B.Sc., Biochemistry **2018-2019**

Semester III

18BCU304A TOOLS AND TECHNIQUES IN BIOCHEMISTRY

Course objective

- The main objective of the course is to understand various techniques used in clinical laboratory.
- The course also provides skills through virtual lab setup.

Course outcomes (CO's)

1. The subject provides the principles and theoretical aspects behind various techniques that are being used in clinical laboratory setup.
2. The course enables the students to get equipped with clinical laboratory tests in better way.

Unit I: Biochemical reagents and solutions

Good laboratory practices: Quality concepts, personal protective equipment. General safety-biological safety, chemical safety and fire safety.Principles of GLP: Test Facility Organization and Personnel, Test Systems, Test and Reference Items, Standard Operating Procedures.Preparation and storage of solutions. Concepts of solution concentration (molarity, molality, normality) and storing solutions. Proper techniques on the use of a pipette, volumetric flask. Use, calibration and maintenance of micropipette.

Unit II: Buffers

Concept of a buffer, buffers solutions, pH indicator, Henderson-Hasselbach equation, working of a pH meter. Measurement of pH – glass electrode, pH scale.

Colorimetry: Colour and absorption spectra. Beer Lamberts – deviation from Beer's law. Working of single cell photoelectric colorimeter.Measurement of extinction.Calibration curve.

Unit III: Spectrophotometric techniques

Principle, instrumentation and applications of UV-visible and fluorescence, FT-IR, NIR, Nuclear Magnetic Resonance (NMR) spectroscopy. Spectroscopy in clinical diagnosis, Raman spectra in clinical diagnosis.

Centrifugation techniques: Principles and techniques of preparative and analytical centrifuge.

Unit IV: Radioactive Isotopes

Radioactive decay, units of radioactivity, detection and measurement of radioactivity – GM counter, Scintillation counter, Autoradiography, biochemical applications of radio isotopes.

Unit V: Virtual labs

Objectives, salient features, the role of Virtual Laboratories in Science Education. Introduction and importance of virtual labs in Biochemistry.Virtual lab for immunological techniques.

SUGGESTED READING

1. Sheehan, D., (2010). Physical Biochemistry: Principles and Applications 2nd ed., Wiley Blackwell (West Sussex), ISBN:978-0-470-85602-4 / ISBN:978-0-470-85603-1.

2. Freifelder, D., (1982). Physical Biochemistry: Applications to Biochemistry and Molecular Biology 2nd ed., W.H. Freeman and Company (New York), ISBN:0-7167-1315-2 / ISBN:0-7167-1444-2.

3. Plummer D. T., (1998). An Introduction to Practical Biochemistry 3rd ed., Tata McGraw Hill Education Pvt. Ltd. (New Delhi), ISBN:13: 978-0-07-099487-4 / ISBN:10: 0-07-099487-0.

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2017 onwards)
DEPARTMENT OF BIOCHEMISTRTY

SUBJECT: TOOLS AND TECHNIQUES IN BIOCHEMISTRY

SEMESTER: III
SUBJECT CODE: 18BCU304A **CLASS: II B. Sc. BC**

LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRTY

S.No	Lecture Duration Hour	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	Good laboratory practices: Quality concepts, personal protective equipment.	T1: 1-4
2	1	General safety-biological safety, chemical safety and fire safety	T2:13-17
3	1	Principles of GLP: Test Facility Organization and Personnel	T1: 17-48
4	1	Test Facility Organization and Personnel	T1: 71-76
5	1	Standard Operating Procedures	T1: 76-97
6	1	Concepts of solution concentration (molarity, molality, normality)	T1: 97-104
7	1	Storage of the prepared solutions	T1: 119-124
8	1	Proper techniques on the use of a pipette, volumetric flask	
9	1	Use, calibration and maintenance of micropipette	
	Total number of hours planned for Unit I=09		
		UNIT-II	
1	1	Concepts of a buffer solution, Preparations of buffers solutions, Universal pH indicators	T1:7-9
2	1	Derivations of Henderson-Hasselbach equation	T1:9-10
3	1	Working principle of a pH meter	T1:10-11

4	1	Working principle of a glass electrode and determination of pH using pH scale	T1:9-11
5	1	Measurement of pH – glass electrode, pH scale	T1:12-14
6	1	Colour and absorption spectra	T1:12-15
7	1	Beer Lamberts – deviation from Beer's law	R1:81-83
8	1	Working of single cell photoelectric colorimeter	
9	1	Measurement of extinction. Calibration curve	
	Total number of hours planned for Unit II=09		
		UNIT-III	
1	1	Principle, instrumentation and applications of UV-visible and fluorescence	T1: 482-493
2	1	Principle, instrumentation and applications FT-IR	T1: 493-507
3	1	Principle, instrumentation and applications NIR	T1: 523-527
4	1	Nuclear Magnetic Resonance (NMR) spectroscopy	T1: 536-546
5	1	Spectroscopy in clinical diagnosis, Raman spectra in clinical diagnosis	T1: 526 -527
6	1	Principles and techniques of preparative and analytical centrifuge	T1: 86-95
	Total number of hours planned for Unit III=06		
		UNIT-IV	
1	1	Radioactive decay, units of radioactivity	T1: 553-561
2	1	Detection and measurement of radioactivity-GM counter	T1: 561-564
3	1	Scintillation counter	T1: 564-567
4	1	Autoradiography	T1: 571-573
5	1	Biochemical applications of radio isotopes	W1
	Total number of hours planned for Unit IV=05		
		UNIT-V	
1	1	Salient features of virtual labs	J1:40-42
2	1	The role of Virtual Laboratories in Science Education	J1:42-43

3	1	Introduction and importance of virtual labs in Biochemistry	J1:43-44
4	1	Applications of Virtual Laboratories in Science Education	J2:4-6
5	1	Introduction of virtual labs in Biochemistry	J2:4-7
6	1	Importance of virtual labs in Biochemistry	J2:5-6
7	1	Usage and applications of Virtual lab for immunological techniques	J2:5-6
	Total number of hours planned for Unit V=07		
Total Planned Hours		**36**	

References:

T1: Wilson, K., and Walker, J., (2010). Principles and Techniques of Biochemistry and Molecular Biology, 7th Low Price Edition, Cambridge University Press, India.

T2: Chatanta DK, Mehra PS (2012) Instrumental Methods of Analysis in Biotechnology. I.K. International Publishing House

T3: Sawhney SK, Singh R (2000) Introductory Practical Biochemistry. Publisher:Narosa

T4: Ajoy Paul A (2011) Textbook of Cell and Molecular Biology. Publisher: Books & Allied Limited, 2011

T5: Ridley (2009) Good Laboratory Practice (GLP) 2nd Edition handbook. Publisher: WHO on behalf of the Special Programme for Research and Training in Tropical Diseases.

W1: Radioisotopes and Their Biomedical Applications, Journal of Biomolecular Research & Therapeutics, **Nida Tabassum Khan. doi:10.4172/2167-7956.1000156**

J1: https://www.sciencedirect.com/science/article/pii/S0263224106001023

Signature of the Staff

UNIT-I (Biochemical reagents and solutions)

SYLLABUS

Good laboratory practices: Quality concepts, personal protective equipment. General safety-biological safety, chemical safety and fire safety. Principles of GLP: Test Facility Organization and Personnel, Test Systems, Test and Reference Items, Standard Operating Procedures. Preparation and storage of solutions. Concepts of solution concentration (molarity, molality, normality) and storing solutions. Proper techniques on the use of a pipette, volumetric flask. Use, calibration and maintenance of micropipette.

Good Laboratory Practices: Quality concepts, personal protective equipment. General safety-biological safety, chemical safety and fire safety. data generation and storage, quality control documents, retention samples, records, audits of quality control facilities. List of Regulations to be followed. Laboratory safety procedure- glass ware, equipment safety, hands protection, precaution to be undertaken to prevent accident and contamination. GLP – an overview and basic information, Scope. Principles of GLP: Test Facility Organization and Personnel, Test Systems, Test and Reference Items, Standard Operating Procedures, Performance of the Study, Reporting of Study Result, Storage and Retention of Records and Materials. Responsibilities in GLP. Implementing of GLP in non GLP analytical laboratory.

The word quality is often used indiscriminately for many different meanings. Quality can be defined as “fitness for use,” “customer satisfaction,” “doing things right the first time,” or “zero defects.” These definitions are acceptable because quality can refer to degrees of excellence. Webster’s dictionary defines quality as “an inherent characteristic, property or attribute.”. Quality control is the science of keeping these characteristics or qualities within certain bounds.

Basic Elements in a Quality System

There are three basic elements in a quality system: Quality Management, Quality Control, and Quality Assurance.

Quality Management: Quality management is the means of implementing and carrying out quality policy. They perform goal planning and manage quality control and quality assurance activities. Quality management is responsible for seeing that all quality goals and objectives are implemented and that corrective actions have been achieved. They periodically review the quality system to ensure effectiveness and to identify and review any deficiencies.

Quality Control: The term quality control describes a variety of activities. It encompasses all techniques and activities of an organization that continuously monitor and improve the conformance of products, processes or services to specifications. Quality control may also include the review of processes and specifications and make recommendations for their improvement. Quality control aims to eliminate causes of unsatisfactory performance by identifying and helping to eliminate or at least narrow the sources of variation. Quality control has the same meaning as variation control of product characteristics. The objective of a quality control program is to define a system in which products meet design requirements and checks and feedback for corrective actions and process improvements. Quality control activities should also include the selecting and rating of suppliers to ensure that purchased products meet quality requirements.

Quality Assurance: The term quality assurance describes all the planned and systematic actions necessary to assure that a product or service will satisfy the specified requirements. Usually this

takes the form of an independent final inspection. The distinction between quality control and quality assurance is stated in an ANSI/ASQ standard: "Quality control has to do with making quality what it should be, and quality assurance has to do with making sure quality is what it should be." The quality assurance function should represent the customer and be independent of the quality control function, which is an integral part of the manufacturing operation.

Personal protective equipment

Always wear proper eye protection in the lab.

– safety glasses or goggles

- Do not wear contact lenses in the lab.
- Wear proper gloves.
- Wear aprons or lab coats.
- Wear closed-toe shoes.
- Wear hearing protection if the noise level isgreater than 85 dBA.
- Wash arms and hands immediately afterworking with allergens, carcinogens,pathogenic organisms, or toxic chemicals.

General safety-biological safety, chemical safety and fire safety

- Avoid working alone.
- Clean up spills.
- Do not store or consume food or beverages in the lab.
- Use proper guards – rotating parts– sharp edges– hot surfaces– machine belts, pto's

- Do not use defective equipment.

Data Generation And Storage, Quality Control Documents, Retention Samples, Records, Audits Of Quality Control Facilities.

Good data and record management are critical elements of the pharmaceutical quality system and a systematic approach should be implemented to provide a high level of assurance that across the product life cycle all GxP records and data are accurate, consistent, trustworthy and reliable.The data governance programme should include policies and governance procedures that address the general principles listed below for a good data management program. These principles are clarified with additional detail in sections below. Applicability to both paper and electronic data. The requirements for good data and recordmanagement that assure robust control of data validity apply equally to paper and electronic data. Organizations subject to GxP should be fully aware that reverting from automated/ computerized to manual/paper-based systems does not in itself remove the need for robustmanagement controls.

Applicability to contract givers and contract acceptors: The principles of these guidelines apply to contract givers and contract acceptors. Contract givers are ultimately responsible for the robustness of all decisions made on the basis of GxP data, including those that are made on the basis of data provided to them by contract acceptors. Contract givers therefore should perform due diligence to assure themselves that contract acceptors have in place appropriate programmes to ensure the veracity, completeness and reliability of provided data. Good documentation practices: To achieve robust decisions and data sets based need to be reliable

and complete. Good documentation practices (GDP) should be followed in order toensure all records, both paper and electronic, allow the full reconstruction of the relatedactivities.

Management governance: To establish a robust and sustainable good data management system it is important that senior management ensure that appropriate data management governance programmes are in place. Elements of effective management governance should include: application of modern quality risk management principles and good data management principles to the current quality management system to integrate those elements that assure the validity, completeness and reliability of data. For example, monitoring of risks and application of appropriate quality metrics can help management gain the awareness necessary for good decision-making to reduce data integrity risks;

- management should ensure personnel are not subject to commercial, political, financial and other organizational pressures or incentives that may adversely affect the quality and integrity of their work;
- management should allocate adequate human and technical resources such that the workload, work hours and pressures on those responsible for data generation and record keeping do not increase errors;
- management should also make staff aware of the importance of their role in ensuring data integrity and the relationship of these activities to assuring product quality and protecting patient safety.

Quality culture: Management, together with the quality unit, should establish and maintain a working environment often referred to as a quality culture that minimizes the risk

noncompliantrecords and erroneous records and data. An essential element is the transparent and open reporting of deviations, errors, omissions and aberrant results at all levels of the organization. Steps should be taken to prevent and detect and correct weaknesses in systems and procedures that may lead to data errors so as to continually the improve scientific robustness of decision making of the organization.

Quality risk management and sound scientific principles: Assuring robust decision making requires valid and complete data, appropriate quality and risk management systems, adherence to sound scientific and statistical principles. For example, the scientific principle of being an objective, unbiased observer regarding the outcome of a sample analysis requires that suspect results be investigated and rejected from the reported results only if they are clearly due to an identified cause. Adhering to good data and record-keeping principles requires that any rejected results be recorded, together with a documented justification for their rejection, and that this documentation is subject to review and retention. Data life cycle. Continual improvement of products to ensure and enhance their safety, efficacy and quality requires a data governance approach to ensure management of data integrity risks throughout all phases of the process by which data are recorded, processed, reviewed, reported, retained, retrieved and subject to ongoing review. In order to ensure that the organization, assimilation and analysis of data into information facilitates evidence based and reliable decision-making, data governance should address data ownership and accountability for data process(es) and risk management of the data lifecycle.

Design of record-keeping methodologies and systems: Record-keeping methodologies and

systems, whether paper or electronic, should be designed in a way that encourages compliance with the principles of data integrity. Examples include but are not restricted to: restricting access to changing clocks for recording timed events; ensuring batch records are accessible at locations where activities take place so that adhoc data recording and later transcription to official records is not necessary;• controlling the issuance of blank paper templates for data recording so that all printed forms can be reconciled and accounted for;Working document restricting user access rights to automated systems in order to prevent (or audit trail) dataamendments;• ensuring automated data capture or printers are attached to equipment such as balances;• ensuring proximity of printers to relevant activities;• ensuring ease of access to locations for sampling points (e.g. sampling points for water systems) such that the temptation to take shortcuts or falsify samples is minimized;• ensuring access to original electronic data for staff performing data checking activities. Maintenance of record-keeping systems. The systems implemented and maintained for both paper and electronic record-keeping should take account of scientific and technical progress. Systems, procedures and methodology used to record and store data should be periodically reviewed and updated as necessary.

QUALITY RISK MANAGEMENT TO ENSURE GOOD DATA MANAGEMENT

All organizations performing work subject to GxP are required by applicable existing WHO guidance to establish, implement and maintain an appropriate quality management system,the elements of which should be documented in their prescribed format such as a quality manual or other appropriate documentation. The quality manual, or equivalent documentation, should include a quality policy statement of management's commitment to an effective quality

management system and good professional practice. These policies should include expected ethics and proper code of conduct to assure the reliability and completeness of data, including mechanisms for staff to report any questions or concerns to management. Within the quality management system, the organization should establish the appropriate infrastructure, organizational structure, written policies and procedures, processes and systems to both prevent and detect situations that may impact data integrity and in turn the risk- based and scientific robustness of decisions based upon that data. Quality risk management is an essential component of an effective data and record validity program. The effort and resource assigned to data and record governance should be commensurate with the risk to product quality. The risk-based approach to record and data management should ensure that adequate resources are allocated and that control strategies for the assurance of the integrity of GxP data are commensurate their potential impact on product quality and patient safety and related decision-making. Control strategies that promote good practices and prevent record and data integrity issues from occurring are preferred and are likely to be the most effective and cost-effective. For example, security controls that prevent persons from altering a master processing formula will reduce the probability of invalid and aberrant data occurring. Such preventive measures, when effectively implemented, also reduce the degree of monitoring required to detect uncontrolled change. Record and data integrity risks should be assessed, mitigated, communicated and reviewed throughout the data life cycle in accordance with the principles of quality risk management.

Example approaches that may enhance data reliability are given in these guidelines but

should be viewed as recommendations. Other approaches may be justified and shown to be equally effective in achieving satisfactory control of risk. Organizations should therefore Working document design appropriate tools and strategies for management of data integrity risks based upon their specific GxP activities, technologies and processes. A data management program developed and implemented, based upon sound quality risk management principles, is expected to leverage existing technologies to their full potential, streamline data processes in a manner that not only improves good data management but also the business process efficiency and effectiveness, thereby reducing costs and facilitating continual improvement.

MANAGEMENT GOVERNANCE AND QUALITY AUDITS

Assuring robust data integrity begins with management which has the overall responsibility for the technical operations and provision of resources to ensure the required quality of GxP operations. Senior management has the ultimate responsibility to ensure an effective quality system is in place to achieve the quality objectives, and that staff roles, responsibilities and authorities, including those required for effective data governance programs, are defined, communicated and implemented throughout the organization. Leadership is essential to establish and maintain a company-wide commitment to data reliability as an essential element of the quality system. The building blocks of behaviours, procedural/policy considerations and basic technical controls together form the basis of a good data governance foundation upon which future revisions can be built. For example, a good data governance program requires the necessary management arrangements to ensure personnel are not subject to commercial, political, financial and other pressures or conflicts of interest that may adversely affect the

quality of their work and integrity of their data. Management should also make staff aware of the relevance of data integrity and importance of their role in protecting the safety of the patient and the reputation of the organization for quality products and services. Management should create a work environment in which staff are encouraged to communicate failures and mistakes, including data reliability issues, so that corrective and preventative actions can be taken and the quality of an organization's products and services enhanced. This includes ensuring adequate information flow between staff at all levels. Senior management should actively discourage any management practices that might reasonably be expected to inhibit the active and complete reporting of such issues. Management reviews and regular reporting of quality metrics facilitate these objectives. This requires designation of a quality manager who has direct access to the highest level of management in order to directly communicate risks so that senior management is aware and can allocate resources to address any issues. To fulfil this role the quality unit should conduct and report to management formal, documented risk reviews of the key performance indicators of the quality management system. These should include metrics related to data integrity to help identify opportunities for improvement. For example: • tracking and trending the occurrence of invalid and aberrant data may reveal unforeseen variability in processes and procedures previously believed to be robust, opportunities to enhance analytical procedures and their validation, validation of processes, training of personnel or sourcing of raw materials and components

Good Laboratory Practice (GLP)

Introduction In the early 70's FDA (United States Food and Drug administration) have

realized cases of poor laboratory practice throughout the United States. FDA decided to check over 40 toxicology labs in-depth. They revealed lot dishonest activities and a lot of poor lab practices. Examples of some of these poor lab practices found were equipment not been calibrated to standard form, therefore giving wrong measurements, incorrect or inaccurate accounts of the actual lab study and incompetent test systems. Although the term "good laboratory practice" might have been used informal already for some time in many laboratories around the world GLP originated in the United States and it had a powerfull effect world wide.

Quality Management in the present context can be considered a modem version of the hitherto much used concept "Good Laboratory Practice" (GLP) with a somewhat wider interpretation. The OECD Document defines GLP as follows: "Good Laboratory Practice (GLP) is concerned with the organizational process and the conditions under which laboratory studies are planned, performed, monitored, recorded, and reported." Thus, GLP prescribes a laboratory to work according to a system of procedures and protocols. This implies the organization of the activities and the conditions under which these take place are controlled, reported and filed. GLP is a policy for all aspects of the laboratory which influence the quality of the analytical work. The result of GLP is that the performance of a laboratory is improved and its working effectively controlled. An important aspect is also that the standards of quality are documented and can be demonstrated to authorities and clients. This results in an improved reputation for the laboratory (and for the institute as a whole). The basic rule is that all relevant plans, activities, conditions and situations are recorded and that these records are safely filed

and can be produced or retrieved when necessary. These aspects differ strongly in character and need to be attended to individually.

Standard Operating Procedures (SOP)

According to EPA(Environmental Protection Agency) GLP regulations, "Raw data" means any laboratory worksheets, records, memoranda, notes, or exact copies thereof, that are the result of original observations and activities of a study and are necessary for the reconstruction and evaluation of the report of that study. Logbooks for recording temperatures or equipment use, repair, and maintenance, field or laboratory notebooks, forms for field or laboratory observations, training reports, computer printouts, recorded data from automated instrument are examples of raw data. It's so hard and not necessary for anyone remember all these details and that's one of the functions of the Standard Operating Procedures (SOPs). 8.1 In FDA it is said that :"If it is not documented..., it did not happen!" or, it's a rumor!" GLPs SOPs Can't do Guarantee "good science", guarantee good documentation, replace common sense, prevent all mistakes (Cobb, 2007). SOPs are written procedures for a laboratories program. They are approved protocols indicating test objectives and methods. Standard Operating Procedures are intended to ensure the quality and integrity of the data generated by the test facility. Revisions to Standard Operating Procedures should be approved by test facility management (OECD, 1998). 8.1.1 They define how to carry out protocol-specified activities. SOPs are most often written in a chronological listing of action steps. They are written to explain how the procedures are supposed to work SOP of routine inspection, cleaning, maintenance, testing and calibration, actions to be taken in

response to equipment failure, analytical methods, definition of raw data, keeping records, reporting, storage, mixing, and recovery of data. (Standard Operating Procedures should have been written and approved by test facility management that are intended to ensure the quality and integrity of the data generated by that test facility. Revisions to Standard Operating Procedures should be approved by test facility management. Each separate test facility unit or area should have at once available current Standard Operating Procedures relevant to the activities being performed therein.

Performance of the study: Performance of the study should be monitorized carefully. All the standards supplied by the GLP should be followed from the beginning of the study to the end by the final report. For each study, a written plan should exist prior to the initiation of the study (Seiler, 2005). The study plan should contain the following information: Identification of the study, the test item and reference item, information concerning the sponsor and the test facility, dates, test methods, issues (where applicable)and records. (OECD, 1998) The study plan should be approved by dated signature of the Study Director and verified for GLP compliance. Deviations from the study plan should be described, explained, recognized and dated in a timely fashion by the Study Director and/or Principal Investigator(s) and maintained with the study raw data. 9.1.1 In the study plan the identification of the study, the test item and reference item information should exist: A descriptive title; a statement which reveals the nature and purpose of the study; Identification of the test item by code or name; The reference item to be used. Information Concerning the Sponsor and the Test Facility should be declared. It should comprise:Name and address of the sponsor, any test facilities and test sites

involved, Study Director, Principal Investigator(s), and the phase(s) of the study delegated by the Study Director and under the responsibility of the Principal Investigator(s) with the date of approval of the study plan by signature of the Study Director, of the study plan by signature of the test facility management and sponsor if required by national regulation or legislation in the country where the study is being performed, the proposed experimental starting and completion dates, reference to the OECD Test Guideline or other test guideline or method to be used, the justification for selection of the test system characterisation of the test system, such as the species, strain, substrain, source of supply, number, body weight range, sex, age and other pertinent information. It should also contain the method of administration and the reason for its choice; The dose levels and/or concentration(s), frequency, and duration of administration/application; detailed information on the experimental design, including a description of the chronological procedure of the study, all methods, materials and conditions, type and frequency of analysis, measurements, observations and examinations to be performed, and statistical methods to be used. Specimens from the study should be identified to confirm their origin. Such identification should enable traceability, as appropriate for the specimen and study. The study should be conducted in accordance with the study plan. All data generated during the conduct of the study should be recorded directly, punctually, correctly, and legibly by the individual entering the data. These entries should be signed or initialled and dated. Any change in the raw data should be made in order to understand the previous entry easily, should indicate the reason for change and should be dated and signed or initialled by the individual making the change. 9.1.2

Computerised system design should always supply for the retention of full audit trails to show all changes to the data without obscuring the original data. It should be possible to associate all changes to data with the persons having made those changes. Reason for changes should be given.

Reporting of study results: All studies generate raw data that are the original data gathered during the conduct of a procedure. They are essential for the reconstruction of studies and contribute to the traceability of the events of a study. Raw data are the results of the experiment upon which the conclusions of the study will be based. Some of the raw data may be used directly, and some of them will be treated statistically. The results and their interpretations provided by the scientist in the study report must be a true and accurate reflection of the raw data. A final report should be prepared for each study. The study report, like all the other scientific aspects of the study, is the responsibility of the Study Director. He/she must ensure that it describes the study accurately. Reports of Principal Investigators or scientists involved in the study should be signed and dated by them. The final report should be signed and dated by the Study Director to indicate acceptance of responsibility for the validity of the data.If necessary, corrections and additions to a final report should be in the form of amendments. Amendments should clearly specify the reason for the corrections or additions and should be signed and dated by the Study Director. The Study Director is responsible for the scientific interpretation included in the study report and is also responsible for declaring to what extent the study was conducted in compliance with the GLP Principles. The GLP

Principles list the essential elements to be included in a final study report. The final

report should include, the following information: A descriptive title; identification of the test item by code or name, characterisation of the test item including purity, stability and homogeneity. Information concerning the sponsor and the test facility should imply;name and address of the sponsor, any test facilities and test sites involved, the study Director, the Principal Investigator(s) and the phase(s) of the study, delegated and scientists having contributed reports to the final report, experimental starting and completion dates. A Quality Assurance Programme statement listing the types of inspections made and their dates, including the phase(s) inspected, and the dates any inspection results should be reported to management and to the Study Director and Principal Investigator(s). This statement should also serve to confirm that the final report reflects the raw data. It should contain the Description of Materials and Test Methods. A summary of results should be given. All information and data required by the study plan;A presentation of the results, including calculations and determinations of statistical significance;An evaluation and discussion of the results and, where appropriate, conclusions. It should imply the location(s) where the study plan, samples of test and reference items, specimens, raw data and the final report are to be stored. 10.1.3 A computerised system to be used in a GLP area should include both the dating and timing of the original entry and the retention of a full audit trail. Such identification could be possible either by the use of personal passwords recognised by the computer or by digital signatures. Furthermore, the system should not accept any changes to data without concomitant entry of a reason or justification. In manual recording the entries made on a sheet of paper can be dated and signed to bear witness to the

validity of data and to accept responsibility. Therefore GLP wants to ensure that data safety and integrity remains the same in electronically as in manually recorded data, irrespective of how they were recorded, and that reconstruction of the way in which the final results and conclusions were obtained remains fully possible The Study Director must sign and date the final report to indicate acceptance of responsibility for the validity of all the data.

Storage and retention of records and materials: Storage and retention of records and materials should be prepared appropriately. The following should be retained in the archives for the period specified by the appropriate authorities : the study plan, raw data, samples of test and reference items, specimens, and the final report of each study records of all inspections performed by the Quality Assurance Programme, as well as master schedules, records of qualifications, training, experience and job descriptions of personnel; records and reports of the maintenance and calibration of apparatus; validation documentation for computerised systems. In the absence of a necessitated retention period, the final arrangement of any study materials should be documented.

Responsibilities in GLP. Implementing of GLP in non GLP analytical laboratory: When implementing GLP in a test facility, and particularly during training, it is important to clearly differentiate between the formal, regulatory use of the term Good Laboratory Practice and the general application of "good practices" in scientific investigations. Since the term "Good Laboratory Practice" is not a trade-mark protected term, any laboratory may consider that it is following good practices in its daily work. This does not comprise GLP compliance. It must be clearly understood that only adherence to, and compliance with, all the requirements of the

OECD GLP Principles constitutes real compliance with GLP. Therefore, the use of similar terminology to describe quality practices outside the scope of GLP proper should be strongly discouraged.

Molarity and Formality

Both molarity and formality express concentration as moles of solute per liter of solution. There is, however, a subtle difference between molarity and formality. **Molarity** is the concentration of a particular chemical species in solution. **Formality,** on the other hand, is a substance's total concentration in solution without regard to its specific chemical form. There is no difference between a substance's molarity and formality if it dissolves without dissociating into ions. The molar concentration of a solution of glucose, for example, is the same as its formality.

For substances that ionize in solution, such as NaCl, molarity and formality are different. For example, dissolving 0.1 mol of NaCl in 1 L of water gives a solution containing 0.1 mol of Na^+ and 0.1 mol of Cl^-. The molarity of NaCl, therefore, is zero since there is essentially no undissociated NaCl in solution. The solution, instead, is 0.1 M in Na^+ and 0.1 M in Cl^-. The formality of NaCl, however, is 0.1 F because it represents the total amount of NaCl in solution. The rigorous definition of molarity, for better or worse, is largely ignored in the current literature, as it is in this text. When we state that a solution is 0.1 M NaCl we understand it to consist of Na^+ and Cl^- ions. The unit of formality is used only when it provides a clearer description of solution chemistry.

Molar concentrations are used so frequently that a symbolic notation is often used to simplify its expression in equations and writing. The use of square brackets around a species indicates that we are referring to that species' molar concentration. Thus, $[Na^+]$ is read as the "molar concentration of sodium ions".

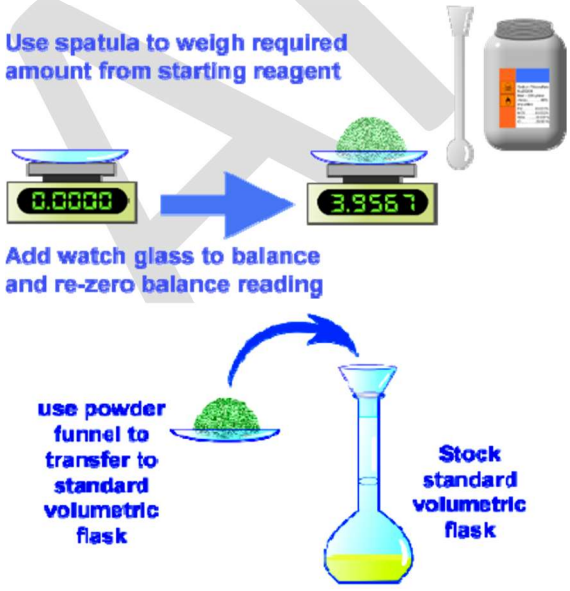
$$\text{Molarity} = \frac{\text{Moles of solute}}{\text{Volume of solution (L)}}$$

$$\text{Moles of solute} = \text{Molarity} \times \text{Volume of solution (L)}$$

$$\text{Volume of solution} = \frac{\text{Moles of solute}}{\text{Molarity}}$$

$$\text{Moles of solute} = \frac{\text{Weight of solute (g)}}{\text{Molecular Weight (g/mol)}}$$

Note that it is the final volume of the solution that is important, not the starting volume of the solvent used. The final volume of the solution might be a bit larger than the volume of the solvent because of the additional volume of the solute. In practice, a solution of known molarity is prepared by weighing an appropriate amount of solute and placing it in a volumetric flask. Enough solvent is added to dissolve the solute, and further solvent is added until an accurately calibrated final volume is reached. The solution is then shaken until it is uniformly mixed as show in figure 1.1.



(b)

(c)

Figure 1.1: Preparing a stock solution of known molarity. (a) A measured number of moles of solute is weighed using analytical balance. (b) Solute is transferred in a volumetric flask. (c) Enough solvent is added to dissolve the solute by swirling and further solvent is carefully added until the calibration mark on the neck of the flask is reached, and the solution is then shaken until uniform.

Molarity can be used as a conversion factor to relate a solution's volume to the number of moles of solute. If we know the molarity and volume of a solution, we can calculate the number of moles of solute. If we know the number of moles of solute and the molarity of the solution, we can find the solution's volume.

Examples 1.1 - What is the molarity of a solution made by dissolving 2.355 g of sulfuric acid in water and diluting to a final volume of 50.0 mL?

Molarity is the number of moles of solute per liter of solution. Thus it's necessary to find the number of moles of sulfuric acid in 2.355 g and then divide by the volume of the solution.

Molar mass of H2SO4 = (2 x 1.0 g/mol) + (1 x 32.1 g/mol) + (4 x 16.0 g/mol) = 98.1 g/mol

$$2.355\,\cancel{g\ H_2SO_4} \times \frac{1\ mol\ H_2SO_4}{98.1\,\cancel{g\ H_2SO_4}} = 0.0240\ mol\ H_2SO_4$$

$$\text{Molarity} = \frac{\text{Moles of solute}}{\text{Volume of solution (L)}} = \frac{0.0240\ mol\ H2SO4}{0.0500\ L} = 0.480\ M$$

The solution has a sulfuric acid concentration of 0.480 M

Examples 1.2 - Hydrochloric acid is sold commercially as a 12.0 M solution. How many moles of HCl are in 300.0 mL of 12.0 M solution?

The number of moles of solute is calculated by multiplying the molarity of the solution by its volume. Moles of HCl = (Molarity of solution) × (Volume of solution L)

$$= \frac{12.0 \text{ mol HCl}}{1 \cancel{\text{L Solution}}} \times 0.300 \cancel{\text{L}} = 3.60 \text{ mol HCl}$$

There are 3.60 mol of HCl in 300.0 mL of 12.0 M solution.

Normality

Normality is an older unit of concentration that, although once commonly used, is frequently ignored in today's laboratories. Normality is still used in some handbooks of analytical methods, and, for this reason, it is helpful to understand its meaning. For example, normality is the concentration unit used in *Standard Methods for the Examination of Water and Wastewater,* and in some *Standard EPA methods* commonly used sources of analytical methods for environmental laboratories.

Normality makes use of the chemical equivalent, which is the amount of one chemical species reacting stoichiometrically with another chemical species. Note that this definition makes an equivalent, and thus normality, a function of the chemical reaction in which the species participates. Although a solution of H_2SO_4 has a fixed molarity, its normality depends on how it reacts.

The number of **equivalents,** *n,* is based on a reaction unit, which is that part of a chemical species involved in a reaction. In a precipitation reaction, for example, the reaction unit is the charge of the cation or anion involved in the reaction; thus for the reaction

$$Pb^{2+}(aq) + 2I^{-}(aq) \longrightarrow PbI_2(s)$$

$n = 2$ for Pb^{2+} because each ion takes two electrons and $n = 1$ for I^- because each ion donate only

one electron. In an acid–base reaction, the reaction unit is the number of H^+ ions donated by an acid or accepted by a base. For the reaction between sulfuric acid and ammonia

$$H_2SO_4(aq) + 2NH_3(aq) \longrightarrow 2NH_4^+(aq) + SO_4^{2-}(aq)$$

we find that $n = 2$ for H_2SO_4 because each molecule donate two ions of H^+ and $n = 1$ for NH_3 because each ion accept one H^+. For a complexation reaction, the reaction unit is the number of electron pairs that can be accepted by the metal or donated by the ligand. In the reaction between Ag^+ and NH_3

$$Ag^+(aq) + 2NH_3(aq) \longrightarrow Ag(NH_3)_2^+(aq)$$

the value of n for Ag^+ is 2 because each ion accept pair of electrons in covalent bonds with Ammonia NH_3 one electron from each covalent bond, for NH3 is $n = 1$ because each molecule of ammonia donate one electron in each covalent bond it form with Ag^+. Finally, in an oxidation–reduction reaction the reaction unit is the number of electrons released by the reducing agent or accepted by the oxidizing agent; thus, for the reaction

$$2Fe^{3+}(aq) + Sn^{2+}(aq) \longrightarrow Sn^{4+}(aq) + 2Fe^{2+}(aq)$$

$n = 1$ for Fe^{3+} because each ion accept one electron in the reduction step and $n = 2$ for Sn^{2+} because each ion donate two electrons in the oxidation step. Clearly, determining the number of equivalents for a chemical species requires an understanding of how it reacts.

Normality is the number of **equivalent weights** (EW) per unit volume and, like formality, is independent of speciation. An equivalent weight is defined as the ratio of a chemical species' **formula weight** (FW) to the number of its equivalents

$$\text{Normality} = \frac{\text{Number of EWs solute}}{\text{liters of solution}}$$

$$\text{Number of EWs solute} = \frac{\text{Weight of solute}}{\text{Equivalent Weight (EW)}}$$

$$\text{Equivalent Weight (EW)} = \frac{\text{Formula Weight (g/mol)}}{\text{n}}$$

$$\text{Normality} = \frac{\text{Weight of solute}}{\text{Formula Weight} \times \text{liters of solution}} \times n$$

Consequently, the following simple relationship exists between normality and molarity.

$$N = n \times M$$

This equation is the simple form to fully understand the normality, you have to be able to determine the number of equivalents and calculate the molarity then use above equation to calculate the normality of the target analyte.

Example 1.3 - Calculate the equivalent weight and normality for a solution of 6.0 M H_3PO_4 given the following reactions:

(a) $H_3PO_4(aq) + 3OH^-(aq) \longrightarrow PO_4^{3-}(aq) + 3H_2O(l)$

(b) $H_3PO_4(aq) + 2NH_3(aq) \longrightarrow HPO_4^{2-}(aq) + 2NH_4^+(aq)$

(c) $H_3PO_4(aq) + F^-(aq) \longrightarrow H_2PO_4^-(aq) + HF(aq)$

For phosphoric acid, the number of equivalents is the number of H^+ ion donated to the base. For the reactions in (a), (b), and (c) the number of equivalents are 3, 2, and 1, respectively. Thus, the calculated equivalent weights and normalities are

(a) $EW = \frac{FW}{n} = \frac{97.994}{3} = 23.665$ $\quad N = n \times M = 3 \times 6.0 = 18.0\ N$

(b) $EW = \frac{FW}{n} = \frac{97.994}{2} = 48.997$ $\quad N = n \times M = 2 \times 6.0 = 12.0\ N$

(c) $EW = \frac{FW}{n} = \frac{97.994}{1} = 97.994$ $\quad N = n \times M = 1 \times 6.0 = 6.0\ N$

Example 1.4 - How many grams of Na_2CO_3 required to prepare 1.0 Liter of 0.05N solution? According to the reaction above (acid-base reaction), each molecule of Na_2CO_3 accept two hydrogen ions (H+) from sulfuric acid, then number of equivalents for Na_2CO_3 n=2.

Formula Weight (FW) for Na_2CO_3 = 105.99 g/mol

$$\text{Equivalents Weight } EW = \frac{FW}{n} = \frac{105.99}{2} = 52.995 \text{ g/mol}$$

$$\text{Normality} = \frac{\text{Number of EWs solute}}{\text{liters of solution}} \longrightarrow \text{Number of EWs solute} = \text{Normality} \times \text{Liter of solution}$$

Number of EWs solute = 0.05 N x 1.0 L = 0.05 mol

$$\text{Number of EWs solute} = \frac{\text{Weight of solute}}{\text{Equivalent Weight (EW)}} \longrightarrow$$

Weight of solute = Number of EWs solute × Equivalent Weight (EW) = 0.05 mol × 52.995 g/mol = 2.65 g

To prepare 0.05N of Na_2CO_3 weigh 2.65 g of Na_2CO_3 and dissolve and complete to volume 1.0 L.

