad, [#] S.K. University, Anantapur*

Introduction

When a beam of monochromatic light passes through a medium, the species present in the medium absorb the light. If the wavelength of the light matches with the difference between a pair of molecular states (allowed transition), the intensity is gradually reduced as the beam progresses through the medium. The reduction in intensity is directly proportional to the concentration of the absorbing species. Beer-Lambert's law is applicable to relate the reduction in intensity with concentration of the absorbing substances in the solution.

When light at the resonance wavelength from the source of initial intensity, I_0, is focused on the cell containing ground state molecules, the intensity is decreased by an amount determined by the concentration of molecules. The light is then directed into the detector where the reduced intensity, I, is measured.

$$\text{Absorbance, } A = \log I_0/I = \epsilon bc \qquad (2.1)$$

where, ϵ is absorptivity, b is the length of the cell and c is the concentration of the absorbing species. A is directly proportional to concentration of the species with a characteristic ϵ, for a cell of constant path length.

Measurement in UV-visible region is extensively carried out to estimate the absorbing species which in turn can be utilized for physico-chemical characterization and analytical determination of molecules. These measuring devices are known as spectrophotometers. The analytical sensitivity of spectrophotometric analysis depends on the magnitude of A, minimum absorbance that can be measured and interferences at the chosen wavelength.

Generally, the molecular absorption spectra are not sharp and often suffer from interferences from other species present in the matrix. Atomic absorption spectroscopy (AAS) is a technique in which molecules are atomized and absorbance by individual atoms is measured. Selectivity of AAS is achieved by choosing the wavelength that corresponds to specific difference of energy states of the atoms. The atomic absorption follows Beer-Lambert's law. The concentration of an element in an unknown sample solution can be determined from the calibration graph obtained by plotting absorbance vs concentration of known standard solutions of the analyte element. The determination of any element with enhanced sensitivity in any form of AAS depends on the availability of more number of ground state atoms.

Atomic emission spectroscopy (AES) is the standard method that is used for most of the metals. In AES, a small part of the sample is vaporized and excited to the point of emission. The energy required for this is provided by thermal, electrical or plasma heating. For example, in ICP-AES, the sample is aspirated into the plasma. The high temperature of the plasma

(~10000K) causes dissociation of the sample into atoms and ions and excites them to higher energy levels. The excited atoms/ions de-excite through thermal or radiative (emission) transitions to the lower energy states. The light emitted in the radiative transition corresponds to specific wavelengths characteristic to the analytes which are measured using suitable detectors (PMT/CCD). The emission intensity is proportional to the analyte concentration.

In this chapter a few experiments on absorption and emission spectroscopy are described. In addition, an experiment on ICP-MS is included.

Experiment 1

Spectrophotometric Determination of Fe in Water Sample using Standard Addition Method.

Fe^{3+} reacts with excess of thiocyanate (SCN^-) to give an intense red colour complex. Absorbance of this $[Fe(SCN)_6]^{3-}$ complex at λ_{max}= 480 nm is proportional to the concentration of Fe^{3+} ion in the sample. Ideally analysis should be carried out using matrix matched standards. However for complex matrix, where it is difficult to get a matrix matched standard, standard addition method of calibration is used. In standard addition method varying amount of standard solutions are added to a fixed aliquot of the sample and the resulting absorbance is measured. Absorbance corrected for volume addition, is proportional to the amount of standard added.

For sample, concentration of Fe^{3+} (C_s) is related to absorbance as

$$A = \varepsilon b C_s \frac{V_s}{V_t} \qquad (2.2)$$

where V_s is the fixed volume of the sample diluted to the total volume V_t.

When standard solution of volume V_k of concentration C_k is added to the fixed volume of the sample V_s and made upto total volume V_t, then the absorbane A_i is given as:

$$A_i = \varepsilon b C_s \frac{V_s}{V_t} + \varepsilon b C_k \frac{V_k}{V_t} \qquad (2.3)$$

First term is a constant for a given sample and the second term varies with V_K. A plot of V_k(Fe^{3+} standard solution added) versus A_i gives a straight line. The slope m and y- intercept i are given as:

$$m = \varepsilon b C_k \frac{1}{V_t} \qquad i = \varepsilon b C_s \frac{V_s}{V_t} \qquad (2.4)$$

Thus,

$$\frac{i}{m} = \frac{C_s V_s}{C_k} \qquad (2.5)$$

From equation 2.5 concentration C_s is calculated as,

$$C_s = \frac{i}{m}\frac{C_k}{V_s} \qquad (2.6)$$

Materials/Chemicals required:

Standards Fe^{3+} solution of 100 µg mL^{-1}, SCN^- solution, 50 mL and 1000 mL volumetric flasks, 1 mL transfer pipette.

Procedure:

1. Prepare standard solution of Fe^{3+} by dissolving 0.864 g ammonium iron(III) sulphate in water. To it add 10 mL concentrated hydrochloric acid and dilute it to 1L. This will give a 100 µg mL^{-1} Fe^{3+} solution.
2. Prepare thiocyanate solution by dissolving 20 g of potassium thiocyanate in 100 mL water.
3. Take six 50 mL volumetric flasks and transfer 30mL of water samples to each flask. Label them as 1, 2, 3, 4, 5 and 6.
4. Add 0, 1, 2, 3, 4, and 5 mL of standard Fe^{3+} solution.
5. Add excess of SCN^- solution (5mL) and 3mL of 4 M nitric acid. Make up to the volume by adding deionised water.
6. Prepare a reagent blank using the same quantities of reagents. In place of 30 mL sample take 30 mL deionised water.
7. Take the reagent blank in reference cell of the instrument. Transfer 2.5 mL aliquot from the flask 1 to the sample cell and measure the absorbance at 480 nm.
8. Repeat step 7 for flasks 2-6 and note the absorbance.

Instrument Model:

Table 2.1: Operating conditions of the Spectrophotometer

Analyte

Wavelength maximum(λ_{max} nm)

Source

Detector

Table 2.2: Observations

Flask No	1	2	3	4	5	6
Absorbance						

Calibration Plot

Plot absorbance (A) as a function of V_k. Obtain the slope m and y-intercept i.

Calculation

Concentration of Fe^{3+} in the sample (C_s) is given by

$$C_s = \frac{i}{m}\frac{C_k}{V_s}$$

Calculate the standard deviation for the three replicate measurements

Measured Concentration of Fe^{3+} in the sample = _____

Comment:

1. Is it possible to calculate ϵ?
2. If there are interfering elements does this procedure work?

Experiment 2

Determination of Complex Ion Composition by Job's Method of Continuous Variation

Composition of complex ions in solutions and their formation constants can be determined by spectrophotometry. Absorbance measurements are performed without disturbing their equilibrium. One of the simpler techniques to determine composition of binary compounds or complexes is the method of continuous variation or the Job's Method. In this method cation solution and ligand solution with identical concentration mixed in such a way that the total volume and total moles of reactants in all the possible mixtures is constant but the mole ratio of reactants vary from 0 to 1. Absorbance of each solution is measured by fixing the wavelength generally around λ_{max} of the mixture. The absorbance starts to increase with the increase of mole ratio. It reaches a maximum value when the complexation is completed and starts to decrease after that. The initial rise is due to the increase in concentration of the complex and later decrease is due to the dilution of the complex. The absorbances are plotted against the mole ratios, which results in an inverted parabola contained in two straight lines starting from mole ratios of 0 and 1. An intersection point is obtained by extrapolating these two lines corresponding to the composition ratio of complex ion.

In this experiment composition of complex ion Fe^{3+} (salicylic acid) will be determined.

Materials/Chemicals required:

Ferric nitrate, salicylic acid, 10 mL Round botton flasks, spectrophotometer, 25 mL volumetric flasks, 1 mL transfer pipette.

Procedure:

1. Prepare standard solution of 0.0025 M Fe^{3+} by dissolving appropriate amount of ferric nitrate in 0.0025 M sulphric acid.
2. Prepare 0.0025 M salicylic acid solution.
3. Take nine 25 mL volumetric flasks and label them as 1 to 9.
4. Transfer 1 mL of Fe^{3+} solution and add 9 mL of ligand solution to bottle 1. Make up the volume.
5. Repeat this to make solutions of ratios 2:8, 3:7 etc.
6. Transfer 2.5 mL aliquot from the flask 1 to the sample cell and measure the absorbance at 525 nm and record the absorbance spectrum in one of the mixtures and λ_{max} is obtained.
7. Repeat step 6 for pure metal solution labeled 0 and pure ligand solution labeled 10
8. Calculate mole ratios of all the 11 solutions and tabulate the absorbance and corresponding mole ratios.
9. Plot absorbance as a function of mole ratios in a linear graph paper,
10. Extrapolate straight line plots on either side to get inflexion point. Note the corresponding mole ratio which gives the composition of complex ion.

Instrument Model:

Table 2.3: Operating conditions of the Spectrophotometer

Analyte

Wavelength maximum(λ_{max} nm)

Source

Detector

Table 2.4: Observations

Flask No	1	2	3	4	5	6	7	8	9
Absorbance									

Absorbance Plot

Plot absorbance (A) as a function of the Mole ratio. Obtain the inflexion point

Calculation

Molar ratio observed : _____

Calculate the composition of complex ion.

Experiment 3

Determination of Fe in Copper Metal by Flame Atomic Absorption Spectrometry (FAAS)

Flame atomic absorption spectrometry (FAAS) is the most common spectroscopic technique for determination of trace elements in aqueous samples. In this technique, sample solution is aspirated into a flame (atomizer) where atoms of analytes are formed. The use of special light sources and careful selection of wavelength allow the specific quantitative determination of individual elements in the presence of others. This technique is advantageous due to its specificity, ease of operation and high sample throughput. A hollow cathode lamp (HCL), selective for the analyte of interest is used as the light source.

Materials/Chemicals required:

Copper metal (2 g), Fe standards of 1 mg mL^{-1} (5 mL), Concentrated HNO_3, Deionised water, 50 mL beakers, 25 mL standard flasks.

Procedure:

1. Accurately weigh about 250 mg of the copper metal and transfer into a 50 mL beaker.
2. Add 10 mL of 1:1 nitric acid, cover with a watch glass and heat slowly on a hot plate till the sample is dissolved.
3. Make-up the digest to 25 mL.
4. Repeat steps 2 to 3 for blank without the sample.
5. Prepare five calibration standard solutions of Fe by stepwise dilution of a 1 mg mL^{-1} Fe stock standard solution.
6. Measure the absorbance of Fe, for the standard as well as sample solutions and blank using the FAAS instrument at _____ nm.

Instrument Model:

Table 2.5: Operating conditions of the FAAS for the determination of Fe

Analyte	
Wavelength (nm)	
Source	
Atomizer	
Type of Monochromator and its resolution	
Detector	

Table 2.6: Observations

	Concentration of Fe taken ($\mu g\ mL^{-1}$)	**Absorbance**	**Concentration of Fe Measured ($\mu g\ mL^{-1}$)**
Standard - 1			
Standard - 2			
Standard - 3			
Standard - 4			
Standard - 5			
Process blank			
Sample 1			
Sample 2			
Sample 3			

Calibration Plot

Plot the absorbance v/s concentration for the five standards. Slope of the calibration plot gives absorbance per unit concentration of analyte.

Calculation

Concentration of Fe in the sample (C) is given by

$$C(mg\ kg^{-1}) = \frac{A_{sam}}{A_{std}} x \frac{DF}{m_{sam}} xV \qquad (2.7)$$

where, A_{sam} is absorbance of sample, A_{std} is absorbance of standard per ($\mu g\ mL^{-1}$) (from calibration plot), DF is the dilution factor(if diluted), V is the final volume made (mL), m_{sam} is the mass of sample in gram.

Calculate the standard deviation for the three replicate measurements

Measured Concentration of Fe in sample =

Calculate the concentration of Fe using a single point absorbance and compare the results.

Experiment 4

Determination of Trace Metals (Fe, Ni, Cu, Cr and Zn) in Environment Water Samples by Flame Atomic Absorption Spectrometry (FAAS).

Metal ions and metal complexes are natural constituents of the environment. Certain metals are essential for plant growth and for animal and human health. At high concentrations, trace metals can become toxic for living organisms and behave as conservative pollutants. Metals enter the environment mainly by two means: (i) natural processes (for example, erosion of rocks, volcanic activity, forest fires) and (ii) processes due to human activities. An anthropogenic activity may add considerable amounts of polluting compounds, which will influence the existing natural aquatic system. For the determination of trace metals in environment waters different spectrochemical methods are used. However, flame atomic absorption spectrometry (FAAS) is one of the most extensively used techniques for the determining various elements with significant precision and accuracy.

Materials:

Environment water samples, Fe, Cu, Ni, Cr and Zn standards of 1 mg mL^{-1} (5 mL each), deionized water, nitric acid, GBC SensAA FAAS instrument

Procedure

- **Preparation of Standards:**

Stock standard of 1mg mL^{-1} prepared from their respective salts or metals of pure form which are traceable to primary standards. From this stock, required standard solutions of Fe, Ni, Cu, Cr and Zn elements were prepared by suitable dilutions.

- **Samples:**

Samples were collected from _____ and filtered through a 0.45µm size filter paper and filterate is taken for the analysis.

1. Switch on the power supply and Instrument.
2. To start the flame AAS, fix the burner as per manual,
3. Switch on air compressor and open acetylene gas cylinder.
4. Fix the hollow cathode lamp in the turret as per manual.
5. Click on Method Window, right click on it, click properties, select FLAME and press OK.
6. Open the method window. Select element and wavelength
7. Enter the concentration of standards.
8. Check the fuel flow.
9. Click on sample window and enter calibration and samples list
10. Open Analysis window and create a new file.
11. Click on instrument window & check gas box status, it should display indicates “ready to ignite”.
12. Switch on exhaust.

13. Ignite the flame using flame ON/OFF button of the instrument.
14. Adjust the flame height by monitoring absorbance of standard to max. Using vertical and horizontal knobs.
15. Place the nebulizer tube in DM water and auto zero the instrument.
16. Start analysis using START icon on the result window.
17. First aspirate the standards into the flame in sequence and draw a calibration graph.
18. Now, aspirate the sample in to the flame and measure the absorbance. From slope of the calibration graph we can determine the concentration of the samples.
19. To stop analysis click STOP icon button on the result window.

Table 2.7: Experimental parameters

Sr.No.	Element	Wavelength (nm)
1	Fe	284.3
2	Cu	324.7
3	Cr	357.9
4	Ni	232.0
5	Zn	213.9

Table 2.8: Calibration

Fe		Cu		Cr		Ni		Zn	
Std ($\mu g\ mL^{-1}$)	A	Std ($\mu g\ mL^{-1}$)	A	Std ($\mu g\ mL^{-1}$)	A	Std ($\mu g\ mL^{-1}$)	A	Std ($\mu g\ mL^{-1}$)	A
Blank		Blank		Blank		Blank		Blank	
0.5		0.5		1.0		0.5		0.2	
1.0		1.0		2.0		1.0		0.5	
2.0		2.0		5.0		2.0		1.0	
4.0		4.0		10.0		4.0		2.0	

Table 2.9: Sample Results

Element		Sample 1	Sample 2	Sample 3
Fe	A			
	Conc.			
	RSD			
Cu	A			
	Conc.			
	RSD			
Cr	A			
	Conc.			
	RSD			
Ni	A			
	Conc.			
	RSD			
Zn	A			
	Conc.			

	RSD			

Concentration of analyte in the sample (C) is given by

$$C(mg\ L^{-1}) = \frac{A_{sam}}{A_{std}} xDF \qquad (2.8)$$

where, A_{sam} is absorbance of sample, A_{std} is absorbance of standard per (µg mL^{-1}) (from calibration plot), DF is the dilution factor

Table 2.10: Standard Addition Recovery

Element	Conc. in Sample (µg mL^{-1})	Conc. Added (µg mL^{-1})	Conc. Expected (µg mL^{-1})	Conc. Obtained (µg mL^{-1})	Recovery(%)
Fe					
Cu					
Cr					
Ni					
Zn					

Recovery (%) =

Table 2.11: Results

Element	Sample 1 (µg mL^{-1})	Sample 2 (µg mL^{-1})	Sample 3 (µg mL^{-1})
Fe			
Cu			
Cr			
Ni			
Zn			

Experiment 5

Determination of Cadmium (Cd) in Biological Reference Material using Graphite Furnace Atomic Absorption Spectrometry

Graphite furnace atomic absorption spectrometry (GFAAS) is one of the most widely used techniques for the determination of toxic analytes at ultra trace levels in various matrices. In this technique, 5 to 20 µL of sample is transferred to a graphite furnace (atomizer) and subjected to a programmed heating cycle. This facilitates atomization and an increased residence time (0.1-1 s) in the observation zone of semi enclosed graphite furnace. The enhanced sensitivity in GFAAS is thus due to longer residence time and higher sample transport efficiency. As it requires small sample volumes, it is one of the alternative techniques available for analysis of biological, toxic chemicals and radioactive materials.