Molality

Molality is used in thermodynamic calculations where a temperature independent unit of concentration is needed. Molarity, formality and normality are based on the volume of solution in which the solute is dissolved. Since density is a temperature dependent property a solution's volume, and thus it's molar, formal and normal concentrations, will change as a function of its temperature. By using the solvent's mass in place of its volume, the resulting concentration becomes independent of temperature.

Molality is defined as mole of solute in Kg of solvent as per equation:

$$\text{molality} = \frac{\text{moles of solute}}{\text{Kg of solvent}}$$

Example 1.5 - What is the molalityof solution made by dissolve 25 g of NaCl in to 2.0 Liter of water. Assume the density of water d = 1.0 g/mL (= kg/L).

Molar mass of NaCl = (1 x 22.99 g/mol) + (1 x 35.45 g/mol) = 58.44 g

$$25\ \text{g NaCl} \times \frac{1\ \text{mol NaCl}}{58.44\ \text{g NaCl}} = 0.428\ \text{mol of NaCl}$$

$$2.0\ \text{Liter water} \times \frac{1.0\ \text{kg}}{1.0\ \text{Liter water}} = 2.0\ \text{kg}$$

$$\text{molality} = \frac{\text{moles of solute}}{\text{Kg of solvent}} = \frac{0.428\ \text{mol NaCl}}{2.0\ \text{kg water}} = 0.214\ \text{m of NaCl}$$

The solution has concentration of NaCl equals to 0.214 m.

Preparing Solutions

Solutions of known concentration can be prepared in a number of different ways depending on the nature of the analyte and/or the concentration required:

- Weighing out a solid material of known purity, dissolving it in a suitable solvent and diluting to the required volume
- Weighing out a liquid of known purity, dissolving it in a suitable solvent and diluting to the required volume
- Diluting a solution previously prepared in the laboratory
- Diluting a solution from a chemical supplier.

Remember to record all masses and volumes used in the preparation of solutions in a laboratory workbook, and to show how you calculated the concentration of the solution. The procedure for preparing a solution by dissolving a solid material is shown in Figure. The procedure for preparing a solution by dilution of a more concentrated solution (either prepared in the laboratory or from a chemical supplier) is shown in Figure.

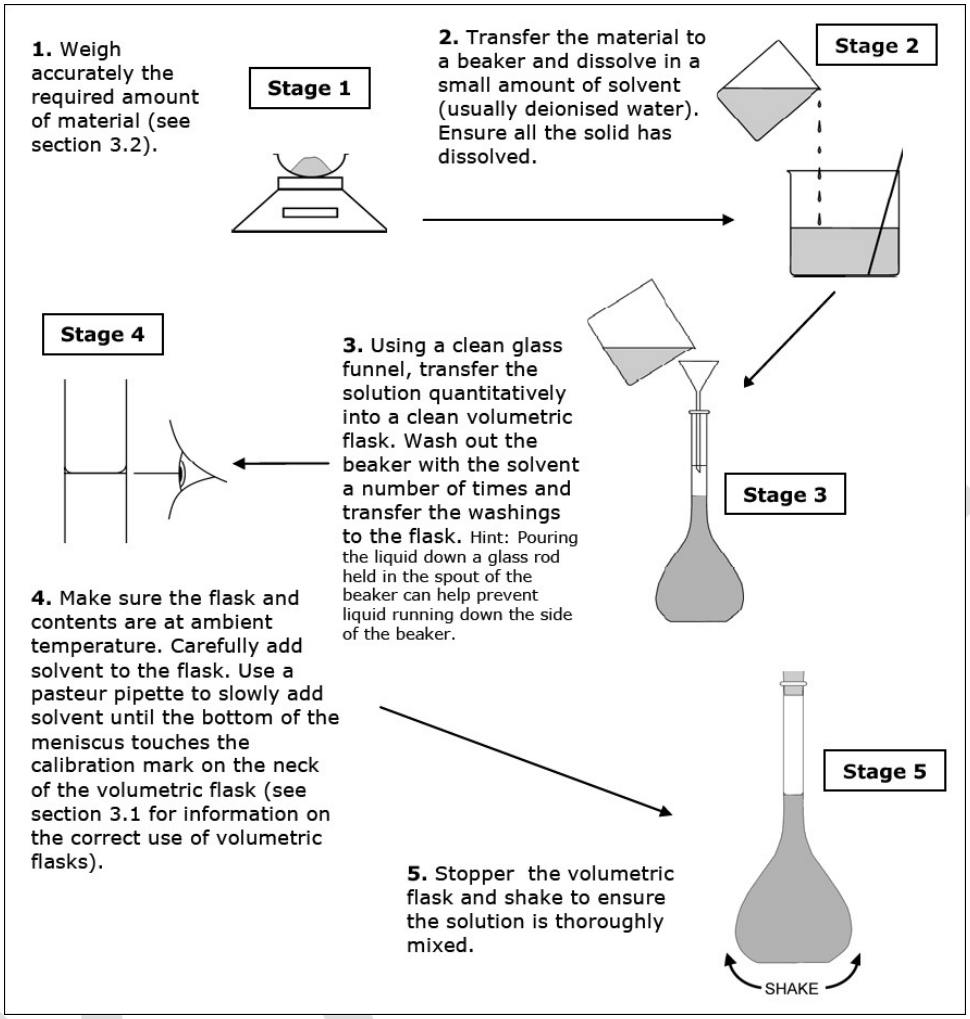


Figure: Procedure for preparing a solution of known concentration from a known amount of a solid material

Note that in some cases the solute may be a liquid rather than solid. The procedure is very similar to that shown in Figure 3-6. The required amount of the liquid is weighed accurately. The liquid is then transferred directly to the volumetric flask containing some of the solvent.

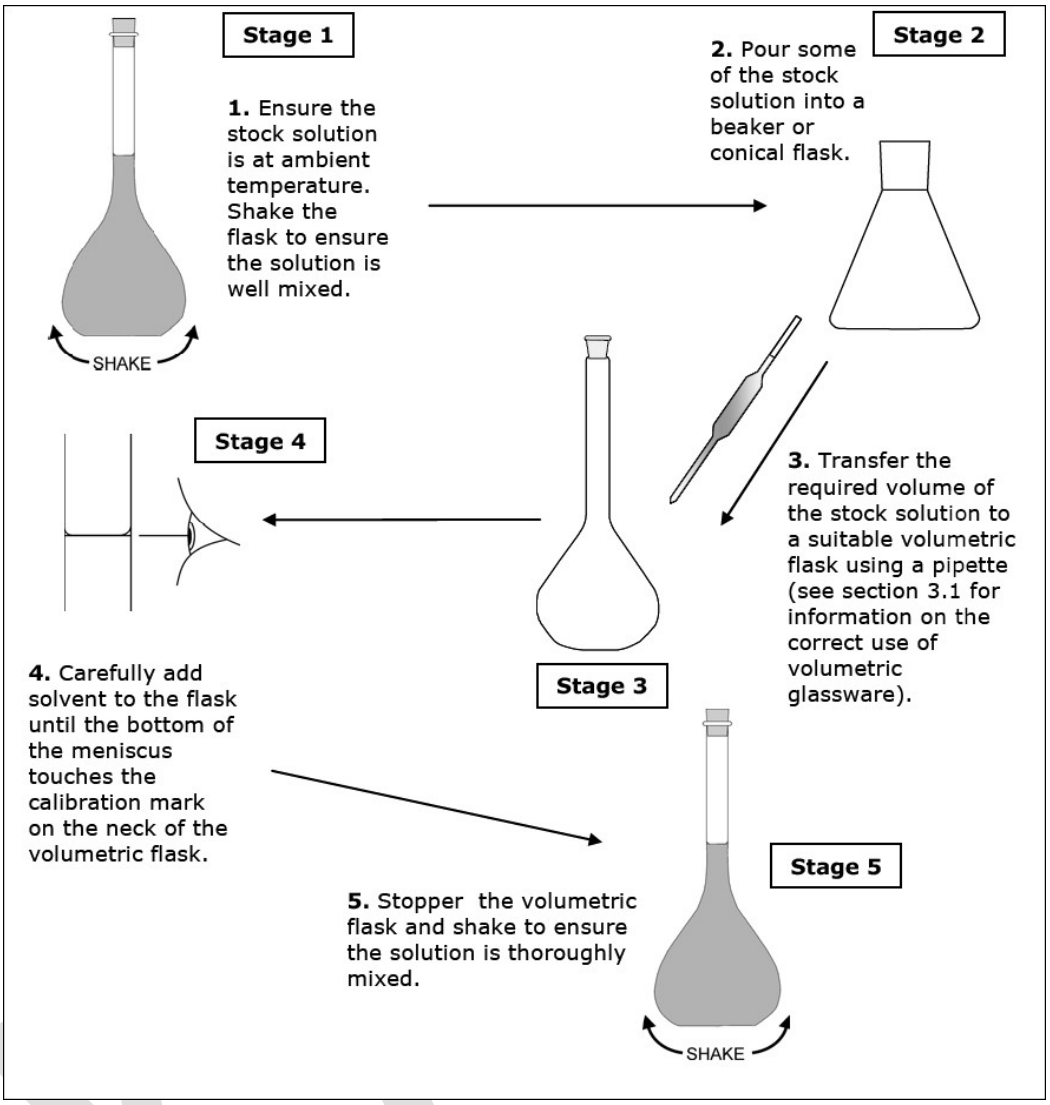


Figure: Procedure for preparing a solution of known concentration by dilution

Labeling and storing solutions

Once you have prepared the solution you need to think about how you will store it and how it will be identified in the future. Remember the following key points:

- Solutions should not be stored in volumetric flasks – transfer them to a suitable container for storage;
- Ensure that solutions are stored correctly. Some solutions will need to be stored in a refrigerator while others may be light-sensitive and need to be stored in amber bottles;

- All solutions should be clearly labeled with the following information:
  1- The name and concentration of the solution;
  2- Date of preparation;
  3. Name of analyst;
  4. Review or expiry date;
  5. Hazard information (if appropriate);
- The label must be securely attached to the container and be written in water insoluble ink.

In some cases, particularly where volatile solvents are used, it is useful to check for any changes in the mass of the solution during storage. After the solution has been prepared, it is transferred to a suitable container and the mass of the sealed container and the solution is recorded. Prior to an aliquot of the solution being used, the container is re-weighed. The mass should not be significantly different from that recorded prior to the solution being stored. After the required volume of the solution has been transferred from the storage container, the solution is reweighed before being returned to storage. If a significant change in mass is observed after the solution has been stored then it should not be used.

VOLUMETRIC FLASKS AND PIPETS

The most common types of volumetric glassware are volumetric pipets and volumetric flasks. These containers are calibrated at a specific temperature to deliver or contain VERY PRECISE amounts of liquid. Heating the glassware or using heated solutions distorts the calibrated volume! Volumetric ("vol") containers are expensive and should be handled with care.

Care of Volumetric Glassware

- Wash glassware with a mild dilute soap solution. Rinse first with tap water, and then deionized (DI) water. If beads of water form on the walls, rewash the glassware. If glassware is to be dried, allow it to drain or use lint-free paper towels.
- Never dry volumetric glassware in an oven! The heat will distort the glass and change the calibrated volumes. Never dry glassware using air jets! The air system contains oil droplets and

fine dust, also the compressed air can break the glass.

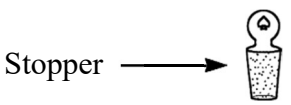
- A **volumetric *flask*** should always be rinsed with a small amount of the *solvent* to be used. This step prevents contamination of the solution from water or other contaminates on the glassware's inside walls and removes the need to dry the flask.
- A **volumetric *pipet*** should always be rinsed with a small amount of the *solution* to be used. This step prevents contamination or dilution of the solution from water or other chemicals on the glassware's inside walls and removes the need to dry the pipet.
- After an experiment is completed, drain all chemicals from glassware, pouring them into marked collection containers in the hoods. Wash the glassware, rinse thoroughly, and return to common storage area at the front of the laboratory.
- Return all broken or cracked glassware to the stockroom for replacement.

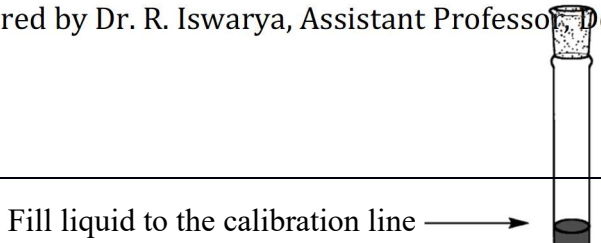
How to Read the Level of Liquid in Volumetric Glassware

A liquid's **meniscus** is the curvature of the liquid surface in a narrow container. The level of **concave** (downward curving) liquid surfaces (ex: water) is read at the bottom of the meniscus. The level of **convex** (upward curving) liquid surfaces (ex: mercury) is read from the top of the meniscus. When the shape of the meniscus is difficult to discern (ex: dark liquids such as purple $KMnO_4$), the liquid level is read from the edge of the liquid. Avoid **parallax error** when reading liquid levels by positioning the eye at the same level as the meniscus. For easier viewing, a buret card (a white card marked with a dark line) can be held behind the glassware to contrast the line between glassware markings and the liquid level.

Volumetric Flasks

A volumetric flask is a flat-bottomed flask with a narrow neck (Figure 1). The top of the neck is specially ground-glass which accommodates a tight-fitting glass stopper or plastic cap. The calibration mark is a single gradation line on the neck indicates the exact volume the flask will contain at a specified temperature marked on the flask (usually 20°C). Prevent warming flask contents (and volume distortions) by handling the flask by the neck instead of the body.





Volumetric flasks are used to make solutions of known concentration by the dissolution of a known mass of solid or the dilution of a more concentrated solution. Before use, always wash the flask and then prerinse with the solvent. Some frequently used volumes in General Chemistry lab are 10.00, 25.00, 50.00, 100.00, and 250.0-mL flasks. At times the zeros to the right of the decimal point are omitted. However, these zeros must always be considered in calculations, as they indicate the accuracy of the volume measurement (i.e., they are significant figures.)

Volumetric Pipet

A volumetric pipet is an elongated glass bulb with two narrow glass stems at the top and bottom of the bulb (Figure 2). The pipet is used "to deliver" a single, fixed volume of liquid at a specific temperature (usually 20.0°C) from one container to another. Some frequently used volumes in General Chemistry lab are 1.00, 5.00, 10.00, and 25.00-mL pipets. Like the vol flasks, the zeros to the right of the decimal point are sometimes omitted but are significant figures. The bottom tip is tapered to deliver a fine stream of liquid and is easily clogged. A single calibration mark on the top stem marks the volume contained at a specific

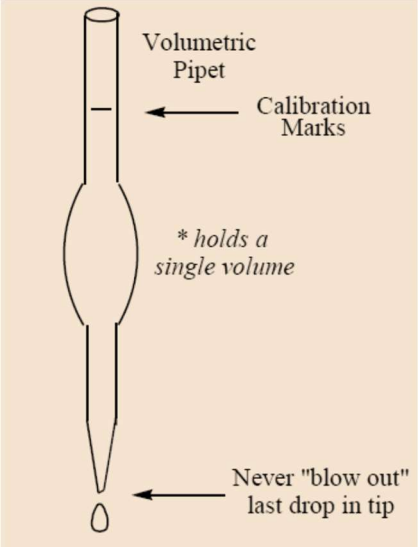


Figure 2: Volumetric Pipet

temperature. Above the calibration mark, stem is open so a suction bulb can be attached to draw liquid into the pipet. *Note:* Never pipet by mouth! Always use a pipet bulb to provide the necessary suction.

First rinse the pipet with a small amount of the solution to remove any water film from the inside walls. To fill the pipet, compress the rubber pipet bulb and fit its Teflon adapter loosely over the top stem of the pipet. Insert the pipet tip into the liquid and slowly release the pressure on the pipet bulb. Allow the liquid level to rise above the calibration mark but do not permit liquid to enter the rubber pipet bulb. Remove the bulb and quickly fit your index finger over the stem. Allow the level of liquid to drop until the meniscus is exactly level with the calibration mark by adjusting the pressure of the index finger. Touch off the hanging drop from the tip of the pipet. (Tissues are not recommended because the paper fibers can draw liquid out of the pipet tip by capillary action.) To transfer the liquid to another container, simply release finger pressure on the pipet stem and allow the liquid to drain freely. When finished, touch off the drop of liquid hanging at the tip into the transferred liquid (it is part of the delivered volume) but do not blow out any liquid remaining inside the tip of the pipet! The pipet has been calibrated to contain this last drop of liquid.

Caution: Never use a pipet to transfer heated solutions or incompletely dissolved solids. The heat will distort the calibrated volume and solids can "condense out" on the colder glass walls of the pipet, clogging the narrow stem and tip.

Mohr (Graduated) Pipets

Like a buret, Mohr pipets have graduated volume markings that increase in value going down the pipet. Mohr pipets are made in two different ways:

(1) *Graduated tip:* Volume markings are present all the way down to the tip (so the pipet can be completely drained). Once a suction bulb has been used to fill this type of Mohr pipet, the level of liquid needs to be lowered so its meniscus falls within the markings and then the initial volume of liquid is recorded. The initial volume can be lowered so that the volume left within the pipet is the desired volume that is to be delivered. Because of the way the markings are

numbered the initial volume needs to be determine by subtracting the desired volume from the pipet's total volume. For example, if 0.40 mL of a stock solution is needed to create a dilute solution, a 1 mL Mohr pipet is used. The stock solution initial level should be 0.60 mL (1.00 – 0.40) so that 0.40 mL is held in the pipet. Allow the liquid to flow out of the pipet under the force of gravity. Like volumetric pipets, you do not force the last drop out.

Clear tip: Volume markings end with a last marking for the total volume of the pipet (so the liquid must be stopped before it goes below this last marking and the final volume must be recorded from the pipet). Like the first type of pipet, once a suction bulb has been used to fill the pipet, the level of liquid needs to be lowered so its meniscus falls within the markings and then the initial volume of liquid is recorded. However, for this type of Mohr pipet, dispensing the liquid requires more coordination because the flow from the pipet has to be stopped once the amount needed is dispensed and before the volume level falls below the last volume marking. *You should fill the pipet with water and practice dispensing small volumes before using the chemicals required in an experiment.*

The Use of Volumetric Pipets

Purpose: Guidance on the use of Class A pipets for precision measurements in package testing.

Introduction: Laboratory glassware is marked to indicate the volume of fluid it will contain when filled to a specific level. The graduations placed on the glassware by the manufacturer differ greatly in the precision they indicate, depending on the Class of the glassware. Class A pipets are manufactured to provide high accuracy and they are required to meet the requirements and tolerances in ASTM E969 − 02 (Reapproved 2012) "Standard Specification for Glass Volumetric (Transfer) Pipets."

Distilled Water: Use distilled water with Type A pipets to avoid contamination and the buildup of minerals within the glassware.

Safety: Below are a few of the main hazards associated with the use of pipets and other laboratory glassware, and the precautions that should be followed to reduce the risk of injury.

Links to additional safety information are provided in the resource section at the end of this paper.

Hazard: *Cuts from…*

1. glass tubing or pipets that break due to forcing plastic or rubber tubing or bulbs onto them;
2. the cleanup of broken glass and other sharp items;
3. broken glass improperly disposed of in ordinary waste bins;
4. flying glass due to explosion or implosion following pressurization or evacuation; and
5. broken/flying glass following breakage due to impact or thermal shock.

Minimize risk:

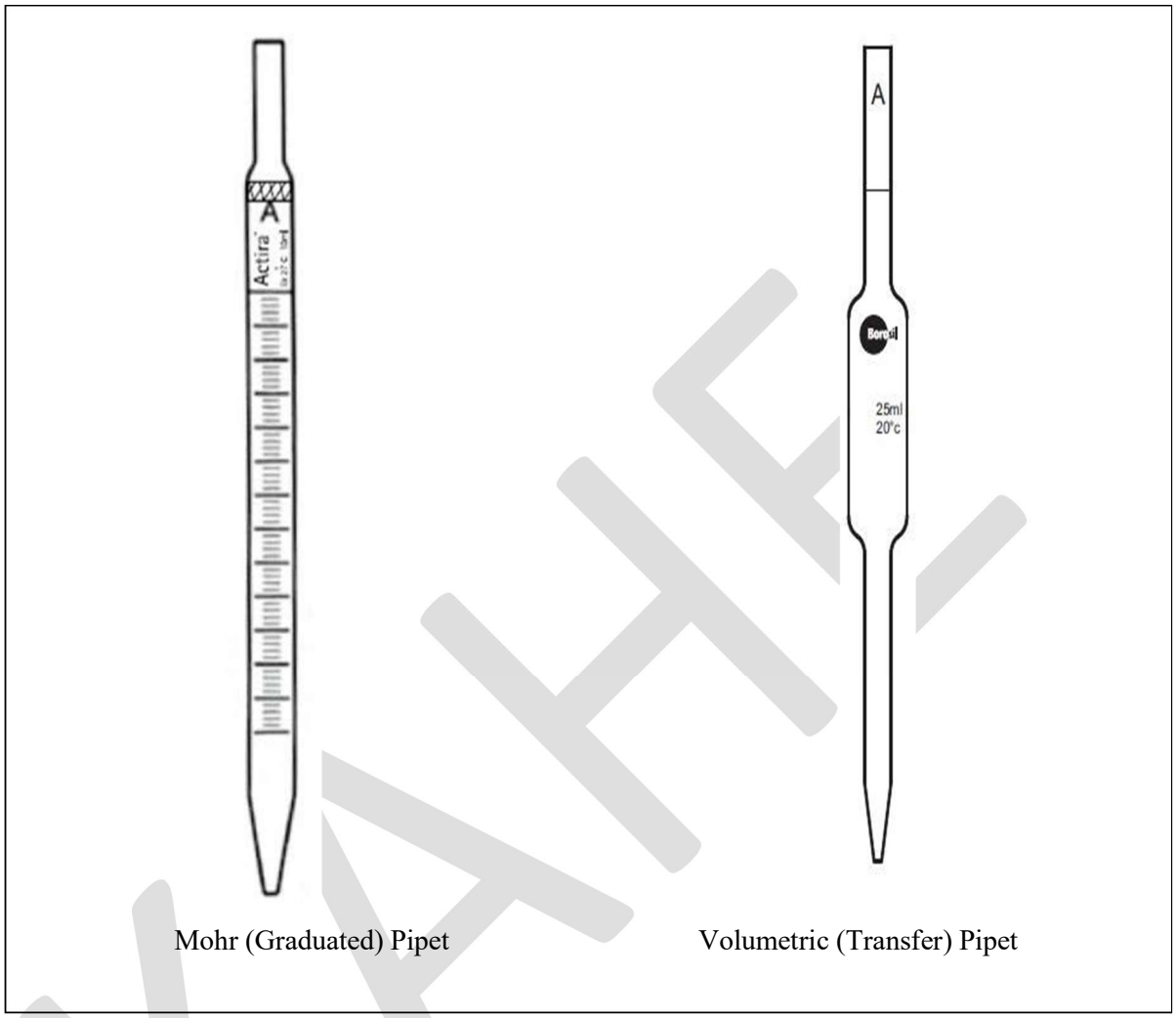
1. Before use, check that all glassware is free from cracks, flaws, or scratches that may cause it to fail in use. Dispose of damaged glassware or have it repaired.
2. Hold beakers, bottles, flasks and other pieces of glassware by the sides and bottoms rather than by the tops. The rims or necks of these items may break if used as a lifting point.
3. Avoid carrying glassware by hand; use a suitable container.
4. Thoroughly clean glassware of all chemical residue (especially if the glassware has contained strong corrosives or reagents) before reusing or sending for repair.
5. Avoid trying to catch falling glassware.
6. Use a brush and dustpan to clean up broken glass. Be especially careful when cleaning broken glass from a sink where water can make sharp edges difficult to see. Use tongs, forceps, or pads of disposable paper towels to pick out pieces.
7. Dispose of glass "sharps" in special containers used solely for this purpose and labeled appropriately. Do not overfill. Do not dispose of broken glass in the ordinary waste bins.

8. Protect hands with gloves, a towel, or tubing holder when inserting glass tubing into bulbs. Lubricate the tube with water or glycerol. Keep hands on pipets close to the bulb and out of line with end of the tube. Do not use excessive force; NEVER push with the palm of the hand.

9. Do not use excessive force. Do not exert force in a direction that will make the glass snap. Think about where the sharp edge of the glass might go if it does break and arrange your grip accordingly. Wrap the glass in a towel or thick layers of paper tissue. Reduce the leverage on pipettes by holding them near the end when fitting fillers. When removing plastic tubing, cut off tubing that does not yield to gentle pressure.

10. Pipets should always be covered [or stored in protective sleeves] when not in use.

Pipets: NIST Handbook 133 requires the use of a Class A Mohr (graduated) Pipet or Class A Transfer Pipet for high precision volumetric measurements. Pictured in Figure 1 below are the two pipets referenced in Handbook 133. On the left is a graduated (Mohr) Pipet and on the right is a Transfer (volumetric) Pipet. A Mohr pipet is calibrated in milliliters (and fractions of a mL) to deliver any amount of water in different capacities including 10 mL, 25 mL and 50 mL sizes. A Transfer (volumetric pipet) is calibrated "to deliver" a specific volume in a single delivery and comes in a variety of sizes including 10 mL, 25 mL, 50 mL, and 100 mL. Both types of pipets are calibrated to deliver the specified volumes at 20 °C (68 °F). When you adjust the quantity of water in a pipet, read the meniscus according to "NIST IR7383 – GMP 3 Good Measurement Practice for Method of Reading a Meniscus Using Water or Other Wetting Liquid, April 2013."



Mohr (Graduated) Pipet Volumetric (Transfer) Pipet

Figure 1. Class A Pipets

It is important to understand the difference in the way Mohr Pipets and Transfer Pipets are emptied because improper emptying will affect the accuracy of a measurement.

Using a Mohr (Graduated) Pipet

A graduated pipet is filled to its marked capacity and the water is dispensed in the different amounts within the range of graduations. The water that remains below the bottom graduation on the pipet must not be dispensed. Figure 2 shows two ways to use a 10 mL graduated pipet to measure 3.2 mL of water. The drawing on the left illustrates that the pipet is emptied to the lowest graduation and no further. If the water below the lowest graduation is emptied into the receiving vessel, the delivered quantity will be excessive. The right-hand drawing shows how to fill the pipet to the zero mark and dispense the 3.2 mL of water from that point using the graduati

ons.

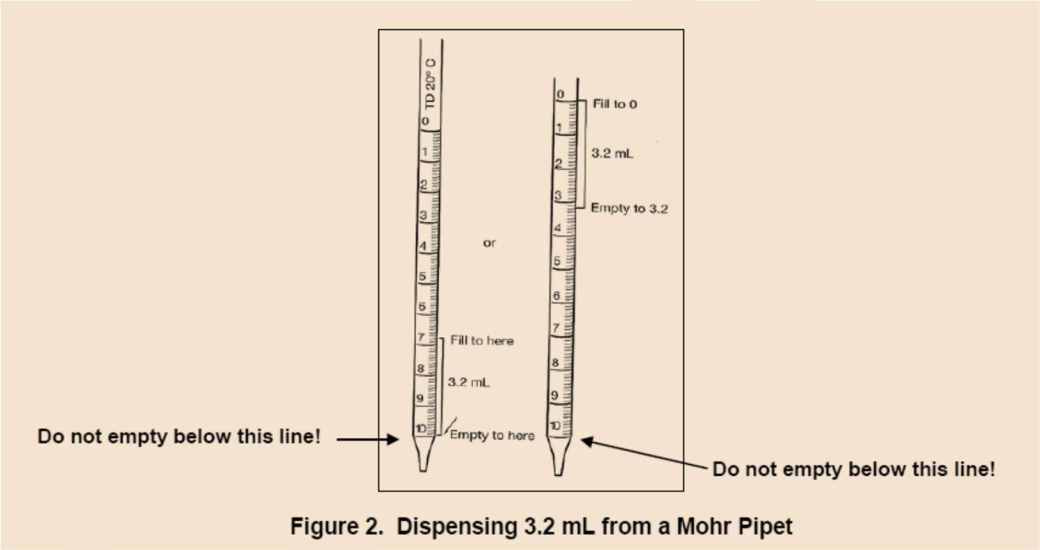


Figure 2. Dispensing 3.2 mL from a Mohr Pipet

Using a Volumetric (Transfer) Pipet

A volumetric (Transfer) pipet delivers its marked capacity in one delivery (capacity is printed on the bulb). These pipets are filled to a level above the capacity mark and then water is released until the meniscus is aligned with the zero graduation. The pipet is then completely emptied into the receiving vessel.

Drying the Tip of a Pipet: Always keep a low-lint laboratory wipe nearby to enable you to keep the outside of a pipet dry and so you can wipe off the tip of the pipet after it is filled. Hold the pipet in vertical position and gently wipe the pipet from top to bottom to dry it.

Filling the Pipet

Wet-Down Procedure: All pipets are calibrated to deliver the marked capacity after they have been wet down with water. You can either fill and empty the pipette several times or partially fill it and turn it so that its interior surface is wet.

Hold the pipet vertical at eye level and insert the tip into the water. Pipets can be filled with an electric-filler or a rubber bulb such as shown in Figure 3.

Do not insert the pipet too far into the bulb or force the bulb on the pipet. Excessive force may cause the pipet to break or to become stuck in the filler[1].

To use the three valve-type bulb, squeeze the A-Valve and compress the bulb to expel the air. Place the tip of the pipet in the water to be measured, and squeeze the S-Valve to fill the pipet with water (the tip of the pipet must be kept under the surface of the water being measured out during the entire time suction is being applied, or air will be sucked into the pipet). When the water is about 20 mm above the calibration mark of the pipet, release S- Valve to stop. Squeeze the E-Valve gently to the calibration mark.

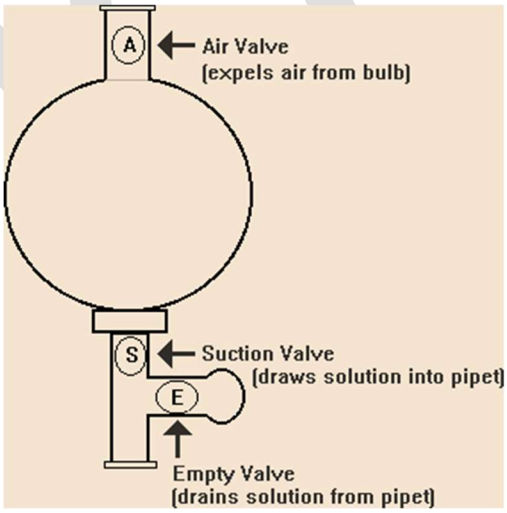


Figure -Way Pipet Filling Bulb

As an alternative, you can also remove the bulb and place your **index finger** on top of the pipet to prevent the water level from falling. By releasing the pressure of your finger, you can allow the water level (meniscus) to fall until it reaches the calibration mark.

DRY THE LOWER OUTSIDE OF THE PIPET AFTER FILLING TO REMOVE ANY EXCESS WATER AT THE TIP.

Dispensing from a Pipet

Place the pipet into the receiving container and release the water to the predetermined amount for Mohr (Graduated Pipets) or the full volume from volumetric (Transfer) pipets.

For volumetric (Transfer) Pipets, place the tip in contact with the wet sidewall of the receiving container (the surface tension created will aid in ensuring a full drainage of the pipet).

Avoid splashing water inside the receiving container (especially when it can end up above the fill capacity or outside of the container).

Drain Time for Volumetric (Transfer) Pipets:

Remove the tip from contact with the sidewall of the receiving vessel within two seconds after the end of the flow of water to complete the delivery. According to ASTM E969[2], NO AFTER DRAINAGE PERIOD IS REQUIRED.

Do not blow any remaining water out of the lower tip of the pipet. Pipets are calibrated assuming a small amount of liquid will remain due to the surface tension of the water inside the tip.

Pipette Function:

Instruments used to handle, measure and dispense liquids. Uses a pressure gradient to draw a liquid.

Types of Pipettes:

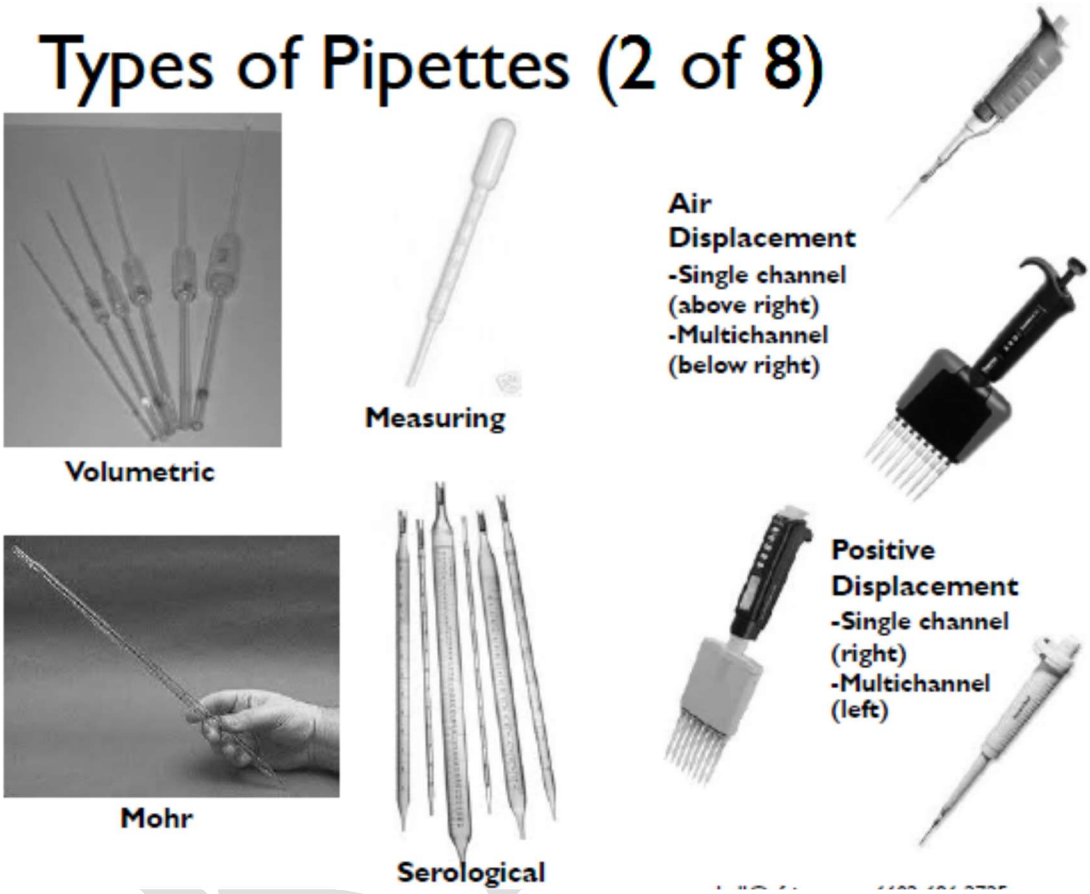
Six types

1. Volumetric
2. Measuring
3. Mohr or Serological
4. Glass/ Pasteur

5. Air Displacement

6. Positive Displacement

Types of Pipettes



POSSIBLE QUESTIONS

UNIT-I

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Write about preparation of stock solution with example.
2. Define the term accuracy.
3. Write a note on Quality Assurance.
4. Define Quality control.
5. Define Molarity with example.

PART-C (6 MARKS)

1. Explain about the Quality control in clinical biochemistry and its classifications.
2. What are the safety regulations carried out in biochemistry laboratories.
3. A solution is prepared by dissolving 10.2g of glucose, C6H12O6, in 405g of water. The final volume of the solution is 414mL. Find the concentration of the solution in units of molality (The molar mass of glucose is 180.16g/mol).
4. Explain the pre-analytical phase of laboratory diagnostic process.
5. Write in detail about the standard operating procedures.
6. Explain about the tragic incident by which rapid implementation of GLP by FDA occurred
7. How will you prepare 0.1M solution of NaCl (Mol.Wt-58.44) in 10ml of distilled water.

Unit I

S. No	Questions	Option 1	Option 2	Option 3	Option 4	Option 5	Option 6	Answer
1	The desire to maintain a safe laboratory environment for all begins with	prevention	ubiquity	microbiology	accidents			**prevention**
2	When a chemical splashes in the eye rinse for	10 seconds	30 seconds	5 minutes	15 minutes			**15 minutes**
3	Which of the following types of personal protective equipment (PPE) is frequently used	safety glasses	lab coats	face shields	all of the above			**all of the above**
4	Chemical, reagents, broth cultures should be pippeted by	mouth	ear	pipette	nose			**pipette**
5	Good work practices include	smelling and tasting chemicals	not washing hand before and after lab	avoiding long hair and loose clothing	using damaged equipment and glassware			**avoiding long hair and loose clothing**
6	What is the name of procedure performed under sterile conditions to eliminate contamination in hopes to obtain a pure culture of one type of microorganism	sterilization technique	aseptic technique	disinfectal technique	pathogen technique			**aseptic technique**
7	After a biohazard spill is covered with paper towels and disinfectant solution, it must sit for	5 minutes	30 minutes	60 minutes	20 minutes			**30 minutes**
8	What is needed as the source of nutrient for the growth and reproduction of microbes	pathogens	bacteria	reagents	media			**media**
9	To prevent the contamination of microscopes and surrounding areas disinfect /clean used slides prepared by students with	70% ethanol and lens paper	acetone and lens paper	5% methylene blue and lens paper	water and lens paper			**70% ethanol and lens paper**
10	Which of the following extinguishers is suitable for a fire involving flammable liquids	carbon dioxide extinguisher (black)	powder extinguisher (blue)	foam extinguisher (cream)	polka dot extinguisher (dotty)			**carbon dioxide extinguisher (black)**
11	GLP is an	Glass ware	FDA regulation	Analytical laboratory	Safety rules			**FDA regulation**
12	Which of the following is the principles of GLP?	Test systems	Reporting of study results	Test and reference substances	All the above			**All the above**
13	Molarity is calculated by the formula	Moles of solute/Litres of solution	B.Moles of solute/Volume of solution (L)	C.Weight of solute (g)/Molecular weight (g/mol)	number of Ews solute/litres of			number of Ews solute/litres of solution
14	SOP is otherwise known as	Standard operating procedures	System operating procedures	Safety operating procedures	Stationary operating procedures			**Standard operating procedures**
15	What a good laboratory must contain?	Area should be free from smoke, smell, dust	Maintenance and calibration data	Air conditional the lab with humidity control	Both A and C			**Both A and C**
16	The prevention of large scale loss of biological intergrity is	Fire safety	Bio safety	Chemical safety	Test systems			**Bio safety**
17	Which of the following is not a laboratory safety rule?	You should never mix acids with bases	You should tie back your long hair	You should never add water to acid	All the above			**You should never mix acid with bases**
18	Which piece of laboratory equipment is best-suited for accurately measuring the volume of a liquid?	Graduated cylinder	Beaker	Erlenmeyer flask	More than one of the above			**Graduated cylinder**
19	Which piece of laboratory equipment can be used to store chemicals for long periods of time?	Burette	Evaporating dish	Beaker	More than one of the above			**Beaker**
20	The independent variable in an experiment is:	The variable you hope to observe in an experiment	The variable you change in an experiment	The variable that isn't changed in an experiment	None of these is correct			**The variable you change in an experiment**
21	Qualitative results refers to	Results that can be observed during an experiment	Results those are difficult to observe during an experiment	Results that require numerical data	None of these is correct			**None of these correct**
22	When drawing a graph that measures family average income over a period of 50 years , the independent variable is	Income	Average	Years	It is impossible to say			**Years**
23	Accuracy is defined as	A measure of how often an experimental value can be repeated	The closeness of a measure value to the real value	The number of significant figures used in a measurement	None of these			**The closeness of a measure value to the real value**
24	How many significant figures are present in the number 10.450?	Three	Four	Five	None of these			**Four**
25	The key component of GLP system of quality is	Quality unit	Quantity unit	Quality reading unit	Quality assurance unit			**Quality assurance unit**
26	Microscope is wiped by using	90% isopropyl alcohol +30% water	distilled water	75 % ethanol	only with water			**90% isopropyl alcohol +30% water**
27	Which one of the followinit is correct?	acid can be added to water	water can be added to acid	both a and b	none of these			**acid can be added to water**
28	Before operating inoculation chamber the palm should be wiped with	Ethanol	distilled water	sanitizer	all of the above			**Ethanol**
29	Which one of the following are GLP regulations on requirements	21CFR58	40CFR160	21CFR211	a and b only			**a and b only**
30	A "class –D" fire extinguisher can be used to treat fires involving which as fuel sources	ordinary combustibles (wood and plastics)	electrical equipment	combustible metals	flammable to combustible liquids			**combustible metals**
31	Which of the following id not a type of firefighting equipment	fire blanket	hose reel	sprinkler	ice cubes			**ice cubes**
32	Why shouldn't carbon dioxide extinguishers be used in confined spaces	they might explode	harmful fumes may be inhaled	they could cause claustrophobia	they might not show up if its dark			**harmful fumes may be inhaled**
33	What is the correct definition of fire	a chemical reaction from which heat and light are emitted	hot orange stuff	mixture of carbon dioxide and nitrogen	a yellow coloured solution			**a chemical reaction from which heat and light are emitted**
34	What is the extraction as practiced in the organic chemistry laboratory	the removal of one solid material from other	the separation of one substance from the another based on solubility	the removal of painful or impacted teeth	none of these			**the removal of one substance from the another based on solubility**
35	Latex gloves	may be reused only if they have not be been permeated	may be reused as long as they are clean	should never be reused	both a and b only			**should never be reused**
36	What is distillation?	distillation is when a liquid is evaporated and then recondensed in another container	distillation is when material heated to melting and then separated	distillation is when a substance is dissolved, heated and then precipitated	none of these			**distillation is when a liquid is evaporated and then recondensed in another container**
37	What piece of laboratory equipment is best suited for accurately measuring the volume of a liquid	graduated cylinder	beaker	Erlenmeyer flask	more than one of the above			**graduated cylinder**
38	What piece of laboratory equipment can be used to store chemical for lor periods of time	burette	evaporating dish	beaker	more than one of the above			**beaker**
39	Qualitative results refer to	results that can be observed during an experiment	results that is difficult to observe during an experiment	results that require numerical data	none of these is correct			**none of these is correct**
40	Accuracy is defined as	a measure of how often an experimental value can be	the closeness of a measured value to the real value	the number of significant figures used in a	none of these			**the closeness of a measured value to the real value**
41	Glassware used to measure 24-hour urine volumes is a:	volumetric flask	beaker	Erlenmeyer cylinder	graduated cylinder			**graduated cylinder**
42	The durable material used to make heat resistant glassware is:	polyethylene	soda lime	polystyrene	borosilicate			**borosilicate**
43	The destruction of all micro-organisms including spores is called:	sanitation	antisepsis	sterilization	disinfection			**sterilization**
44	Cells in a hypertonic solution will:	swell and burst	dehydrate	hemolyze	not be affected			**dehydrate**
45	Which reagent is not routinely used to preserve tissue in a life-like manner:	formic acid	Zenker's fluid	40% formaldehyde dissolved in water	Bouin's fluid			**formic acid**
46	Which piece of histology equipment is not temperature dependent:	tissue processor	microtome	embedding center	water bath			**microtome**
47	A biopsy is:	a removal of biological fluid	the removal of an organ	a post mortem examination	excision of a representative tissue sample			**excision of a representative tissue sample**
48	The liquid portion of blood remaining after a clot has formed is called:	the buffy coat	serum	plasma	lymph			**serum**
49	The shape of a normal erythrocyte is described as:	biconcave disc	spherocyte	polymorphonucleocyte	thin column			**biconcave disc**
50	The tourniquet is:	applied very tightly to the arm	used to increase venous fill	applied about 6-8" above the elbow	tied in a knot to keep it on correctly			**used to increase venous fill**
51	What vein/veins is not used to obtain a venous blood sample:	basilica vein	cephalic vein	medial cubital vein	femoral vein			**femoral vein**
52	The test procedure that uses a Westergren tube is:	erythrocyte sedimentation rate	hematocrit	reticulocyte count	microhematocrit			**erythrocyte sedimentation rate**
53	What areas on an infant are suitable for skin puncture:	any calloused areas of the foot	the second or third finger on either hand	C.the posterior curvature of the heel	the lateral, flat portion of the heel			**the lateral, flat portion of the heel**
54	A disinfectant used on metal surface is:	10% formalin	2% glutaraldehyde	1% hypochlorite	70% isopropyl alcohol			**2% glutaraldehyde**
55	The purpose of heat fixing a bacterial smear is to:	prevent cells from being washed off during staining	causes the cells to absorb the stain more easily	provide a warm temperature for the bacteria to grow	make the cells visible under the			**prevent cells from being washed off during staining**
56	Which Gram stain reagent acts as a mordant to bind the stain to the bacteria:	Lugol's iodine	safranin	acetone-alcohol	Gram's iodine			**Gram's iodine**
57	The autoclave is set at _____ for small loads:	121*C for 50min at 6 p.s.i	130*C for 30min at 30 p.s.i.	121*C for 15min at 15 p.s.i.	121*C for 45min at 15 p.s.i.			**121*C for 15min at 15 p.s.i.**
58	The universally accepted disinfectant for the medical workplace is:	2% glutaraldehyde	1% hypochlorite	10% formalin	70% isopropyl alcohol			**1% hypochlorite**
59	A patient's health card # consists of ____ digits:	4	6	8	10			**10**
60	Xylene is used in:	dehydration of tissues	histology as a clearing agent	attaching cover slips to slides	paraffin wax embedding process			**histology as a clearing agent**

UNIT-II (Buffers)

SYLLABUS

Concept of a buffer, buffers solutions, pH indicator, Henderson-Hasselbach equation, working of a pH meter. Measurement of pH – glass electrode, pH scale.
Colorimetry: Color absorption spectra. Beer Lambert's-deviation from Beer's law. Working of single cell photoelectric colorimeter. Measurement of extinction. Calibration curve.

pH:

pH is a measure of the acidity or basicity of an aqueous solution. Solutions with a pH less than 7 are said to be acidic and solutions with a pH greater than 7 are basic or alkaline. Pure water has a pH very close to 7. The pH scale is traceable to a set of standard solutions whose pH is established by international agreement. Measurement of pH for aqueous solutions can be done with a glass electrode and a pH meter, or using indicators. pH measurements are important in medicine, biology, chemistry, agriculture, forestry, food science, environmental science, oceanography, civil engineering, chemical engineering, nutrition, water treatment & water purification, and many other applications. Mathematically, pH is the negative logarithm of the activity of the (solvated) hydronium ion, more often expressed as the measure of the hydronium ion concentration.

THE HENDERSON-HASSELBALCH EQUATION:

- Dissociation of a weak acid is mathematically described by the Henderson-Hasselbalch equation

  $Ka = [H+][A-] / [HA]$ or $Ka = [H+] \times [A-] / [HA]$

  $\log Ka = \log[H+] + \log \{[A-] / [HA]\}$

  $-\log[H+] = -\log Ka + \log \{[A-] / [HA]\}$

  $pH = pKa + \log \{[A-] / [HA]\}$

- So, if CB = conjugate base and WA = weak acid, then:

  $pH = pKa + \log \{[CB] / [WA]\}$

- This is the Henderson-Hasselbalch equation pH = pKa when [CB]

APPLICATIONS OF THE HENDERSON-HASSELBALCH EQUATION

- Calculate the ratio of CB to WA, if pH is given.
- Calculate the pH, if ratio of CB to WA is known.
- Calculate the pH of a weak acid solution of known.

Concentration

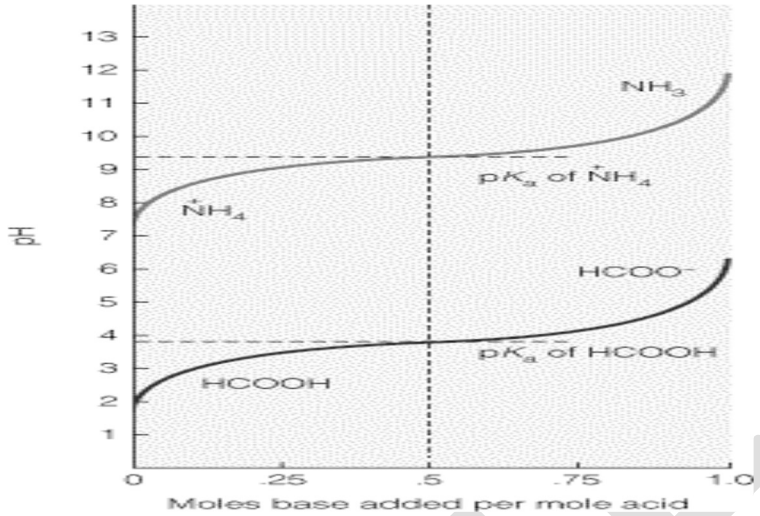
- Determine the pKa of a WA-CB pair.
- Calculate change in pH when strong base is added to a solution of weak acid. This is represented in a titration curve.
- Calculate the pI= [WA]

Titration curve for weak acids

- Initially, [WA] >>> [CB].
- When [WA]=[CB], pH=pKa.
- The central region of the curve (pH+1) is quite flat because: When [CB]/[WA] = 10, pH = pKa +1; When [CB]/[WA] = 0.1, pH = pKa – 1.
- Titration curve is reversible, if we start adding acid, [WA] increases.

Titration of a weak acid with a strong base

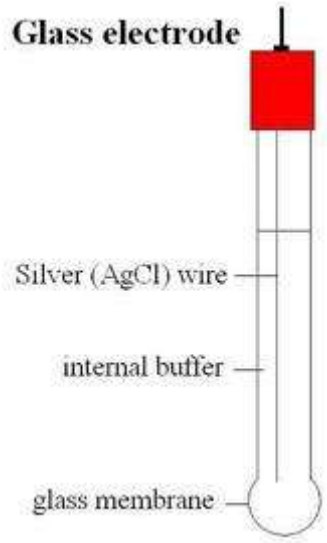
- A weak acid is mostly in its conjugate acid form.
- When strong base is added, it removes protons from the solution, more and more acid is in the conjugate base form, and the pH increases.



- When the moles of base added equals half the total moles of acid, the weak acid and its conjugate base are in equal amounts. The ratio of CB / WA = 1 and according to the HH equation, pH = pKa + log (1) or pH = pKa.
- If more bases are added, the conjugate base form becomes greater till the equivalence point when all of the acid is in the conjugate base form.

DETERMINATION OF pH BY GLASS ELECTRODES:

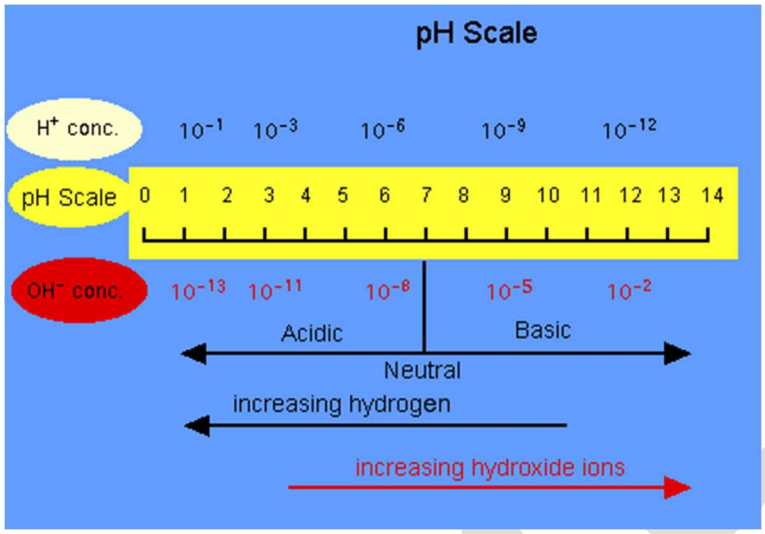
- A glass electrode is a potentiometric sensor made from glass of a specific composition.
- All glass pH electrodes have extremely high electric resistance from, **50 to 500 MOhm.**
- There are different types of pH glass electrode.
- Some of them have improved characteristics for working in very alkaline or acidic medium. But almost all electrodes can operate in the **1 to 12 pH range**.
- A typical pH probe is a combination electrode, which combines both the glass and reference electrodes into one body.
- The pH electrode is essentially a galvanic cell.



- The measuring part of the electrode, the glass bulb on the bottom, is coated both inside and out with a **~10nm layer of a hydrated gel**.
- These two layers are separated by a layer of dry glass and the potential is created by the equilibrium in H^+ ions across the membrane

pH Scale:

The pH scale, (0 - 14), is the full set of pH numbers which indicate the concentration of H^+ and OH^- ions in water. The diagram on the left gives some relationships, which summarizes much of the previous discussion.



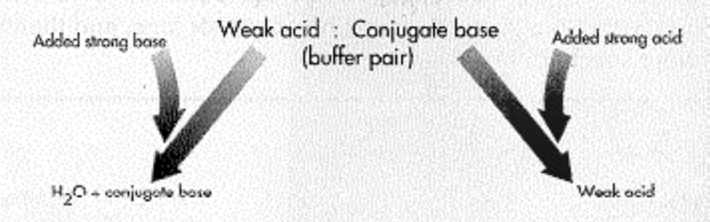
pH Scale Principle: [L SEP]H^+ ion concentration and pH relate inversely. OH^- ion concentration and pH relate directly.

BUFFERS:

- Biological systems use buffers to maintain pH.
- A buffer is a solution that resists a significant change in pH upon addition of an acid or a base.
- Chemically: A buffer is a mixture of a weak acid and its conjugate base
- Example: Bicarbonate buffer is a mixture of carbonic acid (the weak acid) and the bicarbonate ion (the conjugate base): $H_2CO_3 + HCO_3^-$
- All OH^- or H^+ ions added to a buffer are consumed and the overall $[H^+]$ or pH is not altered $H_2CO_3 + HCO_3^- + H^+ \leftrightarrow 2H_2CO^3$

  $H_2CO_3 + HCO_3^- + OH^- \leftrightarrow 2HCO_3^- + H2O$
- For any weak acid / conjugate base pair, the buffering range is its pKa +1.

Mechanism by which Buffers Operate



Example:

$CH_3COOH + CH_3COO^- + OH^- = 2CH_3COO^- + H_2O$ (you get more conjugate base)

$CH_3COOH + CH_3COO^- + H^+ = 2CH_3COOH$ (you get more weak acid)

BUFFER SYSTEM OF BODY FLUIDS AND pH MAINTENANCE:

- The pH (or the amount of hydrogen ions (H^+) in a solution) level of the blood is important in ensuring the proper functionality of biological systems.
- The optimal pH level of the blood is 7.4, which is maintained by three different types of buffer systems working in the body.
- The addition of an acid or a base to a substance changes its pH level.
- A buffer is a solution (or a substance) that has the ability to maintain pH and bring it back to its optimal value.
- It does this by the additional or removal of hydrogen ions.
- Buffers working in the body fluid adjust the pH level of the blood and function to lower pH if its level rises above 7.4 by making the blood slightly more acidic.
- If the pH of blood falls below 7.4, buffers act to take up hydrogen atoms and decrease the acidity of the blood.

Protein Buffer Systems

- Proteins are the most important and widely operating buffers in the body fluid.
- The protein buffer system is an integral component of the body's pH controlling mechanism.
- Protein buffers are either intracellular or extracellular.
- Their functionality is mainly intracellular focused and include haemoglobin (Hb).

- Hb is the protein that functions to transport oxygen within the body.
- Plasma proteins function as buffers but their amount is small in comparison with the intracellular protein buffers.
- Protein buffers include basic group, and acidic protein buffer groups, that act as hydrogen ion depletors or donors to maintain the pH level at 7.4.
- The most well-known protein buffers include 0.1 M NaH2PO4, pH 6.2 (Activation buffer), PBS, pH 7.4 (Alternate Coupling Buffer) and the PBS, 1 percent BSA, pH 7.4 (Assay Buffer).

Phosphate Buffer System

- The phosphate buffer system is comprised of two ions: hydrogen phosphate ions and dihydrogen phosphate ions.
- The pH level of the blood drops below 7.4 when the H^+ ions in the bloodstream increase.
- Hydrogen phosphate ions accept all additional H^+ ions to reestablish the equilibrium between the hydroxide and hydrogen ions in the blood.
- When the pH level of the blood increases above 7.4, the dihydrogen phosphate ions release additional hydrogen ions to reinstate the pH level of the blood to its optimal 7.4.

Bicarbonate Buffer System

- The bicarbonate buffer system functions to maintain the pH level in the blood of mammals.
- It also plays a major role in the formation of acid in the stomach, and to neutralize the pH of chyme that enters the small intestine from the stomach.
- The bicarbonate buffer system manages acid/base imbalances and effectively manages the release of excess carbon dioxide as a bi-product of cellular respiration.

pH indicators:

A **pH indicator** is a halochromic chemical compound added in small amounts to a solution so the pH (acidity or basicity) of the solution can be determined visually. Hence, a pH indicator is a chemical detector for hydronium ions (H_3O^+) or hydrogen ions (H^+) in the Arrhenius model. Normally, the indicator causes the colour of the solution to change depending on the pH.

Indicators can also show change in other physical properties; for example, olfactory indicators show change in their odor. At 25°C, considered the standard temperature, the pH value of a neutral solution is 7.0. Solutions with a pH value below 7.0 are considered acidic and solutions with pH value above 7.0 are basic (alkaline). As most naturally occurring organic compounds are weak protolytes, carboxylic acids and amines, pH indicators find many applications in biology and analytical chemistry. Moreover, pH indicators form one of the three main types of indicator compounds used in chemical analysis. For the quantitative analysis of metal cations, the use of complexometric indicators is preferred, whereas the third compound class, the redox indicators, are used in titrations involving a redox reaction as the basis of the analysis.

pH indicators are frequently employed in titrations in analytical chemistry and biology to determine the extent of a chemical reaction. Because of the subjective choice (determination) of color, pH indicators are susceptible to imprecise readings. For applications requiring precise measurement of pH, a pH meter is frequently used. Sometimes, a blend of different indicators is used to achieve several smooth color changes over a wide range of pH values. These commercial indicators (e.g., universal indicator and Hydrion papers) are used when only rough knowledge of pH is necessary.

Examples: Malachite green, Thymol blue, Methyl yellow, Bromophenol blue, Congo red, Methyl orange, Phenol red, Naphtholphthalein, Phenolphthalein and Thymolphthalein.

Molality:

The molal unit is not used nearly as frequently as the molar unit. **A molality is the number of moles of solute dissolved in one kilogram of solvent**. Be careful not to confuse molality and molarity. A small “m represents molality" whereas molarity is represented by an upper case "M." Note that the solvent must be weighed unless it is water. One liter of water has a specific gravity of 1.0 and weighs one kilogram; so one can measure out one liter of water and add the solute to it. Most other solvents have a specific gravity greater than or less than one. Therefore, one liter of anything other than water is not likely to occupy a liter of space. To make a one molal aqueous (water) solution of sodium chloride (NaCl), measure out one kilogram of water and add one mole of the solute, NaCl to it. The atomic weight of sodium is 23 and the atomic weight of chlorine is

35. Therefore the formula weight for NaCl is 58, and 58 grams of NaCl dissolved in 1kg water would result in a 1 molal solution of NaCl.

Molality (m): $\frac{\text{Weight of a solute in gm/ kg of Solvent}}{\text{Mol. Weight of solute}}$

Molarity:

The molar unit is probably the most commonly used chemical unit of measurement. **Molarity is the number of moles of a solute dissolved in a liter of solution**. Placing 1 mole of a solute into a 1-liter volumetric flask makes a molar solution of sodium chloride. (Taking data from the example above we will use 58 grams of sodium chloride). Water is then added to the volumetric flask up to the one-liter line. The result is a one molar solution of sodium chloride.

Molarity (M): $\frac{\text{Weight of a solute in gm/ Lt of Solvent}}{\text{Mol. Weight of solute}}$

Normality:

There is a relationship between normality and molarity. Normality can only be calculated when we deal with reactions, because normality is a function of equivalents. The example below uses potassium hydroxide (KOH) to neutralize arsenic acid. By studying the reaction it is possible to determine the proton exchange number to determine the normality of the arsenic acid.

Look at the equation $H_3AsO_4 + 2KOH \rightarrow K_2HAsO_4 + 2H_2O$:

Equivalent weight = molar mass/(H+ per mole)

Equivalent = mass of compound / Equivalent weight

And Normality = (equivalents of X)/Liter

And the part that is of interest to you is that Normality = molarity x n (where n = the number of protons exchanged in a reaction).

You probably remember that when a hydrogen atom is ionized and loses its electron, you are left with only a proton. So a hydrogen ion is basically a proton. Let's assume that we have a 0.25 M solution of H_3AsO_4 and want to determine the normality of it if it participates in the reaction

$H_3AsO_4 + 2KOH \rightarrow K_2HAsO_4 + 2H_2O$

When H_3AsO_4 is neutralized by KOH, H_3AsO_4 provides two protons to form $2H_2O$. Note that H_3AsO_4 has three hydrogens, but K_2HAsO_4 only has hydrogen. That means that 2 protons were

exchanged,

Again normality = molarity * n

Remember that normality of the solution is 0.25 mol H_3AsO_4 and there were two protons exchanged (2 equivalents/mole). So, in short, while there is a relationship between the normality of a solution and the molarity of a solution, the normality can only be determined by examining reaction, determining the proton exchange and multiplying molarity by that number.

Normality is particularly useful in titrations calculations. Where N = normality, V = volume, a = the substance on the left of the equation involved in proton exchange, and b=substance on the right of the equation involved in proton exchange: NaVa = NbVb.

Normality (N): $\frac{\text{Amount of substance in gm/ Lt of Solvent}}{\text{Equivalent weight of substance}}$

Mole fraction: moles of target substance divided by total moles involved. The symbol for the mole fraction is the lower-case Greek letter chi, χ. You will often see it with a subscript: χ_{solute} is an example.

Mole fraction (X) : $\frac{\text{Mole of a solute}}{\text{Mole of a solute + mole of a solvent}}$

Example #1: 0.100 mole of NaCl is dissolved into 100.0 grams of pure H_2O. What is the mole fraction of NaCl?

Solution: 100.0 g / 18.0 g mol^{-1} = 5.56 mol of H_2O

Add that to the 0.100 mol of NaCl = 5.56 + 0.100 = 5.66 mol total

Mole fraction of NaCl = 0.100 mol / 5.66 mol = 0.018

What is the mole fraction of the H_2O?

5.56 mol / 5.66 mol = 0.982

Parts-per notation: is a set of pseudo units to describe small values of miscellaneous dimensionless quantities, e.g. mole fraction or mass fraction. Since these fractions are quantity-per-quantity measures, they are pure numbers with no associated units of measurement. Commonly used are **ppm** (parts-per-million, 10^{-6}), **ppb** (parts-per-billion, 10^{-9}), **ppt** (parts-per-

trillion, 10^{-12}) and **ppq** (parts-per-quadrillion, 10^{-15}).

One part per hundred is generally represented by the percent (%) symbol and denotes one part per 100 parts, one part in 10^2, and a value of 1×10^{-2}. This is equivalent to approximately one drop of water diluted into 5 milliliters (one spoonful) or about fifteen minutes out of one day.

One part per thousand should generally be spelled out in full and **not** as "ppt" (which is usually understood to represent "parts per trillion"). It may also be denoted by the millage (‰) symbol. Note however, that specific disciplines such oceanography, as well as educational exercises, do use the "ppt" abbreviation. "One part per thousand" denotes one part per 1000 parts, one part in 10^3, and a value of 1×10^{-3}. This is equivalent to one drop of water diluted into 50 milliliters (ten spoon-fulls) or about one and a half minutes out of one day.

One part per ten thousand is denoted by the permyriad (‱) symbol. In contrast, in finance, the basis point is a quantity with dimensions of (time^{-1}) and is typically used to denote changes in or differences between percentage interest rates. For instance, a change in an interest rate from 5.15% per annum to 5.35% per annum could be denoted as a change of 20 basis points. Although rarely used in science (ppm is typically used instead), one permyriad has an unambiguous value of one part per 10,000 parts, one part in 10^4, and a value of 1×10^{-4}. This is equivalent to one drop of water diluted into half a liter or about nine seconds out of one day.

One part per million (**ppm**) denotes one part per 1,000,000 parts, one part in 10^6, 1/1,000,000 * 100% = 0.0001% (or 1% = 10,000 ppm), and a value of 1×10^{-6}. This is equivalent to one drop of water diluted into 50 liters (roughly the fuel tank capacity of a compact car) or about 32 seconds out of a year.

One part per billion (**ppb**) denotes one part per 1,000,000,000 parts, one part in 10^9, 1/1,000,000,000 * 100% = 0.0000001% (or 1% = 10,000,000 ppb) and a value of 1×10^{-9}. This is equivalent to one drop of water diluted into 250 chemical drums (50 m^3), or about three seconds

out of a century.

One part per trillion (**ppt**) denotes one part per 1,000,000,000,000 parts, one part in 10^{12}, and a value of 1×10^{-12}. This is equivalent to one drop of water diluted into 20 Olympic-size swimming pools (50,000 m^3), or about three seconds out of every hundred thousand years.

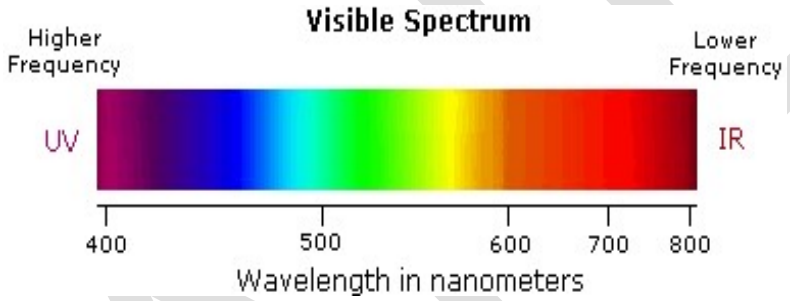
One part per quadrillion (**ppq**) denotes one part per 1,000,000,000,000,000 parts, one part in 10^{15}, and a value of 1×10^{-15}. This is equivalent to 1 drop of water diluted into a cube of water measuring approximately 368 meters on a side (fifty million cubic meters, which is a cube about as tall as the Empire State Building's 102 stories), or two and a half minutes out of the age of the Earth (4.5 billion years). Although relatively uncommon in analytical chemistry, measurements at the ppq level are sometimes performed.

Serial dilution: is the stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. A ten-fold serial dilution could be 1 M, 0.1 M, 0.01 M, 0.001 M... Serial dilutions are used to accurately create highly diluted solutions as well as solutions for experiments resulting in concentration curves with a logarithmic scale. A tenfold dilution for each step is called a **logarithmic dilution** or **log-dilution**, a 3.16-fold ($10^{0.5}$-fold) dilution is called a **half-logarithmic dilution** or **half-log dilution**, and a 1.78-fold ($10^{0.25}$-fold) dilution is called a **quarter-logarithmic dilution** or **quarter-log dilution**. Serial dilutions are widely used in experimental sciences, including biochemistry, pharmacology, microbiology, and physics.

Colorimetry:

Colors and absorption Spectra

The electromagnetic spectrum is comprised of all known types of electromagnetic radiation, including X-rays and radio waves, and is organized by wavelength, which is determined by the energy of the radiation. Shorter wavelengths correspond to higher energy radiation. Visible light is the very small portion of the spectrum (380 nm to 800 nm) that the human eye is sensitive to. The colors of most substances arise from the interaction between visible light and the electrons within molecular bonds. Specifically, the light at specific wavelengths is absorbed, causing these electrons to occupy different energy levels.



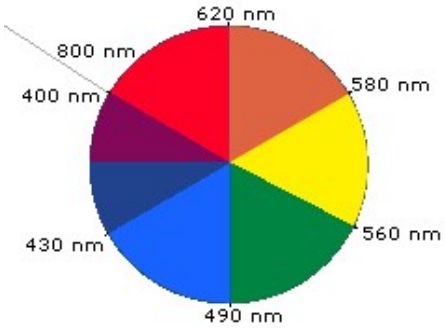
Wavelengths that the molecule doesn't absorb are transmitted, or reflected, to the observer's eye as the perceived color of the molecule, especially when it is dissolved in a transparent solvent such as water. For example, nickel sulfate, NiSO4, dissolved in water appears greenish-blue because it transmits those wavelengths associated with blue-green-yellow portion of the visible spectrum. All other visible wavelengths are absorbed.

- **Violet** : 400 - 420 nm
- **Indigo** : 420 - 440 nm
- **Blue** : 440 - 490 nm
- **Green** : 490 - 570 nm
- **Yellow** : 570 - 585 nm
- **Orange** : 585 - 620 nm
- **Red** : 620 - 780 nm

When white light passes through or is reflected by a colored substance, a characteristic portion of the mixed wavelengths is absorbed. The remaining light will then assume the

complementary color to the wavelength(s) absorbed. The color wheel shown on the right demonstrates this relationship. Here, complementary colors are diametrically opposite each other. Thus, absorption of 420-430 nm lights renders a substance yellow, and absorption of 500-520 nm lights makes it red. Green is unique in that it can be created by absorption close to 400 nm as well as absorption near 800 nm.

Early humans valued colored pigments, and used them for decorative purposes. Many of these were inorganic minerals, but several important organic dyes were also known. These included the crimson pigment, kermesic acid, the blue dye, indigo, and the yellow saffron pigment, crocetin. A rare dibromo-indigo derivative, punicin, was used to color the robes of the royal and wealthy. The deep orange hydrocarbon carotene is widely distributed in plants, but is not sufficiently stable to be used as permanent pigment, other than for food coloring. A common feature of all these colored compounds, displayed below, is a system of extensively conjugated pi-electrons.



The operation of colorimeters is based on the property of colored solutions of absorbing light passing through them. The absorption increases with increasing concentration *c* of the coloring substance. All colorimeter measurements are taken in monochromatic light in the region of the spectrum that is most strongly absorbed by the particular substance in the solution and most weakly absorbed by the solution's other components. Therefore, colorimeters are equipped with light filters; the use of various light filters with narrow spectral ranges for transmitted light makes possible separate determination of the concentration of the various components of the same solution.

Colorimeters are divided into visual and objective (photoelectric) types. In visual colorimeters the light passing through the solution being measured illuminates one part of the

field of vision, and light passing through a solution with a known concentration of the same substance is incident on the other part. By changing the thickness *I* of the layer of one of the solutions being compared or the intensity *I* of the light beam, the viewer attempts to make the color tones of the two halves of the field of vision indistinguishable to the eye. The concentration of the solution under study may be determined from the known relationships for *I, l*, and *c* (the Bouguer-Lambert-Beer law).

Beer's Law

According to Beer's law when monochromatic light passes through the colored solution, the amount of light transmitted decreases exponentially with increase in concentration of the colored substance.

$$I_t = I_o^{e\text{-}KC}$$

Lambert's Law

According to Lambert's law the amount of light transmitted decreases exponentially with increase in thickness of the colored solution.

$$I_t = I_o^{e\text{-}kt}$$

Therefore, together Beer-Lambert's law is:

$$I_E/I_o = e^{-KCT}$$

where,

I_E = intensity of emerging light; I_o = intensity of incident light; e = base of neutral logarithm; K = a constant; C = concentration; T = thickness of the solution

Photoelectric Colorimeter

Photoelectric colorimeters give higher accuracy of measurement than the visual type. Selenium and vacuum photocells, photomultipliers, photoresistors, and photodiodes are used as light detectors in photoelectric colorimeters. The strength of the photocurrent of the detectors is determined by the intensity of the incident light and thus by the extent of absorption of the light beam in the solution (absorption increases with increasing concentration). In addition to photoelectric colorimeters with reading of the photocurrent strength, compensating colorimeters are also common; in this type the difference in the signals corresponding to the standard and test solutions is set to zero (compensated) by an electric or optical compensator (for example, a photometer wedge). The reading in this case is taken from the compensator scale. Compensation

makes possible minimization of the effect on accuracy of measurement conditions, such as temperature and instability of properties of the colorimeter's parts. The readings of colorimeters do not immediately give concentration values for the substances studied. Conversion to concentration values requires the use of calibration curves obtained by measuring solutions of known concentrations.

Colorimeter measurements are relatively simple and rapid. The accuracy of such measurements is often not less than that of more complicated methods of chemical analysis. The lower limits for determinable concentration are 10^{-3} to 10^{-18} moles per liter, depending on the type of substance.

Principles:

Colorimetry is the techniques that are frequently used in biochemical investigations. This involves the quantitative estimation of colors. This measure the quantity of a substance in a mixture, you could use the technique of colorimetry, by allowing the substance to bind with color forming chromogens. The difference in color results in the difference in the absorption of light, which is made use of here in this technique called colorimetry.

- Light from a suitable source is passed through a light filter to select the most appropriate wavelength of light, some of which is then absorbed by the solution held in a special glass cuvette (a sort of 'test tube').
- The amount of light absorbed is called, and measured as, the absorbance which is a function of the coloured solute concentration.

- Most expensive instruments use a double beam system of two cuvets, one is a 'blank' of water and one the actual coloured solution under test, two photocells and sophisticated optics of lenses and mirrors which need not concern as at all.
- Cheaper colorimeters (i.e. in school and illustrated above) allow you to put in a cuvet of 'colourless' water, zero the instrument i.e. set it to read zero absorbance, replace with a cuvet of the coloured solution and simply read of the 'absorbance'.
- The 'zeroing' is necessary because even the apparently 'colourless blank' of glass cuvet and water can absorbed a tiny amount of light. This procedure eliminates this error.
- The filter is chosen to select the band of wavelengths which are most strongly absorbed by the coloured solution e.g. this is illustrated on the diagram above, and in the table below, by using a yellow filter to use in measuring the concentration of a blue coloured solution like copper (II) sulphate or its ammine/amine complex.
- Although the table illustrates the 'complementary' colour relationship between the solution and the filter, in practice it is better to try several filters on a typical concentration of the solution under test to see which filter gives the highest absorption value i.e. gives you maximum sensitivity and hence maximum accuracy in your measurements.

The difference in color results in the difference in the absorption of light, which is made use of here in this technique called colorimetry.

Instrumentation

The instrument use for colorimetry is colorimeter. This apparatus will comprise of the following parts:

1. light source
2. filter (the device that selects the desired wavelength)
3. cuvette chamber (the transmitted light passes through compartment wherein the solution containing the colored solution are kept in cuvette, made of glass or disposable plastic)
4. detector (this is a photosensitive element that converts light into electrical signals)
5. Galvanometer (measures electrical signal quantitatively)

Steps for operating the photoelectric colorimeter:

- Choose the glass filter recommended (see table below) in the procedure and insert in the filter.
- Fill two of the cuvette with blank solution to about three-fourth and place it in the cuvette slot.
- Switch on the instrument and allow it to warm up for 4 – 5 minutes.
- Adjust to zero optical density.
- Take the test solution i another cuvette and read the optical density.
- Take the standard solution in varying concentration and note down the optical density as S1, S2, S3, S4, S5 and so on.
- A graph is plotted taking concentration of standard solution versus the optical density.
- From the graph the concentration of the test solution or the unknown solution can be calculated.

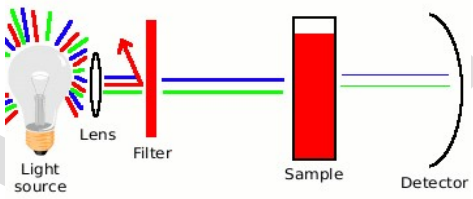


Table for choosing the wavelength of absorption:

Si. No.	Color of the Solution	Colour Absorbed	Wavelength of Absorption
1.	Yellow to Green	Violet	400 nm – 435 nm
2.	Yellow to Orange	Blue	435 nm – 490 nm
3.	Red	Blue to Green	490 nm – 500 nm
4.	Purple	Green	500 nm – 560 nm
5.	Violet	Yellow to Green	560 nm – 580 nm
6.	Blue to Green	Yellow to Orange	580 nm – 650 nm
7.	Bluish Green	Red	650 nm – 700 nm

POSSIBLE QUESTIONS

UNIT-III

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Define Buffer
2. Write the preparations of phosphate buffer.
3. Define pH
4. What are Universal indicators?
5. Write about Reference electrode.

PART-C (6 MARKS)

1. Derive the Henderson-Hasselbalch equation.
2. Explain the importance of pH meter in biochemistry laboratory.
3. Write about the different types electrode used in pH meter.
4. Describe about the universal indicators.
5. Write about the bicarbonate buffer system.
6. Describe the working principle of pH meter and its applications.