Materials/Chemicals required

Biological reference material (sample, 500 mg), Cd standards of 1 mg mL^{-1} (5mL), Concentrated HNO_3, Concentrated $HClO_4$, H_2O_2, Deionized water, 50 mL beakers, 25 mL standard flasks.

Procedure

1. Transfer accurately weighed amount of about 200 mg of the reference material into the cavity of microwave digester.
2. Add 3 mL of nitric acid, 0.5 mL of perchloric acid and pre-digest at room temperature for 20 min and then close the digestion vials. Digest the sample using the optimized temperature program.
3. Cool the digest and add 2 mL of H_2O_2 and repeat the same temperature program.
4. Make-up the digest to 25 mL.
5. Take an aliquot from the step 4 and measure absorbance corresponding to Cd using GFAAS at _____ nm.
6. Repeat steps 2 to 5 for blank without the sample.
7. Prepare five calibration standard solutions of Cd by stepwise dilution of a 1 mg mL^{-1} Cd stock standard solution and measure absorbance corresponding to Cd .

Instrument Model:

Table 2.12. Operating conditions of the GFAAS for the determination of Cd

Analyte	
Wavelength (nm)	
Source	
Atomizer	
Type of Monochromator and its resolution	
Detector	

Table 2.13: Observations

	Concentration of Cd taken (ng mL^{-1})	Absorbance	Concentration of Cd Measured (ng mL^{-1})
Standard - 1			
Standard - 2			
Standard - 3			
Standard - 4			
Standard - 5			
Process blank			
Sample 1			
Sample 2			
Sample 3			

Table 2.14: Spike recovery of cadmium from the digested solution using GFAAS.

Type of sample	Conc. Added (ng mL^{-1})	Absorbance	Conc. Measured (ng mL^{-1})	Recovery* (%)
sample digest	-			
Sample digest + Cd std.1				
sample digest + Cd std.2				

*****Recovery (%) = Conc. Measured/Conc. Added X 100

Calibration Plot

Plot of the absorbance v/s concentration for the five standards. Slope of the calibration plot gives absorbance per unit concentration of analyte.

Calculation

Concentration of Cd in the sample (C) is given by

$$C(\mu g\ kg^{-1}) = \frac{A_{sam}}{A_{std}} x \frac{DF}{m_{sam}} xV \qquad (2.9)$$

where, A_{sam} is absorbance of sample, A_{std} is absorbance of standard per (ng mL^{-1}) (from calibration plot), DF is the dilution factor(if diluted), V is the final volume made (mL), m_{sam} is the mass of sample in grams.

Calculate the standard deviation for the three replicate measurements

Table 2.15: Results of cadmium in the biological reference material samples using GFAAS.

Biological reference material (μg kg^{-1})	
Certified value	Measured value

Experiment 6

Determination of Arsenic in Ground Water using High Resolution Continuum Source Hydride Generation Atomic Absorption Spectrometry (HG-AAS)

Determination of arsenic is very important in the ground water because of its high toxicity. Atomic absorption spectrometry (AAS) is one of the most widely used techniques for the determination of trace analytes in aqueous samples. AAS is a sequential technique which requires a different hollow cathode lamp (HCL) for every analyte. Recent advent in the technology has enabled the use of the continuum source based instrument for multi-elemental analysis using AAS. The high resolution continuum source atomic absorption spectrometry (HR-CS-AAS) is equipped with xenon short arc lamp as a continuum source. A high resolving power echelle monochromator separates the absorption line of interest from the continuum light. It is equipped with flame atomizer with liquid and gas (HG) introduction and also graphite tube atomizer with liquid, solid and gas sampling facilities.

Advantages of HR-CS-AAS

- Sequential multi-elemental analysis.
- Can select any wavelength between 180-800 nm with same intensity.
- No need for separate hollow cathode lamps for different analytes.
- Direct solid sample analysis.

Principle of HGAAS

Hydride generation is a process of efficient separation and transport of gaseous hydrides of elements like arsenic, antimony, selenium, bismuth, germanium, tellurium and tin from aqueous solution into a atomizer. The hydrides (e.g. AsH_3 or SeH_2) are formed by addition of sodium tetrahydroborate ($NaBH_4$) to the acidified samples. The generated gaseous hydrides are separated using gas liquid separator using argon as carrier gas and transported to a electrically heated quartz tube, where they get atomized.

Materials/Chemicals required

Ground water sample (10 mL), Concentrated HCl, Concentrated HNO_3, Sodium tetra hydro borate ($NaBH_4$) (5 g), NaOH (10 g), KI (1 g), Ascorbic acid (2 g), Deionized water, As standard of 1 mg mL^{-1} (5 mL), 50 mL beakers, 25 mL standard flasks.

Procedure

1. Prepare five calibration standard solutions of As by stepwise dilution of a 1 mg mL^{-1} As stock standard solution.
2. Filter groundwater samples using 0.45µm pre-washed membranes and store the samples in PFA container after acidifying with HNO_3 to pH 2.

3. Take 100 mL of sample. Add 2 mL 1:1 HNO_3 and 10 mL 1:1 HCl and heat it in a heating mantle until the volume reduces to 25 mL. Cool and quantitatively transfer the sample to volumetric flask, adjust the volume to 100 mL.
4. Take 10 mL above digested water sample and add 10 mL HCl and 5 mL of 5% KI and 5% ascorbic acid into the flask. Allow the mixture to stand for 1 hour at ambient temperature. Dilute to 100 mL in a volumetric flask after 1 hour.
   [*The efficiency of hydride generation depends on the oxidation state of arsenic. During the digestion all the arsenic is oxidized to As (V), but the hydride forming efficiency of As(III) is more compared to As(V). Hence, pre-reduction of As(V) to As (III) is necessary for efficient determination.*]
5. Place 100 mL of digested water in the auto sample container**.** Keep $NaBH_4$ (0.6%) stabilized with 0.5% NaOH and 7% HCl in the respective compartments of hydride generator and connect to peristaltic pump.
   [*On mixing the sample solution with acidified $NaBH_4$ generates the gaseous arsine (AsH_3). The arsine was separated by using gas liquid separator with argon as a carrier gas and transported to heated quartz cell. Where atomization of arsine takes places and generates arsenic atoms, which absorbs the source radiation giving corresponding absorbance.*]
6. Measure the absorbance of As, for the standard as well as sample solutions and blank using the HR-CS-AAS instrument at _____ nm.

Instrument Model:

Table 2.16: Observations

	Concentration of As taken (ng mL^{-1})	**Absorbance**	**Concentration of As Measured (ng mL^{-1})**
Standard - 1			
Standard - 2			
Standard - 3			
Standard - 4			
Standard - 5			
Process blank			
Sample 1			
Sample 2			
Sample 3			

Table 2.17: Spike recovery of Arsenic from the ground water sample using HG-CS-FAAS.

Type of sample	**Conc. Added (ng mL^{-1})**	**Absorbance**	**Conc. Measured (ng mL^{-1})**	**Recovery* (%)**
sample digest	**-**			
Sample digest + As std.1				
sample digest + As std.2				

*Recovery (%) = Conc. Measured/Conc. Added X 100

Calibration Plot

Plot of the absorbance v/s concentration for the five standards. Slope of the calibration plot gives absorbance per unit concentration of analyte.

Calculation

Concentration of As in the sample (C) is given by

$$C(\mu g\ L^{-1}) = \frac{A_{sam}}{A_{std}} xDF \qquad (2.10)$$

where, A_{sam} is absorbance of sample, A_{std} is absorbance of standard per (ng mL^{-1}) (from calibration plot), DF is the dilution factor.

Calculate the standard deviation for the three replicate measurements.

Measured Concentration of As (ng mL^{-1} or µg L^{-1}) in ground water = _____

Experiment 7

Determination of Cu, Ni and Zn in soil by ICP-OES

Inductively coupled plasma optical emission spectrometry (ICP-OES) is one of the versatile spectroscopic techniques for determination of trace elements in various matrices. In this technique, sample solution is aspirated into a plasma where atoms of analytes are formed and excited. The plasma itself acts as the source. This technique is advantageous due to its multi element capability, sensitivity and high sample throughput.

Materials/Chemicals required:

Soil sample (2 g), Concentrated HF, Concentrated HNO_3, Concentrated $HClO_4$, Cu, Ni and Zn standards of 1 mg mL^{-1} (5 mL each), Deionised water

Procedure

1. Weigh 50 mg of dry soil having particle size less than 2000 µm and dry ash in the microwave muffle furnace to remove the organic content.
2. Dissolve the residue using a microwave digestion system with 3 mL nitric acid, 0.5 mL HF and 0.5 mL perchloric acid. Take it in a platinum dish, evaporate to dryness and made upto 25 mL with 1% nitric acid.
3. Take an aliquot from the step 2 and take emission wavelengths of each element.
4. Emission intensity of Cu, Ni and Zn were measured at the chosen wavelengths using ICP-OES.
5. Repeat steps 2 to 4 for blank without the sample.
6. Prepare five mixed calibration standard solutions of Cu, Ni and Zn by stepwise dilution of a 1 mg mL^{-1} stock standard solutions.

Instrument Model: _____

Table 2.18: Observations

λ_{max} for Cu = _____, Ni = _____ and Zn = _____ nm.

	Concentration of analyte taken (μg mL^{-1})	**Emission intensity (counts)**		
		Cu	**Ni**	**Zn**
Standard – 1				
Standard - 2				
Standard - 3				
Standard - 4				
Standard - 5				
Process blank				
Sample 1				
Sample 2				
Sample 3				

Calibration Plot

Plot of the emission intensity v/s concentration for the five standards for each analyte. Slope of the calibration plot gives emission intensuty (counts) per unit concentration of analyte.

Calculation

Concentration of Analyte in the sample (C) is given by

$$C(mg\ kg^{-1}) = \frac{I_{sam}}{I_{std}} x \frac{DF}{m_{sam}} xV \qquad (2.11)$$

where, I_{sam} is counts of analyte in sample, I_{std} is counts of analyte in standard per (μg mL^{-1}) (from calibration plot), DF is the dilution factor (if diluted), V is the final volume made (mL), m_{sam} is the mass of sample in gram.

Calculate the standard deviation for the three replicate measurements.

Measured Concentration of Cu (mg kg^{-1}) in soil =
Measured Concentration of Ni (mg kg^{-1}) in soil =
Measured Concentration of Zn (mg kg^{-1}) in soil =

Experiment 8

Determination of Uranium in Ground Water by ICP- MS

Inductively coupled plasma mass spectrometry (ICP-MS) is a versatile and widely used tool for identification and determination of the elements in a wide variety of samples. The advantage of this technique over optical spectrometric methods include, simple spectra which are easy to interpret, capability of measuring atomic isotopic ratio, better detection limits and wide linear dynamic range.

Principle of Mass spectrometry

Mass spectrometry is an analytical technique to identify elements or molecules based on their mass/charge (m/z) ratio. Solution samples in the form of aerosols generated with the help of a nebuliser are passed into the plasma. The plasma dissociates the sample into its constituent ions. The ions are then extracted from the plasma and focused into the quadrupole mass analyser by a set of lenses where the ions are separated based on their mass-to-charge ratio. The ions are then detected by an electron multiplier detector.

Materials/Chemicals required

Ground water sample (10 mL), Concentrated nitric acid, U standard of 1 mg mL^{-1} (5 mL), Deionised water

Procedure

1. Filter groundwater samples using 0.45µm pre-washed membranes and store the samples in PFA container after acidifying with HNO_3 to pH 2.
2. Check the ground water samples for total dissolved salts (TDS). For this take 5 mL of the sample in a clean, dry and weighed 25 mL beaker. Dry the sample on a hot plate. After cooling the beaker to room temperature, take its weight.

   $$TDS\ (per\ mL) = (W_2 - W_1)/5 \qquad (2.12)$$

   where, W_1 is the weight of empty beaker, W_2 is the weight of beaker and dried sample

   When TDS is less than 2000 µg mL^{-1} samples can be analysed directly by ICP-MS, other wise dilute the sample to bring the TDS within 2000 µg mL^{-1}.

3. Prepare five calibration standard solutions of U by stepwise dilution of a 1 mg mL^{-1} U stock standard solution.
4. Monitor the counts at mass number 238. The integrated counts per seconds (ICPS) at mass number 238 is proportional to the uranium concentration.

Instrument Model: _____

Table 2.19: Operating conditions of the ICP-MS for the determination of U

Plasma power	
Mass-to-charge ratio	
Cool gas flow	
Auxiliary gas flow	
Nebulizer gas flow	
Detector	

Table 2.20: Observations

	Concentration of U (ng mL^{-1})	**ICPS**
Standard - 1		
Standard - 2		
Standard - 3		
Standard - 4		
Standard - 5		
Blank		
Sample 1		
Sample 2		
Sample 3		

Calibration Plot

Plot of the ICPS v/s concentration for the five standards. Slope of the calibration plot gives ICPS per unit concentration of analyte.

Calculation

Concentration of Uranium in the sample (C) is given by

$$C(\mu g\ L^{-1}) = \frac{ICPS_{sam}}{ICPS_{std}} xDF \qquad (2.13)$$

where, $ICPS_{sam}$ is counts of analyte per second in sample, $ICPS_{std}$ is counts of analyte per second in standard per (ng mL^{-1}) (from calibration plot), DF is the dilution factor.

Calculate the standard deviation for the three replicate measurements.

Measured Concentration of U (ng mL^{-1}) in ground water =

Chapter 3

Chromatographic Methods

Sangita D.Kumar, V. Hima Bindu, Neha Thakur, Ayushi, B.N.Singh, Niyoti Shenoy and Harshla Parab. * JNTU, Hyderabad.*

Introduction

A few methods of chemical analysis are truly specific to a particular analyte. It is often found that the analyte of interest must be separated from the myriad of individual compounds that may be present in a sample otherwise these compounds / species may interfere with the measurement of analyte by either contributing to the signal of the measurement or attenuating the signal. Many separating techniques are used either to remove the interfering compounds / species or to isolate and preconcentrate the analytes. Chromatographic techniques provide the analytical scientist with methods for separation of analytes, their detection and measurement. Chromatography is a physical separation technique in which the components of a mixture are separated by differences in their distribution between two phases: stationary and mobile phase. Mobile and stationary phases are chosen such that the degree of distribution of various components of the sample between these two phases is variable. It involves a sample (or sample extract) dissolution in a mobile phase (which may be a gas, a liquid or a supercritical fluid). The mobile phase is then forced through an immobile, immiscible stationary phase (which may be silica gel, ion exchange resin, molecular sieves etc.). The components of the sample have different affinities for each phase. A component which has a high affinity for the stationary phase will take longer to travel through it than a component which has a lower affinity. As a result of these differential mobilities various analytes of sample are separated from each other as they travel through the stationary phase. Often differences in migration rates separate the components into resolvable zones facilitating their analysis. Depending upon the retention time in the column the components will be eluted from the column at different times. Retention time (tr) is the time interval between the injection of the sample and the appearance of a solute peak at the detector.

Based on the mobile phase used chromatography is divided into three broad classes- (1) Liquid Chromatography (2) Gas Chromatography (3) Supercritical fluid Chromatography. Because of very high resolving power of instrumental liquid chromatography it is called as High performance liquid chromatography (HPLC). HPLC is an analytical technique based on the separation of the components of a mixture in a solution by selective adsorption/partition on the column. There are basically three modes of separation: liquid/solid, liquid/liquid and liquid/ fixed pore size material.

High performance thin layer chromatography (HPTLC) is a technique in which stationary phase consists of a layers of fine sorbent (3-5 µm silica gels) on a planar surface. The separation is based on the different affinities of the solutes for the adsorbent and the developing solvents.

Ion Exchange Chromatography (IEC) is a technique for separation of anions and cations using ion exchange resins. The main principle behind the separation of anions or cations is the difference between the affinities of the ions towards the resin. This affinity and hence separation of ions is governed by two factors a) Charge on the ions b) Size of the ion (hydrated). Ion

chromatography is high performance analytical technique for separation and quantification of anions and cations. The two types of ion chromatographic (IC) techniques in use are - Suppressed IC and Non Suppressed IC. In the case of suppressed IC a suppressor unit is installed after analytical column. It is needed for decreasing the background conductivity arising due to the mobile phase. In non Suppressed IC there is no suppressor; instead a mobile phase is chosen having low conductivity e.g. organic acid salts.

Gas chromatography (GC) is used to separate volatile components in a liquid/gaseous mixture. The different components are separated due to the differences in the adsorption/partition behavior between the carrier gas and stationary phase in the column. Depending upon the retention time in the column the volatile component is eluted at different times and detected by a suitable detector.

The Gas Chromatography/Mass Spectrometry (GC/MS) technique involves separation of components using gas chromatography and detection by mass spectrometry. Direct-coupled interface is the most common method for coupling GC with MS. Here the GC capillary column is inserted directly into the ion source. The identification of compounds is based on comparison of mass spectra with those in a mass spectral library.

Experiment 9

Determination of Pesticides (Organophosphate) in Soil Sample using HPLC

Instrumentation

High Performance Liquid Chromatography (HPLC) instrument consists of reservoirs of mobile phase, a pump, an injector, a separation column, and a detector. The sample mixture is loaded onto the column by means of an injection valve. The different components in the mixture pass through the column at different rates due to their different partition behavior between the mobile liquid phase and the stationary phase. The most widely used detector is UV-visible detector. In this case the chromatogram consists of the absorbance as a function of time. Organophosphate pesticides and their metabolites are separated using a step-gradient mobile phase.

Materials

Acetonitrile (1L), reference standards of Organophosphate pesticides, acetone, hexane, soil (100 g). Volumetric flasks, conical flasks and glass syringe.

Procedure

1. Take 25 g of soil and extract with acetone:hexane (1:1 v/v, 250 mL), agitate on a shaker (30 min.) collect the eluate, filter and concentrate to 100 mL.
2. Prepare a stock solution of the reference standard of pesticide in acetone:hexane.
3. Carry out serial dilutions from the stock reference standards of the pesticides for calibration. Label them as standard 1, standard 2 and standard 3.
4. Run HPLC as per SOP.

5. The initial mobile-phase composition is acetonitrile-water (55-45 v/v), which is held constant from 0.00 to 11.00 min. At 11.10 min, acetonitrile is increased to 65%. From 11.10 min, the gradient is programmed linearly to a final composition of acetonitrile-water (70-30 v/v) at 34.00 min.
6. Separations are performed on a 250 mm **x** 4.6 mm C-18 column with a particle size of ***5*** µm. A guard column (10 mm **x** 4.6 mm) is used along with the analytical column.
7. The flow rate kept is 1.0 mL/min, and injection volume is 20 µL.
8. Absorption spectra of the organophosphate pesticides are obtained by scanning wavelengths in the range 190-350 nm in 2-nm increments.
9. Individual wavelengths are monitored on the basis of sensitivity and/or interference considerations. The individual wavelengths monitored are 202,207,230,250,274, and 314 nm.
10. Again run HPLC as in step 5 and note the observations.
11. Take aliquot of clear solution from step 1 and inject the sample.

Observations

Pressure: _____ MPa
Flow rate: _____ mL min^{-1}
Standard wavelength: _____ nm

Table 3.1: Observations

	Absorbance	Peak Area	Concentration
Standard 1			
Standard 2			
Standard 3			
Sample			

Calculations:

I. Relative retention times and UV absorbance profiles characteristic for each compound are established using the UV-visible detector.
II. Construct the calibration curves by plotting peak area Vs concentration.
III. Calculate the coefficients of determination ($r^2 > 0.995$) for the regression curves.
IV. Determine the concentration in the sample from the calibration plot.

Results:

The concentration of organophosphate pesticides in soil sample is _____ ppm

Experiment 10

Determination of Anions in Aqueous Samples using Ion Chromatography

Principle:

A divalent species (sulphate and hydrogen phosphate) has stronger affinity towards the resin than the monovalent species (fluoride, chloride and nitrate) and hence are held up by the resin for a longer time. Among F^-, Cl^-, NO_3^- i.e. species of same charge F^- ions have the largest hydrated radius due to high charge density. Since hydrated radius is larger, it has least affinity towards the resin and hence F^- is eluted first followed by Cl^- and then NO_3^-. The technique employs eluents like Na_2CO_3, $NaHCO_3$, $Na_2B_4O_7$ or NaOH to separate the sample anions on a low capacity anion exchanger. Low capacity resins permit the separation to be performed with a relatively low eluent concentration. The eluent then flows through a second column (suppressor) which has a high capacity cation exchange resin in the hydrogen form. This converts the eluent into a low conducting weak acid and the sample anions to highly conducting acids. The sample is transported through the column by continuous flow of mobile phase. This process is called *elution*.

Instrumentation

1. Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.
2. Ion chromatograph - Analytical system complete with ion chromatograph and all required accessories anion analytical column, guard column, detectors and data system.

Materials

Sodium carbonate, sodium bicarbonate, sodium/ potassium salts of fluoride, chloride, nitrate, bromide, nitrite, sulphate, Water sample (25mL), Volumetric flasks and plastic syringe.

Procedure

1. Prepare the standard solutions of solutions of anions - fluoride, chloride, nitrate, bromide, nitrite and sulphate by weighing appropriate amount of salt of respective ions and dissolving them in de-ionized water.
2. For preparing 1000 ppm of the standard solution, following weights of corresponding salts are dissolved in 100 mL of de-ionized water.

Table 3.2: Parameters for preparation of standards

Standard solution	Salt	Weight (g)
F^-	NaF	2.210
Cl-	NaCl	1.649
NO_2^-	$NaNO_2$	1.499
Br^-	NaBr	1.288
NO_3^-	$NaNO_3$	1.371
SO_4^{2-}	K_2SO_4	1.8142

3. Appropriate dilutions of stock standards are carried out to prepare multianion stock standard solution (100ppm each). From these, serial dilutions are made to give concentrations in the range of interest.
4. Run IC as per SOP.
5. A small volume of sample, typically 20 to 100 µL, is introduced into an ion chromatograph.
6. The anions of interest are separated and measured using a conductivity detector.
7. Note the observations.

Observations

Mobile phase conductivity: _____ µS
Pressure: _____ MPa
Flow rate: _____ mL min^{-1}

Table 3.3: Observations

ANION (Standard)	Retention time	Peak area Std 1	Peak area Std 2	Peak area Std 3	Precision (SD)
Fluoride					
Chloride					
Nitrite					
Bromide					
Nitrate					
Sulphate					

Table 3.4: Observations

ANION (Sample)	Retention time	Peak area	Concentration mg L^{-1}
Fluoride			
Chloride			
Nitrite			
Bromide			
Nitrate			
Sulphate			

Calculations:

- Tabulate peak area response against the concentration for standard.
- The calibration curves for each anion should have the correlation coefficient ($r^2 \geq 0.995$).
- Determine the concentration of anion in the sample from the calibration plot.

Results:

Report the concentration of fluoride, chloride, nitrate, bromide, nitrite and sulphate in mgL^{-1}

Experiment 11

Determination of Hydrogen in the Gaseous Sample using Gas Chromatography

Instrumentation:

A gas chromatograph consists of carrier gas, a sample injection system, column, detector and a data recording system. The function of sample injection system is to vaporize the sample instantaneously so that sample is introduced as a vapour into the column. Liquid samples are generally injected by a graduated micro syringe through a self sealing rubber septum into a preheated injection port located at the head of the column. Typical liquid sample volumes used with packed column in GC from about 0.1 to 5.0 µL. Gaseous sample can also be injected by similar syringes which have gas tight plunger. The carrier gas transport the sample vapour from injector to the detector via column. The most commonly used carrier gases are helium, nitrogen, hydrogen and argon. The most commonly used detector in GC is thermal conductivity detector (TCD). It consists of electrically heated sensing element. The temperature of the sensing element depends on the surrounding carrier gas. When organic molecules displace some of the carrier gas, it causes a temperature rise in the element which is sensed by a change in the resistance.

Materials:

Hydrogen standard, carrier gas (N_2), Syringes.

Procedure

1. Run GC as per SOP.
2. Nitrogen is used as a carrier gas and the flow rate is kept as 30 mL min^{-1}.
3. Separations are performed on a Molecular sieve 5A^{o}column with a particle size of ***5*** µm.
4. The detector and the injector temperature are held at 100^{o}C while the column oven temperature is held at 180^{o}C.
5. A small volume of standard/sample, typically 10 to 100 µL, is introduced into a gas chromatograph.
6. Hydrogen in the gaseous sample is separated and measured using a thermal conductivity detector.
7. Record the observations in the table below.

Table 3.5: Observations

	Retention time	Peak Area(mV-sec)
Standard		
Standard		
Standard		
Sample		

$$\text{Percentage of purity of sample (gas)} = \frac{\text{Sample area}}{\text{Standard area}} \times \text{purity of standard gas}$$

Result:

The retention time obtained for sample is _____
The peak area obtained for sample is _____
The percentage purity of the sample is _____

Experiment 12

Determination of Hydrocarbons in a Sample by Gas Chromatography (GC)

Instrumentation:

For the detection of hydrocarbons Flame Ionization Detector (FID) is used. The eluted analyte from the column undergoes ionization or combustion in the flame which gives rise to an increase in the current. The output signal from the detector is recorded to obtain the chromatogram. Qualitative analysis is done by measuring retention time of the sample component and comparing it with the retention time of the known compounds. Quantitative analysis is based upon a comparison of either the peak height or the area of the analytical peak with that of standards.

Materials:

Reference material, carrier gas, Syringes.

Procedure:

1. Run GC as per SOP.
2. He gas (purity > 99.99%) is used as a carrier gas and the flow rate is kept as 25-30 mL min^{-1}.
3. Flow rate of the fuel gas is kept as 30 mL min^{-1}
4. The detector temperature is held at 120^{o}C while the column and injection port temperature is held at 80-100^{o}C.
5. Separations are performed on a silica gel column.
6. A small volume of standard/sample, typically 50 to 100 µL, is introduced into a gas chromatograph.
7. Hydrocarbons are separated and measured using FID.
8. Record the observations in the table below.

Table 3.6: Observations

No	Injection volume	Retention time	Peak height
1. Std mixture-CH_4			
2. Std mixture-CH_4			
3. Std mixture-CH_4			
4. H.C.sample-CH_4			
5. H.C.sample-CH_4			
6. H.C.sample-CH_4			

Calculations:

Let the concentration of the methane (**CH_4)** in the standard - A (ppm or in %)
Let the average peak height of the methane - B
Let the average peak height of the methane in a given sample- C
Therefore the concentration of methane in a sample: A/B) x C

In the same way, calculate the concentrations of other hydrocarbon in the given sample using the following data.

Table 3.7: Observations

No	Injection volume	Retention time	Peak height
1.Std mixture-C_2H_6			
2.Std mixture- C_2H_6			
3. Std mixture- C_2H_6			
4. Sample- C_2H_6			
5. Sample- C_2H_6			
6. Sample- C_2H_6			

Table 3.8: Observations

No	Injection volume	Retention time	Peak height
1. Std mixture-C_2H_4			
2. Std mixture- C_2H_4			
3. Std mixture- C_2H_4			
4. Sample- C_2H_4			
5. Sample- C_2H_4			
6. Sample- C_2H_4			

Table 3.9: Observations

No	Injection volume	Retention time	Peak height
1.Stdmixture-C_2H_2			
2.Stdmixture- C_2H_2			
3. Std mixture- C_2H_2			
4. Sample- C_2H_2			
5. Sample- C_2H_2			
6. Sample- C_2H_2			

Results:

Report the concentration of methane, ethane, ethylene and acetylene in the given sample as ppm.

Experiment 13

Determination of Organics in Ground Water using Gas Chromatography / Mass Spectrometry

Instrument

GC and MS instruments are interfaced in two ways: (a) open slit (b) direct coupling interface. In open slit interface the helium is sucked out before the sample goes on to the ionization chamber. Direct-coupling interface is the most common method, where the GC capillary is inserted directly into the ion source via length of fused capillary tubing and a vacuum tight flange. Most commonly used ionization sources are electron impact and chemical ionization in which the sample of interest is ionized with positive or negative charges. Mass analyzers are of many types, but widely used filter is quadruple mass analyzer. Each molecule results in a unique fragmentation pattern. Therefore, the pattern from the mass spectrum can be used to identify the molecule. The identification of compounds is based on comparison of mass spectra with those in a mass spectral library.

Materials

Reference material, carrier gas, Glass syringes

Sample preparation

Take 5 mL of the acidified sample, pH adjusted to 2.0. Extract the sample with 20 mL of dichloromethane. The mixture is taken in a separating funnel and mixed thoroughly for 20 min. Keep it aside for 15 min and then collect the organic layer.

Procedure:

- GC and MS parameter like temperature, flow rate and column are set according to the analytes.
- The automatic tuning is carried out using standard per flouro tributyl amine(PFTB) solution and when the instrument has passed all acceptable ion criteria proceed to step 3.
- The instrument is calibrated with 1-5 µL of standard.
- 1-5 µL of sample is injected.
- The response of MS detector after separation in GC column is monitored.
- The unknown compound is identified with library.
- The data is computerized generated.

Observations

1. Chromatogram
2. Peak report of TIC

Table 3.10: Observations

Peak	R. Time	Area	Area %	Name

Table 3.11: Results

Peak	R. Time	m/z	Area	concentration	Name

Results:

Report the concentration of organic compounds in ground water in ppb.

Chapter 4

NUCLEAR ANALYTICAL TECHNIQUES

A.V.R. Reddy, K.K. Swain, R. Acharya, D.D. Sood, Sanjiv Kumar* and G.L.N. Reddy*

* NCCCM, Hyderabad

Introduction

Nuclear analytical techniques (NATs) involve measurements of radiations from radioisotopes obtained from nuclear reactions or nuclear decay. In NATs, elemental concentrations are determined based on the measurement of intensities of radiations emitted by radioisotopes. There is neither a unique definition nor a sharp boundary for NATs. A number of NATs have been developed, demonstrated and are being deployed. Alpha spectrometry, γ-ray spectrometry, neutron activation analysis (NAA), prompt gamma neutron activation analysis (PGNAA), charged particle activation analysis (CPAA), radioimmunoassay, ion beam analysis (IBA) including nuclear reaction analysis (NRA), Rutherford's back scattering (RBS), particle induced γ-ray emission (PIGE) and other radiotracer based techniques are some of the popular NATs. Additionally, techniques like particle induced X-ray emission (PIXE) and X-ray flourscence (XRF) are kept under NATs as the measurement of X-rays using Si(Li) detector is similar as in some of the NATs. Most of the NATs are non-destructive and capable of simultaneous multi-element concentration determination in a variety of samples.

As nuclear properties like energy of radiations, half life and formation cross section are unique for most of the isotopes, these techniques are not influenced by chemical environment. It is also possible to check the internal consistency, e.g., in NAA, by determining concentration of an element through two or more isotopes of the same element. Detection limits and sensitivities are excellent in NATs, particularly in NAA for most of the elements. NATs are extensively used in many areas like analysis of reference materials, measurement of environmental radioactivity, multielement determination in environmental matrices of different origin and composition, large size sample analysis and use of radioisotopes as tracers. NAA in various forms stands out on accuracy and sensitivity. However, turnaround time for NAA using long-lived activation products is longer compared to XRF as well as spectroscopic techniques.

NATs are used to determine the activity present in an environmental sample or a mineral or induced activity in an activated sample or in a radio-pharmaceutical. The characteristic radiations like α, β, X-rays and γ-rays interact with matter (detector) and produce ionization and excitation which can be quantitatively measured. Silicon surface barrier detector, gas filled detector, NaI(Tl), Si(Li) and HPGe are some of the detectors used for radiation detection and measurement. In addition to good detectors, good electronics and software are important for reliable measurements. Detector calibration for energy and efficiency are requirements for qualitative and quantitative analysis.

Neutron activation analysis (NAA)

When a stable isotope of an element is exposed to neutrons some of the atoms absorb neutrons and are converted to the higher isotope of the element. This reaction is represented as follows:

$$^{A}X\ (n,\gamma)\ ^{A+1}X$$

where A is the mass number of stable isotope. Quite often the isotope ^{A+1}X is radioactive and can be used for quantitative determination of the element by measuring its radioactivity. For example:

$$^{55}Mn\ (\sigma = 13.3\ b) + n \rightarrow {}^{56}Mn^{*} \xrightarrow{\beta^{-},\ t_{1/2} = 2.58\ h} {}^{56}Fe \qquad (4.1)$$

Activity formed at the end of irradiation (A_0) is given by equation (4.2) and can be monitored using high-resolution gamma ray spectrometry.

$$A_0 = N\sigma\ \phi\ (1 - e^{-\lambda t_i}) \qquad (4.2)$$

where N is number of the target atoms of ^{A}X; σ is the neutron absorption cross section of ^{A}X; ϕ is the neutron flux; t_i is the duration of irradiation and λ is the decay constant of radioisotope ^{A+1}X that is formed.

Radioactivity of the irradiated sample can be measured by using a beta counter or a gamma spectrometer. Gamma measurement using a high resolution γ-ray spectrometry is commonly used in NAA as β-counting for multi-isotopes is difficult since β-spectrum is continuous unlike γ-spectrum.

Ion Beam Analysis (IBA)

Rutherford backscattering spectrometry (RBS) is an important ion beam analysis (IBA) technique for analyzing surface and near surface regions of materials. RBS is performed using protons, α-particles and other ion beams obtained from a particle accelerator as probes. It is used for identification as well as quantification of elements (isotopes). It is a simultaneous multielemental technique and is sensitive to light as well as heavy elements. It is used for the determination of thickness of films non-destructively by employing the concept of stopping power.