Unit II

S.No	Questions	Option 1	Option 2	Option 3	Option 4	Option 5	Option 6	Answer
1	What is the normal blood pH	7.8	7.4	6.8	6.4			**7.4**
2	Equivalent weight X Normality=	Amount of substance dissolved in one litre	Amount of substance dissolved in 100 ml	Amount of substance dissolved in 1 ml	Mole Fraction			**Amount of substance dissolved in one litre**
3	(HCl is always fixed	0.1 M	1 M	5 M	Zero			**0.1 M**
4	pKa is	Negative logarithm of Ka	Positive logarithm of Ka	Negative anti- logarithm of Ka	Positive anti-logarithm of Ka			**Negative logarithm of Ka**
5	Amount of substance in grams dissolved in 1 kg of solvent is referred as	1 Molal	1 Molar	1 Normal	1 Percentage			**1 Molal**
6	pH is	Negative logarithm of hydrogen ion concentration	Positive logarithm of hydrogen ion concentration	Negative anti- logarithm of hydrogen ion concentration	Positive anti-logarithm of hydrogen ion concentration			**Negative logarithm of hydrogen ion concentration**
7	10 ml of a solution of NaOH is found to be	4 ml	8 ml	12 ml	16 ml			**16 ml**
8	pH of 1M HCl and 1M CH_3COOH are	same	different	zero	2			**different**
9	Serial dilution results in	Gradual decrease in the concentration of solutes	Gradual increase in the concentration of solutes	doesnot decrease the concentration of solutes	doesnot increase the concentration of solutes			**Gradual decrease in the concentration of solutes**
10	Buffer solution contains	Weak acid and its salt	Strong acid and its salt	only weak acid	only strong acid			**Weak acid and its salt**
11	pKa of carbonic acid is	6.1	7.4	1.2	9.2			**6.1**
12	Buffering action is maximum when the pH	Equals pKa	Higher than pKa	Lower than pKa	Zero			**Equals pKa**
13	Chromatography generally involves	Two phases	single phase	Three phase	No phase			**Two phases**
14	Chromatography can be used to	form mixtures	change mixture commositions	separate mixtures into pure substances	all of these			**separate mixtures into pure substances**
15	One milligram of substance dissolved in one litre is referred as	1 ppm	1 M	1 N	1%			**1 ppm**
16	Amino acids can be determined using	Ninhydrin	Phenolpthalein	Phenol Red	Diazo reagent			**Ninhydrin**
17	Cations have	Positive charge	Negative charge	No charge	It is impossible to predict the charge on a cation			**Positive charge**
18	If I dilute 5 mL of 0.15 M NaCl to a final volume of 5 L, what is	0.00015 M	0.0015 M	15000 M	none of these			**0.00015 M**
19	Buffers keep the pH of a solution from changing by	converting strong acids to weak ones	converting weak acids to strong ones	converting weak bases to strong ones	more than one of the above answers			**converting strong acids to weak ones**
20	Which of the following is not an acid?	HNO3	CH3COOH	H2SO4	All of these are acids			**All of these are acids**
21	Which of the following dye changes from indicate red and yellow in acidic and alkaline environment?	Methyl red	Bromothymol blue	Phenolpthalein	Alizarin yellow			**Methyl red**
22	Which of the following dye changes from indicate yellow and red in acidic and alkaline environment?	Methyl red	Bromothymol blue	Phenolpthalein	Alizarin yellow			**Alizarin yellow**
23	Which of the following dye changes from indicate yellow and blue in acidic and alkaline environment?	Methyl red	Bromothymol blue	Phenolpthalein	Alizarin yellow			**Bromothymol blue**
24	Which of the following dye changes from indicate colourless and red in acidic and alkaline environment?	Methyl red	Bromothymol blue	Phenolpthalein	Alizarin yellow			**Phenolpthalein**
25	Isotonic solutions have	same osmotic pressure	same temperature	exhibit similar chemistry	same charge			**same osmotic pressure**
26	Flow of solvent from dilute to a concentrated solution across a semipermeable membrane is referred as	electrolysis	endosmosis	osmosis	reverse osmosis			**osmosis**
27	For the preparation of 100 ml of 10% SDS, how much of SDS should be used	100 grams	10 grams	100 milligrams	10 milligrams			**10 grams**
28	For the preparation of 10 ml of 10% SDS, how much of SDS should be used	100 grams	1 gram	100 milligrams	10 milligrams			**1 gram**
29	For the preparation of 100 ml of 5% APS, how much of APS should be used	5 grams	50 grams	500 milligrams	60 milligrams			**5 grams**
30	For optimal buffer action, which of the following is essential	pH	Molarity	pH and Molarity	acid			
31	Laboratory concentration of commercially available hydrochloric acid is	5 M	12 M	20 M	24 M			**12 M**
32	Laboratory concentration of	5 M	12 M	18 M	24 M			**18 M**
33	Acidosis is defined as	a blood pH below 7.4	a blood pH greater than 7.4	a blood pH of 7.4	a condition of low H+ concentration			**a blood pH below 7.4**
34	In Universal indicators, red color shows	strong acids	strong alkalis	weak acids	weak bases			**strong acids**
35	Soap is	acidic	alkaline	neutral	basic			**alkaline**
36	Orange juice is	acidic	alkaline	neutral	basic			**acidic**
37	Lemon juice has a pH of	2	6	8	12			**2**
38	An aqueous solution with pH = 0 is	Strongly acidic	Strongly basic	Neutral	weakly basic			**Strongly acidic**
39	Which one of the following types of medicines is used for treating indigestion?	Antibiotic	Analgesic	Antacid	antiseptic			**Antacid**
40	The pH of the gastric juices released during digestion is	less than 7	equal to 7	more than 7	equal to 0			**less than 7**
41	Which of the following is acidic in nature?	lime water	lime juice	Human blood	antacid			**lime juice**
42	Which of the following is used for the dissolution of gold?	Hydrochloric acid	Sulphuric acid	Nitric acid	Aqua regia			**Aqua regia**
43	Which of the following is not an mineral acid	Hydrochloric acid	Sulphuric acid	Citric acid	Nitric acid			**Citric acid**
44	The acid present in lemon is	Tartaric acid	Ascorbic acid	Citric acid	Oxalic acid			**Citric acid**
45	The acid present in grape is	Tartaric acid	Ascorbic acid	Citric acid	Oxalic acid			**Tartaric acid**
46	The acid present in tomato is	Tartaric acid	Ascorbic acid	Citric acid	Oxalic acid			**Oxalic acid**
47	The acid present in apple is	Tartaric acid	Malic acid	Citric acid	Oxalic acid			**Malic acid**
48	The most important buffering system for maintaining proper blood pH is	the charges on the amino acids	the bicarbonate buffer system	phosphate groups of serum phosphoproteins	albumin			**the bicarbonate buffer system**
49	The acid having a highest H+ ion concentration is one with	pH = 7.0	pH = 1.2	pH = 2.3	pH = 8.2			**pH = 1.2**
50	Aqueous solution of which of the following turns blue litmus red?	NaNO3	CuSO4	NH4OH	CH3COONa			**CH3COONa**
51	An element common to all acids is?	Chlorine	Nitrogen	Oxygen	Hydrogen			**Hydrogen**
52	Bases on ionization release	Hydrogen ions	Sodium ions	Chlorine ions	Hydroxide ions			**Hydroxide ions**
53	A solution turns red litmus to blue its pH is likely to be	2	5	7	10			**10**
54	Which of the following represents a base?	KOH	KCl	CH3OH	CH3COOH			**KOH**
55	Fruit juices, such as orange juice contain	Boric acid	Citric acid	Sulphuric acid	Nitric acid			**Citric acid**
56	Which of the following solution will turn phenolphthalein pink?	HCl (aq)	CO2(aq)	KOH(aq)	CH3OH(aq)			**KOH(aq)**
57	A common substance that contains acetic acid is	Vinegar	Ammonia water	Salad oil	Soap			**Vinegar**
58	When HCl (aq) is exactly neutralized by NaOH (aq), the hydrogen ion concentration in the resulting mixture is	Always less than the concentration of the hydroxide ions	Always greater then the concentration of hydroxide ions	Always equal to the concentration of the hydroxide ions	Sometimes greater and sometimes less than the concentration of the hydroxide ions.			**Always equal to the concentration of the hydroxide ions**
59	Which of the following statement is correct about an aqueous solution of an acid and of a base?	Higher the pH, stronger the acid	Higher the pH, weaker the acid	Lower the pH, stronger the base	Acid and base have equal pH			**Higher the pH, weaker the acid**
60	Which of the following is not a base	NaOH	KOH	CH3OH	NH4OH			**CH3OH**

UNIT-III (Spectrophotometric techniques)

SYLLABUS

Principle, instrumentation and applications of UV-visible and fluorescence, FT-IR, NIR, Nuclear Magnetic Resonance (NMR) spectroscopy. Spectroscopy in clinical diagnosis, Raman spectra in clinical diagnosis.
Centrifugation techniques: Principles and techniques of preparative and analytical centrifuge.

SPECTROSCOPY

Properties of Electromagnetic Radiation

Electromagnetic radiation is characterized by a broad range of wavelengths and frequencies, each associated with a specific intensity (or amplitude) and quantity of energy. This interactive tutorial explores the relationship between frequency, wavelength, and energy, and enables the visitor to adjust the intensity of the radiation and to set the wave into motion.

The tutorial initializes with a visible light wave appearing in the window having a wavelength of 650 nanometers (red light) and amplitude of 61 candelas. Energies associated with waves in the tutorial appear beneath the window and are given in units of kJ/mole.

To adjust the wavelength (and simultaneously, the frequency) of the wave, translate either the Wavelength or Frequency sliders to the left or right. As the sliders are relocated, the new values for wavelength and frequency appear above the sliders, the wave color changes to match the value for visible light associated with the wavelength, and the energy associated with the wave appears beneath the tutorial window. The amplitude of the wave can be adjusted with the Amplitude slider, and the resulting intensity values will appear above the slider, measured in units of candelas. In order to stop propagation of the wave, click on the Propagation Stop button located in the lower right-hand side of the tutorial window. The wave can be restarted by again clicking on the button, which changes into a Start button when the wave is halted.

An electromagnetic wave moves or propagates in a direction that is at right angles to the vibrations of both the electric and magnetic oscillating field vectors, carrying energy from its radiation source to undetermined final destination. The two fields are mutually perpendicular. By

convention, and to simplify illustrations, the vectors representing the electric and magnetic oscillating fields of electromagnetic waves are often omitted, although they are understood to still exist.

Whether transmitted to a radio from the broadcast station, heat radiating from the oven, furnace or fireplace, X-rays of teeth, or the visible and ultra-violet light emanating from the sun, the various forms of electromagnetic radiation all share fundamental wave-like properties. Every form of electromagnetic radiation, including visible light, oscillates in a periodic fashion with peaks and valleys, and displaying a characteristic amplitude, wavelength, and frequency that defines the direction, energy, and intensity of the radiation.

The standard unit for all electromagnetic radiation is the magnitude of the wavelength (in a vacuum), which is usually reported in terms of nanometers for the visible light portion of the spectrum. Each nanometer represents one-thousandth of a micrometer, and is measured by the distance between two successive peaks (see Figure 1). The corresponding frequency of the radiation wave, the number of sinusoidal cycles (oscillations or complete wavelengths) that pass a given point per second, is proportional to the reciprocal of the wavelength. Frequency is usually measured in Hertz (Hz) or cycles per second (cps). Thus, longer wavelengths correspond to lower frequency radiation and shorter wavelengths correspond to higher frequency radiation.

The different wavelengths and frequencies of various forms of electromagnetic radiation are fundamentally similar in that they all travel at the same speed--about 186,000 miles per second (approximately 300,000 kilometers per second), commonly known as the speed of light (and identified with the variable **c**). Electromagnetic radiation (including visible light) travels 149 million kilometers (93 million miles) from the sun to Earth in about 8 minutes.

The wavelength of light, and all other forms of electromagnetic radiation, is related to the frequency by a relatively simple equation:

$$n = c/l$$

where c is the speed of light (measured in meters per second), n is the frequency of the light in hertz (Hz), and l is the wavelength of the light measured in meters. From this relationship one can conclude that the wavelength of light is inversely proportional to frequency. An increase in frequency produces a proportional decrease in the wavelength of light with a corresponding increase in the energy of the photons that compose the light. Upon entering a new medium (such

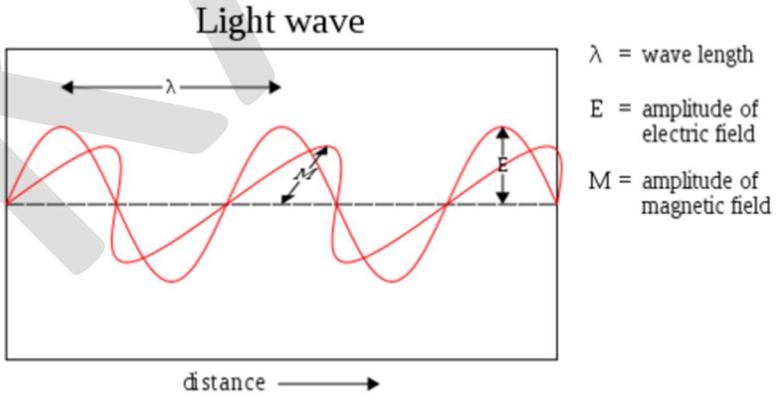
as glass or water from air), the speed and wavelength of light is reduced, although the frequency remains unaltered.

Electromagnetic radiation (EMR) is a form of energy that is produced by oscillating electric and magnetic disturbance, or by the movement of electrically charged particles traveling through a vacuum or matter. The electric and magnetic fields come at right angles to each other and combined wave moves perpendicular to both magnetic and electric oscillating fields thus the disturbance. Electron radiation is released as photons, which are bundles of light energy that travel at the speed of light as quantized harmonic waves. This energy is then grouped into categories based on its wavelength into the electromagnetic spectrum. These electric and magnetic waves travel perpendicular to each other and have certain characteristics, including amplitude, wavelength, and frequency.

General Properties of all electromagnetic radiation:

1. Electromagnetic radiation can travel through empty space. Most other types of waves must travel through some sort of substance. For example, sound waves need either a gas, solid, or liquid to pass through in order to be heard.
2. The speed of light is always a constant. (Speed of light : 2.99792458 x 10^8 m s^{-1})
3. Wavelengths are measured between the distances of either crests or troughs. It is usually characterized by the Greek symbol \(\lambda\).

Waves and their Characteristics



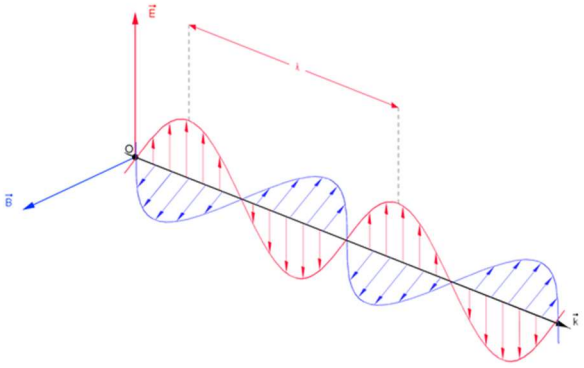


Fig. 1 & 2: Electromagnetic Waves

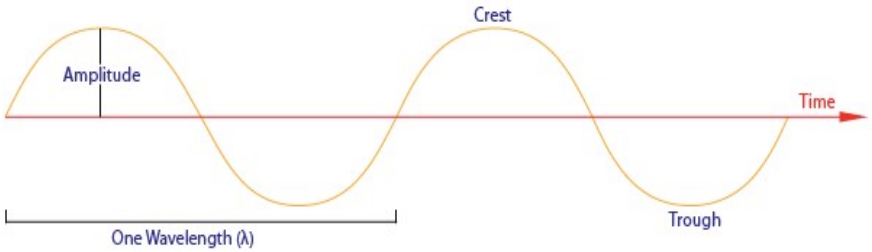
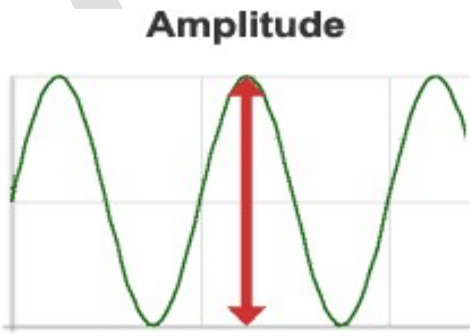


Fig. 3: An EM Wave

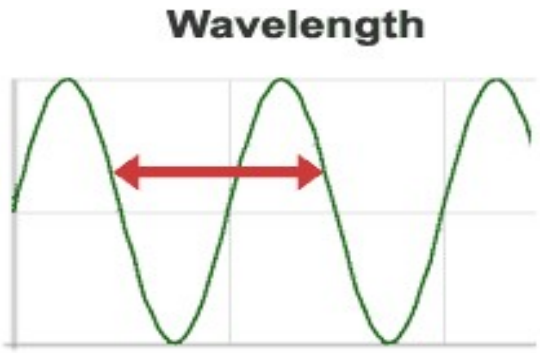
Amplitude



Amplitude is the distance from the maximum vertical displacement of the wave to the middle of the wave. This measures the magnitude of oscillation of a particular wave. In short, the amplitude is basically the height of the wave. Larger amplitude means higher energy and lower

amplitude means lower energy. Amplitude is important because it tells you the intensity or brightness of a wave in comparison with other waves.

Wavelength



Wavelength (\(\lambda\)) is the distance of one full cycle of the oscillation. Longer wavelength waves such as radio waves carry low energy; this is why we can listen to the radio without any harmful consequences. Shorter wavelength waves such as x-rays carry higher energy that can be hazardous to our health. Consequently lead aprons are worn to protect our bodies from harmful radiation when we undergo x-rays. This wavelength frequently relationship is characterized by:

\[c = \lambda\nu \]

where

- c is the speed of light,
- \(\lambda \) is wavelength, and
- \(\nu \) is frequency.

Shorter wavelength means greater frequency, and greater frequency means higher energy. Wavelengths are important in that they tell one what type of wave one is dealing with.

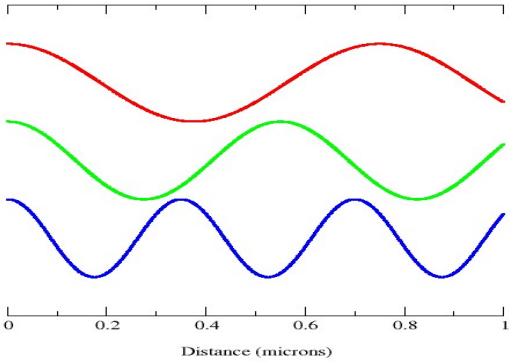
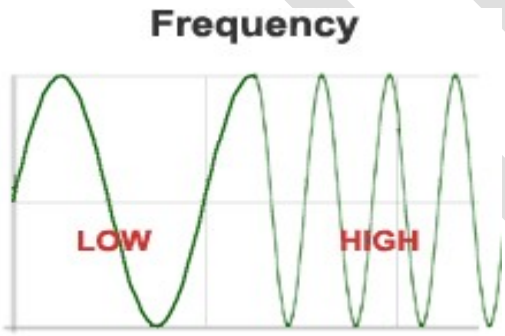


Fig. 4: Different Wavelengths and Frequencies

Frequency



Frequency is defined as the number of cycles per second, and is expressed as sec^{-1} or Hertz (Hz). Frequency is directly proportional to energy and can be express as:

$$ E = h\nu $$

where

- E is energy,
- h is Planck's constant, ($h= 6.62607 \times 10^{-34}$ J), and
- ν is frequency.

Period

Period (T) is the amount of time a wave takes to travel one wavelength. It is measured in seconds (s).

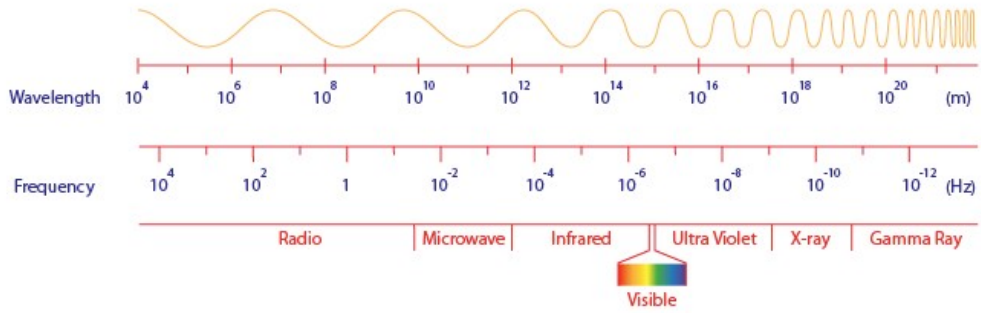
Velocity

The velocity of wave in general is expressed as:

\[velocity = \lambda\nu \]

For Electromagnetic wave, the velocity is 2.99x108m/s or 186,000 miles per second.

Electromagnetic spectrum



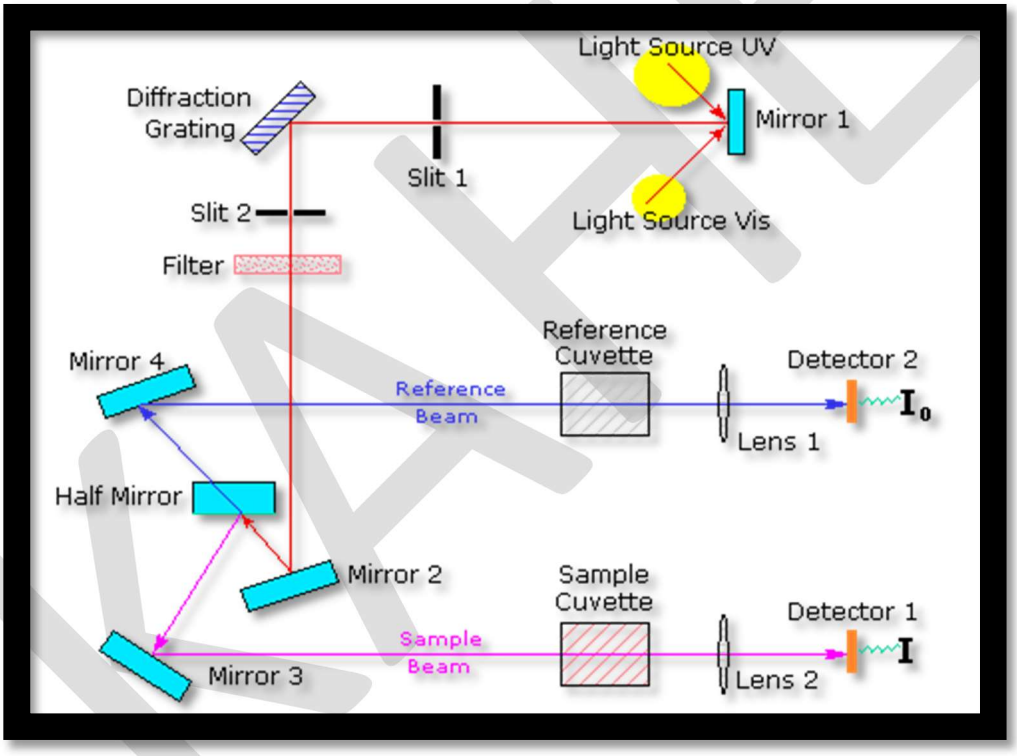
In general, as a wave's wavelength increases, the frequency decreases, and as wave's wavelength decreases, the frequency increases. When electromagnetic energy is released as the energy level increases, the wavelength decreases and frequency decreases. Thus, electromagnetic radiation is then grouped into categories based on its wavelength or frequency into the electromagnetic spectrum. The different types of electromagnetic radiation shown in the electromagnetic spectrum consists of radio waves, microwaves, infrared waves, visible light, ultraviolet radiation, X-rays, and gamma rays. The part of the electromagnetic spectrum that we are able to see is the visible light spectrum.

UV/Visible Spectroscopy

The absorption spectrum (plural, spectra), or more correctly the absolute absorption spectrum, of a compound may be shown as a plot of the light absorbed by that compound against wavelength. Such a plot for a colored compound will have one or more absorption maxima (λmax's) in the visible region of the spectrum (400 to 700 nm). Absorption spectra in the ultraviolet (200 to 400 nm) and visible regions are due to energy transitions of both bonding and nonbonding outer electrons of the molecule. Usually delocalized electrons are involved such as the B bonding electrons of C=C and the lone pairs of nitrogen and oxygen. Since most of the electrons in a molecule are in the ground state at room temperature, spectra in this region give information about this state and the next higher one. As the wavelengths of light absorbed are

determined by the actual transitions occurring, specific absorption peaks may be recorded and related to known molecular substructures.

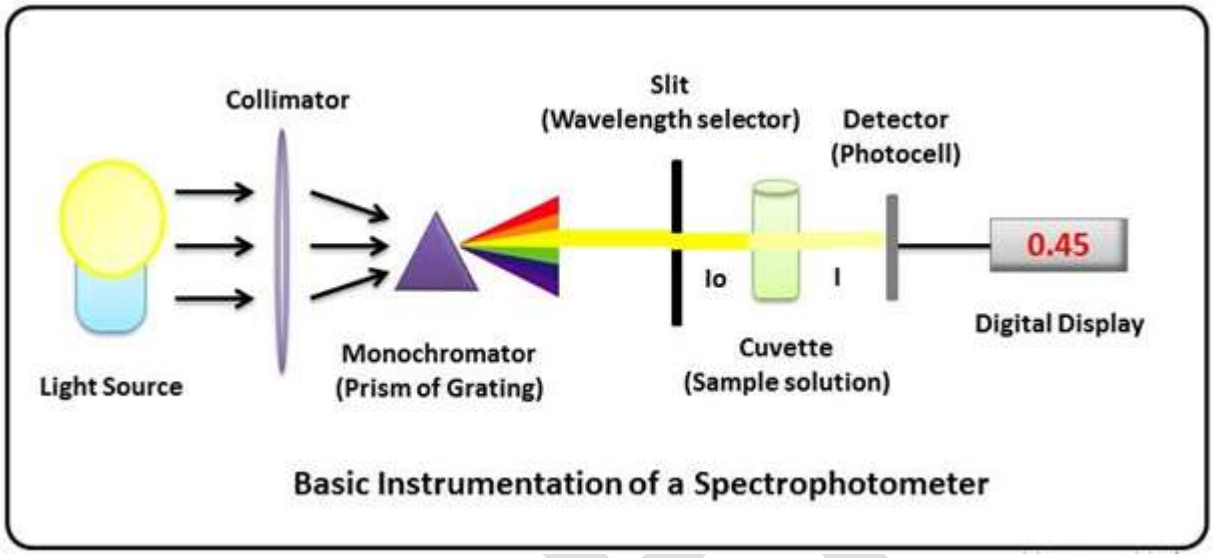
The term chromophore is given to that part of a molecule that gives rise independently to distinct parts of an absorption spectrum, for example the carbonyl group. Conjugated double bonds lower the energy required for electronic transitions and results in an increase in the wavelength at which a chromophore absorbs. This is referred to as a bathochromic shift, whereas a decrease in conjugation, caused for example by protonating a ring nitrogen atom, causes a hypochromic shift which leads to a decrease in wavelength. Hyperchromic and hypochromic effects refer to an increase and a decrease in absorbance respectively.



Instrumentation

To obtain an absorption spectrum, the absorbance of a substance must be measured at a series of wavelengths. Absorption in the visible and ultraviolet regions can be measured by a UV/visible spectrophotometer. UV/Vis spectrometers consist of three basic components, (i) a light source and a mechanism to select a specific wavelength of light in the UV/visible region of

the spectrum, (ii) a chamber where a cuvette containing a test solution can be introduced into the light path, and (iii) a photocell that can determine the amount of light absorbed by the sample (or the intensity of light transmitted through the sample).



Basic Instrumentation of a Spectrophotometer

The light source is usually a tungsten lamp for the visible region of the spectrum, and either ahydrogen or deuterium lamp for ultraviolet wavelengths. Cuvettes are optically transparent cells that hold the material(s) under study and are used to introduce samples into the light path. A reference cuvette optically identical to, and containing the same solvent (and impurities) as the test cuvette is always required for setting the spectrophotometer to read zero absorbance at each wavelength used. For accurate work, the optical matching of the two cuvettes should always be checked. Glass and plastic absorb strongly below 310 nm and are not useful for measuring absorbance below that wavelength. Quartz or silica cells are used when measuring absorption of ultraviolet wavelengths by a solution since they are transparent to wavelengths greater than 180 nm.

Application

- UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analyses, such as transition metal ions, highly conjugated

organic compounds, and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.

- Solutions of transition metal ions can be colored (i.e., absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another.
- The colour of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the colour of a dilute solution of copper sulfate is a very light blue; adding ammonia intensifies the colour and changes the wavelength of maximum absorption (λmax).
- Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water-soluble compounds, or ethanol for organic-soluble compounds. (Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy. Ethanol absorbs very weakly at most wavelengths.)
- Solvent polarity and pH can affect the absorption spectrum of an organic compound. Tyrosine, for example, increases in absorption maxima and molar extinction coefficient when pH increases from 6 to 13 or when solvent polarity decreases.
- While charge transfer complexes also give rise to colours, the colours are often too intense to be used for quantitative measurement.
- The wavelengths of absorption peaks can be correlated with the types of bonds in a given molecule and are valuable in determining the functional groups within a molecule.
- The spectrum alone is not, however, a specific test for any given sample. The nature of the solvent, the pH of the solution, temperature, high electrolyte concentrations, and the presence of interfering substances can influence the absorption spectrum. Experimental variations such as the slit width (effective bandwidth) of the spectrophotometer will also alter the spectrum.
- To apply UV/Vis spectroscopy to analysis, these variables must be controlled or accounted for in order to identify the substances present.

Mass Spectroscopy

In order to measure the characteristics of individual molecules, a mass spectrometer converts them to ions so that they can be moved about and manipulated by external electric and magnetic fields. The three essential functions of a mass spectrometer, and the associated components, are:

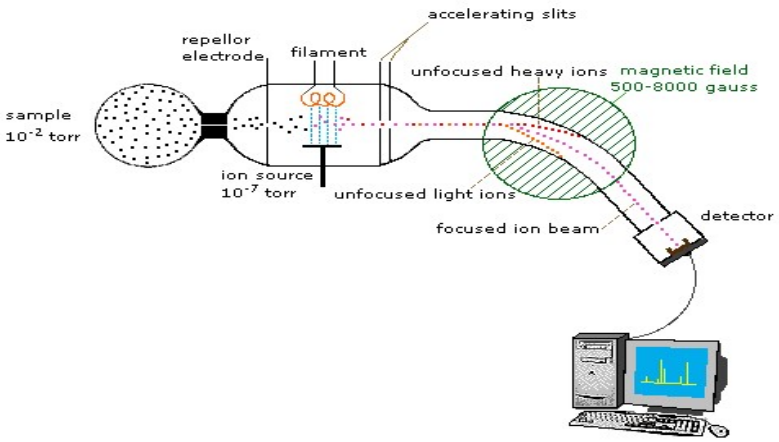
The Ion Source: A small sample is ionized, usually to cations by loss of an electron.

The Mass Analyzer: The ions are sorted and separated according to their mass and charge.

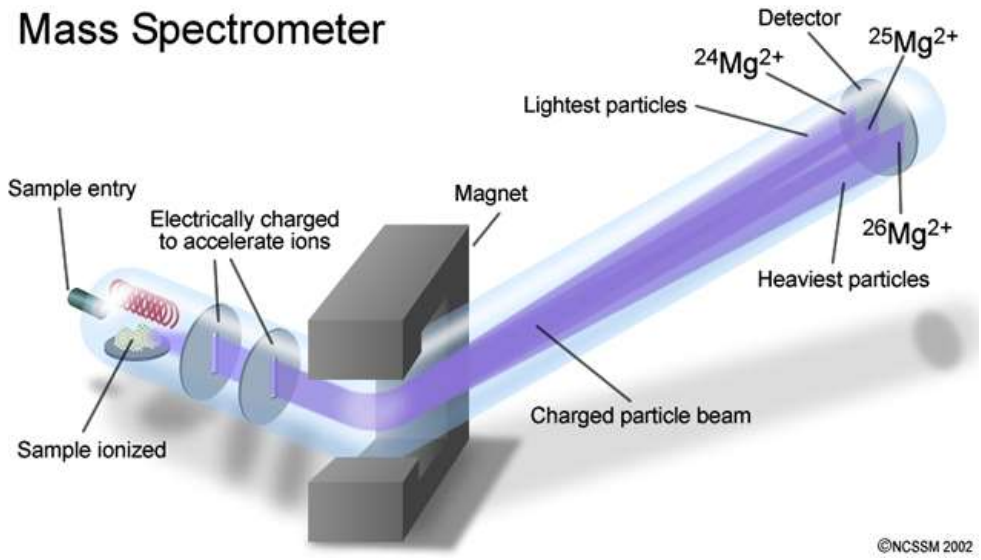
The Detector: The separated ions are then measured, and the results displayed on a chart.

Because ions are very reactive and short-lived, their formation and manipulation must be conducted in a vacuum. Atmospheric pressure is around 760 torr (mm of mercury). The pressure under which ions may be handled is roughly 10^{-5} to 10^{-8} torr (less than a billionth of an atmosphere). Each of the three tasks listed above may be accomplished in different ways. In one common procedure, ionization is effected by a high energy beam of electrons, and ion separation is achieved by accelerating and focusing the ions in a beam, which is then bent by an external magnetic field. The ions are then detected electronically and the resulting information is stored and analyzed in a computer. A mass spectrometer operating in this fashion is outlined in the following diagram. The heart of the spectrometer is the ion source. Here molecules of the sample (black dots) are bombarded by electrons (light blue lines) issuing from a heated filament. This is called an EI (electron-impact) source. Gases and volatile liquid samples are allowed to leak into the ion source from a reservoir (as shown). Non-volatile solids and liquids may be introduced directly.

Cations formed by the electron bombardment (red dots) are pushed away by a charged repeller plate (anions are attracted to it), and accelerated toward other electrodes, having slits through which the ions pass as a beam. Some of these ions fragment into smaller cations and neutral fragments. A perpendicular magnetic field deflects the ion beam in an arc whose radius is inversely proportional to the mass of each ion. Lighter ions are deflected more than heavier ions. By varying the strength of the magnetic field, ions of different mass can be focused progressively on a detector fixed at the end of a curved tube (also under a high vacuum).



When a high energy electron collides with a molecule it often ionizes it by knocking away one of the molecular electrons (either bonding or non-bonding). This leaves behind a molecular ion (colored red in the following diagram). Residual energy from the collision may cause the molecular ion to fragment into neutral pieces (colored green) and smaller fragment ions (colored pink and orange). The molecular ion is a radical cation, but the fragment ions may either be radical cations (pink) or carbocations (orange), depending on the nature of the neutral fragment. An animated display of this ionization process will appear if you click on the ion source of the mass spectrometer.



Nature of Mass Spectra

A mass spectrum will usually be presented as a vertical bar graph, in which each bar represents an ion having a specific mass-to-charge ratio (m/z) and the length of the bar indicates the relative abundance of the ion. The most intense ion is assigned an abundance of 100, and it is referred to as the base peak. Most of the ions formed in a mass spectrometer have a single charge, so the m/z value is equivalent to mass itself. Modern mass spectrometers easily distinguish (resolve) ions differing by only a single atomic mass unit (amu), and thus provide completely accurate values for the molecular mass of a compound. The highest-mass ion in a spectrum is normally considered to be the molecular ion, and lower-mass ions are fragments from the molecular ion, assuming the sample is a single pure compound.

Applications:

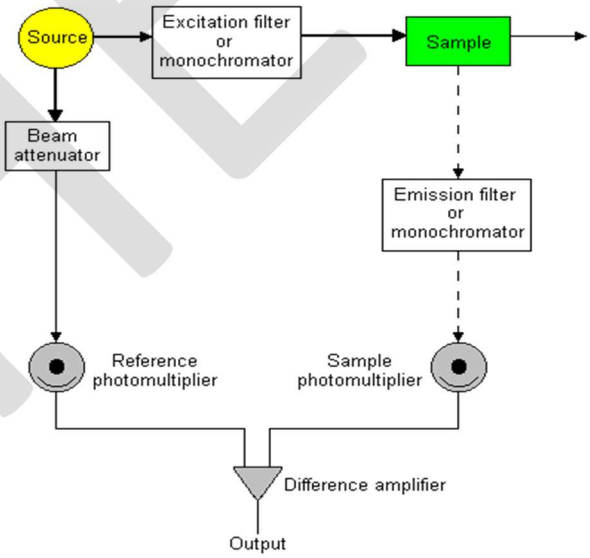
- Mass spectrometry has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation.
- Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum).
- MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds.

- As an analytical technique it possesses distinct advantages such as:
    - Increased sensitivity over most other analytical techniques because the analyzer, as a mass-charge filter, reduces background interference
    - Excellent specificity from characteristic fragmentation patterns to identify unknowns or confirm the presence of suspected compounds.
    - Information about molecular weight.
    - Information about the isotopic abundance of elements.
- Temporally resolved chemical data.

- A few of the disadvantages of the method is that often fails to distinguish between optical and geometrical isomers and the positions of substituent in o-, m- and p- positions in an aromatic ring.
- Also, its scope is limited in identifying hydrocarbons that produce similar fragmented ions.
- Mass spectrometry is also used to determine the isotopic composition of elements within a sample.
- Differences in mass among isotopes of an element are very small, and the less abundant isotopes of an element are typically very rare, so a very sensitive instrument is required.

Spectrofluorimeter

A spectrofluorimeter is an instrument which takes advantage of fluorescent properties of some compounds in order to provide information regarding their concentration and chemical environment in a sample. A certain excitation wavelength is selected, and the emission is observed either at a single wavelength, or a scan is performed to record the intensity versus wavelength, also called an emission spectra.



Instrumental components

Sources

Generally, the source must be more intense than that required for UV-Vis. absorption spectroscopy; magnitude of the emitted radiation is directly proportional to the power of the source.

Filter fluorometers often employ a low-pressure mercury vapour lamp. This source produces intense lines at certain wavelengths. One of these lines will usually be suitable for excitation of a fluorescent sample.

Spectrofluorometers, which need a continuous radiation source, are often equipped with a 75-450 W high-pressure xenon arc lamp.

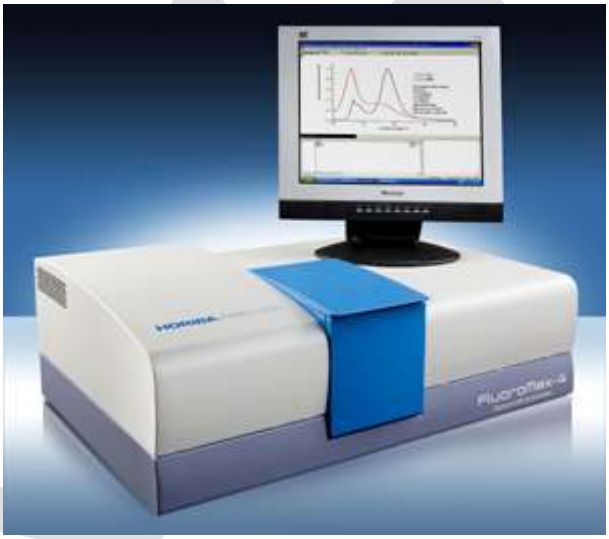
Lasers are sometimes used as excitation sources. A tunable dye laser, using a pulsed nitrogen laser as the primary source can produce monochromatic radiation between 360 and 650 nm. Since the radiation produced *is* monochromatic, there is no need for an excitation monochromator.

Filters and monochromators

Fluorometers use either interference or absorption filters. Spectrofluorometers are usually fitted with grating monochromators.

Detectors

Fluorescence signals are usually of low intensity. Photomultiplier tubes are in common use as detectors. Diode-array detectors are sometimes used.