Experiment 14

Determination of Absolute Activity by High Resolution Gamma ray Spectrometry using High Purity Germanium (HPGe) Detector

The semiconductor detectors such as Ge(Li) and high purity germanium (HPGe) are used for high resolution gamma-ray spectrometric measurements. Germanium detectors have resolution of the order of 1.0 to 2.5 keV in the energy of range 100 to 2500 keV. Due to their excellent resolution, HPGe detectors are widely used in measuring the complex gamma ray spectra of radioactive samples containing many radioisotopes, which can be deconvoluted to obtain activities due to individual gamma ray peaks that are used to calculate the amounts of radioisotopes in the sample. A multichannel analyser (MCA) differentiates the incoming signal into peaks having different energies. To arrive at absolute activities from measured count rates, the efficiency of the detection system at different peak (energies) has to be known. The efficiency of detection decreases logarithmically as a function of energy, very similar to the cross section for photoelectric absorption. The count rate under a photo peak of a gamma ray is directly proportional to its activity and is given by eqn. 4.3.

$$C = A\, a_\gamma\, \varepsilon \qquad (4.3)$$

where C is count rate defined by counts per second (cps), A is disintegration rate per second (dps), a_γ is gamma ray abundance and ε is the absolute detection efficiency of the detector for the energy E. The detection efficiency for the energy in the range of 58 to 1408 keV is determined by measuring the photopeak areas of standard sources of known strengths of radionuclides such as ^{133}Ba, ^{152}Eu, ^{125}Sb and ^{134}Cs. A typical plot of efficiency v/s energy is given in Fig. 4.2. This experiment consists of the following: energy calibration, identification of unknown energy, efficiency calibration and estimation of the strength of the unknown source.

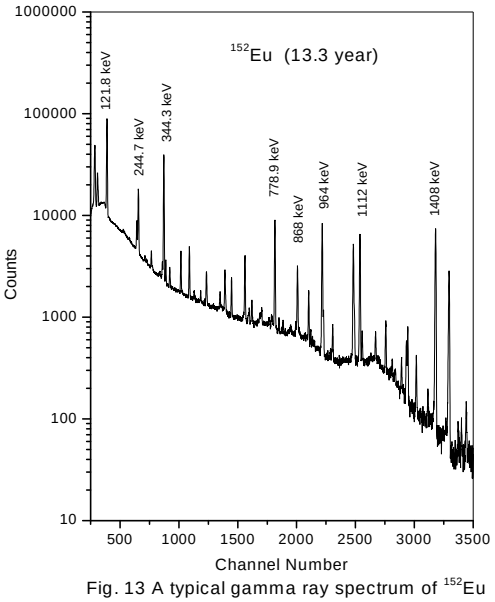


Fig. 13 A typical gamma ray spectrum of ^{152}Eu used for efficiency determination

Material Required

High Purity Germanium (HPGe) detector coupled to a Multi Channel Analyzer (MCA), radioactive sources: ^{241}Am, ^{137}Cs, ^{60}Co, ^{133}Ba, ^{152}Eu, and unknown sources.

Fig. 4.1: Gamma ray spectrum of ^{152}Eu acquired on an MCA using HPGe.

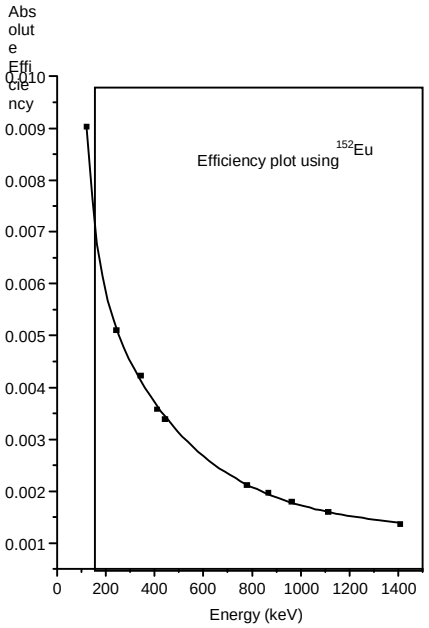


Fig. 4.2: Efficiency of HPGe detector as a function of gamma ray energy

Procedure

- Fill the Dewar of the HPGe detector with liquid nitrogen. The detector should be cooled at least for 2 hours before applying bias (High Voltage).
- Assemble the electronic units, HPGe detector and MCA. Make sure that the connections are made as given in the instruction manual. Note down the settings.
- Switch on the mains supply. After a few minutes, apply high voltage in steps of 100 volts slowly until rated voltage is reached. Allow to stabilize for an hour.
- Fix the plate containing the source in a shelf of the sample holder. Acquire spectrum for 1000 s. Use the same shelf for counting all sources.
- From the spectrum obtain the channel number corresponding to photo peak maximum.
- Repeat this with other sources and tabulate the results.
- Place the standard source of ^{152}Eu in a shelf of sample holder of HPGe detector. Acquire the γ-spectrum for a period of 1000 s and record the spectrum. Your spectrum should look like Fig. 4.1.
- Remove the source and acquire background spectrum for 2000 s.

Date:

Table 4.1: Parameters

Detector	: HPGe	Detector No.	:
Amplifier Model	:	High Voltage	: V
Settings for Multi-channel Analyser (MCA) and Sample details.			
Bias (to reduce Background)	: V	MCA Mode	: PHA
Amp Coarse gain	:	Amp Fine gain	:
Time Constant	:	Calibration standard	: ^{152}Eu
Shelf number	:	Sources	:^{241}Am,^{137}Cs, ^{60}Co

Table 4.2: Peak positions for energy calibration

S. No.	Source	E in keV	Centroid Channel No.
1	^{241}Am	60	
2	^{137}Cs	662	
3	^{60}Co	1172	
		1332	

Identification of gamma ray energy of an unknown source

Obtain a calibration graph by plotting channel No. on X-axis and the corresponding energy on Y-axis. It should be a straight line of the type E = mX + c, where X is the channel number corresponding to energy (E) and m and c are slope and intercept respectively. Calculate the calibration constants m and c:

m = _____ keV/channel and c = _____ keV

1. Determine the peak positions from the spectrum obtained using unknown source.
2. From the calibration plot, note the energy corresponding to the channel for each peak of the unknown source. It may be noted that most of the MCA systems have built in programmes for energy calibration.

Determination of detection efficiency of HPGe detector as a function of gamma ray energy.

Calculate peak areas corresponding to different gamma rays of ^{152}Eu. Record the channel number, area and FWHM for each peak in the table given below.

1. Calculate efficiency using the relation between dps and cps: $\varepsilon = C/Aa$ (Note: Calculate the dps as on the date of experiment using decay correction).
2. Plot efficiency ε as a function of energy E in keV on a semilog graph paper taking ε on Y-axis and E on X-axis.
3. Calculate the gamma abundances for the peaks at 411.1 keV and 867.3 keV. Your values should be 0.022 and 0.042 respectively
4. Find full width at half the maxima (FWHM) for at least four peaks.
5. Using gamma ray compilations, find the unknown nuclide. Also find its dps.
6. Fit the efficiency (ε) and energy (E) using eqn 4.4.

$$\ln \varepsilon = a + b \ln(E) + c (\ln(E))^2 \qquad (4.4)$$

where a, b, c are constants.
Source strength of ^{152}Eu: dps on:

Counting time: s

Table 4.3 : Peak areas for different gamma ray peaks of ^{152}Eu

Energy keV	γ-ray abundance (a_γ)	Channel No.	FWHM keV	Peak area counts	Efficiency
121.8	0.282				
244.7	0.074				
344.2	0.264				
778.9	0.130				
963.4	0.145				
1112.8	0.135				
1408.1	0.207				
1460					

Estimation of un**known activity**

1. Calculate peak area corresponding to 1460 keV.
2. Calculate its absolute activity using equation 4.3.

This activity is due to the γ-ray of ^{40}K, which is present in soil, plant, rocks, milk products etc.

Experiment 15

Determination of Radioactivity in Surface Soil, Cement and Fly Ash

Uranium and thorium present in soil/rock contribute to the presence of radioactivity in a variety of matrices we come across in our daily life. Some processes lead to enhanced concentration of radioisotopes. For example, burning of coal leads to higher levels of radioactivity in coal fly ash (activity / unit mass) than in coal. In addition, coal fly ash enters the respiratory system easily and results in internal exposure. This experiment aims at providing first hand information on the levels of radioactivity in surface soil, cement and coal fly ash.

Materials required

NaI(Tl) counter set up, different types of solid samples such as surface soil, cement and coal fly ash (100 g each), sample holders (7.5 cm dia x 1 cm depth, with cover) - 8 nos., standard soil sample prepared by mixing 200 mg each of natural uranium and thorium nitrates in 10 g of surface soil, and a weighing balance.

Procedure

1. Assemble the electronic units of NaI(Tl) with single channel analyzer and make connections as given in the instruction manual. Note down the settings.
2. Switch on the mains supply. After a few minutes put on the 'EHT'. Allow to stabilize for half an hour.
3. Calibrate the NaI(Tl) detector for energy using standards ^{137}Cs and ^{60}Co.
4. Adjust the gain and set the base line voltage and window width so as to measure γ-rays upto 2 MeV energy.
5. Keep the empty sample holder, along with the cover, touching the face of the detector and count background for 30 min. Calculate the background count rate per second (C_b).
6. Similarly obtain background count rate per second (C_b) for each sample holder.
7. Take one of the sample holders with cover and weigh it. Fill 10g of standard soil sample in this sample holder and cap it.
8. Mount this sample touching the face of the detector. Note the counting time required to accumulate 10000 counts. From this calculate counts per second per kg of material (C_s).
9. Take another sample holder. Weigh it and fill surface soil sample in this sample holder up to the brim and cap it. Weigh the sample holder filled with surface soil. Note the weight of the surface soil.
10. Mount the sample touching the face of the detector. Note the counting time required to accumulate 10000 counts. From this calculate counts per second per kg of material (C_s).
11. Repeat steps 9 and 10 with the samples of coal fly ash and cement.

Date:

Table 4.3: Parameters

Detector	: NaI(Tl)	Detector No.	:
Amplifier Model	:	High voltage	: 700 V
Settings for Single Channel Analyser (SCA):			
Mode	: Integral	Amplifier Gain	:
Base line	: V	Window	: V (to count upto 2 MeV γ-rays)
Position	: Sample touching the detector	Sources	:Standard and environmental Samples
Function	: Counts	Preset Counts	: 10,000
Background/ 1800s	:	Background/s ($C_{b)}$	:

Table 4.4: Counting Data for environmental samples.

S.No.	Sample	Wt. of sample, g	Counting time, s	Specific counts rate (cps/kg), C_s	Corrected (cps/kg) C_s-C_b	Mean Activity (Bq/kg)
1	Empty holder					
2	Standard Soil (10g)					
3	Surface soil					
4	Coal Fly Ash					
5	Cement					

Calculations

Background correction: Subtract the background count rate from the count rates of the samples and standard.

Total specific activity in the standard (A_S) = Bq (Calculated from Wts of U and Th)
Specific count rate measured (C_S) =
Conversion factor (CF) = A_S/C_S =

Obtain absolute count rate of the environmental solid samples by multiplying the corrected count rates with CF[1].

1 All the values you have obtained are based on the assumption that the ratio of U/Th ratio in the sample is same. as that of the standard

Experiment 16

Determination of Manganese in Steel by Neutron Activation Analysis

A standard consisting of a known amount of the element that is to be analyzed, is prepared. It is irradiated along with the sample to be analyzed under identical conditions. Then eqn. (4.2) changes to

$$A_{sam} = N_{sam}\sigma\ \phi\ (1-e^{-\lambda t_i}) \qquad (4.5)$$

$$A_{std} = N_{std}\sigma\ \phi\ (1-e^{-\lambda t_i}) \qquad (4.6)$$

where, A_{sam} and A_{std} are activities of analyte for sample and standard respectively.

After applying corrections for cooling period, ratio of the equations (4.5) and (4.6) gives

$$\frac{m_{sam}}{m_{std}} = \frac{cps_{sam}}{cps_{std}}\ x\ \frac{D_{sam}}{D_{std}} \qquad (4.7)$$

where, cps indicates count rate per second and D is decay correction factor. Thus, m_{sam}, the mass of the element of interest in the sample is determined.

Materials required

Manganese salt, thin aluminium foils, fine mild steel filings, mounting plates, gamma counting set up (NaI(Tl) or HPGe with analyzer) and semi log graph papers.

Procedure

1. Assemble the electronic units of NaI(Tl) detector and counting set up and make connections as given in the instruction manual. Note down the settings.
2. Switch on the mains supply. After a few minutes, apply high voltage in steps of 100 V. Allow to stabilize for half an hour.
3. Take a known amount of manganese salt in an aluminium wrapper of known weight. Wrap it properly and label it as 'std'.
4. Prepare the sample by taking a known amount of the mild steel filings containing an unknown quantity of manganese. Wrap this in another aluminium foil and label it as 'sam'.
5. Keep standard and sample together in another aluminium foil and place them in an irradiation can (Fig. 6.2).
6. Irradiate in a position of a nuclear reactor having a suitable neutron flux for a time duration t, to form adequate activity for obtaining good counting statistics. Other neutron sources such as ^{252}Cf, Pu- Be or Am-Be, can also be used.
7. Cool the sample and the standard for 20 minutes or more depending on the activity level.
8. Calibrate γ-spectrometer using a single channel analyzer.
9. Locate the base line voltage and window width suitable to count 847 keV region which is the. most abundant γ ray of ^{56}Mn (see Experiment 4, Chapter 3 for details).

10. Count the standard and the sample for manganese activity in a single channel or multichannel analyser using settings 847 keV gamma peak.

Date:
Wt. of the standard: % or ppm of Mn in std. :
Wt. of Mn in standard (W_{sam}) : Wt. of mild steel filings (W_{sam}) :
Wrapped and sealed in : Neutron source:
Neutron flux:
Settings:
Detector: NaI(Tl) High voltage: 700 V
Single channel analyser : Window: -
Base line: Gain:
Background counts: --- /1800 s C_b /300 s:-

Table 4.5: Observations

Standard				Sample			
Sl. No.	Start time	Counts (C_{std}) 300 s	Corrected counts 300 s	Sl. No	Start time	Counts (C_{sam}) 300 s	Corrected counts 300 s
1				1			
2				2			
3				3			
4				4			
5				5			
6				6			

Calculations

- Plot the activity of standard and sample separately as a function of time and check the half-lives of the activities formed. Take activity on y-axis and time on x- axis on a semi log paper.
- From the plots note down the activity of both the sample and standard at zero time.
- A_{sam} = A_{std} =
- Calculate % of Mn in the sample using eqn. 4.7

% Mn = 100 x m_{sam}/W where W is the weight of the sample irradiated.

Comments

1. Find out the minimum amount of manganese you can determine using this technique.
2. Comment on the versatility of this method by comparing with conventional quantitative analysis or spectrophotometric analysis.
3. What will happen if the sample contains Cr or Ni ?
4. For multielement analysis, a multielement standard has to be irradiated along with the sample. HPGe based high-resolution gamma ray spectrometry is used for measuring activities due to different radioisotopes produced. The standards can after be prepared, or preferably obtained from institutions like the IAEA, NIST and IRMM.

Note: ^{56}Mn is obtained from ^{56}Fe(n,p)^{56}Mn reaction, which is an interference but it needs high energy neutrons. If Mn is present in ppm level, it needs correction from Fe.

Experiment 17

Multielement Determination in Soil by Single Comparator NAA.

In a variety of materials like soil, sediment, cereals and biological samples are quite often interested in the determination of a wide range of elements. Relative method described in Experiment 16 is frequently used for multi element determination. However, it requires a priori knowledge of the elements, present in the samples to prepare standards suitable for analysis. Single comparator method, also known as k_0NAA, is useful in such cases as the procedure involves the use of only one comparator (like Au, Mn and Sc). This comparator is irradiated with the sample and ratios of activities of different radioisotopes formed, with respect to the comparator are used for calculating the elemental concentrations. However, this method requires input data for many parameters. Three main inputs are the ratios of sub cadmium to epicadmium flux (f), epithermal neutron flux shape parameter (α) and the detection efficiencies of the individual gamma rays. The concentration of ith element (Ci) in the sample is calculated by using equation 4.8.

$$Concentration\ (C_i) = \frac{\left[\frac{cps}{SDCW}\right]_i}{\left[\frac{cps}{SDCw}\right]_*} x \frac{\left(f + Q_0(\alpha)^*\right)}{\left(f + Q_{0i}(\alpha)\right)} x \frac{1}{k_0} x \frac{\varepsilon^*}{\varepsilon_i} \qquad (4.8)$$

where ‘i’ and ‘*’ refer to the analyte and the comparator respectively, A is the peak area (specific count rate) under the characteristic γ-ray corrected for cooling and saturation, k_0 is a nuclear constant and the values are available in the literature, and Q_0 is the ratio of infinitely dilute resonance integral cross section and thermal neutron cross section.

The objective of this experimental is the multi elemental determination in a soil sample. Accurately weighed samples of soil and gold (single comparator) are irradiated for 4 hours in a reactor position for which the value of f are predetermined.

Sample and standard are cooled for about 2 hours and assayed for γ-activity using HPGe based high resolution γ-ray spectrometry. A fixed sample to detector geometry is used for which detection efficiency as a function of γ-energy has been predetermined. Peak areas under different γ-rays are compared with the peak area of 411 keV peak of ^{197}Au. From these the concentrations of different elements are obtained using equation 4.8.

Note: For each set of experiments, concentrations of elements in a standard reference material of similar matrix are determined to validate the method and for soil samples, IAEA standard IAEA RM Soil-7 is used.

Materials required

Soil sample, polythene sheets, thin aluminium foil, gold solution standard, mounting plates and HPGe based gamma spectrometer with MCA

Procedure

1. Assemble HPGe and the electronic units as per instructions. Note down the settings.
2. Ensure that the dewar of HPGe detector is filled with liquid nitrogen about 2 hours before the experiment.
3. Switch on the main supply. After a few minutes start applying higher voltage at the rate of 100V/min. After reaching the rated voltage, allow it to stabilize for half an hour.
4. Acquire γ- spectrum of a standard like ^{152}Eu.
5. Analyze for peak position and calibrate the analyzer for energy. Note down the calibration constants and save the spectrum.
6. Open the irradiation can with irradiated sample, check for loose contamination, (remove it for detected) and mount the sample on a perplex plate.
7. Follow the same procedure for making gold comparator sample.
8. Acquire the γ-spectrum of standard after placing the standard in the efficiency-precalibrated position. Label the spectrum and save.
9. Similarly acquire γ-spectrum of the sample and save the spectrum.
10. Depending upon the half-lives of the radioisotopes produced and the level of radioactivity, follow the spectrum for a few hours to a few days.

Tabulate all Data

Calculation

Comparison and comments

1. Analyse all spectra for peak areas. Correct the peak areas for decay, cooling and Saturation to obtain normalised count rates (A_p^i) for all the isotopes.
2. Similarly calculate the normalised peak area for the standard (A_p^*). Calculate concentration of all elements in the sample using equation 4.8.

Experiment 18

Determination of Thickness of Films by Rutherford Backscattering Spectrometry (RBS)

Rutherford backscattering spectrometry (RBS) is an important ion beam analysis (IBA) technique for analyzing surface and near surface regions of materials. The present experiment aims to determine the composition and thickness of a silicon dioxide/indium sulphide film. Apart from the methodology used in the determination of thickness, the note also mentions some of the salient analytical features of the technique.

Materials required

3 MV Tandetron (HVEE, Europa) equipped with a duoplasmatron source (358), a Cs sputter ion source (860) and an experimental end station comprising a standard RBS scattering chamber pumped down to 10^{-6} torr by a turbomolecular pumping system. Detection cum data acquisition system consisting of surface barrier detectors, preamplifier, amplifier and PC-based multichannel analyzer.

Procedure

A well-collimated beam of protons or alpha particles of energy E_0 is impinged on the sample mounted on a sample manipulator inside the scattering chamber. The scattering chamber is maintained at a vacuum of ~ 10^{-6} mbar during irradiation. The backscattered particles are detected by a surface barrier detector placed inside the scattering chamber at a backward angle of ~170^o.The detector pulses are processed by a combination of preamplifier and amplifier. The data is acquired by a PC based Multi Channel Analyser and spectral analysis is performed by SIMNRA, an international data analysis software package for RBS analysis.

Typical irradiation parameters:

Beam energy: 1 to 5 MeV
Beam current: < 10 particle nA
Beam diameter : 0.5 mm to 2 mm

Determination of thickness:

The film thickness can be determined by the relation,

$$\Delta E = [S_o]t \qquad (4.9)$$

where, t = film thickness (microns) and
$[S_o]$ = stopping cross section factor as given by the relation

$$[S_o] = \left[\frac{K}{Cos\theta_1} \frac{dE}{dX}\bigg|_{E0} + \frac{1}{Cos\theta_2} \frac{dE}{dX}\bigg|_{KE0} \right] \qquad (4.10)$$

where, K = kinematic factor, dE/dX = stopping power (keV/micron), ΔE = width of the backscattered signal for the element under consideration (in keV) θ_1 = 0 (for normal incidence), θ_2 = angle between surface normal and detector

Nature of analysis: Non-destructive
Range: 0.02 – 15 μm
Calib: Primary method, No calibration
LOD: 0.02 μm

Thickness:
Minimum thickness: ~ 0.02 μm
Probing depth: ~ 15 μm
Uncertainity: 5 %

Rapid analysis
Sample size: 5 mm x 5 mm – 10 mm x 10 mm;
Thickness of the substrate: < 2 mm.

Interference: Prominent among high Z elements

Calculations

Chapter 5

THERMAL AND ELECTROCHEMICAL METHODS OF ANALYSIS

A.K. Satpati, B.B. Kalekar, N. Raje, Avanti Singh, T.S. Reddy [#] and A.V.R. Reddy
[#] S.K. University, Anantapur

Thermal Analysis

Thermal analysis deals with the study of physical properties of materials like mass, energy, dimension, elastic modulus and electrical conductivity, as a function of a temperature or time in specified environment under controlled heating rate. Each technique is identified with the parameter measured as a function of temperature. Among the various thermoanalytical techniques Thermogravimetry (TG), Differential Thermal Analysis (DTA), Differential Scanning Calorimetry (DSC) and Evolved Gas Analysis (EGA) have been routinely employed for the assessment of thermal stability and nature of thermal decomposition behavior of the various materials at high temperatures. These techniques are utilized for the assessment of kinetic parameters like activation energy and pre-exponential factor for the various decomposition reactions that gives insight to the reaction mechanism. Thermal studies find extensive application in nuclear science and technology besides polymers, cement, ceramics, rubber, paint, pharmaceuticals and clay industries.

When a sample is subjected to a constant heat in a controlled environment, the mass of the sample may change depending on the reaction. A plot of mass change as a function of temperature is known as thermogram (Fig.5.1) and the instrument that measures mass change as a function of temperature is called thermogravimeter.

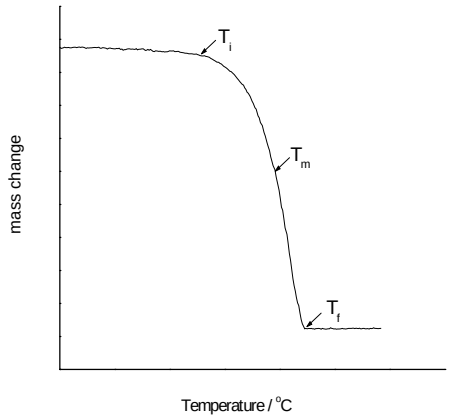


Fig 5.1 Idealized TG curve

TG curve can be horizontal plateau, single or multiple weight loss steps depending on the nature of sample. Horizontal plateau indicates thermal stability of the sample against the temperature. TG signal responds to either gas evolution or absorption. The evolution of gas will cause mass loss of the sample, whereas consumption of gas will show increase in the mass of sample. Decomposition, adsorption, sublimation and degradation reactions cause the mass loss and absorption and oxidation reactions results in the mass gain of sample.

This quantitative technique and provides information on the stoichiometry of the reaction.

The TG curve is associated with inception temperature (T_i), maximum rate temperature (T_m) and final temperature (T_f). The temperature T_i, refers to that temperature at which the cumulative mass change of the sample exceeds the sensitivity of the recording system and plot begins to show the departure from horizontal line. T_m represents maximum rate of the decomposition reaction. T_f represents the temperature at which the reaction is complete.

In Differential Thermal Analysis sample and reference materials are subjected to identical thermal regime as well as environment during the DTA measurement. Temperature difference between sample and thermally inert reference material is measured as function of temperature or time in DTA technique. Alumina, glass beads, silicon carbide are generally used as the reference materials for the solid samples. Generally phase transitions, dehydration reaction, reduction and decomposition reactions produce endothermic effects. Crystallization, oxidation and some decomposition reactions, however, produce exothermic effects.

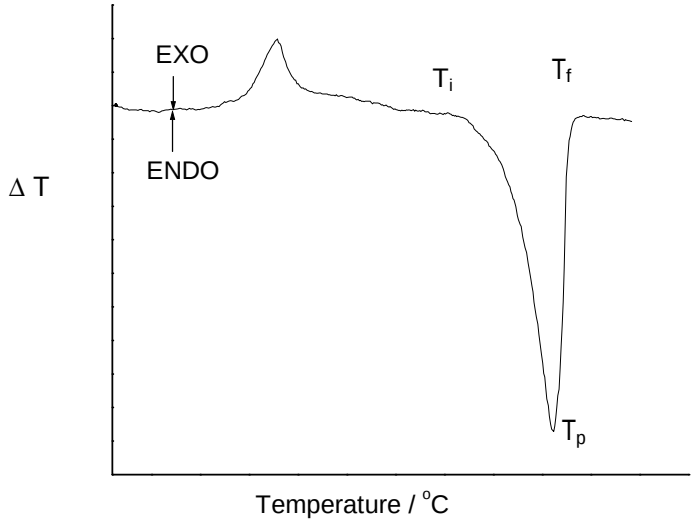


Fig. 5.2: Idealized DTA curve

The DTA peak exhibit inception temperature (T_i), peak temperature (T_p) and final temperature (T_f). T_i is on-set of the reaction and indicated by the deviation from the baseline. T_p represent maximum rate of the reaction and T_f indicate completion of the reaction. The heat of reaction (ΔH) can be determined from DTA curve using the following equation.

$$\Delta H = K \times A \qquad (5.1)$$

TG as well DTA techniques give information on thermal behavior of samples at high temperatures.

Experiment 19

Determination of $\Delta H_{melting}$ of Indium using DTA Technique

DTA curve is utilized to calculate various parameters mentioned above.

Instrument

Simultaneous TG-DTA thermal analyzer with facility of gas manifold, non-isothermal measurements at different heating rates, etc.

Materials Required

Crucibles, indium metal, alumina, etc.

Procedure

1. Open the gas cylinder/s, adjust the appropriate flow rate.
2. Weigh reference and sample materials in crucibles and load crucibles on the TG-DTA carrier assembly.
3. Switch on the computer and enter the parameters heating rate, temperature range and atmosphere, etc., in the data acquisition programme. Acquire the data in the desired temperature range.
4. Obtain the plots in mass loss (%) V_s temperature for TG and diff. thrmo EMF (mV) V_s temperature for DTA curve.
5. Calculate area obtained under the melting endotherm of indium metal.
6. When furnace is cooled, reduce the gas flow, close gas cylinder/s and switch off the instrument.

Observations

Wt. of reference material : mg
Wt. of indium metal : mg
Wt. of potassium tetraoxalate : mg
Flow rate of purge gas : $mL.min^{-1}$

Calculations

1) **$\Delta H_{melting}$ of indium**
ΔH **=** K x A
$\Delta H_{melting}$ = x = J/g
$\Delta H_{melting}$ = 28.4 J/g (reported)

Experiment 20

TG and DTA Techniques to Study of Reaction Mechanism of Potassium Tetraoxalate at Elevated Temperatures

Thermal decomposition scheme of Potassium tetraoxalate is given in equations 5.2 to 5.5

$$KH_3(C_2O_4)_2.2H_2O \rightarrow KH_3(C_2O_4)_2 + 2H_2O \uparrow \quad (5.2)$$

$$KH_3(C_2O_4)_2 \rightarrow KHC_2O_4 + H_2C_2O_4 \uparrow \quad (5.3)$$

$$2KHC_2O_4 \rightarrow K_2C_2O_4 + H_2C_2O_4 \uparrow \quad (5.4)$$

$$K_2C_2O_{4\,(s)} \rightarrow K_2C_2O_{4\,(l)} \quad (5.5)$$

Calculate theoretical % mass loss for the above reactions. % mass loss is calculated for the Ist reaction.

$$\% \text{ mass loss} = \frac{2 \times \text{M.W. of water}}{\text{M.W. of } KH_3(C_2O_4)_2 \,.\, 2H_2O} \times 100 \quad (5.6)$$

Similarly, calculate % mass loss for reactions 5.3 and 5.4. Reaction 5.5 indicates melting of potassium oxalate which can be determined using DTA measurement. The extrapolated on set temperature of the e transition represents the melting of potassium oxalate.

Materials Required

Crucibles, potassium tetraoxalate, alumina, etc.

Procedure

1. Open the gas cylinder/s, adjust the appropriate flow rate.
2. Weigh reference and sample materials in crucibles and load crucibles on the TG-DTA carrier assembly.
3. Switch on the computer and enter the parameters heating rate, temperature range and atmosphere, etc., in the data acquisition programme. Acquire the data in the desired temperature range.
4. Obtain the plots in mass loss (%) V_s temperature for TG and diff. thrmo EMF (mV) V_s temperature for DTA curve. Calculate experimental mass loss % for various reaction of thermal decomposition of potassium tetraoxalate. Determine melting temperature from melting endotherm of potassium oxalate.
5. When furnace is cooled, reduce the gas flow, close gas cylinder/s and switch off the instrument.

Observations;

Experimental mass loss values are determined and noted in table 5.1. Theoretical mass loss on the premise that reactions (1) to (4) take place on the thermal decomposition of this compound. Enter the values in the table.

Table 5.1:Thermogravimetric wt. loss data of potassium tetraoxalate

Sr. No.	Reaction	Temperature range (^oC)	Theoretical (% mass loss)	Experimental (% mass loss)
1.				
2.				
3.				

Melting point of potassium oxalate obtained from the DTA curve: ^oC
Compare and comment on the % mass loss values.

Electrochemical Methods

Electrochemistry deals with the chemical changes due to passage of an electric current subsequent to the application of potential. Different phenomena of electrochemistry are used in energy devices, fuel cells and sensors, to study biological systems and in materials research. Present experiments are aimed to introduce some of the electroanalytical techniques to determine diffusion coefficient, a basic physical parameter and to determine concentration of metal ions in solutions. Diffusion coefficient is important in studying the diffusivity of toxic heavy metals ions in condensed phases, soils and water bodies. Diffusivity of biomolecules in biological matrices is important in the study of the effect of drug and toxins in biological systems. Cyclic voltammetric experiments will be used to determine the diffusion coefficient of cadmium ions in acidic solution. Determination of heavy metal ions in water bodies is an important issue due to their toxicity mostly due to their effect on the central nerves system. Differential pulse anodic stripping voltammetry will be used for the determination of Pd and Cd in water samples. In the following sections two techniques; cyclic voltammetry and the differential pulse anodic stripping voltammetry have been introduced.

Experiment 21

Determination of the Solubility Product Constant of AgCl

Electromotive force (E) of a purpose-built electrochemical cell can be used for the determination of the solubility product constant. An electrochemical cell with Ag (+) and Ag-AgCl (-) electrode can be used to determine the solubility product of AgCl(K_{sp}).

$$Ag\left|AgCl;KCl(c_2)\right|\left|AgNO_3(c_1)\right|Ag$$

Ag electrode immersed in the solution of $AgNO_3$ represents the positive terminal of the cell (cathode), and the silver-silver chloride electrode is a reference electrode (anode). Both the half cells are connected with a salt-bridge to provide electrical contact.

According to the Nernst equation, the E of this concentration cell is given by

$$E = \frac{RT}{F} \ln \frac{a_1}{a_2} \qquad (5.7)$$

where a_1 and a_2 are the activities of Ag^+ in cathode and anode solutions respectively.
For dilute solutions, eqn. 5.7 can be written as

$$E = \frac{RT}{F} \ln \frac{C_{AgNO_3} \gamma_{AgNO_3} C_{KCl} \gamma_{KCl}}{K_{sp}} \qquad (5.8)$$

where γ_{AgNO_3} and γ_{KCl} may be approximated as the mean ionic activities $\gamma_\pm$ of solutions and calculated using the Debye – Hückel limiting law:

$$\log \gamma_\pm = -0.511 \left| z_+ z_- \right| \sqrt{I} \qquad (5.9)$$

where z_+ and z_- are the charge numbers of cation and anion respectively and I is the ionic strength of the solution. The ionic strength of a solution is a function of the concentration of all ions present in a solution and is given by

$$I = \frac{1}{2} \sum_i^n C_i Z_i^{\,2} \qquad (5.10)$$

where c_i is the molar concentration of i-ion (in mol dm), z_i is the charge number of that ion, and the sum is taken over all ions in the solution.

By keeping the concentrations of $AgNO_3$ and KCl the same ($C_{AgNO_3} = C_{KCl} = c$) and $\gamma_{AgNO_3} = \gamma_{KCl} \approx \gamma_\pm$ Eqn 5.8 can be simplified and rearranged into the form

$$\ln K_{sp} = 2 \ln C + 2 \ln \gamma_\pm - \frac{EF}{RT} \qquad (5.11)$$

Materials Required:

potentiometer – pH-meter,Electrochemical cell with Ag and Ag-AgCl electrodes, volumetric flasks (25 mL (2), 10 mL (9)), pipettes, beakers, $AgNO_3$ solution (0.025 mol.dm^{-3})
KX (KCl, KBr, KI), distilled water

Procedure:

1. Prepare 25 ml of the solution KX (KCl, KBr , KI) at the molar concentration 0.025 mol dm^{-3}. This is the stock solution.
2. Prepare 3 solutions, each of the volume 10 mL, with the concentration 0.0125, 0.00625 and 0.003125 mol dm^{-3}, respectively, by dilution of the KX stock solution.
3. Prepare 3 solutions, each of the volume 10 mL, with the concentration 0.0125, 0.00625 and 0.003125 mol dm^{-3}, respectively, by dilution of the $AgNO_3$ solution.