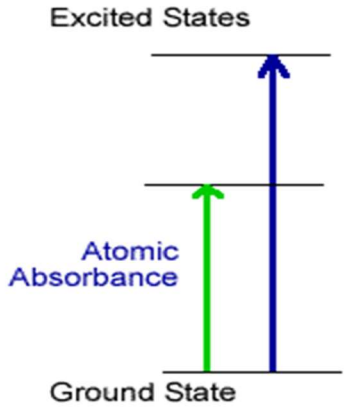


Applications:

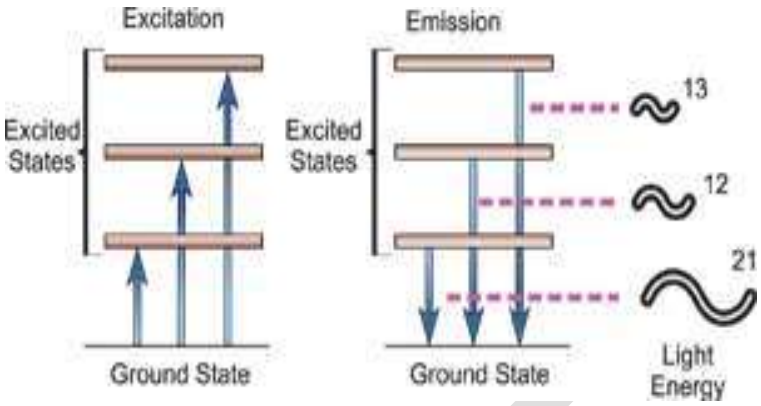
- Generally, spectrofluorometers use high intensity light sources to bombard a sample with as many photons as possible.
- This allows for the maximum number of molecules to be in an excited state at any one point in time.
- The light is either passed through a filter, selecting a fixed wavelength, or a monochromator, which allows a wavelength of interest to be selected for use as the exciting light.

Atomic Spectroscopy

Atomic spectroscopy exploits different energetic transitions experienced by atoms that are associated with either the absorption or emission of photons. When these transitions involve the excitation and relaxation of the valence (outer or bonding) shell electrons of metal atoms and ions, the corresponding photons have energies within the ultraviolet and visible regions of the spectrum. A good example of this is the dark absorption lines in the solar spectrum, which are caused by heavier elements present in the outer layers of the sun.



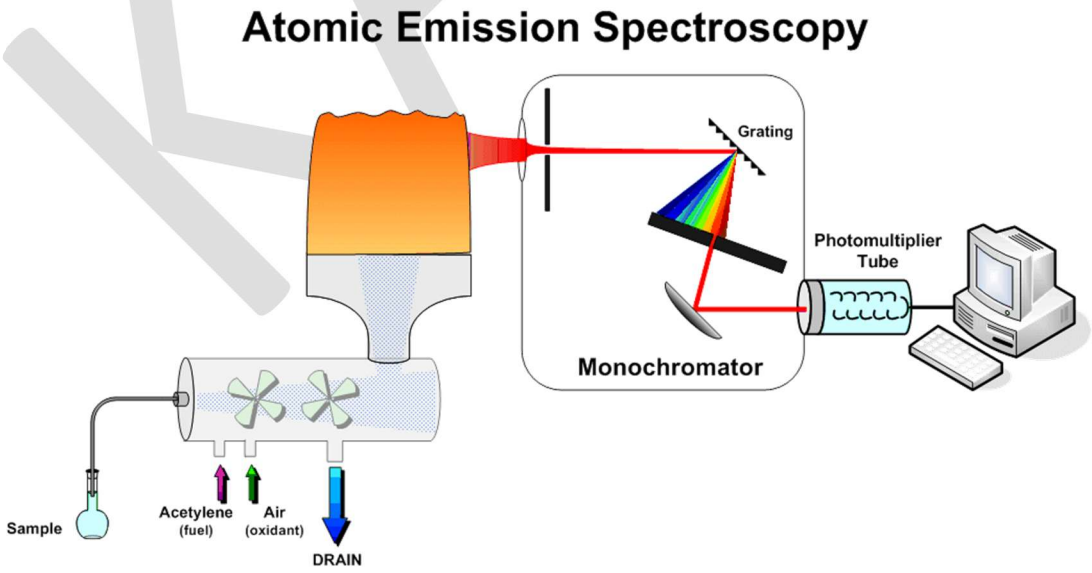
The figure shows a high energy photon with Ephoton = hν being absorbed, resulting in a 2s→3s electron excitation; similarly, a 3d→3p electron relaxation results in the emission of a lower energy photon. By convention, the change in electron energy ΔE = Ef − Ei, where f and i refer to the final and initial states, respectively; so ΔE = Ephoton, and the sign of Ephoton tells you whether the photon is being absorbed or emitted. Since Ef and Ei depend on the number electrons and protons within an atom (or monatomic ion), the wavelengths associated with atomic absorption and emission are considered characteristic for a particular element.



Absorption and Emission:

In atomic absorption (AA) spectroscopy, absorption of a photon results in excitation of an electron from a lower to higher energy atomic orbital (AO). An instrument measures the absorbance, A, which is defined as the logarithm of the ratio of incident to transmitted radiant power of the photon beam, A = log(P0 ÷ P), at a wavelength specific to the element of interest. Samples are typically analysed using a flame atomic absorption spectrophotometer.

In atomic emission (AE) spectroscopy, thermal or electrical energy from an arc, flame, spark, or plasma is used to excite and electron from a lower to higher energy AO; when the excited electron returns to its original AO (i.e. the ground state), it may do so by emitting a photon. The instrument measures the intensity, I, of these emitted photons as a function of wavelength.



Because AO energies are well-defined, atomic absorption and emission spectra consist of discrete, narrow lines. This allows the concentration of metallic elements in different samples to be determined selectively, with lower limits at or below 1 mg/L (1 ppm). Techniques such as graphite furnace atomic absorption spectrophotometry (GFAAS) allow concentration to be measured down to µg/L (ppb) levels. Actual limits-of-detection vary withelement, technique, and sample matrix.

Applications:

- Atomic spectroscopy has many useful applications. Since the emission spectrum is different for every element, it acts as an atomic fingerprint by which elements can be identified.
- Some elements were discovered by the analysis of their atomic spectrum.
- Helium, for example, was discovered while scientists were analyzing the absorption spectrum of the sun.

- Emission spectrum is especially useful to astronomers who use emission and absorption spectra to determine the makeup of far away stars and other celestial bodies.

Spectrofluorimeter Instrumentation

Fluorescence spectroscopy works most accurately at very low concentrations of emitting fluorophores. UV/Vis spectroscopy, in contrast, is least accurate at such low concentrations. One major factor adding to the high sensitivity of fluorescence applications is the spectral selectivity. Due to the Stokes shift, the wavelength of the emitted light is different from that of the exciting light. Another feature makes use of the fact that fluorescence is emitted in all directions. By placing the detector perpendicular to the excitation pathway, the background of the incident beam is reduced.

The schematic of a typical spectrofluorimeter has two monochromators, one for tuning the wavelength of the exciting beam and a second one for analysis of the fluorescence emission. Due to the emitted light always having a lower energy than the exciting light, the wavelength of the excitation monochromator is set at a lower wavelength than the emission monochromator. The better fluorescence spectrometers in laboratories have a photon-counting detector yielding very high sensitivity. Temperature control is required for accurate work as the emission intensity of a fluorophore is dependent on the temperature of the solution. Two geometries are possible for the measurement, with the ° arrangement most commonly used. Pre- and post-filter effects can arise owing to absorption of light prior to reaching the fluorophore and the reduction of emitted radiation. These phenomena are also called inner

filter effects and are more evident in solutions with high concentrations. As a rough guide, the absorption of a solution to be used for fluorescence experiments should be less than 0.05. The use of microcuvettes containing less material can also be useful. Alternatively, the front-face illumination geometry can be used which obviates the inner filter effect. Also, while the ° geometry requires cuvettes with two neighbouring faces being clear (usually, fluorescence cuvettes have four clear faces), the front- face illumination technique requires only one clear face, as excitation and emission occur at the same face. However, front-face illumination is less sensitive than the ° illumination.

Applications

There are many and highly varied applications for fluorescence despite the fact that relatively few compounds exhibit the phenomenon. The effects of pH, solvent composition and the polarisation of fluorescence may all contribute to structural elucidation. Measurement of fluorescence lifetimes can be used to assess rotation correlation coefficients and thus particle sizes. Non-fluorescent compounds are often labeled with fluorescent probes to enable monitoring of molecular events. This is termed extrinsic fluorescence as distinct from intrinsic fluorescence where the native compound exhibits the property. Some fluorescent dyes are sensitive to the presence of metal ions and can thus be used to track changes of these ions in invitro samples, as well as whole cells. Since fluorescence spectrometers have two monochromators, one for tuning the excitation wavelength and one for analyzing the emission wavelength of the fluorophore, one can measure two types of spectra: excitation and emission spectra. For fluorescence excitation spectrum measurement, one sets the emission monochromator at a fixed wavelength ($_{em}$) and scans a range of excitation wavelengths which are then recorded as ordinate (x-coordinate) of the excitation spectrum; the fluorescence emission at $_{em}$ is plotted as abscissa. Measurement of emission spectra is achieved by setting a fixed excitation wavelength ($_{exc}$) and scanning a wavelength range with the emission monochromator. To yield a spectrum, the emission wavelength lem is recorded as ordinate and the emission intensity at $_{em}$ is plotted as abscissa.

Fourier transform infrared spectroscopy (FTIR)

Is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time.

The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual

spectrum. Fourier transform spectroscopy is a less intuitive way to obtain the same information. Rather than shining a monochromatic beam of light at the sample, this technique shines a beam containing many frequencies of light at once, and measures how much of that beam is absorbed by the sample. Next, the beam is modified to contain a different combination of frequencies, giving a second data point. This process is repeated many times. Afterwards, a computer takes all these data and works backwards to infer what the absorption is at each wavelength.

The beam described above is generated by starting with a broadband light source one containing the full spectrum of wavelengths to be measured. The light shines into a Michelson interferometer a certain configuration of mirrors, one of which is moved by a motor. As this mirror moves, each wavelength of light in the beam is periodically blocked, transmitted, blocked, transmitted, by the interferometer, due to wave interference. Different wavelengths are modulated at different rates, so that at each moment, the beam coming out of the interferometer has a different spectrum.

As mentioned, computer processing is required to turn the raw data (light absorption for each mirror position) into the desired result (light absorption for each wavelength). The processing required turns out to be a common algorithm called the Fourier transform (hence the name, "Fourier transform spectroscopy"). The raw data is sometimes called an "interferogram".

There are three principal advantages for an FT spectrometer compared to a scanning (dispersive) spectrometer.

- The multiplex or Fellgett's advantage. This arises from the fact that information from all wavelengths is collected simultaneously. It results in a higher Signal-to-noise ratio for a given scan-time. For a spectrum with m resolution elements, this increase is equal to the square root of m. alternatively; it allows a shorter scan-time for a given resolution. In practice multiple scans are often averaged, increasing the signal-to-noise ratio by the square root of the number of scans.
- The throughput or Jacquinot's advantage. This results from the fact that in a dispersive instrument, the monochromator has entrance and exit slits which restrict the amount of light that passes through it. The interferometer throughput is determined only by the diameter of the collimated beam coming from the source. Although no slits are needed, FTIR spectrometers do require an aperture to restrict the convergence of the collimated beam in the interferometer. This is because convergent rays are modulated at different frequencies as the path difference is varied. Such an aperture is called a Jacquinot stop.[1] For a given resolution and wavelength this circular aperture allows more light through than a slit, resulting in a higher signal-to-noise ratio.

- The wavelength accuracy or Connes advantage. The wavelength scale is calibrated by a laser beam of known wavelength that passes through the interferometer. This is much more stable and accurate than in dispersive instruments where the scale depends on the mechanical movement of diffraction gratings. In practice, the accuracy is limited by the divergence of the beam in the interferometer which depends on the resolution.

Another minor advantage is less sensitivity to stray light that is radiation of one wavelength appearing at another wavelength in the spectrum. In dispersive instruments, this is the result of imperfections in the diffraction gratings and accidental reflections. In FT instruments there is no direct equivalent as the apparent wavelength is determined by the modulation frequency in the interferometer

Nuclear magnetic resonance (NMR)

NMR is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope of the atoms; in practical applications, the frequency is similar to VHF and UHF television broadcasts (60– 1000 MHz). NMR allows the observation of specific quantum mechanical magnetic properties of the atomic nucleus. Many scientific techniques exploit NMR phenomena to study molecular physics, crystals, and non-crystalline materials through nuclear magnetic resonance spectroscopy. NMR is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI).

All isotopes that contain an odd number of protons and/or neutrons (see Isotope) have an intrinsic magnetic moment and angular momentum, in other words a nonzero spin, while all nuclides with even numbers of both have a total spin of zero. The most commonly studied nuclei are 1H and 13C, although nuclei from isotopes of many other elements (e.g. 2H, 6Li, 10B, 11B, 14N, 15N, 17O, 19F, 23Na, 29Si, 31P, 35Cl, 113Cd, 129Xe, 195Pt)

have been studied by high-field NMR spectroscopy as well.

Instrumentation

Schematically, an analytical NMR instrument is very similar to an EPR instrument, except that instead of a klystron generating microwaves two sets of coils are used to generate and detect radio frequencies. Samples in solution are contained in sealed tubes which are rotated rapidly

in the cavity to eliminate irregularities and imperfections in sample distribution. In this way, an average and uniform signal is reflected to the receiver to be processed and recorded. In solid samples, the number of spin–spin interactions is greatly enhanced due to intermolecular interactions that are absent in dissolved samples due to translation and rotation movements. As a result, the resonance signals broaden significantly. However, high-resolution spectra can be obtained by spinning the solid sample at an angle of ° (magic angle spinning).

The sophisticated pulse sequences necessary for multidimensional NMR require a certain geometric layout of the radio frequency coils and sophisticated electronics. Advanced computer facilities are needed for operation of NMR instruments, as well as analysis of the acquired spectra.

Applications

Medicine

The application of nuclear magnetic resonance best known to the general public is magnetic resonance imaging for medical diagnosis and magnetic resonance microscopy in research settings, however, it is also widely used in chemical studies, notably in NMR spectroscopy such as proton NMR, carbon-13 NMR, deuterium NMR and phosphorus-31 NMR. Biochemical information can also be obtained from living tissue (e.g. human brain tumors) with the technique known as in vivo magnetic resonance spectroscopy or chemical shift NMR Microscopy.

Chemistry

By studying the peaks of nuclear magnetic resonance spectra, chemists can determine the structure of many compounds. It can be a very selective technique, distinguishing among many atoms within a molecule or collection of molecules of the same type but which differ only in terms of their local chemical environment. NMR spectroscopy is used to unambiguously identify known and novel compounds, and as such, is usually required by scientific journals for identity confirmation of synthesized new compounds.

Purity determination

NMR is primarily used for structural determination, however it can also be used for purity determination, providing that the structure and molecular weight of the compound is known. This technique requires the use of an internal standard of a known purity. Typically this standard will have a high molecular weight to facilitate accurate weighing, but relatively few protons so as to give a clear peak for later integration e.g. 1,2,3,4-tetrachloro-5-nitrobenzene. Accurately weighed portions of both the standard and sample are combined and analyzed by NMR.

Flow cytometer

Modern flow cytometers are able to analyze several thousand particles every second, in "real time," and can actively separate and isolate particles having specified properties. A flow cytometer is similar to a microscope, except that, instead of producing an image of the cell, flow cytometry offers "high-throughput" (for a large number of cells) automated quantification of set parameters. To analyze solid tissues, a single-cell suspension must first be prepared.

A flow cytometer has five main components:

- a flow cell - liquid stream (sheath fluid), which carries and aligns the cells so that they pass single file through the light beam for sensing
- a measuring system - commonly used are measurement of impedance (or conductivity) and optical systems - lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers (argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals
- a detector and Analogue-to-Digital Conversion (ADC) system - which converts analogue measurements of forward-scattered light (FSC) and side-scattered light (SSC) as well as dye- specific fluorescence signals into digital signals that can be processed by a binary computer
- an amplification system - linear or logarithmic
- a computer for analysis of the signals.

The process of collecting data from samples using the flow cytometer is termed 'acquisition'. Acquisition is mediated by a computer physically connected to the flow cytometer, and the software which handles the digital interface with the cytometer. The software is capable of adjusting parameters (e.g., voltage, compensation) for the sample being tested, and also assists in displaying initial sample information while acquiring sample data to ensure that parameters are set correctly. Early flow cytometers were, in general, experimental devices, but technological advances have enabled widespread applications for use in a variety of both clinical and research purposes. Due to these developments, a considerable market for instrumentation, analysis software, as well as the reagents used in acquisition such as fluorescently labeled antibodies has developed.

Modern instruments usually have multiple lasers and fluorescence detectors. The current record for a commercial instrument is ten lasers and 18 fluorescence detectors. Increasing the

number of lasers and detectors allows for multiple antibody labeling, and can more precisely identify a target population by their phenotypic markers. Certain instruments can even take digital images of individual cells, allowing for the analysis of fluorescent signal location within or on the surface of cells.

Applications

The technology has applications in a number of fields, including molecular biology, pathology, immunology, plant biology and marine biology. It has broad application inmedicine (especially in transplantation, hematology, tumor immunology and chemotherapy, prenatal diagnosis, genetics and sperm sorting for sex preselection). Also, it is extensively used in research for the detection of DNA damage, caspase cleavage and apoptosis. In marine biology, the autofluorescent properties of photosynthetic planktoncan be exploited by flow cytometry in order to characterise abundance and community structure. In protein engineering, flow cytometry is used in conjunction with yeast displayand bacterial display to identify cell surface-displayed protein variants with desired properties.

CENTRIFUGATION TECHNIQUES

Introduction:

- The centrifuge is an instrument, which is used to spin substances at high speed.
- It is often used to separate particles present in a liquid.
- The mixture is placed in a tube that pivots so that when the machine starts to move, the tube can swing out horizontally.
- The centrifugal force tries to push the mixture away from the centre of centrifuge.

BASIC PRINCIPLES OF SEDIMENTATION:

- This method is based on the principle of sedimentation.
- From everyday experience, the effect of sedimentation due to the influence of the Earth's gravitational field (g = 981 cms-2) versus the increased rate of sedimentation in centrifugal field (g>981 cms-2) is apparent.
- To give a simple but illustrative example, crude sand particles added to a bucket of water travel slowly to the bottom of the bucket by gravitation, but sediment much faster when the bucket is swung around in a circle.
- Similarly, biological structures exhibit a drastic increase in sedimentation when they undergo acceleration in a centrifugal field.
- The relative centrifugal field is usually expressed as a multiple of the acceleration due to gravity.
- Below is a short description of equations used in practical centrifugation classes.
- The more dense a biological structure is the faster it sediments in a centrifugal field.
- The more massive a biological particle is, the faster it moves in a centrifugal field.
- The denser the biological buffer system is, the slower the particle will move in a centrifugal field.

- The greater the frictional coefficient is, the slower a particle will move.
- The greater the centrifugal force is the faster the particle sediments.
- The sedimentation rate of a given particle will be zero when the density of the particle and the surrounding medium are equal.
- When particle sediments, it must displace some of the solution in which it is suspended, resulting in an up thrust on the particle equal to the weight of the liquid displaced.
- If a particle is assumed to be a sphere of known volume and density, then the net force(f) is experienced when the centrifugal force at an angular velocity of ω radians/sec is given by:

$$S = \text{Volume} \times \text{Density} \times \omega^2 r$$

Or,

$$F = 4/3\ \Pi\ r\ \ p3\ (\rho p - \rho m)\ \omega 2\ r$$

Where,

$4/3\ \Pi\ r\ p3$ = Volume of sphere of radius 'r'.
ρp = Density of the particle.
ρm = Density of the suspended medium.
ν = Distance of the particle from the center of rotation.
ω = Angular velocity of rotor.

DESKTOP CENTRIFUGES:

- Centrifuges are essential devices, each of the clinical laboratories.
- This may be a desktop or floor-type, refrigerated or cooling.
- Centrifuge is mainly used to create the fractionation due to fluid samples with high g forces of spin.
- Desktop centrifuge designed to meet the laboratory setting to limited space requirements.
- These are used to separate liquid solid;, also dealing with blood samples used.
- Desktop centrifuge is in a variety of different specifications, sizes and abilities. Independent Electoral Commission Centra, Beckman and Adams is a leading manufacturer of desktop centrifuge.

Desktop centrifuge with innovative features

- Desktop centrifuge important feature is to break the power system strong guarantee automatic acceleration and deceleration.
- Another important feature is their silence and vibration free operation.
- The device is designed with a full timer, brushless motor and has a clear lid safety switch.
- It also has a maintenance-free brushless motor; with power interrupt the user's security design.
- The device and the cooling or not cooling at different speeds to choose from.
- Refrigeration unit is an independent unit, but the time dependence of their work, because it's centrifuge for power.
- The device has a multi-functional, four rotor, test tubes and bottles can accommodate all possible sizes.
- Desktop centrifuge have such characteristics:
- Removable rotor bowl for easy cleaning.
- Reagents, and consumables can be controlled.

- Speed control knob.
- Keep the programs running longer.
- Temperature, velocity and time display settings
- Find the real purchasing the best product distributor
- To analyze the working conditions before purchasing, quality and warranty desktop centrifuge specifications.
- A leading supplier of laboratory bench centrifuge at low cost high quality products, and to ensure service for years.

Desktop Centrifuges

LARGE CAPACITY REFRIGERATED CENTRIFUGES

- Controlled by microcomputer, touching panel and LED display.
- The compressor imported from Europe operating without Freon for environment protection, pre-cooling when power on.
- The brushless direct drive motor with high start torque and frequency inversion.
- Imbalance, over-speed, over-temperature and door interlocking protection function for the safety of people and instrument.
- The parameter can be changed at operating state.
- Automatic computing and setting RCF.adjustable rise-and-fail speed from grade 0 to 9
- Fitting to the adapters of 5ml.7ml .10ml.15ml.30ml.50ml, etc.
- Widely used in the filed of radicalization immunity, biochemistry, pharmacy, blood separation and purification
- Hold-on, transient centrifuge.

ANALYTICAL AND PREPARATIVE ULTRACENTRIFUGATION:

Ultracentrifuge:

- The ultracentrifuge is a centrifuge optimized for spinning a rotor at very high speeds, capable of generating acceleration as high as 2,000,000 g (approx. 19,600 km/s^2).
- There are two kinds of ultracentrifuges, the preparative and the analytical ultracentrifuge. Both classes of instruments find important uses in molecular biology, biochemistry and polymer science.

Analytical ultracentrifuge

- In an analytical ultracentrifuge, a sample being spun can be monitored in real time through an optical detection system, using ultraviolet light absorption and/or interference optical refractive index sensitive system.
- This allows the operator to observe the evolution of the sample concentration versus the axis of rotation profile as a result of the applied centrifugal field.
- With modern instrumentation, these observations are electronically digitized and stored for further mathematical analysis.
- Two kinds of experiments are commonly performed on these instruments: sedimentation velocity experiments and sedimentation equilibrium experiments.
- Sedimentation velocity experiments aim to interpret the entire time-course of sedimentation, and report on the shape and molar mass of the dissolved macromolecules, as well as their size-distribution.

- The size resolution of this method scales approximately with the square of the particle radii, and by adjusting the rotor speed of the experiment size-ranges from 100 Da to 10 GDa can be covered.
- Sedimentation velocity experiments can also be used to study reversible chemical equilibria between macromolecular species, by either monitoring the number and molar mass of macromolecular complexes, by gaining information about the complex composition from multi-signal analysis exploiting differences in each components spectroscopic signal, or by following the composition dependence of the sedimentation rates of the macromolecular system, as described in Gilbert-Jenkins theory.
- Sedimentation equilibrium experiments are concerned only with the final steady-state of the experiment, where sedimentation is balanced by diffusion opposing the concentration gradients, resulting in a time-independent concentration profile. Sedimentation equilibrium distributions in the centrifugal field are characterized by Boltzmann distributions.
- This experiment is insensitive to the shape of the macromolecule, and directly reports on the molar mass of the macromolecules and, for chemically reacting mixtures, on chemical equilibrium constants.
- The kinds of information that can be obtained from an analytical ultracentrifuge include the gross shape of macromolecules, the conformational changes in macromolecules, and size distributions of macromolecular samples.
- For macromolecules, such as proteins, which exist in chemical equilibrium with different non-covalent complexes, the number and subunit stoichiometry of the complexes and equilibrium constant constants can be studied.

Preparative ultracentrifuge

- Preparative ultracentrifuges are available with a wide variety of rotors suitable for a great range of experiments.
- Most rotors are designed to hold tubes that contain the samples.
- Swinging bucket rotors allow the tubes to hang on hinges so the tubes reorient to the horizontal as the rotor initially accelerates.
- Fixed angle rotors are made of a single block of metal and hold the tubes in cavities bored at a predetermined angle.
- Zonal rotors are designed to contain a large volume of sample in a single central cavity rather than in tubes.
- Some zonal rotors are capable of dynamic loading and unloading of samples while the rotor is spinning at high speed.
- Preparative rotors are used in biology for pelleting of fine particulate fractions, such as cellular organelles (mitochondria, microsomes, and ribosomes) and viruses.
- They can also be used for gradient separations, in which the tubes are filled from top to bottom with an increasing concentration of a dense substance in solution.
- Sucrose gradients are typically used for separation of cellular organelles.
- Gradients of caesium salts are used for separation of nucleic acids.
- After the sample has spun at high speed for sufficient time to produce the separation, the rotor is allowed to come to a smooth stop and the gradient is gently pumped out of each tube to isolate the separated components.

APPLICATIONS OF ULTRACENTRIFUGATION:

- The analytical ultracentrifuge has found the following wide applications in Biology.
- To determine relative molecular mass of macromolecules such as, proteins and DNA.
- To investigate the purity of DNA preparations, viruses and proteins.
- To detect conformational changes in macromolecules such as DNA and protein.
- Characterize assembly and disassembly mechanisms of bio molecular complexes
- Determine subunit stoichiometries
- Thermodynamic and hydrodynamic information
- Measure equilibrium constants and thermodynamic parameters for self- and hetero-associating systems.

Sedimentation velocity

- Sedimentation velocity is an analytical ultracentrifugation (AUC) method that measures the rate at which molecules move in response to centrifugal force generated in a centrifuge.
- This sedimentation rate provides information about both the molecular mass and the shape of molecules. In some cases this technique can also measure diffusion coefficients and molecular mass.
- In the biotechnology industry sedimentation velocity is used much more frequently than sedimentation equilibrium and thus when biotech scientists say "AUC" or "analytical ultracentrifugation" they typically really mean "sedimentation velocity".
- Sedimentation velocity is particularly valuable for:verifying whether a sample is entirely homogeneous in mass and conformation detecting aggregates in protein samples and quantifying the amount of aggregate comparing the conformations for samples from different lots, manufacturing processes, or expression systems (comparability studies), or comparing different engineered variants of the same protein/peptide,establishing whether the native state of a protein or peptide is a monomer, dimer, trimer, etc.,determining the overall shape of non-glycosylated protein and peptide molecules in solution, measuring the distribution of sizes in samples which contain a very broad range of sizes, detecting changes in protein conformation, for example partial unfolding or transitions to "molten globule" states.studying the formation and stoichiometry of tight complexes between proteins (for example receptor-ligand or antigen-antibody complexes)
- In the sedimentation velocity method a sample is spun at very high speed (usually 40-60 K rpm) in an analytical ultracentrifuge. The high centrifugal force rapidly depletes all the protein from the region nearest the center of the rotor (the meniscus region at the air/solution interface), forming a boundary which moves toward the outside of the rotor with time (see example below), until finally all the protein forms a pellet at the outside of the cell.
- The concentration distribution across the cell at various times during the experiment is measured while the sample is spinning, using either absorbance or refractive index detection in our Beckman ProteomeLab XL-I.
- A major advantage of this method over sedimentation equilibrium is that experiments usually require only 3-5 hours, as opposed to the several days typical of sedimentation equilibrium. Thus sedimentation velocity can be used with samples that are too labile for sedimentation equilibrium.

- The major drawback relative to sedimentation equilibrium applies to interacting systems (proteins that reversibly self-associate or protein-protein complexes), where the non-equilibrium nature of the measurement can lead to significant changes in species distributions over the course of an experiment. Further, for interacting systems it is generally more difficult and less accurate to derive binding constants (Kd's) from sedimentation velocity data.
- An important strength of sedimentation velocity is its ability to study samples over a fairly wide range of pH and ionic strength conditions (and often directly in formulation buffers), and at temperatures from 4 to 40 °C. The amount of protein required depends on the application, but each sample is usually ~0.45 ml at typical protein concentrations of 0.1-1 mg/ml (45-450 micrograms total).
- Protein concentration can range as low as ~10 micrograms/ml or as high as ~40 mg/ml in some cases (but generally the concentration should be 2 mg/mL or below). Up to 3 samples can be run at one time.A sedimentation velocity case study: a monoclonal antibody.
- The graph below shows scans across the centrifuge cell, recording the absorbance at 280 nm versus position within the cell. These scans were taken starting at 13 minutes after initiating a run at 45,000 rpm (the black data set in the graph), and then every ~12 minutes thereafter (blue, green, cyan, etc.).
- The sharp vertical spike at 6.02 cm indicates the position of the air-solution meniscus. In the first data set the sedimentation of the antibody has already depleted its concentration in the region near the meniscus and formed a sedimentation boundary.

- At later times in the run the depleted region expands and the boundary moves away from the center of the rotor, until by the time of the last data set the concentration of antibody has dropped to essentially zero throughout the upper half of the cell.
- The rate at which the sedimentation boundary moves is a measure of the sedimentation coefficient of the protein. The sedimentation coefficient depends on the molecular weight (larger proteins sediment faster) and also on molecular shape. Unfolded proteins or one with highly elongated shapes will experience more hydrodynamic friction, and thus will have smaller sedimentation coefficients than a folded, globular protein of the same molecular weight.
- The minimum width of the sedimentation boundary is related to the diffusion coefficient of the molecule; the presence of multiple species with similar sedimentation coefficients will cause the boundary to be broader than expected on the basis of diffusion alone. In this case the majority of the boundary is reasonably narrow, but the slow rise of the data on the right side of the boundary suggests the presence of some faster moving species.
- When viewed as in the graph above the data is difficult to interpret. What we often want to know is how much material is sedimenting at various sedimentation coefficients. By taking many scans close together in time (the graph above shows only a small number of the scans), subtracting them in pairs, and doing some mathematical manipulation these data can be transformed into the sedimentation coefficient distribution, g(s*), which is shown below.
- This distribution resembles a chromatogram, and in many ways is similar to a size-

exclusion chromatogram except the peaks come in the opposite order. Like a chromatogram, the area under each peak gives the total amount of that species.

- For this antibody sample we see only one distinct peak, centered at a sedimentation coefficient of ~6.5 S, which corresponds to the native antibody 'monomer' (really a covalent heterotetramer of 2 light and 2 heavy chains). A sedimentation coefficient of 6.5 S is actually rather low for a 150 kDa species, which is consistent with high hydrodynamic friction from its highly asymmetric, non-globular 'Y' shape.
- Although we see only a single peak in the g(s*) distribution, a more detailed analysis quickly reveals that this sample is not homogeneous. The red curve is a fit of these data as a single species. This fit clearly fails to account for the data over the region from 8-12 S, indicating the presence of some dimer and possibly also some trimer.
- The fact that the distribution has not returned fully to zero at 12 S also suggests that there may also be small amounts of even faster sedimenting species present.
- From the width of the main peak we can also calculate the apparent diffusion coefficient of the monomer. In turn, from the ratio of sedimentation coefficient to diffusion coefficient we can calculate a mass of 151 kDa for this species, which matches the expected value well within 3-5% error expected for masses determined in this fashion.
  Lastly, we can apply a new analysis algorithm to further enhance the resolution of the species distribution.
- In this figure the full distribution is shown in the main graph, while the inset magnifies the vertical scale by 10X in order to better show the minor components. With the enhanced resolution we now see a fully baseline-resolved dimer peak at ~9.4 S (4.7% of the total protein), and small peaks at ~13.7 S and 17.5 S (1.8% and 1.1%, probably trimer and tetramer). In addition there is 0.7% of a low mass contaminant at 2 S (possibly free light chain).
- Thus from this one velocity experiment we have been able to quantify the amount and mass of the main component, the content and sedimentation coefficients of 3 aggregates and a low mass contaminant, and to obtain information about the conformation of the main component.

Other applications

A sedimentation coefficient distribution for a sample of adenovirus (~150 MDa, used to deliver vectors for gene therapy). This illustrates the broad range of sizes and molecule types that can be studied using this technique. The inset shows a 20-fold expanded scale to allow the many minor peaks to be seen. This material turns out to be quite heterogeneous, having only 57% of the material as the main peak (virus monomer), plus many rapidly-sedimenting viral aggregates and some slowly-sedimenting species that may be fragments or empty capsids.

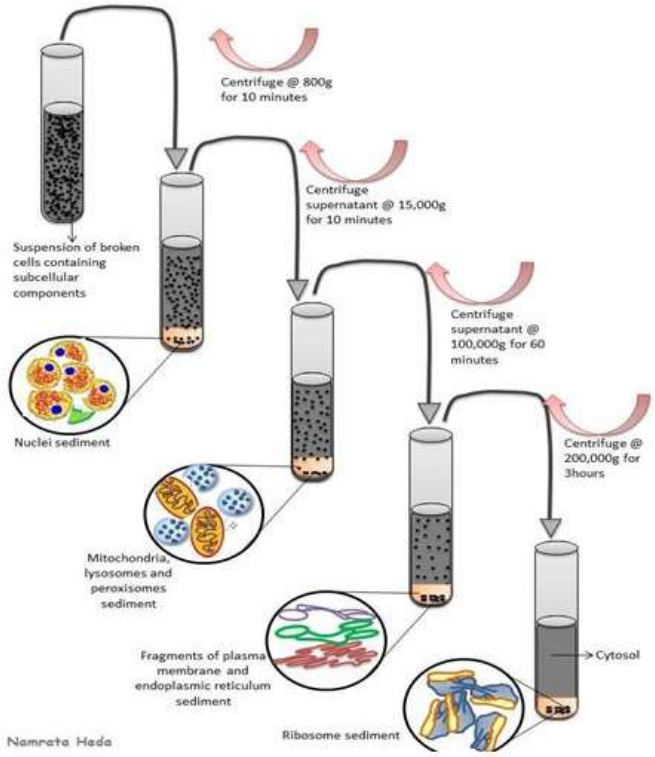
SEPARATION OF CELL ORGANELLES:

- To study the functions of any organelle in depth, it is first necessary to isolate it in a relatively pure form, free of concentration by other organelles.
- The process of separation of cell organelles is known as subcellular fractionation.
- As a first step toward isolating a specific organelle, organs (liver, brain, and kidney) are homogenized in a suitable homogenizing medium at 4º C.
- The resulting suspension, containing many contact organelles, is known as a homogenate.

- Fractionation of the contents of a homogenate is done by a classical biochemical technique called differential centrifugation.
- This methodis based upon the differences in the sedimentation rate of particles of different size and density.
- This method uses a series of four different centrifugation steps at successively greater speeds.
- Each step yields a pellet and a supernatant.
- The supernatant from each step is subjected to centrifugation in the next step.
- This procedure provides four pellets, namely, nuclear, mitochondrial, lysosomal and microsomal fractions.
- At the end of each step, the pellet is washed several times by resuspending in the homogenization medium followed by recentrifugation under the same conditions.
- This procedure minimizes contamination of other subcellular organelles and gives a fairly pure preparation of pellet fraction.
- The purity of organelles obtained by differential centrifugation is measured by estimating some marker activity.
- A marker is one that is almost exclusively present in one particular organelle.
- A marker may be an enzyme molecule or a biochemical compound.
- Various fractions, their functions and markers

S. No	Organelle	Function	Marker
1.	Plasma membrane	Regulates entry and exit of compounds	5' Nucleotidase
2.	Nucleus	Site of DNA-directed RNA synthesis	DNA
3.	Mitochondrion	Citric acid cycle, ammonia release	DHases
	for urea formation.		
4.	Lysosome	Site of many hydrolases.	Acid phosphatase
5.	ER	Oxidation of many xenobiotics	Glucose6-pase.
6.	Cytosol	Enzymes of glycolysis.	LDH

- The microsomal fraction contains mostly a mixture of smooth endoplasmic reticulum and free ribosomes.
- The contents of the final supernatant correspond approximately to those of Cytosol.



Fractionation of Cells

Although biochemical analysis requires disruption of the anatomy of the cell, gentle fractionation techniques have been devised to separate the various cell components while preserving their individual functions. Just as a tissue can be separated into its living constituent cell types, so the cell can be separated into its functioning organelles and macromolecules. In this section we consider the methods that allow organelles and proteins to be purified and analyzed biochemically.

Organelles and Macromolecules Can Be Separated by Ultracentrifugation:

- Cells can be broken up in various ways: It can be subjected to osmotic shock or ultrasonic vibration, forced through a small orifice, or ground up in a blender. These procedures break many of the membranes of the cell (including the plasma membrane and membranes of the endoplasmic reticulum) into fragments that immediately reseal to form small closed vesicles. If carefully applied, however, the disruption procedures leave organelles such as nuclei, mitochondria, the Golgi apparatus, lysosomes, and peroxisomes largely intact.
- The suspension of cells is thereby reduced to thick slurry (called a homogenate or extract) that contains a variety of membrane-enclosed organelles, each with a distinctive size, charge, and density. Provided that the homogenization medium has been carefully chosen (by trial and error for each organelle), the various components—including the vesicles derived from the endoplasmic reticulum, called microsomes—retain most of their

original biochemical properties.

- The different components of the homogenate must then be separated. Such cell fractionations became possible only after the commercial development in the early 1940s of an instrument known as the preparative ultracentrifuge, in which extracts of broken cells are rotated at high speeds. This treatment separates cell components by size and density: in general, the largest units experience the largest centrifugal force and move the most rapidly.
- At relatively low speed, large components such as nuclei sediment to form a pellet at the bottom of the centrifuge tube; at slightly higher speed, a pellet of mitochondria is deposited; and at even higher speeds and with longer periods of centrifugation, first the small closed vesicles and then the ribosomes can be collected.
- All of these fractions are impure, but re-suspending the pellet and repeating the centrifugation procedure several times can remove many of the contaminants.