4. Fill the (+) compartment of the electrochemical cell with the $AgNO_3$ solution of the lowest concentration c=0.003125 mol dm^{-3} (V~5 ml).
5. Fill the (-) compartment of the electrochemical cell with the KX solution of the same concentration (c=0.003125 mol dm^{-3}) (V~5 ml). Add few drops of $AgNO_3$ solution with the same concentration as is the KX in the compartment (-). Mix the solution gently. Due to the reaction $AgNO_3 KCl \rightarrow AgCl\downarrow KNO_3$, a low soluble AgCl is formed and a turbidity of the solution is observed.
6. Measure the electromotive force of the cell *E* (mV).
7. Drain both compartments of the cell, wash them with the distilled water.
8. Repeat the procedure for each prepared concentration i.e. 0.00625, 0.0125, and 0.025 mol dm^{-3}.
9. After finishing the experiment, wash carefully both the compartments of the cell with distilled water, and dip both the electrodes in distilled water.

Data treatment:

1. Calculate the ionic strength *I* of prepared solutions according to the eqn. 5.10.
2. Calculate the mean ionic activity coefficients using Debye - Hückel limiting law (eqn. 5.9).
3. Calculate the solubility product constants K_{sp} for each measured *E* using the eqn. 5.11. (R=8.314 $JK^{-1}mol^{-1}$, F=96485 C mol^{-1})
4. Calculate the average value of K_{sp}.
5. Calculate the standard deviation of K_{sp} according to

$$s_D = \sqrt{\frac{\sum_{i=1}^{n} (K_{spi} - \overline{K}_{sp})^2}{n-1}}$$

where i=1 - n, and n=4 is the number of values.

Table 5.2: Experimental data

T = ...°C

Sl. No.	c($AgNO_3$)=c(KX) (mol.dm^{-3})	E (V)	I (mol.dm^{-3})	γ (±)	K_{sp} (mol.^{2} dm^{-6})
1	0.003125				
2	0.00625				
3	0.0125				
4	0.025				

Experiment 22

Determination of Diffusion Co-efficient of Cadmium Ions in HCl Medium by using Cyclic Voltammetry

Experimental Method

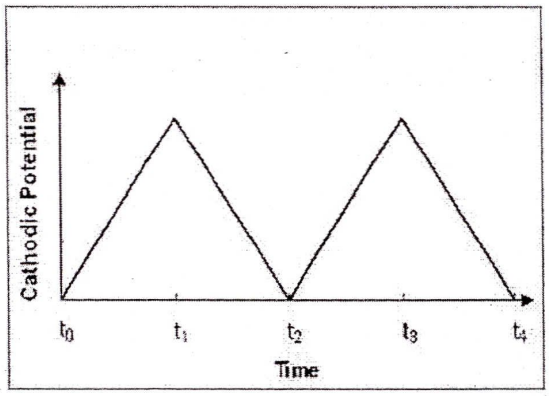


Fig.5.4 : Cyclic voltammetry waveform applied to the electrode

In cyclic voltammetry, the electrode potential ramps linearly versus time as shown in Fig. 5.4. This ramping is known as the experiment's scan rate (V/s). The potential is applied between the reference electrode and the working electrode, and the current is measured between the working electrode and the counter electrode. As the waveform shows, the forward scan produces a current peak for any analyte that can be reduced (or oxidized depending on the initial scan direction) through the range of the potential scanned. The current will increase until the potential reaches the reduction potential of the analyte and then falls off as the concentration of the analyte is depleted close to the electrode surface. If the redox couple is reversible then when the applied potential is reversed the current. Where it will reach the potential that will reoxidize the product formed in the first reduction reaction, and produce a current of reverse polarity from the forward scan. This oxidation peak will usually have a similar shape to the reduction peak. As a result, information about the redox potential and electrochemical reaction rates of the compounds is obtained. A typical cyclic voltagram is shown in Fig. 5.5.

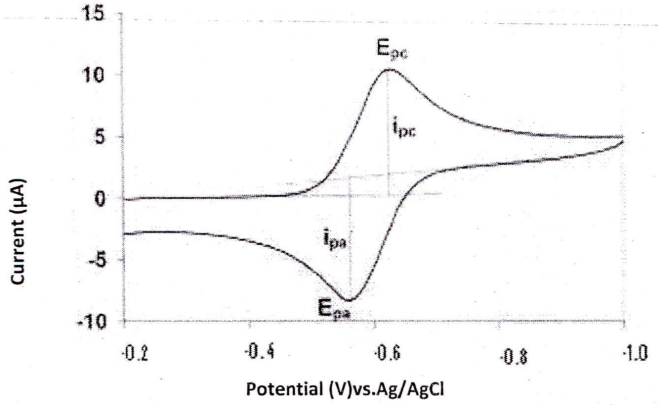


Fig. 5.5 : Typical cyclic voltammogram where i_{pc} and i_{pa} show the peak cathodic and anodic current respectively for a reversible reaction.

Characteristics of the Technique

The utility of cyclic voltammetry is highly dependent on the analyte being studied. The analyte has to be redox active within the experimental potential window. It is highly also desirable to the analyte to display a reversible wave. A reversible wave is when an analyte is reduced or oxidized on a forward scan and is then reoxidized or rereduced in a predictable way on the return scan as shown in Fig. 5.4.

In an ideal reversible system at 25^{o}C, the difference in the cathodic and the anodic peak peak potentials for an electron transfer process is given by,

$$\Delta E_p, = |E_{pc} - E_{pa}| = \frac{57 \text{ mV}}{n} / n \qquad (5.12)$$

where n is the number of electron transferred in the process.

For an irreversibility process, because of the slow electron transfer kinetics, ΔE_p exceeds the expected value. While an electron transfer electronic may appear reversible at slow scan rates, increasing the sweep rate may lead to increasing values of $\Delta E_{p,}$ a sign of Irreversibility. Hence to detect reversibility and irreversibility of process and to obtain rate constants, ΔE_p is measured for different sweep rates. There are some other quantitative information obtained from the cyclic voltammetric measurements by applying the analysis procedure using the **Randles – Sevcik equation:**

$$i_p = 0.4463\ n\ F\ A\ C\ (n\ F\ v\ D / R\ T)^{1/2} \qquad (5.13)$$

In this equation, **n** is the number of electrons appearing in half – reaction for the redox couple, **v** is the rate at which the potential is swept (V s^{-1}), **F** is Faraday's constant (96485 C M^{-1}), **T** is the absolute temperature (K), and **D**, is the analyte's diffusion coefficient (cm^2 S^{-1}). Note that if the temperature is assumed to be 25^{o}C (298.15 K), the Randles-Sevcik equation can be written in a more concise form,

$$i_p = 0.4463\ n\ F\ A\ C\ (n\ F\ v\ D / R\ T)^{1/2} \qquad (5.14)$$

where the constant is understood to have units (i.e., 2.687×10^5 C mol^{-1} $V^{-1/2}$)

Typical cathodic waves taken different scan rates of 200, 150, 100, 75, and 50 mV s^{-1} are shown in Fig. 5.6A. As expected, the peak current increases with increase in the voltage scan rates. The ***Randles-Sevcik equation*** predicts that the peak current should be proportional to the square root of the sweep rate. From the slope of the liner plot as shown in Fig. 5.6B, the diffusion coefficient can be calculated if the electrode area, A, and the concentration, C, is known.

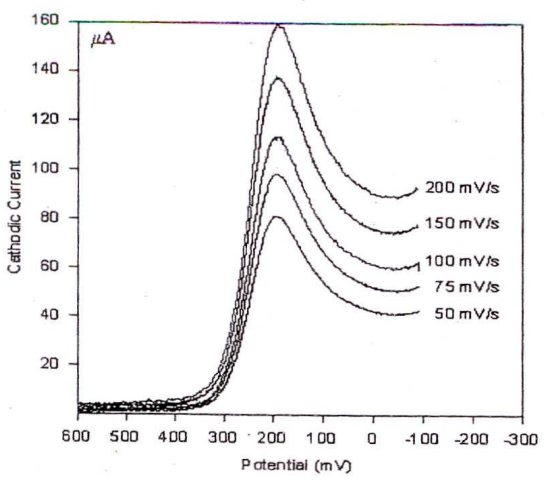


Fig. 5.6A:

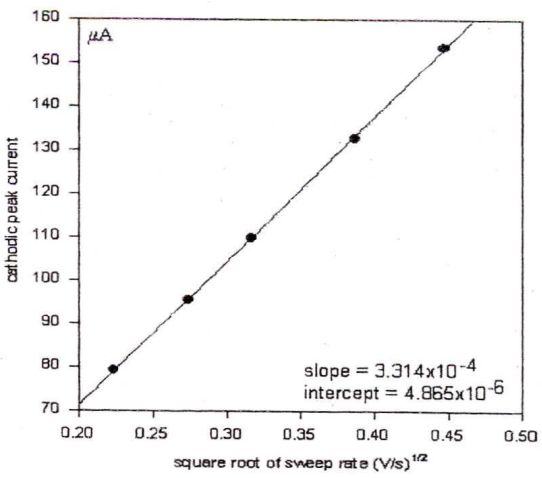


Fig. 5.6B

Fig. 5.6. (A) Liner sweep voltammetry of a typical electroreduction process at different scan rates; (B) plot from the Randle-Sevcik analysis.

Experiment

- Cyclic voltammetry of Cd^{2+} will be performed in 0.1 M HCl medium at site mercury drop electrode (SMDE) in the potential range from -0.2V to -0.8V at various scan rates ranging from 25 mV s^{-1} to 500mV^{-1}.

- Experimental Observation records

Table 5.3: Observations

S. No.	Sweep rate (mV^{s-1})	Square root of Sweep rate $(V/s)^{1/2}$	Cathodic peak current (A)
1	25	0.1581	
2	50	0.2236	
3	100	0.3123	
4	200	0.4472	
5	300	0.5477	
6	400	0.6325	
7	500	0.7071	

The peak current when plotted against the square root of scan rates, is supposed to follow the straight line as in eqn 5.14.

$$D = [Slope / (2.687 \times 10^5 n^{3/2} AC)]^2 \quad (5.15)$$

In this case n=2 for a given concentration of Cd^{2+} and known electrode area, diffusion coefficient D will be calculated from the equation 3. Expected value of D is ~$6.057x10^{-6}$ cm^2 s^{-1}.

Experiment 23

Determination of Cu, Pb and Cd in Water Sample by Differential Pulse Anodic Stripping Voltammetry (DPASV)

Toxic elements like copper, cadmium and lead in water in trace levels can be determined simultaneously by DPASV. **Anodic stripping voltammetry** is a voltammetric method for quantitative determination of specific ionic species. The analyte of interest is electroplated on the working electrode during a deposition step, and oxidized from the electrode during the stripping step. The current is measured during the stripping step. The oxidation of species is registered as a peak in the current signal at the potential at which the species begins to be oxidized. The stripping step can be either linear, staircase, squarewave, or pulse.

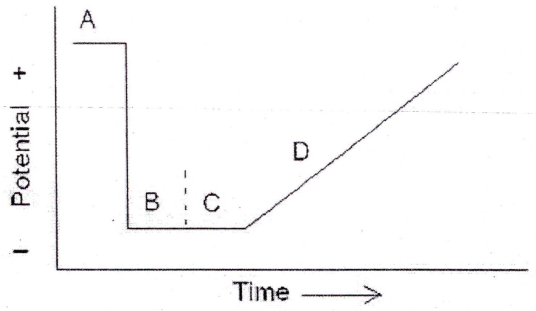


Fig. 5.7: Variation of potential as a function of time in four steps,
***A**: Cleaning step, **B**: Electroplating step, **C**: Equilibrium step, **D**: Stripping step*

The concentration of the analyte in the Hg electrode, C_{Hg}, is given by:

$$C_{Hg} = i_1 \, t_d \, / \, n \, F \, V_{Hg}$$

where i_1 is the limiting current during reduction of the metal, t_d is the duration of accumulation, n is the number of moles of electrons transferred in the half reaction, F is the Faraday constant (96,487 coulombs $mole^{-1}$ of e^-), and V_{Hg} is the volume of the electrode. The expression for current produced by anodic stripping depends on the type of Hg electrode, but is directly proportional to the concentration of the analyte concentrated into the electrode. The main advantage of stripping analysis is the pre-concentration of the analyte into the electrode before making the actual current measurement. Detection of concentrations as low as 10^{-10} M is possible by Anodic Stripping Voltammetry.

Anodic stripping voltammetry usually incorporates 4 steps if the working electrode is a mercury film or mercury drop electrode. The solution is stirred during the first two steps at a repeatable rate. The first step is a cleaning step. In the cleaning step, the potential is maintained at a more oxidizing potential than the analyte of interest for a period of time in order to fully remove it from the electrode. In the second step, the potential is maintained at a lower potential to reduce the analyte and deposit it on the electrode. After the second step, stirring is stopped. In the third step the electrode is kept at the lower potential to allow the deposited material to distribute more evenly in the mercury. If a solid inert electrode is used, this step is not required. The last step involves raising the working electrode to a higher potential (anodic),

and stripping (oxidizing) the analyte. As the analyte is oxidized, it gives off electrons which is measured as current.

Stripping analysis is an analytical technique that involves (i) preconcentration of a metal phase onto a solid electrode surface or into Hg (liquid) at negative potentials and (ii) selective oxidation of each metal phase species during an anodic potential sweep.

Table 5.3: Observations

	Peak Current (nA)		
Concentration of the standard ppb	Copper	Cadmium	Pb
10			
30			
40			
50			
100			
Slope	------nA/ppb	------nA/ppb	------nA/ppb
Sample peak current (nA)			
Sample Concentration (ppb)			
Remarks			

Comments:

Chapter 6

ENVIRONMENTAL ANALYTICAL CHEMISTRY

K. Venkatesh, R.K. Singhal, V. Hima Bindu[#], R.K. Reddy[$], P.K. Sharma, D.K. Avad, H. Basu, S.V. Rao, G.C. Chourasia* and A.C. Sahayam* .*
*[#] JNTU, Hyderabad, $ S.K. University, Anantapur, * NCCCM, Hyderabad.*

Introduction

Environment, a composite term for the condition in which organisms live, consists of air, water, food and sunlight. It keeps changing. Man, continuously strives to improve the quality of and in the process interferes with the environment. This affects the environment, besides continuous changes that occur in the nature. Revolutionary advancements in science and technology over the last two centuries affected the environment; and this has been recognized in the second half of last decade. Regulations have been proposed to monitor the environment. Analytical chemistry has become a tool for environmental monitoring and a new branch of science, environmental analytical chemistry has taken shape in which analytical techniques have been used to measure changes brought about in the environment.

Human effort for the advancements in Science and Technology resulted in contamination of the environment. Environmental contamination could be understood as an addition of material in unwanted/ unexpected compartment of the environment like water, soil, air and land resulting in alteration in the composition of the environment. If these alterations are unfavorable and results in degradation of quality of life, then the contamination is termed as “Environmental Pollution”. These pollutants can migrate from one compartment to another and need to be monitored on a regular basis. Monitoring the air we breathe, the land we live on, the soil that is used for various purposes and the water we drink and use for agriculture etc. is essential. As the subject is vast and evolving, a large number of areas have emerged over the last few decades, concerns are many. In this book, a few experiments to measure the quality of air, water, soil and sediments using chemical analysis and analytical instrumentation are included.

Experiment 24

Estimation of Ammonia in Water using Kjeldahl Method

Presence of nitrogen is of great significance in sanitary engineering practices; determination of various forms of nitrogen was done to assess its bacteriological quality. Presence of organic and ammonia nitrogen is accepted as a chemical evidence of recent organic pollution. Concentration of ammonia in waters and wastewaters is estimated by Kjeldahl method.

Principle:

In the presence of sulphuric acid and mercuric sulphate catalyst organically bound nitrogen gets converted into ammonium sulphate. Potassium sulphate is added to raise the

boiling point of sulphuric acid from 345-370^{o}C. The digestate is diluted, made alkaline with NaOH and distilled. The liberated ammonia is absorbed in boric acid. The absorbed ammonia is determined by titration.

Materials / Chemicals Required

Digestion reagent- K_2SO_4+H_2SO_4, mercuric sulphate, Phenophthalein indicator, Sodium hydroxide-sodium thiosulphate reagent, mixed indicator, Indicating boric acid and 6N NaOH.

Procedure:

(A) Digestion

1. Take suitable volume of well-mixed sample in Kjeldahl flask.
2. Add 50ml digestion reagent and continue digestion for 30 min.
3. Cool and dilute with distilled water to 150/300ml depending on the capacity of the flask.

(B) Distillation

11. Place the flask in its proper position in distillation apparatus and turn on heat.
12. Add 0.5ml Phenophthalein reagent followed by NaOH-$Na_2S_2O_3$ reagent till pH is above 8.3.
13. Distill and collect 200ml distillate in 50ml boric acid.
14. Titrate the distillate with 0.02N sulphuric acid till the indicator turns to pale lavender color.
15. Carry a blank through all the steps and apply necessary correction.

Calculation

NH_3 in mg/l = (A-B) X 280/ ml of the sample
A= ml of 0.02 N H_2SO_4 required for sample
B= ml of 0.02 N H_2SO_4 required for blank

Remarks:

Eventhough, the Kjeldahl method for determining the ammonia contents in water samples is tedious and time consuming as it involves steps like digestion, distillation, and titration. Still, it is used extensively because it remains a highly reliable technique with well-established routines. But, using Kjeldahl's procedure only 2-3 samples can be analyzed per day. Now, there are methods available for rapid and accurate determination of ammonia contents e.g. ion chromatography, ISE etc.

Ion chromatography (IC) is an analytical tool for separation and quantitative determination of trace quantities of the ions in presence of large amounts of similar ions or matrix. A simple, accurate and rapid analytical methodology for the determination of ammonium content in process streams has been developed using IC. The quantitative determination of ammonium content is accomplished by cation exchange chromatography with conductometric detection. The advantage of using this method is that ammonium ion is separated from other cations (Na^{+}, Ca^{+2}, Mg^{+2}) and measured. The ammonium content in the concentration range of ppm to g L^{-1} can be determined. The method has a high throughput and 10-15 samples can be analysed per day. The ion chromatography method has been validated using Kjeldahl's method.

Experiment 25

Estimation of Mass Concentration of Aerosols

Calculation of the total mass concentration of atmospheric aerosols (0.05->25 μm) by studying their physical properties from mass concentrations of nuclei, accumulation and coarse mode aerosols depending on their sizes using Quartz Crystal microbalance and estimate the percentage relative contribution of nuclei, accumulation and coarse mode particles concentration to the total aerosol mass concentration.

Principle:

Quartz Crystal Microbalance (QCM) is a cascade Impactor that has the potential to provide near real-time measurements of total as well as size segregated mass concentration of aerosols. It is designed (Fig. 6.1) based on the principle of inertial impaction. When air containing aerosols (particles), is sucked by means of a pump and directed towards a collecting surface or substrate, particles in the stream having inertia large enough will impact on the substrate while those with lesser inertia will follow the airflow.

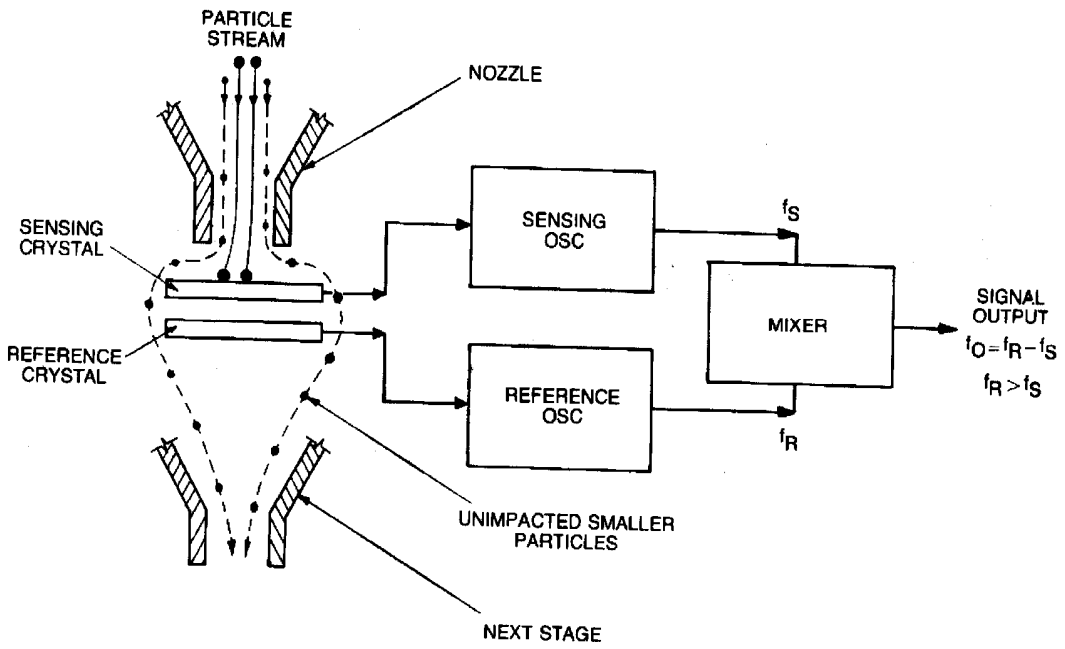


Fig. 6.1: QCM

Materials/ Chemicals Required:

Quartz Crystals, control unit and Sensing stack assembly and cascade Impactors

Method:

Quartz Crystal sensors are used in each stage as mass monitors to provide real-time mass collection data. There are two crystals in each of the Impactor stages, one for collecting

aerosols and another as a reference. The data from the QCM sensing stack are processed in a microprocessor based control unit, which then provides printouts of the mass concentration in each stage and a histogram of the relative mass concentrations of each size range.

The two frequencies one from the sensing and other from reference crystals, are mixed so that the beat frequency emanating from the mixer is a lower frequency in the 2 to 4 KHz range. This beat frequency changes in accordance with the frequency change of the sensing crystal caused by mass collection and increases as particles continue to impact on the crystal. The output beat frequency is, therefore the signal whose changes are proportional only to the mass loading on the sensing crystal.

Analysis of Data:

The instrument sucks in the ambient air and segregates the aerosols in accordance with the aerodynamic diameter into one of its ten size bins. For spherical particles of a density (ρ), the aerodynamic diameter (D_a) and particle diameter (D_p) are related as

$$D_p = D_a/\sqrt{\rho} \qquad (6.1)$$

QCM provides mass concentration of the particles collected in each stage (m_{ci}) as a function of particle diameter assuming a value of 2 g cm^{-3} for ρ. Accordingly, it yields mass concentration in ten size bins; the 50% cut-off diameter and the mean diameter (d_i) of these bins is given in the below Table 6.1. Stage 1 collects all the particles with diameter >25 μm, and hence no mean diameter is assigned to that stage.

Table 6.1

Stage number	Particle diameter (μm)	
	At 50% cut-off Diameter (d_{pi})	Geometric mean diameter (d_{gi})
1	25	---
2	12.5	17.58
3	6.4	8.94
4	3.2	4.53
5	1.6	2.26
6	0.8	1.13
7	0.4	0.566
8	0.2	0.283
9	0.1	0.141
10	0.05	0.071

Total mass concentration (M_t) is separated into M_c, M_a and M_n as

$$M_t = M_c + M_a + M_n \qquad (6.2)$$

Where M_a is the mass concentration in the accumulation size range, M_n is the mass concentration in nuclei mode particle range and M_c in the coarse size range.

$$M_c = \sum_{i=2}^{5} m_{ci} \quad M_a = \sum_{i=6}^{8} m_{ci} \quad M_n = \sum_{i=9}^{10} m_{ci} \qquad (6.3)$$

where i is the stage number of the QCM and m_{ci} is the measured mass concentration in that i^{th} stage.

Mean Mass concentration of Coarse mode aerosols = _ _ _ _ _ _ _ $\mu g/m^3$
Mean Mass concentration of Accumulation aerosols = _ _ _ _ _ _ _ $\mu g/m^3$
Mean Mass concentration of Nuclei mode aerosols = _ _ _ _ _ _ _ $\mu g/m^3$
Total mass concentration of aerosols = _ _ _ _ _ _ _ $\mu g/m^3$

Table 6.2: Aerosol mass concentration in different size bins

Sample	Different Stages($\mu g/m^3$)										Total $\sum_{i=1}^{10} m_{ci}$
	Stage-1 >25.0 μm_{c1}	Stage-2 12.5 μm_{c2}	Stage-3 6.4 μm_{c3}	Stage-4 3.2 μm_{c4}	Stage-5 1.6 μm_{c5}	Stage-6 0.8 μm_{c6}	Stage-7 0.4 μm_{c7}	Stage-8 0.2 μm_{c8}	Stage-9 0.1 μm_{c9}	Stage-10 0.05 μm_{c10}	
14:00											
15.00											
16.00											
17.00											

Table 6.3: Percent relative contribution of different mode of particles to the total mass concentration

Particle mode (μm)	14:00 hrs	15:00 hrs	16:00 hrs	17:00 hrs
Coarse				
Accumulation				
Nucleation				

Experiment 26

Effect of Synoptic Meteorology on Aerosols

Calculation of the effective radius, the mass mean radius, mass concentrations of aerodynamic size of particulate matter (PM_{10}, $PM_{2.5}$) from QCM measurements and study the changes in them associated with the effect of synoptic meteorology.

The effective radius (R_{eff}) of a polydispersive aerosol system is equivalent to the radius required for a monodispersion to exhibit the same total scattering characteristics as the polydispersion. It is defined as the ratio of the third moment to the second moment of the aerosol size distribution (or the ratio of total volume to total area). From the size segregated mass concentration m_{ci}, measured at each size bin, the corresponding volume V_{ci} and area a_{ci} are estimated. The volume and area concentration of aerosols are estimated from the corresponding mass concentrations of individual size bins. The effective radius strongly depends on the size distribution. Mass mean radius is estimated as the mass weighted mean radius. Mainly two size fractions PM_{10} (particulate matter whose aerodynamic diameter is less than 10 μm) and $PM_{2.5}$ (particulate matter whose aerodynamic diameter is less than 2.5 μm) are of interest, especially in view of clean air regulations. PM_{10} may be emitted directly (primary

particulate) or from chemical or thermal reactions in the atmosphere (secondary particulate). It is commonly referred to as inhalable or thoracic particles as they can penetrate into the thoracic compartment of the human respiratory tract. With an increment of inhalable particulate by 10 μg m^{-3} above a base level of 20 μgm^{-3} results in a linear increase in health impacts. With this view, the long-term data collected using the QCM have been examined for PM_{10} and $PM_{2.5}$ concentrations.

Materials:

Quartz Crystals, control unit and Sensing stack assembly, cascade Impactors, 240V ac power supply, wind vane, Anemometer, Thermohygrometer sensors.

Procedure and Data Analysis:

Raw data from QCM provide the total mass concentration (M_t) as well as the size segregated mass concentration (m_{ci} for the i^{th} size bin) of each size bin, for each measurement. Besides yielding characteristic information by themselves, they can also be used to derive physically meaningful parameters describing the aerosols.

The volume and area estimates are used to estimate the effective radius of aerosols R_{eff}.

$$R_{eff} = \frac{\sum_{i=2}^{10} V_{ci}}{\sum_{i=2}^{10} a_{ci}} \qquad (6.4)$$

In estimating R_{eff}, eqn. 6.4, the summation is made only over stages 2-10. Stage 1 is not considered because it collects all particles with size exceeding 25 μm and hence that stage cannot be assigned a meaningful mean radius. And the mass weighted mean radius is estimated as

$$R_m = \frac{1}{2}\frac{\sum_{i=2}^{10} d_{gi} m_{vi}}{\sum_{i=2}^{10} m_{vi}} \qquad (6.5)$$

where d_{gi} is the geometric diameter of each stage.

Particulate matter (PM) is the mass of aerosols suspended in unit volume of air in the atmosphere which is similar to that of M_t. PM_{10} are those particles whose aerodynamic diameter is less than 10 μm and $PM_{2.5}$ are those particles whose aerodynamic diameter is less than 2.5 μm. From the size segregated mass concentration provided by the QCM, PM, PM_{10} and $PM_{2.5}$ are estimated based on the aerodynamic diameters. It is known that most of the PM_{10} is contributed by the QCM stages from 3 to 10m while 5 to 10 contribute mostly to the $PM_{2.5}$. Accordingly from the QCM data (m_{ci} as a function of i), PM_{10}, $PM_{2.5}$ are estimated as

$$PM_{10} = \sum_{i=3}^{10} m_{ci} \qquad PM_{2.5} = \sum_{i=5}^{10} m_{ci} \qquad (6.6)$$

Plots obtained between time Vs effective radius, mass mean radius, PM_{10} and $PM_{2.5}$ and variation of meteorological parameters with time will be like Fig. 6.2 (a), (b) and (c).

Enter the observations and calculations in Tables 6.4 and 6.5.

Table 6.4: Aerosol mass concentration in different size bins

Sample	Different Stages($\mu g/m^3$)										Total $\sum_{i=1}^{10} m_{ci}$
	Stage-1 >25.0 μm_{c1}	Stage-2 12.5 μm_{c2}	Stage-3 6.4 μm_{c3}	Stage-4 3.2 μm_{c4}	Stage-5 1.6 μm_{c5}	Stage-6 0.8 μm_{c6}	Stage-7 0.4 μm_{c7}	Stage-8 0.2 μm_{c8}	Stage-9 0.1 μm_{c9}	Stage-10 0.05 μm_{c10}	
14:00											
15.00											
16.00											
17.00											

Table 6.5: Variation of meteorological parameters with time

Time (hrs)	WS (m/s)	WD (deg)	Temp (^oC)	RH (%)
14:00				
14:15				
14:30				

16:30				
16:45				
17:00				

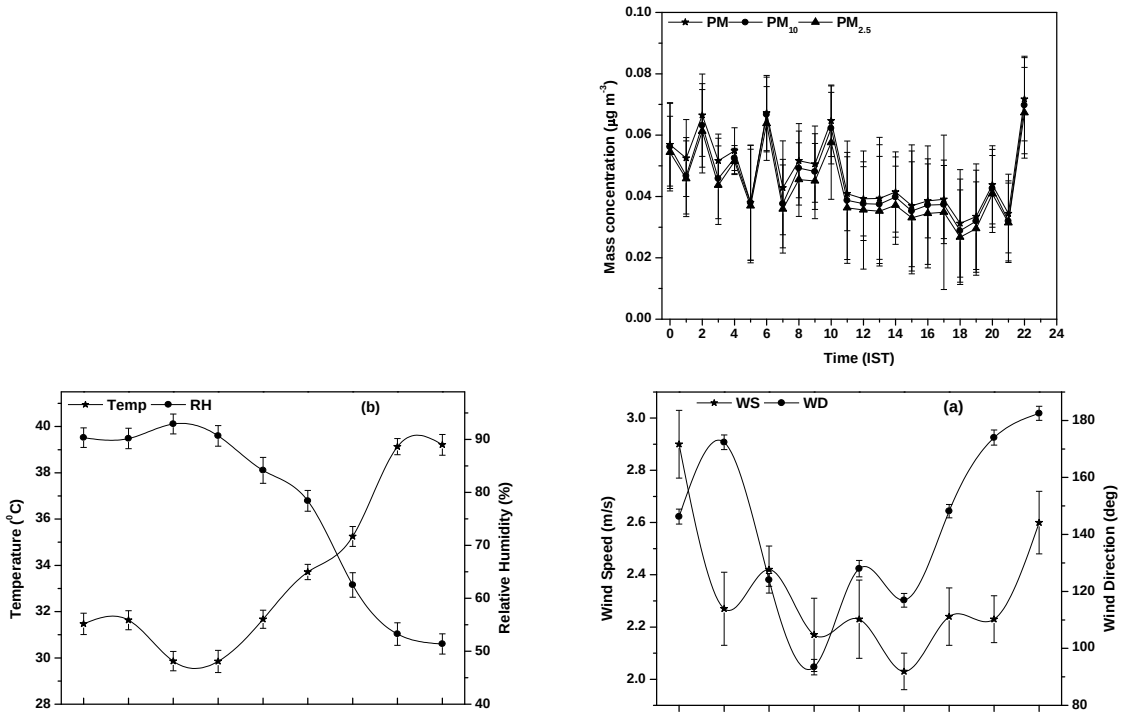


Fig 6.2 (a), (b) and (c)

Result:

The effective radius and mass mean radius of near surface aerosols are calculated from the measurements of QCM and observed the changes in them due to effect of prevailing meteorology. The aerodynamic diameter of the particulate matter (PM) in the size range PM_{10} and $PM_{2.5}$ are also determined.

Experiment 27

Analysis of BOD and DO in Waste Water Sample

(A) BIOCHEMICAL OXYGEN DEMAND (BOD)

Biochemical Oxygen Demand (BOD) refers to the amount of oxygen that would be consumed if all the organics in one liter of water were oxidized by bacteria and protozoa. It is a standard method for indirect measurement of the amount of organic pollution (that can be oxidized biologically) in a sample of water. BOD test procedure is based on the activities of bacteria and other aerobic microorganisms (microbes), which feed on organic matter in presence of oxygen. The result of a BOD test indicates the amount of water-dissolved oxygen (expressed as parts per million or milligrams per liter of water) consumed by microbes incubated in darkness for five days at an ambient temperature of 20°C. Higher the BOD, higher the amount of pollution in the test sample. For the contaminants that cannot be oxidized biologically, chemical oxygen demand (COD) method is used.

Materials/Chemicals Required

Incubator, Al foil, Dissoved oxygen test kit.