The preparative ultracentrifuge:

- The sample is contained in tubes that are inserted into a ring of cylindrical holes in a metal rotor. Rapid rotation of the rotor generates enormous centrifugal forces, which cause particles in the sample to sediment. The vacuum reduces friction, preventing heating of the rotor and allowing the refrigeration system to maintain the sample at 4°C.
- Centrifugation is the first step in most fractionations, but it separates only components that differ greatly in size. A finer degree of separation can be achieved by layering the homogenate in a thin band on top of a dilute salt solution that fills a centrifuge tube. When centrifuged, the various components in the mixture move as a series of distinct bands through the salt solution, each at a different rate, in a process called velocity sedimentation.
- For the procedure to work effectively, the bands must be protected from convective mixing, which would normally occur whenever a denser solution (for example, one containing organelles) finds itself on top of a lighter one (the salt solution). This is achieved by filling the centrifuge tube with a shallow gradient of sucrose prepared by a special mixing device. The resulting density gradient—with the dense end at the bottom of the tube—keeps each region of the salt solution denser than any solution above it, and it thereby prevents convective mixing from distorting the separation.
- When sediment through such dilute sucrose gradients, different cell components separate into distinct bands that can be collected individually. The relative rate at which each component sediments depends primarily on its size and shape—being normally described in terms of its sedimentation coefficient, or s value. Present-day ultracentrifuges rotate at speeds of up to 80,000 rpm and produce forces as high as 500,000 times gravity. With these enormous forces, even small macromolecules, such as tRNA molecules and simple enzymes can be driven to sediment at an appreciable rate and so can be separated from one another by size.
- Measurements of sedimentation coefficients are routinely used to help in determining the size and subunit composition of the organized assemblies of macromolecules found in cells.The ultracentrifuge is also used to separate cellular components on the basis of their buoyant density, independently of their size and shape. In this case the sample is usually sedimented through a steep density gradient that contains a very high concentration of

sucrose or cesium chloride.

- Each cellular component begins to move down the gradient but it eventually reaches a position where the density of the solution is equal to its own density. At this point the component floats and can move no farther. A series of distinct bands is thereby produced in the centrifuge tube, with the bands closest to the bottom of the tube containing the components of highest buoyant density. This method, called equilibrium sedimentation, is so sensitive that it is capable of separating macromolecules that have incorporated heavy isotopes, such as 13C or 15N, from the same macromolecules that contain the lighter, common isotopes (12C or 14N).
- In fact, the cesium-chloride method was developed in 1957 to separate the labeled from the unlabeled DNA produced after exposure of a growing population of bacteria to nucleotide precursors containing 15N; this classic experiment provided direct evidence for the semiconservative replication of DNA.
- Studies of organelles and other large subcellular components isolated in the ultracentrifuge have contributed enormously to our understanding of the functions of different cellular components. Experiments on mitochondria and chloroplasts purified by centrifugation, for example, demonstrated the central function of these organelles in converting energy into forms that the cell can use.
- Similarly, resealed vesicles formed from fragments of rough and smooth endoplasmic reticulum (microsomes) have been separated from each other and analyzed as functional models of these compartments of the intact cell.
- An extension of this approach makes it possible to study many other biological processes free from all of the complex side reactions that occur in a living cell, by using purified cell-free systems. In this case, cell homogenates are fractionated with the aim of purifying each of the individual macromolecules that are needed to catalyze a biological process of interest.
- For example, the mechanisms of protein synthesis were deciphered in experiments that began with a cell homogenate that could translate RNA molecules to produce proteins. Fractionation of this homogenate, step by step, produced in turn the ribosomes, tRNAs, and various enzymes that together constitute the protein-synthetic machinery.
- Once individual pure components were available, each could be added or withheld separately to define its exact role in the overall process. A major goal today is the reconstitution of every biological process in a purified cell-free system, so as to be able to define all of its components and their mechanism of action.

POSSIBLE QUESTIONS

UNIT-IV

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Write about the Basic Terminology of Sedimentation rate?
2. Define Absorbance with example.
3. What is the difference between settling and sedimentation?
4. What are the two types of rotors found in high-powered centrifuges?
5. What is the meaning of the word “derated”?
6. What is the beer lambert law? What are the correlations derived from it.]
7. What is chemiluminescence? What are its uses in diagnostic?
8. What is fluorescence? How is it used in diagnostics?

PART-C (6 MARKS)

1. Define Svedberg equation. Explain in detail about the principles behind centrifugation.
2. Explain in detail on Density gradient centrifugation.
3. Explain in detail on isopycnic centrifugation.
4. Explain in detail on rate zonal centrifugation.
5. What is the principle of colorimetry? Draw a simple labelled diagram of a colorimeter
6. What is the principal of a flame photometerr?
7. Write a short note on spectrophotometry?
8. Write in detail on FTIR.
9. Explain about NMR.
10. What is the process involved in flow cytometer?

Unit III

S.No.	Questions	Option 1	Option 2	Option 3	Option 4	Option 5	Option 6	Answer
1	Which of the following are the primary colours	Red, Blue, Yellow	Red, Green, Violet	Yellow, Green, Blue	Red, Green,			**Red, Green,**
2	A colorimeter is used to .	determine the heat of a reaction	the absorbance of a coloured compound in	store the heat from a chemical reaction	determine the heat of			**the absorba**
3	The amount of light is	Beer's law	Lambert's law	Beer and Lambert's Law	Planck's			
4	The intensity of the color is directly proportional to the concentration of the	Beer's law	Lambert's law	Beer and Lambert's Law	Planck's law			**Beer's law**
5	The correct order for the basic features of a mass spectrometer is_____	acceleration, deflection, detection, ionisation	ionisation, acceleration, deflection, detection	acceleration, ionisation, deflection, detection	acceleration, deflection, ionisation,			**ionisation, acceleration,**
6	Which one of the following statements about ionisation in a	gaseous atoms are ionised by bombarding them	atoms are ionised so they can be accelerated	atoms are ionised so they can be deflected	it doesn't matter how much			**it doesn't matter**
7	Beer's Law states that	absorbance is proportional to both	absorbance is proportional to the log	absorbance is equal to P0/P	absorbance is equal to			**absorbance is**
8	A UV-VIS spctrophotometer has -----	Two	Three	One	Four			**Two**
9	In colorimetry, Beer-Lamberts law is used to evaluate	quantitative measurements	qualitative measurements	absorbance measurements	adsorption spectrum			**quantitative measure**
10	According to the Beer-Lambert law, on which of the following does	Colour of the solution	Solution concentration	Distance that the light has travelled through the sample	Extinction coefficient of the			**Colour of the solution**
11	What is the name of an instrument used to measure the absorbance	Coulometer	Colourmeter	Colorimeter	Calorimeter			**Colorimeter**
12	The optically transparent cells are made up of	Cuvettes	test tubes	Vials	Microtip			**Cuvettes**
13	The source of visible radiation in spectrophotometer is lamp.	hydrogen	deuterium	tungsten filament	mercury			**hydrogen**
14	Flourimeter employs a ---- vapour lamp.	Tungsten	Hydrogen	Mercury	Deuterium			**Mercury**
15	Cuvettes used in spectrophotometer is having an optical path length of cm.	2	3	0.5	1			**1**
16	Which of the following is used as light source in colorimeter?	Hydrogen lamp	Deuterium lamp	Tungsten lamp	Sodium lamp			**Tungsten lamp**
17	In colorimeter, the bandwidth is selected by .	filter	monochromator	prism	gratings			**filter**
18	Lambert's law is applicable to	concentrated	dilute	very dilute	both concentrate			**concentrated**
19	Colorimetry is a form of _____.	Photometry	Electrophoresis	Chromatography	Spectrofluorimetry			**Photometry**
20	_____ is a spectroanalytical procedure for the quantitative determination of chemical elements	Atomic absorption spectroscopy	Spectrofluorimetry	Chromatography	Electrophoresis			
21	Atomic absorption spectrometry analyze _____ in biological fluids	metals	pigments	metabolites	compound			**metals**
22	The absorbed wavelengths in atomic absorption spectrum appear as	dark background	dark lines	light background	light lines			**dark lines**
23	The lines which appear in absorption and emission spectrum are	same	different	very different	far apart			**same**
24	The background in atomic absorption spectrum is	bright	dark	brown	purple			**bright**
25	Ionization of analyte atoms in flame/plasma can be suppressed by:	EDTA or other complexing agents	Addition of KCl to the matrix	Addition of oxyanions such as sulfate or phosphate	Internal standards methods			**Addition of KCl to the**
26	The visible portion of the electromagnetic spectrum occurs between	1 and 10	10 and 100	340 and 800	800 and 1200			**340 and 800**

27	Which part of the spectrophotometer is adjusted to select the desired wavelength?	light source	filter	sample	photodetector			**filter**
28	FT-IR stands for_____	Fourier transform infrared spectroscopy	Focal transform infrared spectroscopy	Fourier transmission infrared spectroscopy	Fourier transcription infrared spectrosco			**Fourier transform infrared**
29	Using a standard curve, if you know the absorbance of an unknown sample, what else can be determined about the unknown?	the wavelength of maximum absorbance	the molecular weight of the sample	the concentration of the samples	the identity of the sample			**the concentration of the samples**
30	Blank contains the ___ but not the dissolved chemical.	Solvent	solute	filter	absorption spectrum			**Solvent**
31	Basically, what is the function of an absorption spectrum?	It converts light energy into electrical	It is a graph of a chemical relating the absorbance to wavelength	It is a graph of a chemical relating the absorbance to concentration	It is the amount of radiation retained by			**It is a graph of a chemical**
32	Basically, what is the path of light through a spectrophotometer?	meter, photodetector, filter, sample, light source	meter, filter sample, photodetector, light source	light source, filter, sample, photodetector, meter	light source, sample, filter, photodetector, meter			**light source, filter, sample, photodetector, meter**
33	The ratio of transmitted light (l) to that of incident light (I_o) is referred to as	transmittance	absorbance	incidence	radiance			**transmittance**
34	The working of colorimeter is based on the principle of	Beer's law	Lambert's law	Beer - Lambert's Law	Planck's law			**Beer - Lambert's Law**
35	The spectrophotometer primarily differs from colorimeter by covering	200-400 nm	400-800 nm	300-700 nm	400-500 nm			**200-400 nm**
36	Which of the following techniques would be used to detect a metabolite labelled with ^2H?	Infra red spectroscopy	Nuclear magnetic resonance spectroscopy	Scintillation counting	Mass spectrometry			**Mass spectrometry**
37	_____ is used to determine the structural formula of an unknown chemical compound	Infra red spectroscopy	Nuclear magnetic resonance spectroscopy	Scintillation counting	Mass spectrometry			**Mass spectrometry**
38	Who was credited for the invention of the mass spectrometer?	J.J. Thompson	Ernest Rutherford	James Chadwick	Neils Bohr			**J.J. Thompson**
39	Visible wavelengths cover a range from approximately	400 to 800 nm	300 to 700 nm	200 to 700 nm	300 to 600 nm			**400 to 800 nm**
40	_____ has a shorter wavelength than the visible violet light	Ultraviolet radiation	Infrared radiation	visible spectrum	electromagnetic spectrum			**Ultraviolet radiation**
41	_____ has a longer wavelength than visible	Ultraviolet radiation	Infrared radiation	visible spectrum	electromagnetic			**Infrared**
42	Sunlight consists of the entire	Ultraviolet radiation	Infrared radiation	visible spectrum	electromagnetic spectrum			**electromagnetic spectrum**
43	Centrifuge is used to	separate different solvent layers	separate particles from liquid suspension	make homogenous suspension	vortex the sample			**separate particles from liquid suspension**
44	The visible _____ light has a wavelength of about 570 nm	yellow	red	violet	indigo			**yellow**
45	The visible _____ light has a wavelength of about 510 nm	green	red	violet	indigo			**green**

46	The visible _____ light has a wavelength of about 650 nm	green	red	violet	indigo			**red**
47	The visible _____ light has a wavelength of about 475 nm	green	Blue	violet	indigo			**blue**
48	The visible _____ light has a wavelength of about 445 nm	green	blue	violet	indigo			**indige**
49	The visible _____ light has a wavelength of about 400 nm	green	Blue	violet	indigo			**Violet**
50	The _____ light is a mixture of the colors of the visible spectrum	green	white	violet	indigo			**white**
51	The visible colors from shortest to longest wavelength are:	violet, blue, green, yellow, orange, and red	blue, violet, green, yellow, orange, and red	green, violet, blue, orange, yellow, and red	red, blue, green, yellow, orange, and violet			**violet, blue, green, yellow, orange, and red**
52	Which one of the following has the shortest wavelength	Ultraviolet light	Infrared light	visible spectrum	electromagnetic spectrum			**Ultraviolet light**
53	Energy with wavelengths too short to see is	bluer than blue	redder than red	greener than green	brighter than bright			
54	The visible indigo light has a wavelength of about nm	200	800	700	445			**445**
55	The centrifugal force pushes	the mixture outwards	the mixture reverse	the mixture inwards	the mixture up and down			**the mixture outwards**
56	The visible red light has a wavelength of about nm	650	475	445	425			**650**
57	The visible orange light has a wavelength of about nm	590	475	445	425			**590**
58	Preparative centrifuge is used to	to prepare samples for further analysis	to quantify the samples	to analyse the samples	to prepare and analyse the samples simultaneously			**to prepare samples for further analysis**
59	Energy with wavelengths too short to see is	bluer than blue	redder than red	greener than green	brighter than bright			**bluer than blue**
60	Flourimeter employs a ---- vapour lamp.	Tungsten	Hydrogen	Mercury	Deuterium			**Mercury**

UNIT-IV

SYLLABUS

Radioactive Isotopes: Radioactive decay, units of radioactivity, detection and measurement of radioactivity -GM counter, Scintillation counter, Autoradiography, biochemical applications of radio isotopes.

RADIOACTIVE ISOTOPES

Atomic structure

An atom is composed of a positively charged central nucleus inside a much larger cloud of negatively charged electrons. The mass of an atom is concentrated in the nucleus, even though it accounts for only a small fraction of the total size of the atom. Atomic nuclei are composed of two major particles, protons and neutrons. Protons are positively charged with a mass approximately 1850 times greater than that of an electron. The number of protons present in the nucleus is known as the atomic number (Z), and it determines what the element is, for example six protons is carbon. Neutrons are uncharged particles with a mass approximately equal to that of a proton. The sum of protons and neutrons in a given nucleus is the mass number (A). Thus

$$A = Z + N$$

where N is the number of neutrons present.

Since the number of neutrons in a nucleus is not related to the atomic number, it does not affect the chemical properties of the atom. Atoms of a given element may not necessarily contain the same number of neutrons. Atoms of a given element with different mass numbers (i.e. different numbers of neutrons) are called isotopes. Symbolically, a specific nuclear species is represented by a subscript number for the atomic number, and a superscript number for the mass number, followed by the symbol of the element. For example:

$$^{12}_{6}C \quad ^{14}_{6}C \quad ^{16}_{8}O \quad ^{18}_{8}O$$

However, in practice it is more conventional just to cite the mass number (e.g. ^{14}C). The number of isotopes of a given element varies: there are three isotopes of hydrogen (^{1}H, ^{2}H and ^{3}H), seven

of carbon (^{10}C to ^{16}C inclusive) and 20 or more of some of the elements of high atomic number.

ATOMIC STABILITY AND RADIATION

In general, the ratio of neutrons to protons will determine whether an isotope of an element is stable enough to exist in nature. Stable isotopes for elements with low atomic numbers tend to have an equal number of neutrons and protons, whereas stability for elements of higher atomic numbers requires more neutrons. Unstable isotopes are called radioisotopes. They become stable isotopes by the process of radioactive decay: changes occur in the atomic nucleus, and particles and/or electromagnetic radiation are emitted.

TYPES OF RADIOACTIVE DECAY

There are several types of radioactive decay; only those most relevant to biochemists are considered below. A summary of properties is given in Table 14.1.

Table 14.1 **Properties of different types of radiation**

Alpha	Beta	Gamma, X-rays and Bremsstrahlung
Heavy charged particle	Light charged particle	Electromagnetic radiation (em)
More toxic than other forms of radiation	Toxicity same as em radiation per unit of energy	Toxicity same as beta radiation per unit of energy
Not penetrating	Penetration varies with source	Highly penetrating

Decay by negatron emission

In this case a neutron is converted to a proton by the ejection of a negatively charged beta (b) particle called a negatron (β^-):

$$\text{Neutron} \rightarrow \text{proton} + \text{negatron}$$

To all intents and purposes a negatron is an electron, but the term negatron is preferred, although not always used, since it serves to emphasise the nuclear origin of the particle. As a result of negatron emission, the nucleus loses a neutron but gains a proton. The mass number, A, remains constant. An isotope frequently used in biological work that decays by negatron emission is 14C.

$$^{14}_{6}C \rightarrow ^{14}_{7}N + \beta^{-}$$

Negatron emission is very important to biochemists because many of the commonly used radionuclides decay by this mechanism. Examples are: 3H and 14C, which can be used to label any organic compound; 35S used to label methionine, for example to study protein synthesis; and 33P or 32P, powerful tools in molecular biology when used as nucleic acid labels.

Decay by positron emission

Some isotopes decay by emitting positively charged b-particles referred to as positrons (β^{+}). This occurs when a proton is converted to a neutron:

$$\text{Proton} \rightarrow \text{neutron} + \text{positron}$$

The nature of radioactivity Positrons are extremely unstable and have only a transient existence. Once they have dissipated their energy they interact with electrons and are annihilated. The mass and energy of the two particles are converted to two g-rays emitted at 180_ to each other. This phenomenon is frequently described as back-to-back emission. As a result of positron emission the nucleus loses a proton and gains a neutron, the mass number stays the same. An example of an isotope decaying by positron emission is ^{22}Na:

$$^{22}_{11}Na \rightarrow ^{22}_{10}Ne + \beta^{+}$$

Positron emitters are detected by the same instruments used to detect g-radiation. They are used in biological sciences to spectacular effect in brain scanning with the technique positron emission tomography (PET scanning) used to identify active and inactive areas of the brain.

Decay by alpha particle emission

Isotopes of elements with high atomic numbers frequently decay by emitting alpha (a) particles. An a-particle is a helium nucleus; it consists of two protons and two neutrons (4He2þ). Emission of a-particles results in a considerable lightening of the nucleus, a decrease in atomic number of 2 and a decrease in the mass number of 4. Isotopes that decay by a-emission are not frequently encountered in biological work although they can be found in instruments such as scintillation counters and smoke alarms. Radium-226 (^{226}Ra) decays by a-emission to radon-222 (222Rn), which is itself radioactive. Thus begins a complex decay series, which culminates in the formation of ^{206}Pb:

$$^{226}_{88}\mathrm{Ra} \rightarrow {}^{4}_{2}\mathrm{He}^{2+} + {}^{222}_{86}\mathrm{Rn} \rightarrow\rightarrow\rightarrow {}^{206}_{82}\mathrm{Pb}$$

Alpha emitters are extremely toxic if ingested, due to the large mass and the ionising power of the a-particle.

Electron capture

In this form of decay a proton captures an electron orbiting in the innermost K shell:

$$\text{proton} + \text{electron} \rightarrow \text{neutron} + \text{X-ray}$$

The proton becomes a neutron and electromagnetic radiation (X-rays) is given out.

Example:

$$^{125}_{53}\mathrm{I} \rightarrow {}^{125}_{52}\mathrm{Te} + \text{X-ray}$$

Decay by emission of g-rays

In some cases a- andb-particle emissionalsogive rise tog-rays (electromagnetic radiation similar to, but with a shorter wavelength than, X-rays). The g-radiation has low ionising power but high penetration. For example, the radiation from 60Co will penetrate 15 cm of steel. The toxicity of g-radiation is similar to that of X-rays.

Example:

$$^{131}_{53}\mathrm{I} \rightarrow {}^{131}_{54}\mathrm{Xe} + \beta^{-} + \gamma$$

RADIOACTIVE DECAY ENERGY

The usual unit used in expressing energy levels associated with radioactive decay is the electron volt. One electron volt (eV) is the energy acquired by one electron in accelerating through a potential difference of 1 V and is equivalent to 1.6×10^{19} J. For the majority of isotopes, the term million or mega electron volts (MeV) is more applicable. Isotopes emitting a-particles are normally the most energetic, falling in the range 4.0 to 8.0 MeV, whereas b- and g-emitters generally have decay energies of less than 3.0 MeV. The higher the energy of radiation the more it can penetrate matter and the more hazardous it becomes.

RATE OF RADIOACTIVE DECAY

Radioactive decay (measured as disintegrations per minute, d.p.m.) is a spontaneous process and it occurs at a rate characteristic of the source, defined by the rate constant (l, the fraction of an isotope decaying in unit time, t^{-1}). Decay is a nuclear event so l is not affected by temperature or pressure. The number of atoms disintegrating at any time is proportional to the number of atoms of the isotope (N) present at that time (t). Clearly, the number of atoms N, is always falling (as atoms decay) and so the rate of decay (d.p.m.) falls with time. Also, the slope of the graph of number of unstable atoms present, or rate of decay (d.p.m.) against time, similarly falls. This means that a graph of radioactivity against time shows a curve, called an exponential decay curve (Fig. 14.1). The mathematical equation that underpins the graph shown is as follows:

$$\ln N_t/N_0 = -\lambda t$$

where l is the decay constant for an isotope, Nt is the number of radioactive atoms present at time t, and N0 is the number of radioactive atoms orginally present. You will notice the natural logarithm (ln) in the equation; this means if we were to plot log d.p.m. against time we would get a graph with a straight line and a negative slope (gradient determined by the value of l).

In practice it is more convenient to express the decay constant in terms of half-life ($t_{1/2}$). This is defined as the time taken for the activity to fall from any value to half that value (see Fig. 14.1). When Nt in equation 14.1 is equal to one-half of N0 then t will equal the half-life of the isotope. Thus

$$\ln 1/2 = -\lambda t_{1/2}$$
$$\text{or } t_{1/2} = 0.693\lambda$$

The values of $t_{1/2}$ vary widely from over 10^{19} years for lead-204 (^{204}Pb) to 3×10^{-7} seconds for polonium-212 (212Po). The half-lives of some isotopes frequently used in biological work are given in Table 14.2. The advantages and disadvantages of working with isotopes of differing half-lives are given in Table 14.3.

Table 14.2 **Properties of radioisotopes commonly used in the biological sciences**

Property	^{3}H	^{14}C	^{35}S	^{32}P	^{33}P	^{125}I	^{131}I
$t_{1/2}$	12.3 years	5730 years	87.4 days	14.3 days	25.4 days	59.6 days	8.04 days
Mode of decay	β	β	β	β	β	X (EC) and Auger electrons	γ and β
Max β energy (MeV)	0.019	0.156	0.167	1.709	0.249	Auger electrons 0.035	0.806
ALI[a]	480 (Mbq)[b]	34 (Mbq)	15 (Mbq)	6.3 (Mbq)	14 (Mbq)	1.3 (Mbq)[c]	0.9 (Mbq)[c]
Maximum range in air	6 mm	24 cm	26 cm	790 cm	49 cm	>10 m	>10 cm
Shielding required	None	1 cm acrylic	1 cm acrylic	1 cm acrylic	1 cm acrylic	Lead 0.25 m or lead-impregnated acrylic	Lead 13 mm
γ dose rate (μSv h^{-1} from 1 GBq at 1 m)	–	–	–	(β dose rate 760 μSv, 10 cm from 1 MBq)	–	41	51
Čerenkov counting	–	–	–	Yes	–	–	–

Notes: [a]Annual limit on intake, based on a dose limit of 20 mSv using the most restrictive dose coefficients for inhalation or ingestion.
[b]Bound ^{3}H.
[c]Based on dose equivalent limit of 500 mSv to thyroid.

Table 14.3 **The advantages and disadvantages of working with a short-half-life isotope**

Advantages	Disadvantages
High specific activity (see Section 14.3.3) makes the experiment more sensitive	Experimental design; isotope decays during time of experiment
Easier and cheaper to dispose of	Cost of replacement for further experiments
Lower doses likely (e.g. in diagnostic testing of human subjects)	Frequently need to calculate amount of activity remaining

UNITS OF RADIOACTIVITY

The Syste`me International d'Unite´s (SI system) uses the becquerel (Bq) as the unit of radioactivity. This is defined as one disintegration per second (1 d.p.s.). However, an older unit, not in the SI system and still frequently used, is the curie(Ci). This is defined as the quantity of radioactive material in which the number of nuclear disintegrations per second is the same as that in 1 g of radium, namely 3.7×10^{10} (or 37 GBq). For biological purposes this unit is too large and the microcurie(mCi) and millicurie (mCi) are used. It is important to realise that the units Bq and Ci refer to the number of disintegrations actually occurring in a sample not to the disintegrations detected, which generally will be only a proportion of the disintegrations occurring. Detected decays are referred to as counts (i.e. counts per second or c.p.s.).

INTERACTION OF RADIOACTIVITY WITH MATTER

a-Particles

These particles have a very considerable energy (3‑8 MeV) and all the particles from a given isotope have the same amount of energy. They react with matter in two ways: they cause excitation (energy is transferred from the a-particle to orbital electrons of neighbouring atoms, these electrons being elevated to higher orbitals, but eventually fall back, emitting energy as photons of light) and they ionise atoms in their path (the target orbital electron is removed, thus the atom becomes ionised and forms an ionpair, consisting of a positively charged ion and an electron). Because of their size, a-particles have slow movement and double positive charge.

They cause intense ionisation and excitation and their energy is rapidly dissipated. Despite their initial high energy, a-particles frequently collide with atoms in their path and so the radiation is not very penetrating (a few centimetres through air).

Negatrons

Negatrons are very small and rapidlymoving particles that carry a single negative charge. They interact with matter to cause ionisation and excitation exactly as with a-particles. However, due to their speed and size, they are less likely than a-particles to interact with matter and therefore are less ionising and more penetrating. Another difference between a-particles and negatrons is that negatrons are emitted over a range of energies. Negatron emitters have a characteristic energy spectrum (see Fig. 14.5b below). The maximum energy level (Emax) varies fromone isotope to another, ranging from0.018MeV for 3H to 4.81 MeV for 38Cl. The difference in Emax affects the penetration of the radiation and therefore the safety measures that are required: b-particles from 3H can travel only a few millimetres in air, whereas those from 32P can penetrate over 1m of air. Therefore radiation shields are needed when working with 32P.

γ-Rays and X-rays

These rays (henceforth collectively referred to as g-rays for simplicity) are electromagnetic radiation and therefore have no charge or mass. They cause excitation and ionisation. They interact with matter to create secondary electrons that behave as per negatron emission.

Bremsstrahlung radiation

When high atomic number materials absorb high energy b-particles, the absorber gives out a secondary radiation, an X-ray, called bremsstrahlung radiation. For this reason, shields for 32P use low-atomic-number materials such as acrylic.

DETECTION AND MEASUREMENT OF RADIOACTIVITY

There are three commonly used methods of detecting and quantifying radioactivity. These are based on the ionisation of gases, on the excitation of solids or solutions, and the ability of radioactivity to expose photographic emulsions (i.e. autoradiography).

Methods based upon gas ionisation

If a charged particle passes through a gas, its electrostatic field dislodges orbital electrons from atoms sufficiently close to its path and causes ionisation (Fig. 14.2). The ability to induce ionisation decreases in the order A > b > γ (10 000 : 100 : 1)

If ionisation occurs between a pair of electrodes enclosed in a suitable chamber (Fig. 14.2) a pulse (current) flows. Ionisation counters like those shown in Fig. 14.2 are sometimes called proportional counters (‘proportional’ because small voltage changes can affect the count rate). The Geiger-Mu¨ ller counter (Figs. 14.3, 14.4a) has a cylindrical-shaped gas chamber and it operates at a high voltage. This makes the instrument less dependent on a stable voltage, so the counter is cheaper and lighter. In ionisation counters, the ions have to travel to their respective electrodes; other ionising particles entering the tube during this time (the so-called ‘dead time’) are not detected and this reduces the counting efficiency.

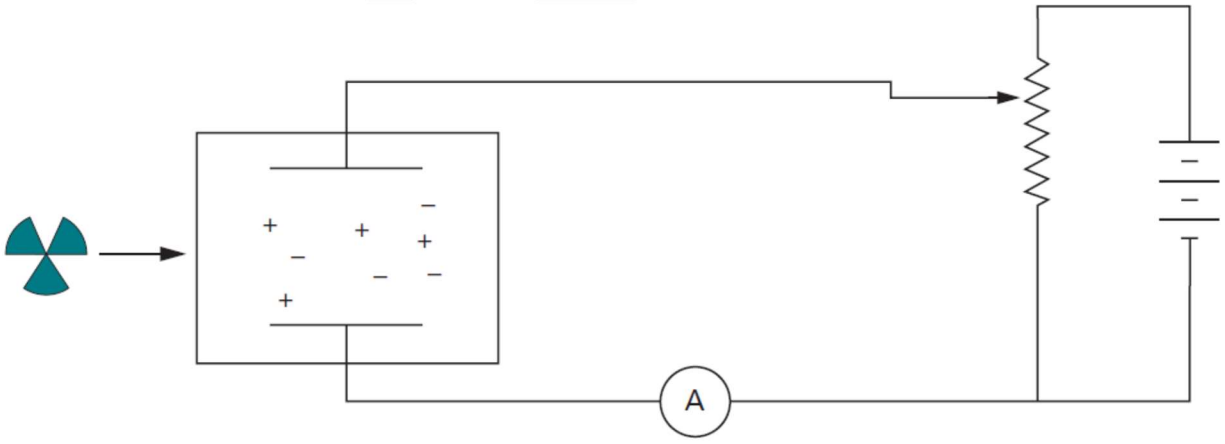


Fig. 14.2 Detection based on ionisation.

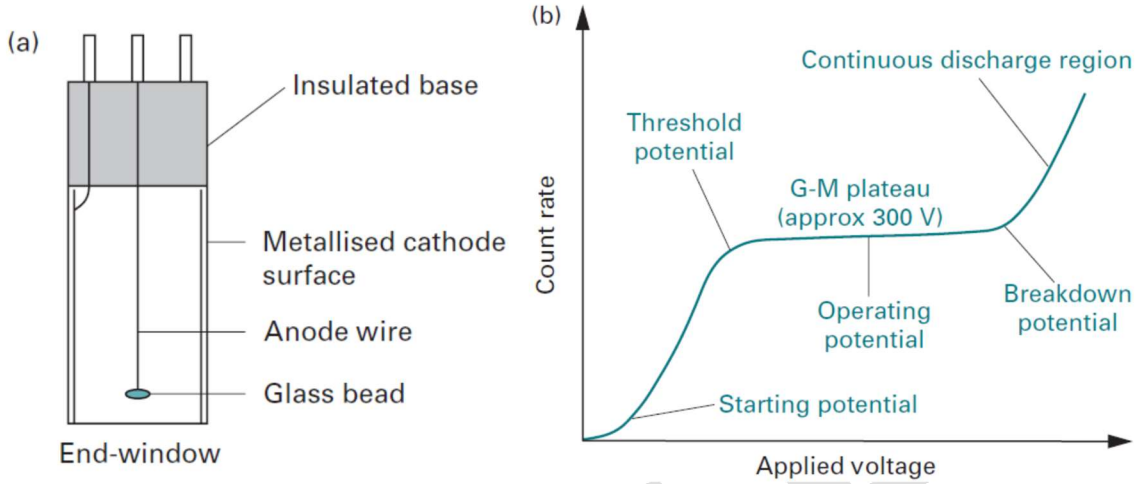


Fig. 14.3 (a) The Geiger-Mu¨ ller (G-M) tube and (b) the effect of applied voltage on count rate.

Ionisation counters are used for routine monitoring of the laboratory to check for contamination. They are also useful in experimental situations where the presence or absence of radioactivity needs to be known rather than the absolute quantity, for example quick screening of radioactive gels prior to autoradiography, checking that a labelled DNA probe is where you think it is (and not down the sink!) or checking chromatographic fractions for labelled components.

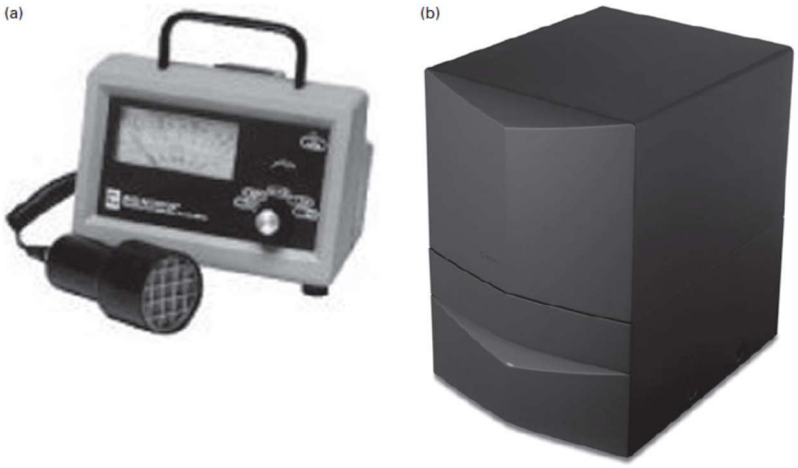


Fig. 14.4 (a) Geiger-Mu¨ ller bench monitor; (b) liquid scintillation counter

Methods based upon excitation

Radioactive isotopes interact with matter in two ways, ionisation and excitation. The latter effect leads an excited atom or compound (known as a fluor) to emit photons of light. The process is known as scintillation. When the light is detected by a photomultiplier, it forms the basis of scintillation counting. Essentially, a photomultiplier converts the energy of radiation into an electrical signal, and the strength of the electric pulse that results is directly proportional to the energy of the original radioactive event. This means that two, or even more, isotopes can be separately detected and measured in the same sample, provided they have sufficiently different emission energy spectra. The mode of action of a photomultiplier is shown in Fig. 14.5a, and the energy spectrum of a b-particle emitter in Fig. 14.5b.

Types of scintillation counting

There are two types of scintillation counting, which are illustrated diagrammatically in Fig. 14.6. In solid scintillation counting the sample is placed adjacent to a solid fluor (e.g. sodium iodide). Solid scintillation counting is particularly useful for g-emitting isotopes. This is because they can penetrate the fluor. The counters can be small handheld devices with the fluor attached to the photomultiplier tube (Fig. 14.5.a), or larger bench-top machines with a well-shaped fluor designed to automatically count many samples (Fig. 14.6.a). In liquid scintillation counting (Fig. 14.6b; see also Figs. 14.4b, c), the sample is mixed with a scintillation fluid containing a solvent and one or more dissolved fluors. This method is particularly useful in quantifying weak b-emitters such as 3H, 14C and 35S, which are frequently used in biological work. Scintillation fluids are called cocktails' because there are different formulations, made of a solvent (such as toluene or diisopropylnaphthalene) plus fluors such as 2,5-diphenyloxazole (PPO), 1,4-bis(5-phenyloxazol-2-yl)benzene (nicknamed POPOP, pronounced as it reads: 'pop op') or 2-(40-t-butylphenyl)-5-(400-bi-phenyl)-1,3,4-oxydiazole (butyl-PBD). Cocktails can be designed for counting organic samples, or may contain detergent to facilitate counting of aqueous samples.

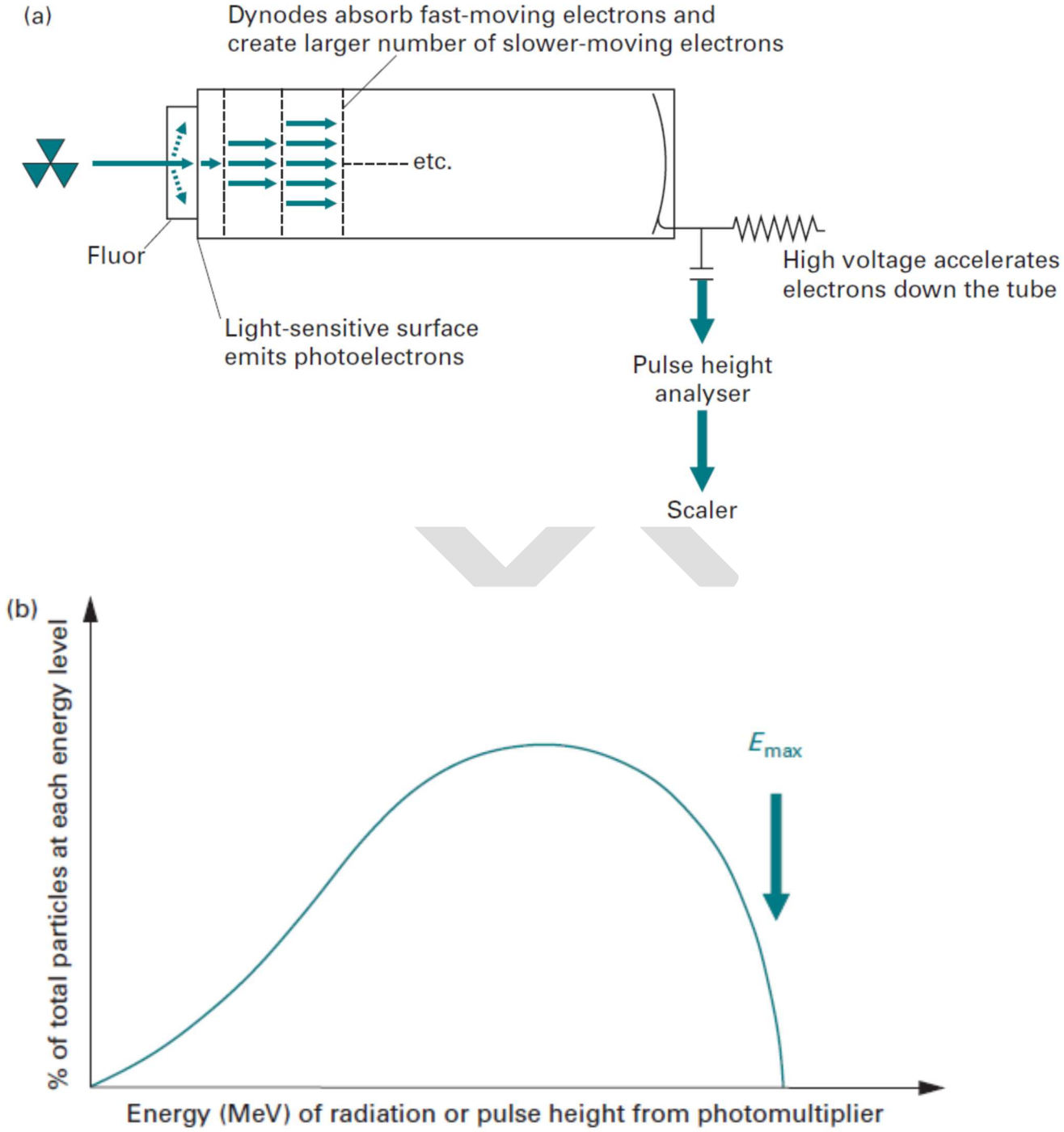


Fig. 14.5 (a) The mode of action of a photomultiplier and (b) the energy spectrum of a typical β-emitter.

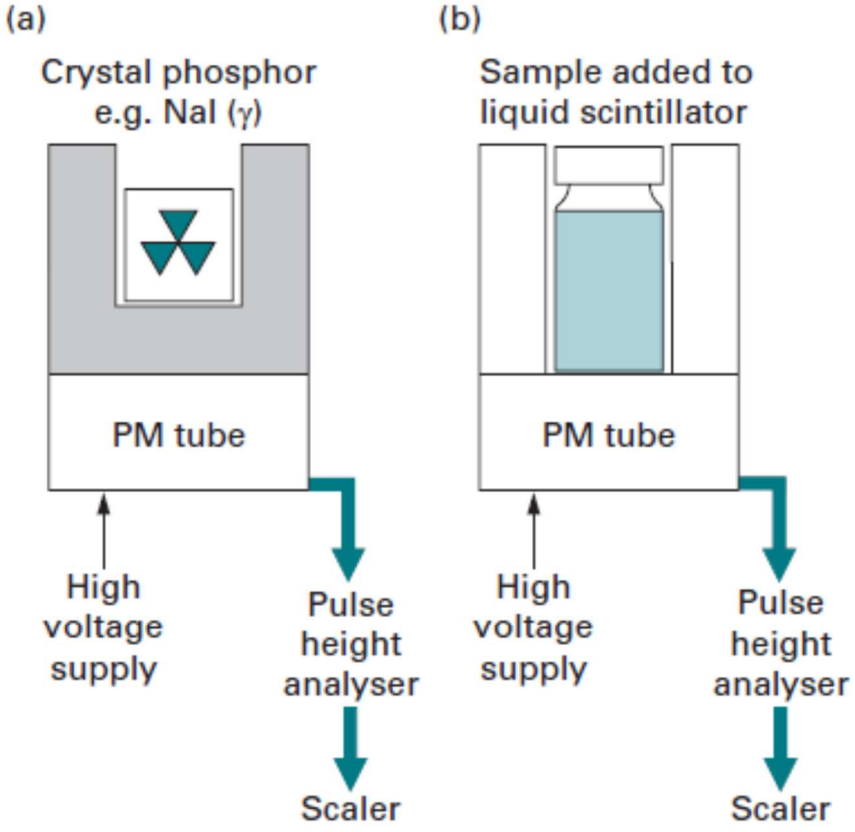


Fig. 14.6 Diagrammatic illustration of (a) solid and (b) liquid scintillation counting methods.

Advantages of scintillation counting

Scintillation counting is widely used in biological work and it has several advantages over gas ionisation counting:

· fluorescence is very fast so there is effectively no dead time

· counting efficiencies are high (from about 50% for low-energy b-emitters to 90% for high-energy emitters)

· the ability to count samples of many types, including liquids, solids, suspensions and gels

· the general ease of sample preparation

· the ability to count separately different isotopes in the same sample (used in dual-labelling experiments)

· highly automated (hundreds of samples can be counted automatically and built-in computer

facilities carry out many forms of data analysis, such as efficiency correction, graph plotting, radioimmunoassay calculations, etc.).

Disadvantages of scintillation counting

No technique is without disadvantages, so the following have to be considered or overcome in the design of the instruments:

· cost of the instrument and cost per sample (for scintillation fluid, the counting vials and disposal of the organic waste)

· potentially high background counts; this is due to photomultiplier noise but can be compensated for by using more than one tube (noise is random, but counts from a radioactive decay are simultaneous, the coincident counts only are recorded)

· 'quenching': this is the name for reduction in counting efficiency caused by coloured compounds that absorb the scintillated light, or chemicals that interfere with the transfer of energy from the radiation to the photomultiplier (correcting for quenching contributes significantly to the cost of scintillation counting)

· chemiluminescence: this is when chemical reactions between components of the samples to be counted and the scintillation cocktail produce scintillations that are unrelated to the radioactivity; modern instruments can detect chemiluminescence and subtract it from the results automatically

· phospholuminescence: this results from pigments in the sample absorbing light and re-emitting it; the solution is to keep the samples in the dark prior to counting.

AUTORADIOGRAPHY

Ionising radiation acts upon a photographic emulsion or film to produce a latent image much as does visible light. This is called autoradiography. The emulsion or film contains silver halide crystals. As energy from the radioactive material is dissipated the silver halide becomes negatively charged and is reduced to metallic silver, thus forming a particulate latent image. Photographic developers show these silver grains as a blackening of the film, then fixers are used to remove any remaining silver halide and a permanent image results. It is a very sensitive

technique and has been used in a wide variety of biological experiments. A good example is autoradiography of nucleic acids separated by gel electrophoresis (see Fig. 14.10).

Suitable isotopes

In general, weak b-emitting isotopes (e.g. 3H, 14C and 35S) are most suitable for autoradiography, particularly for cell and tissue localisation experiments. This is because the energy of the radiation is low. The sample must be close to the film, the radiation does not spread out very far and so a clear image results. Radiation with higher energy (e.g. 32P) give faster results but poorer resolution because the higherenergy negatrons produce much longer track lengths, exposing a greater surface area of the film, and result in less discrete images. This is illustrated in Fig. 14.10, showing autoradiography with three different isotopes.

Choice of emulsion and film

Autoradiography emulsions are solutions of silver halide that can be made to set solid by the inclusion of materials such as gelatine. This can be used for example for autoradiography of microscope slides. X-ray film is the alternative and is used for gels (as shown in Fig. 14.10). Films differ in sensitivity; advice on what to use is provided by the manufacturers.

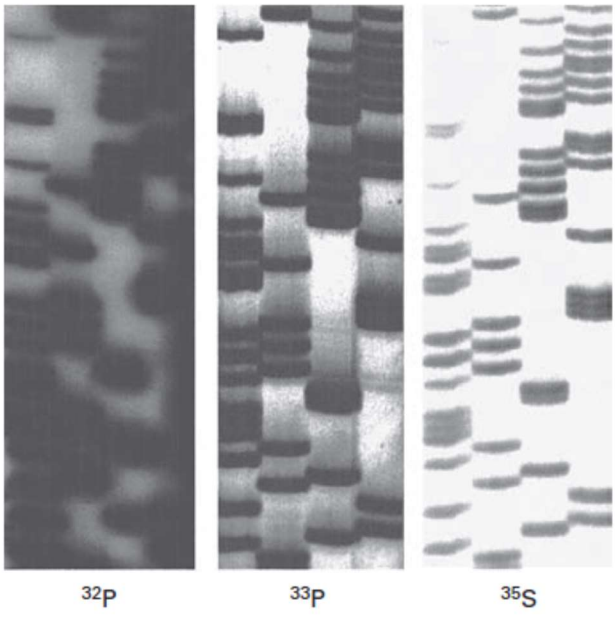


Fig. 14.10 Three autoradiographs showing the use of different radioisotopes in DNA sequencing.

The isotope with the highest energy (32P) leads to the poorest resolution because the radiation spreads out further, making the DNA bands appear thicker. The lowest energy radiation (from 35S) gives the best resolution.

Direct autoradiography

In direct autoradiography, the X-ray film or emulsion is placed as close as possible to the sample and exposed at any convenient temperature. Quantitative images are produced until saturation is reached. The shades of grey in the image are related to a combination of levels of radiation and length of exposure until a black or nearly black image results. Isotopes with an energy of radiation equal to, or higher than, 14C (Emax¼ 0.156 MeV) are required. The higher the energy the quicker the results.

Fluorography

If low-energy b-emitters are used it is possible to enhance the sensitivity several orders of magnitude by using fluorography. A fluor (e.g. PPO or sodium silicate) can be used to enhance the image. The b-particles emitted from the isotope will cause the fluor to become excited and emit light, which will react with the film. This has been used for example for detecting radioactive nucleic acids in gels. The fluor is infiltrated into the gel following electrophoresis; the gel is dried and then placed in contact with a preflashed film (see below).

Intensifying screens

Intensifying screens are used when obtaining a fast result is more important than high resolution. It is useful for example in gel electrophoresis or analysis of membrane filters where high-energy b-emitters (e.g.32P-labelled DNA) or g-emitting isotopes (e.g. 125I-labelled protein) are used. The intensifying screen consists of a solid phosphor, and it is placed on the other side of the film from the sample. High-energy radiation passes through the film, causes the phosphor to fluoresce and emit light, which in turn superimposes its image on the film. The reduction in resolution is due to the spread of light emanating from the screen.

Low-temperature exposure

When intensifying screens or fluorography are used the exposure should be done at low temperature. This is because the kinetics of the film’s response are affected. The light is of low intensity and a back reaction occurs that cancels the latent image. Exposure at low temperature (-

70ºC) slows this back reaction and will therefore provide higher sensitivity. There is no point in doing direct autoradiography at low temperature as the kinetic basis of the film's response is different.

Preflashing

The response of a photographic emulsion to radiation is not linear and usually involves a slow initial phase (lag) followed by a linear phase. Sensitivity of films may be increased by preflashing. This involves a millisecond light flash prior to the sample being brought into juxtaposition with the film and is often used where high sensitivity is required or if results are to be quantified.

Quantification

Autoradiography is usually used to locate rather than to quantify radioactivity. However, it is possible to obtain quantitative data directly from autoradiographs by using digital image analysis. Quantification is not reliable at low or high levels of exposure because of the lag phase (see preflashing above) or saturation, respectively. Preflashing combined with fluorography or intensifying screens create the best conditions for quantitative working.

BIOCHEMICAL APPLICATIONS OF RADIO ISOTOPES

Biochemical analysis

Biochemical assays are used to detect the presence and absence of radioisotopes. Therefore radioactive isotopes are used to label biological molecules. Such assays estimate the concentration of different constituents of plasma, body fluids, urine, blood etc. This technique is called radioimmuno-assays. An example is iodine bioassay which uses gamma emitters' radionuclides of Iodine-125 and Iodine-131 that accrues inside thyroid. Therefore gamma detector can be used to quantify the iodine content (uptake and intake) of the person's thyroid. The amount of measured radioiodine in the thyroid is compared with the Annual Limit on Intake (ALI).

Urinalysis

Radioisotopes are eliminated from the body in body fluids. By determining the active contents in urine one can analyse the uptake and intake of a specific radionuclide.

Tracer studies

Radioisotope is used for biological labelling of cells or entities for identification or tracing specific molecules in an organism. S 35 P 32 and I 125 are widely used radioisotopes used for labelling.

Carbon dating

Radioactive carbon-14 decay could be used to estimate the age of organic materials. For example carbon dating revealed that the burial cloth of Jesus Christ originated during the medieval times between A.D. 1260-1390. Similarly mummified remains found frozen in the Italian Alps were at least 5000 years old.

Potassium dating

Radio potassium-40 decays to stable 40Ar. Thus, by measuring relative ratio of 40K and 40Ar in rocks enable us to determine the age of rocks since its formation.

Clinical diagnostic

Positron Emission Tomography (PET) and PETCT make use of radionuclides emitting positron particle that is injected in to the target cell or tissue. Radionuclide decay release positron particles which interact with the nearby negatively charged particle resulting in the emission of gamma rays which is detected by a PET or gamma camera to give an exact image of the target.

Radionuclide Therapy (RNT)

This therapy makes use of radioisotopes that emits radiations upon their decay. These emitted radiations are used to target specific cancerous cells, tumours etc. to control their abnormal growth or completely eradicate it. For example cobalt-60 is use as a source of gamma radiation for radionuclide therapy, gamma knife radiosurgery and brachytherapy. Similarly targeted alpha therapy uses alphaemitting radionuclide such as Bi-213, Lead-212, and Boron-10 to for treating pancreatic, ovarian and melanoma cancers.

Sterilizing

Sterilization of surgical instruments such as syringes, gloves, clothing and instruments using gamma mitting radionuclides including Cobalt-60, Cs-137 etc.

Radiopharmaceuticals Incorporation of radioisotopes to biologically active substances is

introduced into body in order to observe the functioning of an organ functioning or a metabolic path way etc. For example Yttrium-90 and Iodine-131 is used as radiopharmaceuticals for the treatment of non-Hodgkin's lymphoma and hyperthyroidism respectively.

POSSIBLE QUESTIONS

UNIT-V

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Define radioactive decay?
2. Write a short note about the units of radioactivity?
3. Give a short note radioactivity detection?
4. What is autoradiography?
5. Name few biochemical applications of radioisotopes?

PART-C (6 MARKS)

1. Explain in detail about the detection and measurement of radioisotopes.
2. Give a detail account on the biochemical applications of radioisotopes
3. Write a elaborate note on radioactive decay and units of radioactivity.

Unit IV

S.No	Questions	Option 1	Option 2	Option 3	Option 4	Option 5	Option 6	Answer
1	Isotope of hydrogen	dueterium	proton	electron	neutron			**dueterium**
2	Radio active isotope of hydrogen is	dueterium	proton	tritium	neutron			**tritium**
3	How many types of	2	3	4	8			**3**
4	Most commonly used radioisotopes in biochemistry are	α emitters	β emitters	γ emitters	both α and β emitters			**β emitters**
5	Isotopes of an element have different numbers of	electron	proton	neutron	None of the above			**neutron**
6	Half life of ^{32}P is	12 days	24 days	14 days	10 days			**14 days**
7	Number of isotopes which ox	3	5	9	2			**3**
8	Isotopes have same	atomic mass	atomic number	molecules	atoms			**atomic nu**
9	Properties in which isotopes	Chemical	Physical	electrical	mechanical			**Physical**
10	Most commonly used radioisotopes in the study nucleic acid is	2H	3H	14C	32P			**32P**
11	Which one of the following is a weak β emitter?	2H	125I	14C	32P			**14C**
12	Which one of the following is a strong β emitter?	2H	3H	14C	32P			**32P**
13	Most commonly used radio isotope in the study of metabolic pathways is	2H	3H	14C	32P			**14C**
14	which one of the following isoope is used in carbon dating	2H	3H	14C	32P			**14C**
15	Most commonly used radio isotope in radio immuno assay is	2H	125I	14C	32P			**125I**
16	^{125}I is a	α emitter	β emitter	γ emitter	both α and β emitters			**γ emitter**
17	Radio active isotope used in the study of cellular function and bone formation is	125I	3H	47Ca	32P			**47Ca**
18	Radio active isotope used in radio therapy of tumors	125I	137 Cs	47Ca	32P			**137 Cs**
19	Name the radio isotope used in the treatment of Grave's disease	131I	137 Cs	47Ca	32P			**131I**
20	Who is the father of diagnostic radiography?	Wilhelm Conrad Roentgen	Marie Curie	Frederick Soddy	Francis William Aston			**Wilhelm Conrad Roentgen**
21	Who developed the theory of radioactivity?	Wilhelm Conrad Roentgen	Marie Curie	Frederick Soddy	Francis William Aston			**Marie Curie**
22	Which of the following element was discovered by Marie Curie?	Polonium	47Ca	11C	Both A and C			**Poloniu m**
23	The formula of half life (t1/2) is	0.392/λ	0.693/λ	0.125/λ	0.852/λ			**0.693/λ**
24	Which one of the following is a positron emitter?	11C	125I	47Ca	137Cs			**11C**
25	The proportionality constant (λ) is also known as	Falcon constant	decay constant	energy constant	random constant			**decay constant**
26	Half-life of radioactive sample is known as the time required for	100% decay of the sample	one-half of the sample to decay	three-fourth of the sample to decay	None of the above			**one-half of the sample to decay**
27	What is the basic unit of radioactivity?	Curie (Ci)	nano meter (nm)	Joule (J)	watt (W)			**Curie (Ci)**
28	Which one of the following is a radioactivity detection method	IF spectroscopy	Geiger Muller counter	FT-IR	Mass spectrometr y			**Geiger Muller counter**
29	Autoradiography is known as	detection and localization of electrolyte in a biological system	detection and localization of radioactive substance in a biological system	detection and localization of pathogens in biological system	Treatment method			**detectio n and localizati on of radioacti ve substanc e in a biologic al**
30	When unstable nuclei undergo radioactive decay, they emit three type of radioactivity. Which is **not** one of them?	alpha	beta	gamma	delta			**delta**
31	Radioactivity is a ------ process.	Spontaneous	Random	reversible	Spontaneo us and random			**Spontan eous and random**
32	Which type of radioactive decay does not change the atomic number?	alpha	beta	gamma	All forms of radioactive decay affect the atomic number			**gamma**

33	Phenomena of radioactivity w	Wilhelm Conrad Roentgen	Henri Becquerel	Frederick Soddy	Francis William Aston			**Henri Becquerel**
34	Whether or not a nuclear fission reaction becomes self-sustaining depends on the release of	energy	neutrons	protons	electrons			**neutrons**
35	Particles that are helium nuclei are	alpha particles	beta particles	gamma particles	there are no particles that are helium nuclei			**alpha particles**
36	What is called when two atomic nuclei are combined?	nuclear fission	nuclear fusion	nuclear decay	chain reaction			**nuclear fusion**
37	Quick electron emissions are called	alpha decay	beta decay	gamma decay	radioactive half-life			**beta decay**
38	PET in radiography stands for	Positron Emission Topology	Poly Ethelene Tetran	Positron emission tomography	Poly Ethelene Glycol			**Positron emission tomography**
39	Radioactivity that takes the	alpha	beta	gamma	Zeta			**gamma**
40	What form of radioactive decay reduces the atomic number by 2?	alpha decay	beta decay	gamma decay	None of the above			**alpha decay**
41	Particles that are un affected	protons	neutrons	leptons	bosons			**leptons**
42	Radiations emitted by radioa	alpha	beta	gamma	all of the above			**all of the above**
43	Elements undergo radioactive decay when proton number becomes greater than	50	40	83	73			**83**
44	Which of the following is **not** a radioisotope?	Carbon-14	B.tritium	Carbon-13	Sulphur-35			**Carbon-13**
45	Which pair of isotopes are lil	Carbon-12 and carbon-14	Carbon-12 and carbon-13	Hydrogen and deuterium	Nitrogen-14 and			**Hydroge**
46	Which of the following	Infra red spectroscopy	Nuclear magnetic	Scintillation counting	Mass			**Infra**
47	Which of the following isotop	Fluorine-18	Carbon-11	tritium	Carbon-14			**Carbon-11**
48	What is detected during posit	Positrons	Electrons	Neutrons	Photons			**Photons**
49	Radius of nucleus ranges fro	10-15 m	10-15 m to 10-14 m	10-10 m	10-10 m to 10-6 m			**10-15 m to 10-14 m**
50	Number of protons in an ato	chemical properties	physical properties	magnetic properties	electrical properties			**chemical properti**
51	Most of space in an atom is	filled with positive charge	empty	filled with negative charge	filled with neutrons			**empty**
52	A proton is made up of	one up quark and two down quarks	an up quark and down antiquark	two up quarks and a down quark	strange quark and an anti-strange quark			**two up quarks and a down quark**
53	The reading taken on a ratem	background noise	background sound	background rate	backgroun d count			**backgro und count**
54	Alpha particles are	It causes high cholesterol	It causes cancer	It causes mutation	It causes			**It**
55	The process of determining t	smoke detecting	carbon dating	irradiation	radioactive dating			**carbon dating**
56	Alpha, beta and gamma radiations have different penetrating powers. Select from the following options, the correct answer for increasing penetrating power.	Gamma beta alpha	Beta gamma alpha	Alpha beta gamma	Alpha gamma beta			**Alpha beta gamma**
57	Which one of the following s	The time taken for half of the atoms in a sample to decay	High energy electromagnetic radiation with high penetrating ability	The spontaneous breakup of unstable nuclei, followed by the emission of radiation	Different atoms of same element having different mass numbers			**Differen t atoms of same element having different mass numbers**
58	Radioactive decay is also expressed in terms of	Specfic activity	Original activity	Special activity	Disintegrat ion activity			**Specfic activity**
59	Data from radiation counter are in	A.Mega becquerel (MBq)	Counts per minute (cpm)	Tera becquerel (TBq)	mCi/mmol e			**Counts per minute (cpm)**
60	Microcurie (μCi) is reprensented as	2.2×106min-1	2.2×109min-1	2.2×105min-1	2.2×106mi n-1			**2.2×106 min-1**

UNIT-V (Virtual labs)

SYLLABUS

Virtual labs: Objectives, salient features, the role of Virtual Laboratories in Science Education. Introduction and importance of virtual labs in Biochemistry. Virtual lab for immunological techniques

The role of Virtual Laboratories in Science Education

Information technology has provided new innovations to sustain constructing an artificial educational environment by means of computers. Certain artificial environments sometimes go beyond natural environments, such as simulations and virtual reality, which is a sophisticated educational technology emerging for less than a decade (Hamit, 1993). Virtual reality is distinguished by unique sorts of interaction, that responds to users' behaviors and actions. Unlike traditional multi-media, virtual reality offers a distinctive level of interaction. Hence, virtual reality is considered to be a new model of computer-based learning, that provides the individual learner with a wider range of scientific vision (Chow & Andrews, 2007). This kind of educational technology provides an advanced individualized learning perfectly meets the educational needs and provides a high level of flexibility and freedom from constrains of time and place (Barbour & Reeves, 2009). One of the most important features of virtual reality is the easily and continuous material update aiming to attain learning objectivity and interest (Al-Shanak & Doumi, 2009).

With the increasing popularity of virtual educational technology, globally and locally, the development of virtual learning environment became an important field of science which has its own basics and principles. On observing the reality of science education in Arab region today, the learner is constrained to the theoretical method in acquiring knowledge, rarely allowed to apply these knowledge practically. This is due to several reasons including; the lack of laboratory devices, the risks that may result from applying some scientific experiments, and the high cost of materials (Al-Raid, 2008).

The above display demonstrates the need to apply virtual learning environment in teaching science. The current paper will tackle this issue, highlighting the following themes:

- What is meant by: virtual reality, virtual environment, and virtual learning?
- What are the characteristics and features of virtual learning systems?
- What are the characteristics and possibilities of virtual learning environment of science?
- What is a virtual lab?
- Why the need arises to virtual labs? The importance of the paper:

The importance of the current study is demonstrated in its relevance and advantage to various aspects of educational process, which could be stated as follows:

- The current paper contributes to present definitions to the following terms: (virtual reality, virtual education, virtual laboratory, virtual instructor).
- Investigates the importance of scientific virtual environment.
- Presents recommendations and suggestions.

During the last decades, the information and communication technology has witnessed a rapid development in all fields. The resources of knowledge became various and numerous. The course of science is obviously connected with technology, both cognitively and practically. Yet, educational professionals consider the importance of integrating information and communication technology in science learning, as to facilitate studying many scientific phenomena that cannot be studied experimentally due to its danger, high cost, or lack of time to complete the experiment. Furthermore, it will help the student in investigation and searching, which are considered the main aims of teaching science. (Al-Shaie 2006, P.2. Dillon, 2007).

The fields of internet-based learning are diverse, including virtual laboratories of science, which are considered the main underpinning in practical electronic learning, seeing that virtual labs closely resemble real labs (Al-Baiati, 2006, 13, Salamah, 2007,11). Moreover, a technology-enriched environment would greatly enhance students' motivation and develop positive attitude towards the course. Subsequently, the academic achievement would be enhanced. Several studies emphasized the vital role of virtual labs in developing academic achievement, providing awareness of scientific concepts, and modifying misconceptions. For example, (AlSharhan, 2009, Lal and Al-Gindi, 2009, Hartinez 2003).

So, this paper aims at discussing the modern technology of virtual laboratory, as a model of E-learning and its role in teaching science. This paper will discuss the following:-

- The concept of virtual lab.
- The components of virtual lab.
- The characteristics of virtual lab.
- Virtual labs constrains.

1. Virtual Lab Concept

It was defined as "laboratory experiment without real laboratory with its walls and doors. It enables the learner to link between the theoretical aspect and the practical one, without papers and pens. It is electronically programmed in computer in order to simulate the real experiments inside the real laboratories." Harry & Edward, 2005). In addition, it was defined as "A virtual studying and learning environment aims at developing the lab skills of students. This environment is located on one of the internet pages. Usually, this page has main page & many links, which are related to laboratory activities & its achievements (Zaitoon, 2005, 65). Through the above mentioned definitions, the virtual lab can be defined as virtual studying and learning environment that stimulates the real lab. It provides the students with tools, materials and lab sets on computer in order to perform experiments subjectively or within a group at anywhere and anytime. These experiments are saved on CDS or on web site.

2. The Components of Virtual Lab

The main components of the virtual labs are determined to have the following: (Al-Baiati, 2006 M, 28-32, Dillon, 2007).

1- The lab sets & equipments - The virtual lab is considered integral to the traditional lab but not an alternative to it. The existence of the traditional lab is very necessary, but in lower numbers and requirements, which help in the possibility of using it by several users outside the lab.

2- Computer devices - They are represented in personal computers, which are linked to the local net or to the international net so that the student can work directly in the lab, or distantly at anywhere and anytime.

3- Communication network & the related hardware - In case of performing experiments electronically, all the sets should be linked to the computer, because the link between the users

with lab will be through digital communication.

4- The Programs of the Virtual Lab:- These programs are represented in the simulation programs, which are designed by professionals. It is necessary to design this program in an interesting and attractive form; as these programs were designed to attract students' attentions and urge them to complete the experiment. This is maintained by the animation techniques, video, and the three dimensions pictures.

5- Co-operation Programs & Management - These programs are concerned with the method of managing the lab and the ones who perform the experiment, including students and researchers. These special programs register students in the lab program and determine the kinds of access that should be provided to each user in the different experiments.

6- Technical Staff - It is important to have a technical team to support educators in preparing and assessing scientific materials. In addition to evaluating the program to determine its efficacy.

3. The Characteristics of Education in Virtual Lab

Professionals confirmed certain characteristics of the virtual lab. They are as follow; (Harry and Edward, 2005- Zaitoon 2005- Carneva;le, 2003).

- Creating new intellectual model in education better than the real, and more beautiful than the imagination.
- Knowledge-building and inculcate information.
- Encouraging and guiding students.
- Registering students' information and evaluating them automatically.
- Performing experiments, which are difficult to be performed in the traditional lab due to its danger and high cost.
- Reducing the learning time spent in the traditional lab.
- Develop an exploration based on scientific assumptions and processes.
- Permanently updated.

4. Virtual lab constrains

Among the impediments to the virtual lab are :- (Canevale, 2003,2) (Zaitoun 2005,166)

- The scarcity of the virtual labs, which rely on Arabic language.

- The lack of real interaction between hardware, tools, instructors & colleagues.
- The need for computers and tolls with special standards.
- The need for specialized working staff, instructors and curriculum experts for designing and production.
- The lack of lab social skills gained in the virtual lab compared to the real lab.

5.1 The Characteristics of Education in consideration of School Lab & Virtual Lab:

Educators made a comparison between the school's lab & virtual lab. The researcher recorded it in the following table (Lal, Zakaria, Al-Jindi, Aliaa, 2005, Abdul Aziz, 2008, Al-Zharani, 2008).

Table 1: the characteristics of education in consideration of school lab & Virtual lab.

No	Characteristics of education in consideration of School's Lab	Characteristics of education in consideration of Virtual Labs
1	Closed educational environment	Flexible & opened educational environments.
2	The book & the teacher are the main sources of knowledge	Education depends on varied resources & multimedia.
3	Separating between the theoretical & practical, and between the real & imagination	The integral between the theoretical & practical aspect in virtual situations that stimulates reality.
4	The standardized official education	Continuous learning lifetime
5	Teaching the whole class in large group.	Teaching the whole class through small or individual groups.
6	The traditional method	Varied methods of teaching
7	The individual differences are not considered	The individual differences are considered
8	The teacher positivism, but negativism of the learner.	Positive and active participation from both instructor and learner.
9	Verbal teaching methods	Varied teaching & learning methods.

The Relationship between Virtual Labs & Electronic Education

(Al-Baiati 2006,13) assures that the virtual labs are considered to be the main support in electronic learning in the scientific & applied field. This is through using different electronic programs that simulate the experiments on computer by using different pictures and drawings, which express the experiment to be performed.

(Al-Shehri 2009, P.56) mentioned that the electronic learning is the umbrella term, under which the virtual lab are lying.

Thus, there are three types of electronic learning in consideration of virtual labs; (Zaitoun,2005,163) (AL Shaie, 2006,443) (Martinez, 2003).

1- Synchronous electronic learning through the virtual lab.

2- Asynchronous electronic learning through the virtual lab.

3- The intermixed or programmed learning through the virtual lab.

5. Some of the Related Studies that Handled The Virtual Lab Concept

(Al-Shehri, 2009), which showed the positive effect of using the virtual labs on providing the student with the laboratory experiment skills in the biology course of 3rd secondary school students, in Jeddah. (Al-Mahmadi, 2008), which showed the effectiveness of the virtual lab in developing the academic achievement of the female students of 2nd secondary grade, in chemistry. (Sebas Tinanfotis and Gal Ring, 2008). The project aimed at using learning environment depends on electronic virtual labs in order to support the learning process in the academic achievement of science course intermediate school. The study has revealed that using virtual labs encourage to modify the wrong concepts. (Tracey, 2007). This study aimed at studying students' onions, at the University of Northern Illinois, U.S.A, concerning the virtual biological labs. It revealed that 86,9% of the students support the virtual labs.

THE VIRTUAL LABORATORY AND INTERACTIVE SCREEN EXPERIMENTS

The concept of a virtual laboratory in the physical sciences is one with many ramifications. These may relate to the purpose a virtual laboratory is seeking to address, its mode of delivery, the scope of delivery, the experience of both students and tutors, and indeed the suitability of an activity for implementation in a virtual laboratory. The concept also excites opinion, both for and against, in many educational circles. In this article, we will explore these concepts, and others, in the context of a particular class of virtual laboratory, the interactive screen experiment. Before embarking on detailed discussions, we must first define for ourselves what we mean by a virtual laboratory, understand what value it can bring, and importantly what it cannot (and indeed must not) do. In the most general terms, a virtual laboratory is a computer-based activity where students interact with an experimental apparatus or other activity via a computer interface. Typical examples which come to mind include a simulation of an experiment, whereby a student interacts with programmed-in behaviours, and a remote-controlled experiment where a student interacts with real apparatus via a computer link, yet the student is remote from that apparatus.

We should distinguish the latter case from a computer-controlled experiment, where a student will directly control an apparatus in his or her vicinity via a computer interface (figure 1). This gives us a definition of a virtual laboratory – *A virtual laboratory is one where the student interacts with an experiment or activity which is intrinsically remote from the student or which has no immediate physical reality.* The latter part of this definition may seem to imply that a virtual laboratory can have no physical reality behind it at all. For example, in a simulation of gravity we might code for behaviour different to the familiar inverse square law (if only to explore the consequences of such a "universe"). We will see however, that this need not be the case, and indeed as we shall see, the whole concept of the interactive screen experiment is to bring as close a connection to reality as possible, to as many students as possible, to the virtual laboratory.

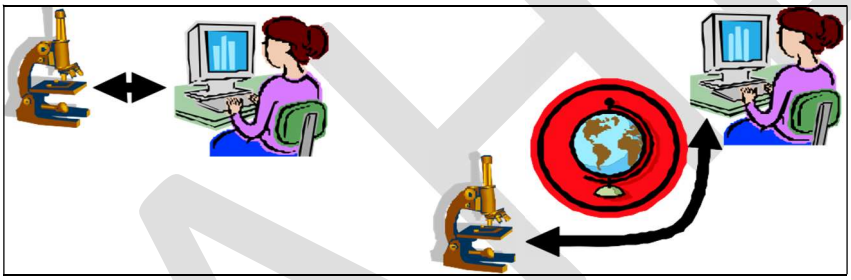
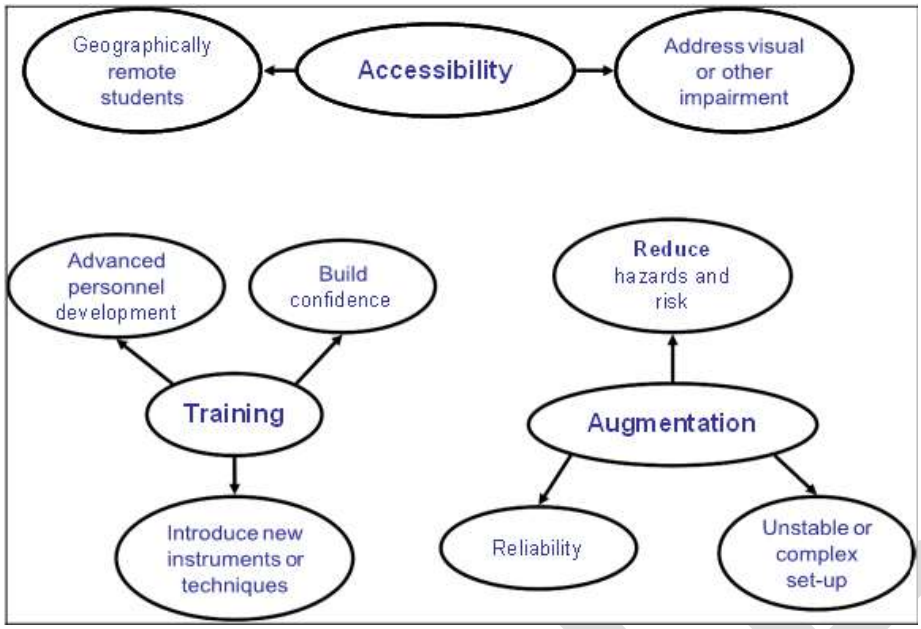


Figure 1. The distinction between (a) a computer-controlled experiment and (b) a remote controlled experiment. The latter case is an example of a virtual laboratory

Having established the concept of a virtual laboratory and examined the properties of interactive screen experiments, we will examine in detail the benefits such resources can bring. In summary, the key areas of benefit are; accessibility, training and augmentation. Some specific examples in each of these areas are given in figure 2; it is of particular note that one frequently perceived "benefit" – that of *replacing* real laboratories – is missing. This is simply because it is not a benefit at all. Nothing can replace the experience of working hands-on with apparatus and equipment, hence, although better than no experience, the virtual laboratory should not be perceived as providing a full experience.

Figure 2. Some of the benefits of a virtual laboratory



THE INTERACTIVE SCREEN EXPERIMENT

In the introduction, we have discussed the definition of a virtual laboratory. It now falls to us to examine the concept of the interactive screen experiment in such a way as to distinguish it from other forms of virtual resource, and to understand the benefits interactive screen experiments can bring.

Figure 3. An example of a simple interactive screen experiment.

In its broadest sense, we can define an interactive screen experiment as a highly interactive movie of an experiment, filmed as that experiment was being performed. By highly interactive, we do not simply mean the movie is capable of being moved forward or backward at different rates – this is trivial interactivity, and would provide minimal educational benefit. It is better perhaps to take a specific example. Figure 3 shows a screen- shot of a simple interactive screen experiment illustrating the relationship between the extension of a spring and the tension in the spring.

In this example, the user "interacts" with the movie (the interactive screen experiment) by "clicking" as normal on the dial of the force-meter, and "turning" it by "dragging" it round using the computer mouse or other control device. The dial then rotates as would the real example, with the spring extending or contracting depending on the direction of rotation. Simultaneously, the force indicated (equivalent to the tension in the spring) is shown by the pointer. In the previous section, we distinguished interactive screen experiments from simulations. This example will serve to strengthen this distinction. In the case of a simulation, a programmer would code the behaviour of each element. For example, the spring might be given the behaviour of its extension being proportional to its tension – a straight-forward Hooke's Law case. In the case of the interactive screen experiment though, the images presented on the screen are taken from a *real* experiment, recorded as it was being performed. The interactivity (the "turning of the dial") arises from coded behaviours governing how the movie switches between recorded frames as a result of user action. In consequence, the outcome of the interactive screen experiment illustrates the *real* physics of the phenomenon, rather than some idealised representation.

EDUCATIONAL BENEFIT OF INTERACTIVE SCREEN EXPERIMENTS

Interactive screen experiments contain within themselves significant technological interest. However, this is of no value if these resources deliver no educational benefit. In this section, we will explore in more detail the benefits identified in the introduction and figure 2.

Firstly, we will examine accessibility, which may manifest itself in two ways – either students may have reduced dexterity or other attributes which limit their ability to carry through a real experiment, or they may be physically unable (due either to mobility issues or geographic location) to attend a laboratory class. The benefit of an interactive screen experiment in the second case is clear. The experiment is effectively "delivered" to the student in his or her own environment, and using equipment familiar to the student. The first case is less clear, until one realises that in producing the interactive screen experiment, one is at liberty to include non-standard means of controlling the virtual apparatus. Returning to the spring example in figure 3, we have discussed its control in terms of "grabbing" and "turning" the force dial using a mouse or similar pointer control device. However, we may include a keyboard control, whereby a student may turn the dial simply by key presses. One can conceive of other input and control

methods such as voice input or custom interfaces. Clearly, these may not be a "default" component of an interactive screen experiment, as individual requirements vary widely. Accessibility is not limited to input, but extends to output. In the spring example, it is evident that a student with a visual impairment may have difficulty reading the extension or force scales (indeed, many students with good eyesight may have similar difficulty). In a real experiment, the obvious solution would be to improve the lighting and provide magnification. Again, this is straightforward to implement via enhanced resolution or magnified images in the interactive screen experiment as appropriate.

In the context of geographical location and/or mobility issues, the use of an interactive screen experiment may provide a substitution for a real experiment. This may seem like using the idea to replace real laboratories, and indeed this is true to a limited extent. We should recognise though that for the student unable to attend a real laboratory for whatever valid reason, a well-designed interactive screen experiment can provide an appropriate substitute.

Moving on, a common experience of students, especially those new to experimental science, is that of entering a laboratory and being faced with the intimidatingly unfamiliar. Although we may try to prepare students with instruction manuals and preparatory work, these approaches cannot address the fundamental "newness" of the laboratory experience. Closely focused interactive screen experiments can yield significant benefit here through providing training and practice in the use of instrumentation, apparatus and techniques. For example, prior to a laboratory class, part of the preparatory work might be to conduct an interactive screen experiment based on a new piece of equipment the students would be expected to use, or indeed based on the entire experiment, allowing a "preview" to gain familiarity. Consequently, students would enter the laboratory with enhanced skills, improving their ability to achieve the intended outcomes of the class.

The impression might be taken from the above that the training aspect of interactive screen experiments applies only to the "novice". However, all practitioners in science have recourse to the unfamiliar at times (indeed, this is a defining characteristic of the scientific researcher). It is quite within the scope of the interactive screen experiment concept to provide advanced training. Finally, we arrive at the concept of augmentation as a third benefit of

interactive screen experiments. This concept covers a range of sub-topics, illustrating enhanced applicability, as shown in figure 2. The benefits in the case of hazardous experiments need not be stressed. This also provides a case where an interactive screen experiment can justifiably replace a real experiment.

Another case where replacement is justified is in experiments with unstable or complex set- ups. Here, the student may focus on the learning outcomes of the resource without distraction from procedures or activities beyond the students' abilities or outside of the learning context of the experiment. Allied with this class are experiments relating to rare events, such as solar or lunar eclipses. Again, a resource may be created enabling the student to experience and investigate the event in a timely manner.

As a final example of augmentation, we come to the use of interactive screen experiments in post-experiment learning. Students quickly discover that real experiments do not always work in the way they expect – either through mistakes or lack of experience on the students' part or on malfunction of equipment. In such cases, a student may re-visit the experiment via an interactive screen experiment, in order to reinforce his or her experience either by gaining additional data or simply to observe expected behaviours.

WHAT MAKES A GOOD INTERACTIVE SCREEN EXPERIMENT?

The question of what makes a good interactive screen experiment is not one with a simple answer. However, we may generate a number of criteria an interactive screen experiment should have in order to provide an effective learning experience.