Procedure:

The BOD test takes 5 days to complete and is performed using a dissolved oxygen test kit. The BOD level is determined by comparing the DO level of a water sample taken immediately with the DO level of a water sample that has been incubated in a dark location for 5 days. The difference between the two DO levels represents the amount of oxygen required for the decomposition of any organic material in the sample and is a good approximation of the BOD level.

1. Take 2 samples of water
2. Record the DO level (ppm) of one immediately using the method described in the dissolved oxygen test. Determination of DO is described in Experiment 28 B
3. Place the second water sample in an incubator in complete darkness at 20^{o}C for 5 days. If you don't have an incubator, wrap the water sample bottle in aluminum foil or black electrical tape and store in a dark place at room temperature (20^{o}C or 68 °F).
4. After 5 days, take another dissolved oxygen reading (ppm) using the dissolved oxygen test kit.
5. Subtract the Day 5 reading from the Day 1 reading to determine the BOD level.
6. Record your final BOD result in *ppm*.

Water quality is assessed using Table 6.6

Table 6.6: BOD Level and Water Quality

BOD Level (in ppm)	Water Quality
1 - 2	Very Good. There will not be much organic waste present in the water supply
3 - 5	Moderately Clean
6 – 9	Somewhat Polluted. Usually indicates organic matter is present and bacteria are decomposing this waste
10 or greater	Very Polluted. Contains organic waste

(B) DETERMINATION OF DISSOLVED OXYGEN (DO):

The sample is treated with manganese sulfate, alkaline-iodide-azide reagent and finally sulfuric acid. The first two chemicals combine with dissolved oxygen to form a compound which, when acid is added, releases free iodine (from the potassium iodide). Because the amount of iodine released is equal to the amount of oxygen present, the sample can be titrated with either sodium thiosulfate to determine the amount of dissolved oxygen present. Under specific conditions, the amount of sodium thiosulfate used is equivalent to the amount of dissolved oxygen present in the sample.

In the first step, manganese (II) sulfate (at 48% of the total volume) is added to an environmental water sample. Next, potassium iodide (15% in potassium hydroxide 70%) is added to create a pinkish-brown precipitate. In the alkaline solution, dissolved oxygen will oxidize manganese (II) ions to the tetravalent state.

$$2\ MnSO_4(s) + O_2(aq) \rightarrow 2\ MnO(OH)_2(s) \qquad (6.7)$$

$MnO(OH)_2$ appears as a brown precipitate.

$$4\ Mn(OH)_2(s) + O_2(aq) + 2\ H_2O \rightarrow 4\ Mn(OH)_4(s) \qquad (6.8)$$

The second part of the Winkler test reduces (acidifies) the solution. The precipitate will dissolve back into solution. The acid facilitates the conversion by the brown, Manganese-containing precipitate of the Iodide ion into elemental Iodine.

The $Mn(SO_4)_2$ formed by the acid converts the iodide ions into iodine, itself being reduced back to manganese(II) ions in an acidic medium.

$$Mn(SO_4)_2 + 2\ I^-(aq) \rightarrow Mn^{2+}(aq) + I_2(aq) + 2\ SO_4^{2-}(aq) \qquad (6.9)$$

Thiosulfate solution is used, with a starch indicator, to titrate the iodine.

$$2\ S_2O_3^{2-}(aq) + I_2 \rightarrow S_4O_6^{2-}(aq) + 2\ I^-(aq) \qquad (6.10)$$

Materials/Chemicals Required

Incubator, Al foil, Dissoved oxygen test kit, Manganous sulfate solution, Alkaline potassium iodide-sodium azide solution, concentrated Sulfuric acid, Starch indicator solution, Sodium thiosulfate ($Na_2S_2O_3.\ 5H_2O$), 0.025 N, Distilled or deionized water.

Procedure:

1. Collect the sample to be tested in a 300 mL BOD bottle taking special care to avoid adding air to the liquid being collected. Fill bottle completely and add stopper.
2. Remove bottle stopper and add 1 mL of the manganese sulfate solution at the surface of the liquid.
3. Add 1 mL of the alkaline-potassium iodide-sodium azide solution at the surface of the liquid.
4. Replace the stopper, avoid trapping air bubbles and shake well by inverting the bottle several times. Repeat shaking after floc has settled halfway. Allow floc to settle a second time.
5. Add 1 mL of concentrated sulfuric acid by allowing the acid to run down the neck of the bottle above the surface of the liquid.
6. Restopper, rinse the top of the bottle to remove any acid and shake well until the precipitate has dissolved.
7. Titrate a volume of treated sample, which corresponds to 200 mL of the original sample. This corrects for the loss of some sample during the addition of reagents. This volume calculated using the formula:
   mL of sample to titrate = 200 x [300/(300-2)] = 201 mL
8. Pour 201 mL of sample from the BOD bottle into an Erlenmeyer flask.
9. If the solution is reddish-brown in color, titrate with 0.0250 N sodium thiosulfate or 0.0250 N PAO until the solution is a pale yellow (straw) color. Record the amount of titrant used. Add a small quantity of starch indicator and proceed to step 11.
10. If the solution has no reddish-brown color, or is only slightly colored, add a small quantity (approximately 1 mL) of starch indicator. If no blue color develops, there is zero dissolved oxygen. If a blue color develops, proceed to step 11.
11. Titrate with 0.0250 N sodium thiosulfate or 0.0250 N PAO to the first disappearance of the blue color. Record the total number of mL of sodium thiosulfate.

Calculations:

Calculate the concentration of DO in the sample using the following formula:

mg/L DO = (mL titrant x normality of titrant x 8000)/equivalent volume of sample titrated (6.11)

Example:

If 10.0 mL of titrant are used, then:

mg/L DO = (10.0 x .025 x 8000)/200 = 10.0

If the normality of the titrant is exactly 0.025 N and an equivalent volume of 200 mL of sample is titrated, the amount of oxygen dissolved in the original sample (in mg/L) will be equal to the number of mL of sodium thiosulfate used in the titration.

Interferences

In the determination of DO by the Winkler method, there are various materials which cause interferences, including iron salts, organic matter, excessive suspended matter, sulfide, sulfur dioxide, residual chlorine, chromium, cyanide, and certain oxidizing and reducing agents.

If the sample contains 5 mg/L or more of ferric iron salt, add potassium fluoride as the first reagent in the azide modification. Alternately, eliminate ferric iron (Fe III) interference by using 90% phosphoric acid (H_3PO_4) instead of sulfuric acid for acidification.

Precautions

The BOD blank (a BOD bottle full of dilution water containing only the required nutrients, but not any seed) must not show a DO, or dissolved oxygen, depletion of more than 0.2 mg/L after the five-day incubation period. A drop of more than 0.2 mg/L indicates some type of contamination or calibration error.

The seed, or the microorganisms added to industrial wastes or disinfected wastewater effluent samples to break down the organic compounds, should contribute 0.6 to 1.0 mg/L DO uptake per BOD bottle.

Ideally, sample dilutions should show about a 50% DO decrease after the 5-day incubation period. At a minimum, there should be at least a 2.0 mg/L DO change between the initial and the final reading. There should also be a residual DO of at least 1.0 mg/L.

The blank, seed determination, and glucose-glutamic acid standard should be run every time a BOD analysis is performed. If any of these basic BOD requirements are not met, then the test is considered invalid and remedial action is needed.

Experiment 28

Determination of Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is used as a measure of oxygen requirement of a sample that is susceptible to oxidation by strong chemical oxidant. The dichromate method is preferred because of its superior oxidizing ability, applicability to a wide variety of samples and ease of manipulation. Oxidation of most organic compounds is 95-100% of the theoretical value.

Materials/Chemicals Required

Volumetric flasks (250mL and 1000 mL), Electric hot plate, Pipettes (10, 25, and 50ml capacity), Burette, (50 mL - 0.1 mL accuracy), Magnetic stirrer and stirring bars, Potassium dichromate ($K_2Cr_2O_7$) 0.25N, Sulphuric acid (H_2SO_4) / silver sulphate (Ag_2SO_4) solution, Mercuric sulphate ($HgSO_4$) crystals, Silver sulphate crystals, Ferrous ammonium sulphate (FAS) [$Fe(NH_4)_2(SO_4)_2$], approximately 0.01N, Ferroin indicator (1, 10-phenanthroline and ferrous ammonium sulphate)

Procedure

1. Dissolve 12.259g of oven-dried (primary standard grade dried at 103^{o}C to a constant weight) potassium dichromate in distilled water and dilute to 1 litre volume in a volumetric flask.
2. Use 1 g of mercuric sulphate ($HgSO_4$) to complex 100 mg chloride (2,000 mg/L).

3. Dissolve 1.485g of 1,10-phenanthroline monohydrate and 0.695g of ferrous ammonium sulphate heptahydrate in distilled water and dilute to approximately 100 mL.
4. Dissolve 39g reagent grade ferrous ammonium sulphate hexahydrate in distilled water. Add 20ml of concentrated sulphuric acid (H_2SO_4). Cool and dilute to exactly 1 litre in a volumetric flask using distilled water.
5. Standardize ferrous ammonium sulfate (FAS) daily by the following procedure:
6. Dilute 10 mL of standard potassium dichromate ($K_2Cr_2O_7$) solution to 100 mL with distilled water. Slowly add 30 mL of concentrated sulphuric acid and cool to room temperature. Titrate with ferrous ammonium sulphate titrant, using 2 to 3 drops (0.10 to 0.15 mL) of Ferroin indicator.
7. Place a 10 mL sample in a conical flask. The blank is prepared using 10 mL of distilled water.
8. Add 1g of mercuric sulphate ($HgSO_4$), and 10 mL of 0.25N potassium dichromate ($K_2Cr_2O_7$) to both sample and blank.
9. Add 20 mL of concentrated sulphuric acid to the sample as well as blank. The function of the mercuric sulphate is to bind or complex chlorides. One gram may not be required if the chloride concentration is low.
10. Always add acid slowly down the side of the flask while mixing to avoid overheating.
11. Add silver sulphate crystals to complex any short chain aliphatic compounds present.
12. After thorough mixing, incubate the flasks on hot plate at 150^{o}C for 2hrs depending on the ease of oxidation of organic materials.
13. A reagent blank has to be treated in the same way as sample
14. After heating for 2 hrs, cool the flasks to room temperature and add 4 to 5 drops of Ferroin indicator.
15. Titrate against 0.1 N ferrous ammonium sulphate to the first red-brown endpoint.

Caution: Use care in titration. The endpoint is very sharp and may be reached rapidly.

Calculation

COD (mg/L) = (a-b)(N) x 8,000 / sample size (mL) (6.12)

Where:
a = mL $Fe(NH_4)_2(SO_4)_2$ used for blank
b = mL $Fe(NH_4)_2(SO_4)_2$ used for sample
N = normality of FAS titrant ($Fe(NH_4)_2(SO_4)_2$)
mL sample = the actual volume of sample used before dilution

Sources of Error

1. The largest error is caused by using a nonhomogeneous sample. Every effort should be made to blend and mix the sample so that solids are never excluded from any aliquot.
2. Always use the largest sample practical and use the largest glassware that is in keeping with good laboratory practice.
3. Use volumetric flasks and volumetric pipettes with a large bore.
4. The $K_2Cr_2O_7$ oxidizing agent must be precisely measured. Use a volumetric pipette and use the same one each time if possible.
5. When titrating, be certain that the burette is clean and free of air bubbles.
6. Always read the bottom of the meniscus and position the meniscus at eye level.

Experiment 29

Analysis of Fluoride in Ground Water and Potable Water

Ground-water is the most dependable, widespread and in most parts the only source of water in India. Despite being a relatively safe source for human consumption from bacterial load point of view, groundwater suffers from various chemical and mineral contaminations like fluoride, arsenic, iron and nitrates. Dissolution from fluoride-bearing minerals and volcanic rocks contaminates the groundwater with fluoride. Till 1999, as many as 17 states of India have been identified with the problem of excessive fluoride in groundwater- most severely affected amongst these are Andhra Pradesh, Rajasthan and Gujarat. Long term consumption of such water (fluoride above 1ppm) can cause damaged and discolored teeth (dental fluorosis) and debilitating bone ailments (skeletal fluorosis) which are irreversible. The only way out is to prevent the intake of fluoride. The first and most obvious step towards this is identification of safe sources of groundwater. A quick, easy and affordable layman's kit is needed for this purpose.

A method for the estimation of fluoride based on the bleaching of zirconium-xylenol orange complex has been developed at BARC. Zirconium forms several complexes with xylenol orange. In this method, the metal to ligand ratio has been optimised. Acidity and dye concentration have been optimised such that distinct colours are produced at three different levels (deficient, normal and toxic) of fluoride. Zirconium ions, which get polymerized at a lower acidity, affect colour development. De-polymerization of zirconium ions has been achieved using specific treatment, which helps in instantaneous colour development. Stability of the reagent solution prepared has been tested. Purity of the dye is also very important in getting colour contrast and stability. This simplifies the procedure that a common man can so easily adapt it for field-testing.

Procedure:

Preparation of fluoride standards

Stock solution of 10mg/L fluoride standard was prepared by dissolving 0.0221gms of sodium fluoride in 1000 mL DI water. Standards of 0.5mg/L, 1mg/L and 2mg/L were prepared from 10mg/L stock solution by appropriate dilution with water.

Preparation of standard color solutions

Take 4 mL of 0.5, 1.0 and 2.0 mg/L fluoride standard solutions in 3 graduated polypropylene test tubes respectively and add 1 mL of the reagent into each. 0.5mg/L fluoride standard solution gives pink color, 1mg/L gives brownish pink and 2 mg/L solution gives yellow color. The colors are stable for more than six months.

Testing procedure for water samples

(i) Step-I:(qualitative estimation or screening test) : Take 4ml of water sample in graduated test tube and add 1 mL of test reagent and compare the resultant solution color with standard color solutions. If it is pink, fluoride level is deficient (less than 1 mg/L), if it is brownish pink, fluoride level is normal (around 1 mg/L) and if it is yellow, fluoride level is toxic (2 mg/L or above).

(ii) Semi Quantitative Estimation: If in Step-I, the color of the resultant solution is yellow (> 2.0 mg/L fluoride), to find out the exact fluoride content draw one ml of yellow solution in to another graduated test tube and dilute it slowly with the Dilution Reagent* till yellow color solution turns to the color of 1 mg/L standard i.e. brownish pink. The total volume of the solution in the graduated test tube in ml at this point is the actual concentration of fluoride of the corresponding water sample in mg/L.

**Dilution Reagent:* Dilution reagent was prepared by mixing distilled or de-ionzed water (fluoride free) and the test reagent in a 4:1 ratio depending on the required quantity.

Inferences:

Pink or Magenta colour	:	Fluoride content < 1 ppm
Brownish Pink colour	:	Fluoride content ~ 1 ppm
Yellow colour	:	Fluoride content > 2 ppm

Annex I

Fundamental Constants

Parameters	Symbol	Value	SI Unit
Speed of light in vacuum	c	2.99792458×10^{8}	$M\ s^{-1}$
Electronic charge	e	1.602176×10^{-19}	C
Plank constant	h	$6.6260693 \times 10^{-34}$	J s
Boltzman constant	k	$1.3806505 \times 10^{-23}$	$J\ K^{-1}$
Avogadro number	A	6.0221415×10^{23}	mol^{-1}
Atomic mass unit (amu)	u	$1.66053886 \times 10^{-27}$	kg
Faraday constant	F	96485.3383	$C\ mol^{-1}$
Molar gas constant	R	8.314472	$J\ mol^{-1}\ K^{-1}$
Gravitational constant	G	6.6742×10^{-11}	$m^3\ kg^{-1}\ s^{-2}$
Acceleration due to gravity	g	9.806650	$m\ s^{-2}$
Electronic rest mass	m_e	$9.1093826 \times 10^{-31}$	kg
Proton rest mass	m_p	1.67262×10^{-27}	kg
Neutron rest mass	m_n	$1.6749286 \times 10^{-27}$	kg
Bohr magneton	μ_B	$9.274009491 \times 10^{-24}$	$J\ K^{-1}$
Nuclear magneton	μ_N	$5.05078343 \times 10^{-27}$	$J\ K^{-1}$
Electron magnetic moment	μ_e	$-9.28476412 \times 10^{-24}$	$J\ K^{-1}$
Proton magnetic moment	μ_p	$1.41060671 \times 10^{-26}$	$J\ K^{-1}$
Neutron magnetic moment	μ_n	$-0.96623645 \times 10^{-26}$	$J\ K^{-1}$

Annex II

Conversion factors

1 Atomic mass unit (u)	= 931.4940430 MeV
Electron rest mass (m_e)	= 0.510998918 MeV
Proton rest mass (m_p)	= 938.272029 MeV
Neutron rest mass (m_n)	= 939.565360 MeV
1 Joule	= 6.7005361×10^{9} u = $6.24150947 \times 10^{18}$ eV = 38846×10^{-4} k Cal
1 eV	= $1.60217653 \times 10^{-12}$ erg
Number of seconds per day	= 86400
1 atmosphere pressure (atm)	= 101325 Pa = 1.01325 bar = 760 torr
Temperature corresponding to 1 eV	= 1.160450×10^{4} K