One criterion is that of number of adjustable parameters. The concept that an interactive screen experiment should provide as close an experience to reality as possible is only effectively realisable in cases with relatively few variable parameters. This is evident when one recalls that the interactive screen experiment is composed of a set of images, each being a point in the experiment's "parameter space" with interaction between images controlled via software. In the case of the spring experiment in figure 3, there is only one parameter; hence the parameter space is simply a one-dimensional array of images. In the case of two parameters, the space is two-dimensional and so on. Clearly, the number of images can grow rapidly, with implications for

production and delivery, which will be discussed later. The solutions are to limit the number of parameters, or to choose a restricted parameter set which although does not include the full range of states at least includes those states relevant to the experiment in hand. In many respects, this latter case is not generally restrictive, since we can cover the parameters typically encountered in a real experiment. However, it would exclude "pathological" situations such as driving an experiment to destruction!

In many physics-related experiments, a normal outcome is that one particular set of parameters always produces the same result. That is, the experiment is deterministically reproducible. Such a situation makes for an excellent interactive screen experiment, although at first sight it would appear to exclude experiments with significant statistical variation such as radioactivity or extension to life sciences. This is not though the case, and strategies for tackling such experiments will be discussed in the next section.

So far, we have dealt with issues of applicability and usability of interactive screen experiments. However, we must not lose sight of the fact that these resources must first be delivered to the student! The first criterion here is that the interactive screen experiment must be platform independent. That is, it must work on all computer systems, be they owned by the student or by the institute they are studying with. This can be achieved by ensuring standard, readily available and easily (and legally) installable support software is used and, where necessary, ensuring versions appropriate to different platforms are produced. Ensuring the platform independence of an interactive screen experiment is clearly an issue of quality control and testing.

Given that an interactive screen experiment is platform independent, it is still necessary to deliver it. In the context of an educational institution, this is trivial since the resource can readily be made available on the institute's own systems. The situation is not so clear for independent or distance learning students, who will typically be remote from central resources and will most likely be using their own or public (e.g., library) computing. The main limitations here are ones of data transfer rate and resource file size. Assuming an internet-based delivery method, the time taken to acquire a resource is evidently limited by the student's connection speed. This speed varies widely between countries, and indeed within a country, especially

between urban and rural areas. Related to this is the resource file size. A large, many parameter and image-intensive interactive screen experiment will not only take a significant time to acquire, but may also stretch the resources available on the student's computer (this may be especially true in a public computing area). A good interactive screen experiment is therefore one which can be easily accessed and placed on the student's computer within a reasonable time and which does not over-stretch the student's computer.

All of the above criteria are addressed at the design and development stage. The parameter space issue requires a consideration of the learning outcomes and ultimate resource size and delivery methods need to be considered in the context of the target audience. Evidently, a resource aimed at training and education in the developing world, where internet connection speeds may be limited or absent and high computing power may not be widespread must take greater account of delivery than one aimed at a developed world clientèle.

THE FUTURE FOR INTERACTIVE SCREEN EXPERIMENTS

The discussion so far has revolved around physics-based experiments which are deterministic in nature (that is, a given set of parameter values produce a well-defined outcome). As indicated in the previous section, not all experiments or experiences follow this pattern. For example, observing radioactive count rates in absorption or decay experiments frequently results in statistical variation of counts about some mean value, with the departures from the mean reflecting normal statistical behaviour. It may seem that such an experiment cannot be implemented as an interactive screen experiment, but this would be a wrong assessment. Classically, an interactive screen experiment is an array of behaviour-linked images, as discussed previously, with each image taken from a real state of the experiment. Similarly, each reading of a rate meter or similar instrument may be regarded as an individual state of the experiment. A statistical experiment may therefore be implemented by providing a sufficiently large database of readings which may be accessed randomly by the visual elements of the experiment.

So, statistical variation can be addressed. By realising this, the door is opened for a widening of the use of interactive screen experiments into topics beyond physics, and indeed science. With such fundamental barriers down, the application of interactive screen experiments will be boundless, limited only by technological aspects of creation and delivery, and the

imagination of their creators. One can easily imagine experiments created for the physical, life and geological sciences, but in addition, one can conceive of "experiments" (or perhaps they should now be called "experiences") targeted at traditionally non-science topics. How about an on-screen archaeological dig? A virtual examination and restoration of a work of art? Or an interactive social sciences study? All are possible with the right motivation and input.

Interactivity and a "Virtual Biochemistry Laboratory"

Media have gradually become an ubiquitous part of the environment in most developing countries. Young persons are today immersed in media to an extent that might seem frightening to some elders. A fairly recent Stanford University study of a cross-section national random sample of American adolescents aged 8 to 18 years has indicated that their daily exposure of media messages approaches 8 hours per day! Although TV is the dominant medium, the use of computers and video games follow close behind. [D.F. Roberts, "Media and Youth: Access, Exposure and Privatization", *Journal of Adolescent Health*, 2000; **27S**: 8-14] This implies that by the time American youngsters are finishing high school, they have spent more hours in front of a TV or computer screen - close to 20,000 hours - than they have spent in a classroom, or about 14,000 hours!

Should not future educational efforts take this into account?

It must be the envy of any ambitious teacher to watch the intense concentration and attention displayed by a young person playing an interesting and captivating computer game. What if we could tap into the psychological or physiological mechanisms behind this behavior and make use of these in more "serious" learning situations? Putting texts and figures on a computer screen – essentially digitizing old textbooks - is obviously not enough. Interactivity is the hallmark of the computer games and would be a powerful component of whatever "e-learning" system we use.

Interactivity and Learning

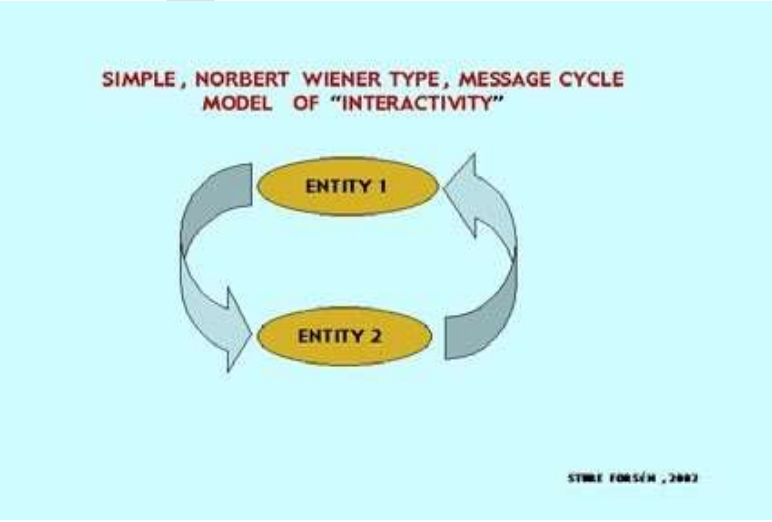
As part of the celebrations in the year 2001 of the first 100 years of Nobel Prizes, an educational "Internet" production called "The Virtual Biochemistry Laboratory" (VBL) has been developed and launched. From the outset, the production was planned to be interactive. I will describe this

production in more detail below – but I would first like to take a brief and very personal look into the history of interactive computer programming.

It struck me that before writing a chapter dealing with interactivity – or at least what I naively regarded as interactivity - I should perhaps look into what the computer and Internet linked community regarded as ”interactivity”. I therefore performed a ”Google” search, using first the phrase ”interactivity,” and then “definition of interactivity”. You may be surprised to learn that the first search yielded 360,000 hits and the second 45,000.

This result probably reflects the circumstance that ”interactions” in a broad sense really is an ubiquitous activity. Any discussion between persons could be regarded as an interaction. In the literature, interactions, in the sense of such a ”give-and–take” situation, have a long history. Plato’s dialogues and Galileo’s dialogues on the Ptolemaic and Copernican view of the solar system can be said to be examples of this approach.

Interaction in the sense of being able to influence a process or phenomenon has probably a much shorter history. I will stick to the view of interactivity as a kind of general message loop – entities 1 and 2 connected in a ”message” or ”control” loop. This kind of interactive approach is obviously not new in the history of learning and has been implemented with great success many decades ago by leading science museums in the world – for example the Science Museum in London and the Exploratorium in San Francisco, to mention just two.



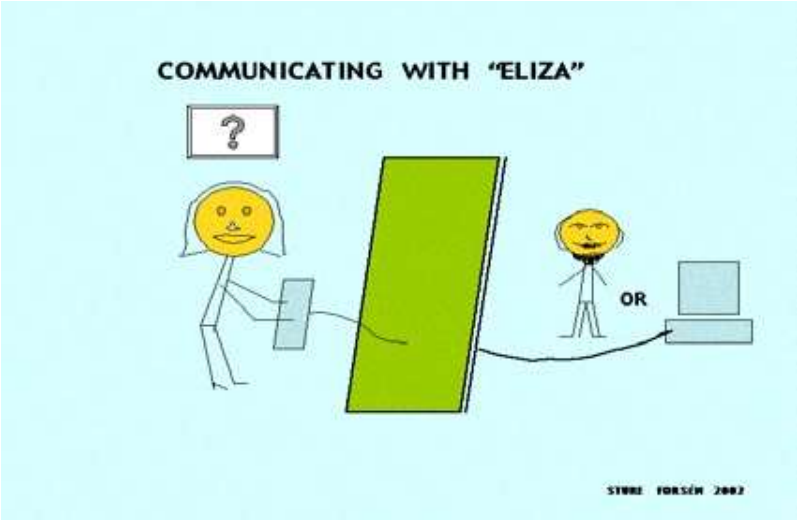
In the domain of computers, interactive programming also has a long history. I would like to go back to the days after the Second World War when modern computers and computing were in its infancy. That did not prevent a few brilliant pioneers to address some very basic and philosophical questions. In 1951 Alan Turing – sometimes called ”the father of artificial intelligence” - wrote a paper in which he described a test of ”true artificial intelligence”. He had in mind an interactive situation in which an interrogator was asked to try to distinguish a programmed computer from a real life person hidden from the interrogator, through the way they responded to his questions. Turing himself guessed that it would take some 50 years before an average interrogator would have a 70% chance of making the correct identification within 5 minutes.

But did Turing make a correct guess? Many computer scientists would probably tend to agree with him – but there is, in fact, a most remarkable exception: the interactive computer program called ”Eliza”, named after the cockney salesgirl who was picked up and ”educated” by Professor Higgins in the play ”Pygmalion” (or ”May Fair Lady” as musical) by George Bernhard Shaw.

This program was designed in 1966 by Joseph Weizenbaum at the MIT to mimic a psychotherapist. The visitor or ”patient” is sending statements – ”talking” - to the distant programmed computer and receives a reply on a printer or a display. The ”Eliza” program was, and still is, astonishingly successful.

Weizenbaum was even horrified to find that many visitors formed strong emotional bonds with ”her”, and wanted being alone in the room when they ”talked” to Eliza. Some professional psychiatrists were even inclined to let ”Eliza” treat their patients! Even if ”Eliza” uses a kind of faked interaction and in some sense merely ”echoes” the input, albeit in a most clever way, it has on several occasions passed the ”Turing test”!

Although ”Eliza” now is 36 years old – she has also been ”face lifted” on a few occasions – she still continues to captivate those who encounter her for the first time. Obviously Weizenbaum hit some very basic strings in human behavior.



In the early 1960s the first carefully designed system for ”Computer-Based Education” was put together at the Urbana Campus of the University of Illinois by a group of creative persons headed by Don Bitzer. The system was called ”PLATO” and was probably one of the first timesharing systems used publicly. Although PLATO was a success from the start, it had many shortcomings that later were removed. The system that can be said to be the father of many subsequent learning systems was eventually purchased by Control Data Corporation, and its offspring is now commercially available.

All kinds of interactive simulation programs and systems were also developed in the 1960s and 1970s – not the least for use in different military fields, e.g. war games and flight simulations. They were often hybrid digital and analog systems. Many of the current systems of this kind are extremely sophisticated, very powerful and educational.

With the increased acceptance and use of the Compact Disc (CD) in the late 1970s and early 1980s and the availability of relatively cheap desktop computers, the entertainment industry realized the potential of this combination for interactive games. The field took off with astonishing speed. The market seemed, and still seems, almost limitless. And today we have interactive video games of all kinds – adventure, combat, gambling, driving, management simulation, role playing, sports, strategy, etc., etc.

The "Virtual Biochemistry Laboratory" at the "Nobel e-Museum"

This brings me to the very beginnings of the "Virtual Biochemistry Laboratory" at the Nobel e-Museum. In the late 1990s I did spend many late nights slowly working my way through the great adventure game "MYST". I was most impressed by the excellent graphics; how you could move around in the graphic environment and the clever way clues were introduced to the visitor.

Not long after my MYST experience, Prof. Nils Ringertz, whom I knew from the period when we both were involved in Nobel Committee work at the Royal Academy of Sciences and the Karolinska Institute respectively, asked me if I could think of a way to illustrate chemistry and past Nobel Prizes in Chemistry in his new "Electronic Nobel Museum", as the Nobel e-Museum was then called. He had seen in what direction the electronic media was moving and realized that an Internet based "Nobel Museum" would be a most timely idea. The Nobel name would hopefully make this virtual museum attractive and it could perhaps be made into a rich source of knowledge and information in all Prize areas. He and a small group of dedicated younger coworkers had already made an impressive start despite virtually no external funds.

I was most intrigued by Nils Ringertz's proposal. After some pondering, I realized that it would be next to impossible to cover the whole of modern chemistry – one hade to restrict oneself. Biochemistry seemed like a possible area. Since I had for many years been teaching biophysical chemistry at Lund University, I settled for a "Virtual Biochemistry Laboratory", where a visitor would be able to move around and encounter all kinds of techniques and instruments that were found in real modern labs. And most, if not all, of these techniques and instruments had some connection with a Nobel Prize – not only in Chemistry, but also in Physics and Physiology or Medicine. And of course, I imagined that the visitor could interact and play with these instruments and be able to perform different kinds of "virtual" experiments.

And, to make the lab more exciting for game addicted youngsters, experiments in the lab could perhaps go totally wrong - a poorly balanced ultracentrifuge could, for example, blow to pieces! I had seen the indentations in the walls of the old The Svedberg laboratory at Uppsala where this had actually happened on several occasions.

My first synopsis in 1998 was finally based on the idea that the visitor was presented with

a mysterious protein sample – the nature of which he or she had to unravel using the equipment in the virtual biochemistry lab. But one would also be able to learn about the basis of the different methods or instruments, learn about the historical development of the methods and the involvement of different Nobel Laureates, etc. And to guide the visitors through the lab, I had the idea to introduce a helpful and well-informed lab engineer, named ”Virtual Eva” and modeled after a superb lab engineer I hade collaborated with for many years at Lund University.

I also thought that the laboratory environment could be based on digital photos of real biochemistry labs, and also that the instruments could be similarly based on digital photos of real ones.

After Nils Ringertz and I had secured funds in 1999 for a ”Young Scholars Program” at the Electronic Nobel Museum from the generous “Knut and Alice Wallenberg Foundation”, honest work begun. It soon became clear to me that interactive CD games and Internet based interactive biochemistry labs are not playing in the same league, when it comes to communication speed, memory requirements, etc. You can forget pixel-based graphics and thus realistic digitized pictures of real environments. The MYST atmosphere would be lost.

And if I wanted ”Virtual Eva” to be able to talk to the visitor, the streaming of her voice had to be synchronized with the pictures – and with Eva’s own bodily movements.

My original idea had also been to have the ”Virtual Biochemistry Lab” in three different versions of increasing levels of difficulty. One version – probably the most difficult one to produce - one for kids of the age 8 to 12 or so, one for the age group 14 to 18, and one for college or university students 19 and up. In the end, this became too ambitious and we finally settled for a not-too-well defined ”high school/college” age group. We have since found out that the visitors actually cover a fairly wide distribution in ages – there seems to be something there for everyone.

A small group of interactive-production developers and graphic artists were employed and the ”VBL Group” after a while consisted of Eskil Janson, Frida Westholm, Mats Danielsson and myself. Also Jan Strandh was involved in the beginning, but he later became involved 100% with other projects in the ”Nobel e-Museum” as the official name soon became. Later on Debbie

Strand joined the group while Frida moved on to other tasks.

After many trials we finally decided to abandon many of the original ideas of the synopsis. It became too complicated to use a single protein sample in all lab environments. Instead, we decided to let the laboratory consist of a number of rooms, each dedicated to a certain method or technique, for example chromatography, electrophoresis, amino acid sequencing, X-ray diffraction, NMR, etc. The visitors would be able to perform experiments with the different instruments, and interactivity would be built in so that the experimental conditions could be altered and the outcome changed accordingly. This important aspect of the VBL turned out to be technically very demanding and time consuming to implement.

The lab environment became vector based instead of pixel based. The body movement of our talking ”Virtual Eva” became standardized. It took a long time and considerable help from expert colleagues at Stockholm, Umeå and Linköping (cf Acknowledgements) to design protocols for the interactive experiments. For the production we used ”Shockwave” with Flash graphics and compressed sound. Databases, etc. were written in Perl.

Eva’s lectures, in the beginning, tended to become too long and had to be drastically shortened and subdivided. I learned from an experienced film producer about how to put together ”story boards”, combining text and outlines of the pictures that should go together. After several auditions, we finally found an American girl, temporarily living in Stockholm, who could give us the voice of ”Virtual Eva”.

The complexity of our undertaking gradually dawned upon us, but we learned as we went along. Had we known what we now know, the ”Virtual Biochemistry Lab” could have been produced much quicker and in a much more efficient way. We jokingly said that, ”with proper planning Rome could have been built in one day!”

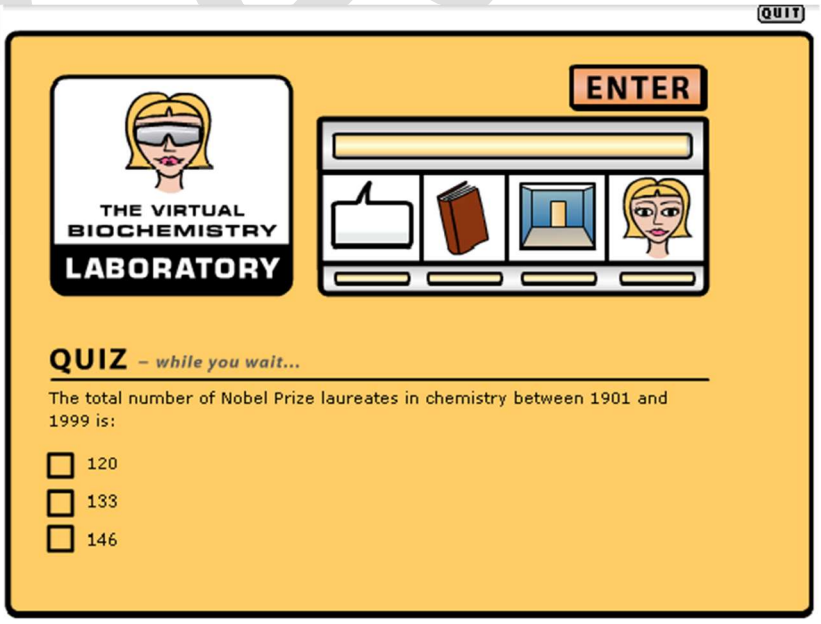
The product was gradually put together. We had tests on early versions with young students – both Swedish and foreign. They gave us most valuable feedback and we modified accordingly. They all liked the interactive parts – not surprisingly – and we worked them over repeatedly. To make a long story short, we finally put the product on the Internet in May 2001 at the official Nobel web site http://www.nobel.se/chemistry/educational/vbl/index.html.

To date the "Virtual Biochemistry Lab" has been accessed by some 30,000 visitors. We have also had reviews of the product from many individuals and journals. I had the opportunity to present the virtual lab at a "National Meeting of the American Chemical Society" at San Diego in April 2001. The official ACS Journal "Chemical and Engineering News" had a page describing the virtual lab in July, 2001 ["Biochemistry: The Game" in *Chem. & Eng. News*, **79,** p.42 (2001)]. By and large, the reviews have been very encouraging despite some remaining bugs.

But it seems somewhat meaningless to just talk about the Virtual Biochemistry Lab, it feels like trying to describe a piece of music in words. Therefore, I will now give you a live demonstration.

A Tour Through the Virtual Biochemistry Laboratory

When we connect to the VBL Internet address it will take some time to download the data. With a broadband connection it will take only a few seconds, but for visitors with modem and phone connections it may take a minute or two. With the brief attention span of many young visitors in mind we right away challenge the visitor with a number of quizzes while he/she downloads.



Then we step into the ”Entrance Hall” of the VBL and are faced with a billboard. This allows us either to enter as a guest, for example, if we are a first time visitor or we may decide to log in with a name, real or fictitious. This we may use next time we enter and all our activities the previous visit will be recalled – in particular the problems and challenges we may have solved during the previous lab tour. Challenges? Yes, there are a number of challenges for the interested visitor as we soon will see. But we decide this time to enter as ”Guest”.

Now, we are greeted by our helpful guide ”Virtual Eva”, who carries a box or bag and a book under her arms. Now, we may click on these and will learn that the book contains a number of help functions – a glossary of terms, a map of the VBL, possibilities to change different parameters like sound levels, adjust for our level of communication rate, etc. The bag has several compartments, which apparently, are for storing something – perhaps sample tubes of some ”missions”. Missions? We turn around – by using the same approach that was used in ”MYST” – and take a look at the peculiar ”Challenge Board” on one of the walls of the entrance hall.

We may be flabbergasted for a while until we remember that moving the cursor to Eva’s head makes her give you instructions or hints on what to do next.

Eventually, we learn that the Challenge Board contains eleven ”Missions” that we may store in our bag and recall when we have entered the relevant room in the lab. Let us store one of

the challenges we find on one of the cogwheels – it concerns finding the largest protein molecule in mixture which we have to separate using gel filtration – whatever that is. Hopefully, we will learn this later on in the labs!

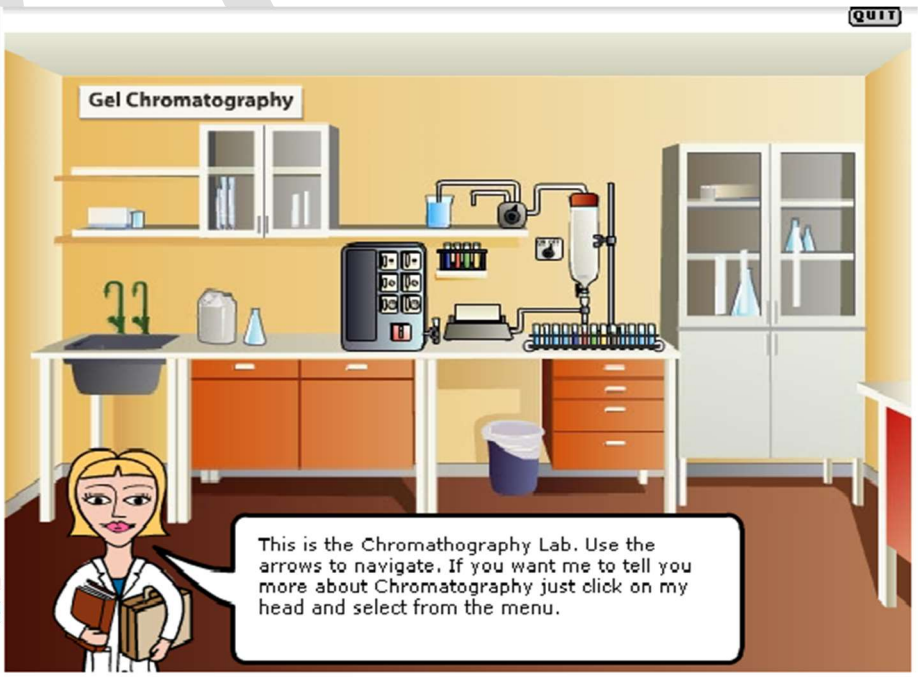
The labs? Well, one of the doors of the entrance hall is labeled ”Laboratories” so we enter through that. Now we are in a six-cornered ”square” – if there is such an ”oxymoron” – with six doors to labs dedicated to different methods used in a modern biochemical laboratory.

We may choose any of these and have a look. Let us enter through the door marked ”Separation Hall” and go further through the ”Chromatography” entrance. Here we learn from Eva that the lab contains equipment for three different kinds of chromatographical experiments – affinity chromatography, gel filtration and ion exchange. But what is ”chromatography”? Let us listen to Eva presenting an introductory ”lecture” – we click on her head and select this one.

To save memory space in the visitors' local computer the lectures are downloaded first when we select them. OK, here comes Eva!

Now we may actually do some interactive experimenting!

Due to lack of time at my lecture here, let us assume that we have listened to Eva explaining the basics of a technique called ”gel filtration”. This is in essence based on the use of porous gel particles with pore sizes of the order of a few nanometers. These gel particles will be



impenetrable to protein molecules with diameters exceeding the pore size but penetrated by protein molecules with smaller diameter. Using a column filled with such porous gel particles, you may separate protein molecules with different sizes from each other – the large ones will go straight through the column while small proteins will be retarded since they spend some time diffusing about inside the gel particles.

You may try this method for yourself. Let us take a look at the equipment on the bench that seems to involve a kind of refrigerator with pictures of columns. By clicking on the equipment we have it close by. Eva suggests that we should try to separate a sample by one of the many columns – they apparently have different pore sizes and we will try to find one that gives us optimal separation.

After some trial and error we will find that the column with the largest pores gives us good separation of the proteins in the sample – we can now see that it actually contained four different proteins. The one that comes out first from the column is the largest. You may remember that the "Mission" we selected on the "Challenge Board" told us to choose the largest protein in the separated mixture and put a test tube of his into Eva's bag. So let us take the red test tube and put it into Eva's bag. First mission completed we hope – you may check this by returning to the "Challenge Board" and put it to test. A strong applause tells you that you have succeeded.

Lets now take a look at some of the other labs. We go out into the main hall and enter into the NMR laboratory. We are now standing in front of an looking impressive-instrument.

What are all these components? We move our cursor to the different parts – here is a superconducting magnet, here a preamplifier for weak signals from the sample in the magnet, and here is a consol that apparently contains some electronic equipment. But what on earth is NMR? We click on Eva's head and listen for a while to her introductory lecture. Are you any wiser?

NMR is surely among the more advanced biophysical tools used in the VBL. But we do not expect the visitors to become experts after a visit – hopefully, they have become aware of its potential and may like to seek out more information from other sources.

Let us finish our brief tour – it will take many hours to go through the entire lab and perform all the possible experiments – and look at how one can determine the sequence of amino acids in a protein. Don't be afraid – you will be introduced to this topic very smoothly!

Final Comments

The VBL is a production that certainly may be further developed and improved. The production is still fairly advanced in comparison with other Internet based interactive productions, but we would love to be able to prepare an upgraded and extended version. One of the reasons why we

are unable to do this at present is bluntly the lack of financial resources. It may seem odd for a ”museum group” connected with the Nobel Foundation, however the statutes of the Foundation restricts the use of the capital earnings from its funds. These may only be used for expenses in connection with the Nobel Prizes – for the awards themselves, the outlays in connection with the selection of the Laureates, the central administration and for the Nobel week in Stockholm and Oslo.

As mentioned above, the Virtual Biochemistry Laboratory is part of the ”Wallenberg Young Scholars Program” at NeM. The grant financing the implementation of this program is regarded as a one time grant and no additional support is expected. We are currently looking for sponsors among multinational chemical industries that have an interest in the education of young future scientists and engineers.

POSSIBLE QUESTIONS

UNIT-V

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Write a short note on Virtual laboratory?
2. What is Xenograft?
3. What is virtuality?
4. What are immunoglobulin’s?
5. What are interleukins?

PART-C (6 MARKS)

1. Explain in detail about the Virtual Laboratories in Science Education.
2. Describe about the Virtual lab for immunological techniques with an example.
3. What makes a good interactive screen experiment?
4. Write about the salient features of Virtual Laboratories in Science Education?
5. Write about the various components of Virtual Laboratory.
6. Write in detail on the necessity of learning with virtual Laboratory tools.
7. Explain in detail about the importance of virtual laboratories in biochemistry.
8. How virtual learning will be useful for biochemistry laboratory.

Unit V

S.No	Questions	Option 1	Option 2	Option 3	Option 4	Option 5	Option 6	Answer
1	Which of these is not an advantage of a virtual	Team members can be recruited for their specific	No overhead costs associated with physical buildings.	Conflict can be resolved easily using face-to-face meetings	Low transport or commuting costs.			Conflict can be resolved easily using face-to-face meetings
2	What is characteristic for the location of a virtual team?	In the same building	In the same industry	In the same country	Remotely			Remotely
3	Which of the following is a mandatory object in virtual laboratory?	Pipettes	Computer devices	Chemicals	Glasswares			Computer devices
4	How does centrifugation works?	Through dripping particles	Through spinning	By keeping large particle in the centre and smaller on the outside	By separating particles into different tubes			By keeping large particle in the centre and smaller on the outside
5	VBL stands for	Virtual Book Library	Virtual Biochemistry Lab	Various Book Libraries	Various Biochemistry lab			Virtual Biochemistry Lab
6	What is the wavelength range for UV spectrum of light?	400 nm – 700 nm	700 nm to 1 mm	0.01 nm to 10 nm	10 nm to 400 nm			10 nm to 400 nm
7	Beer Lambert's law gives the relation between which of the following?	Reflected radiation and concentration	Scattered radiation and concentration	Energy absorption and concentration	Energy absorption and reflected radiation			Energy absorption and concentration
8	In which of the following ways, absorption is related to transmittance?	Absorption is the logarithm of transmittance	Absorption is the reciprocal of transmittance	Absorption is the negative logarithm of transmittance	Absorption is a multiple of transmittance			Absorption is the negative logarithm of transmittance
9	Beer's law states that the intensity of light decreases with respect to	Concentration	Distance	Composition	Volume			Concentration
10	The representation of Beer Lambert's law is given as A = abc. If 'b' represents distance, 'c' represents concentration and 'A' represents absorption, what does 'a' represent?	Intensity	Transmittance	Absorptivity	Admittance			Absorptivity
11	The enzyme in ELISA is present in the?	Conjugate	Microplate	Buffer	water			Conjugate
12	A standard microplate in an ELISA has?	94 wells	96 wells	98 wells	92 wells.			96 wells
13	The method used to estimating insulin is?	Electrophoresis	Spectrophotometer	Kinetic estimation.	Radioimmuno assay			Radioimmuno assay
14	Accuracy is defined as	a measure of how often an experimental value can be repeated	the closeness of a measured value to the real value	the number of significant figures used in a measurement	none of these			the closeness of a measured value to the real value
15	Transmittance is given as T = P/Po. If Po is the power incident on the sample, what does P represent?	Radiant power transmitted by the sample	Radiant power absorbed by the sample	Sum of powers absorbed and scattered	Sum of powers transmitted and reflected			Radiant power transmitted by the sample
16	What is the unit of molar absorptivity	L mol-1 cm-1	L gm-1 cm-1	Cm	No unit			L mol-1 cm-1
17	Which of the following is not true about Fourier Transform Infrared (FTIR) spectrometer?	It is of non-dispersive type	It is useful where repetitive analysis is required	Size has been reduced over the years	Size has increased over the years			Size has increased over the years
18	Which of the following is not the function of the drive mechanism in Fourier Transform Infrared Spectrophotometer?	Movement of mirror to obtain a satisfactory interferogram	Acquire a good interferogram pattern	Allow 50% of the beam to pass	Keep the speed of the moving mirror constant			Allow 50% of the beam to pass
19	Which of the following is not the advantage of Fourier Transform Spectrometers?	Signal to noise ratio is high	Information could be obtained on all frequencies	Retrieval of data is possible	Easy to maintain			Easy to maintain
20	Which of the following has to be computed to determine transmittance and absorbance at various frequencies?	Ratio of signal and noise	Ratio of sample and reference spectra	Sample spectra	Reference spectra			Ratio of sample and reference spectra
21	Which of the following is the reference that is generally used in FTIR interferometer?	air	NaCl solution	Alcohol	Base solution			air
22	Why is the computer necessary in Fourier Transform Spectrometer?	To display the detector output	To process the detector output	To determine the amplitude	To determine the frequency			To process the detector output
23	The intensity of an absorption band is always proportional to which of the following factor?	Atomic population	Molecular population of the initial state	Molecular population of the final state	Temperature			Molecular population of the initial state
24	On which factors the vibrational stretching frequency of diatomic molecule depend?	Force constant	Atomic population	Temperature	Magnetic field			Force constant
25	In which unit Force constant is not expressed?	Dynes cm-1	dyne Å-1	Nm-1	kp			kp
26	The vibrations, without a center of symmetry are active in which of the following region?	Infrared but inactive in Raman	Raman but inactive in IR	Raman and IR	Inactive in both Raman and IR			Raman and IR
27	The frequency of vibration of a bond is a function of which factor?	Force constant of the bond	Masses of the atoms involved in bonding	Force constant of the bond and Masses of the atoms	Bond order			Force constant of the bond and Masses of the atoms
28	How is the wavelength controlled in an FTIR spectrometer?	By a Michelson Interferometer	By a computer	By a laser	By calibration with a standard sample			By a laser
29	What type of technique is FTIR spectroscopy?	A dispersive technique	An emission technique	An absorbance technique	A UV-Vis technique			An absorbance technique
30	How is the detector on the Mattson RS/1 FTIR spectrometer cooled?	With water	With liquid nitrogen	With a fan	It's not cooled			It's not cooled
31	What does the spectrum of Nitrogen(N2) look like?	The same as that of air	It has only p- and r-branches	The same as that of carbon monoxide	It doesn't have one!			It doesn't have one!

32	What occurs when the moving mirror in an FTIR spectrometer is the same distance from the beamsplitter as the static mirror?	Constructive interference	Constructive interference	Radio interference	The spectrum is measured			Constructive interference
33	Which infrared technique can measure two spectra at once?	FTIR spectroscopy	FTIR-ATR spectroscopy	Dispersive infrared spectroscopy	FTIR microscopy			Dispersive infrared spectroscopy
34	In what region of the spectrum does infrared radiation occur?	At the low-energy end	Between the visible and ultraviolet regions	Between the visible and microwave regions	Between the visible and x-ray regions			Between the visible and microwave regions
35	What occurs when a molecule absorbs infrared radiation?	It warms up	It flies around	It spins faster	It vibrates faster			It vibrates faster
36	What is distillation?	distillation is when a liquid is evaporated and then recondensed in another container	distillation is when material heated to melting and then separated	distillation is when a substance is dissolved, heated and then precipitated	Separation of particles from a suspension			distillation is when a liquid is evaporated and then recondensed in another container
37	Which of the following statements is wrong?	UV absorption is attributable to electronic transitions.	UV spectra provide information about valence electrons	IR absorption is attributable to transitions between rotational energy levels of whole molecules	NMR spectrometers use radiofrequency electromagnetic radiation			IR absorption is attributable to transitions between rotational energy levels of whole molecules
38	Which of the following statements regarding IR spectroscopy is wrong?	Infrared radiation is higher in energy than UV radiation	Infrared spectra record the transmission of IR radiation	Molecular vibrations are due to periodic motions of atoms in molecules, and include bond stretching, torsional changes, and bond angle changes	Infrared spectra give information about bonding features and functional groups in molecules			Infrared radiation is higher in energy than UV radiation
39	Which of the following statements is wrong?	A conventional mass spectrometer employs high energy UV radiation	A conventional mass spectrometer does not employ a spectrophotometric detector.	Conventional mass spectrometry does not always require samples of high purity	A mass spectrum does not show signals due to uncharged radicals			A conventional mass spectrometer employs high energy UV radiation
40	Which of the following is not true about Absorption	It involves transmission	Scattering is kept minimum	Reflection is kept maximum	Intensity of radiation leaving the substance			Reflection is kept maximum
41	Glassware used to measure 24-hour urine volumes is a:	volumetric flask	beaker	Erlenmeyer cylinder	graduated cylinder			graduated cylinder
42	The durable material used to make heat resistant	polyethylene	soda lime	polystyrene	borosilicate			borosilicate
43	The destruction of all micro-organisms including spores is called:	sanitation	antisepsis	sterilization	disinfection			sterilization
44	The distance between the centers of the peaks of doublet is called as?	Coupling constant	Spin constant	Spin-spin coupling	Chemical shift			Coupling constant
45	During the motion, if the centre of gravity of molecule changes, the molecule possess	Electronic energy	Vibrational energy	Rotational energy	Translational energy			Translational energy
46	The region of electromagnetic spectrum for nuclear magnetic resonance is	Microwave	Radio frequency	Infra Red	UV rays			Radio frequency
47	Select the correct statement from the following option	Spectroscopic methods require less time and more amount of sample than classical methods	Spectroscopic methods require more time and more amount of sample than classical methods	Spectroscopic methods require less time and less amount of sample than classical methods	Spectroscopic methods require more time and less amount of sample than classical methods			Spectroscopic methods require less time and less amount of sample than classical methods
48	The transition zone for Raman spectra is	Between vibrational and rotational levels	Between electronic levels	Between magnetic levels of nuclei	Between magnetic levels of unpaired electrons			Between vibrational and rotational levels
49	The criteria for electronic spin resonance is	Periodic change in polarisability	Spin quantum number of nuclei > 0	Presence of unpaired electron in a molecule	Presence of chromophore in a molecule			Presence of unpaired electron in a molecule
50	In 500 × g, what does g represent in accordance to centrifugation?	Gravitational force	Centrifugal force is 500 times greater than earthly gravitational force	Centrifugal force is 500 times less than earthly gravitational force	Centrifugal force is 500 times same as that of earthly gravitational force			Centrifugal force is 500 times greater than earthly gravitational force
51	Which of the following is not a type of centrifugation?	Hydro cyclone	Tubular centrifuge	Microfiltration	Disk stack separator			Microfiltration
52	At what speed do you centrifuge blood?	2200-2500 RPM	2200-2500 RPM	1000-1500 RPM	4000 RPM			2200-2500 RPM
53	Which of the following centrifugation is used to separate certain organelles from whole cell?	Rate-zonal centrifugation	Normal centrifugation	Differential centrifugation	Isopycnic centrifugation			Differential centrifugation
54	Which of the following is used as a media for density gradient?	Agarose	Luria broth	Ficoll	Propylene glycol			Ficoll
55	From the following which is the type of filtration centrifuge?	Screen/scroll centrifuge	Tubular centrifuge	Decanter centrifuge	Separator centrifuge			Screen/scroll centrifuge
56	Which of the following is used in uranium enrichment?	Tubular centrifuge	Disk-stack centrifuge	Gas centrifuge	Zippe-type centrifuge			Gas centrifuge
57	What is a rate-zonal centrifugation?	Based on separation of particles by mass	Based on separation of particles by density	Based on separation of particles on solubility	Based on separation of particles on size			Based on separation of particles on size
58	After centrifugation when sublimate settles, clear liquid	can be allowed to rest	can be allowed to form crystals	can be decanted off	can be evaporated			can be decanted off
59	After centrifugation, sublimate	dissolves completely	remain suspended in a liquid	settles at bottom	depends upon pH of sublimate			settles at bottom
60	Which of the following is an effective way of purifyingliquids containing	crystallization	decanting	centrifuging	separating funnel			centrifuging