LABORATORY TECHNIQUES

Aparna Mathur
Souvik Sur

LABORATORY TECHNIQUES

LABORATORY TECHNIQUES

Aparna Mathur
Souvik Sur

BLACK PRINTS
NEW DELHI

LABORATORY TECHNIQUES

Aparna Mathur
Souvik Sur

This edition published by **BLACK PRINTS INDIA INC.**,
Murari Lal Street, Ansari Road, Daryaganj, New Delhi-110002

ALL RIGHTS RESERVED
This publication may not be reproduced, stored in a retrieval system or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the publishers.

Edition: 2022 (Revised)

ISBN: 978-93-82036-38-8

BLACK PRINTS
Excellence in Academic Publishing

Editorial Office: 116-A, South Anarkali, Delhi-110051.
Ph.: 011-22415687

Sales & Marketing: 4378/4-B, Murari Lal Street, Ansari Road, Daryaganj, New Delhi-110002.
Ph.: +91-11-23281685, 41043100 Fax: +91-11-23270680

Production: A 2/21, Site-IV, Sahibabad Industrial Area Ghaziabad, U.P. (NCR)
e-mail: blackprintsindia@gmail.com

CONTENTS

CHAPTER 1

A PRIMER FOR RESEARCHERS ON ENSURING LABORATORY SAFETY

Souvik Sur, Assistant Professor
College of Engineering, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email Id- souviksur@hotmail.com

ABSTRACT:

The introduction to laboratory safety is a crucial aspect of scientific research and experimentation. This chapter provides an overview of the fundamental principles and practices required to ensure the safety of personnel, the environment, and research outcomes in a laboratory setting. It covers essential topics such as hazard identification, risk assessment, safety equipment, emergency procedures, and compliance with regulatory standards. Understanding and implementing laboratory safety measures is essential for preventing accidents, protecting individuals, and maintaining the integrity of scientific investigations. Safety practices and standards at the University of New England designed to protect laboratory employees, while they are working in a laboratory, from harm due to potential exposure to hazardous chemicals. This chapter applies to all laboratories that use hazardous materials and supersedes the Hazard Communication chapter of the Safety Manual.

KEYWORDS:

Compliance, Emergency Procedures, Environment, Hazard Identification, Laboratory Safety.

INTRODUCTION

Laboratories play a pivotal role in scientific research, experimentation, and discovery. While these controlled environments facilitate groundbreaking discoveries, they also harbor various risks and hazards that can pose serious threats to the safety of personnel, the integrity of experiments, and the environment. Therefore, it is essential to establish a strong foundation in laboratory safety practices and protocols. The Introduction to Laboratory Safety chapter serves as the cornerstone for ensuring safe and responsible conduct within a laboratory setting. It lays the groundwork for understanding the potential hazards, assessing risks, and implementing preventative measures to mitigate these risks effectively. This introductory chapter equips individuals with the knowledge and skills necessary to navigate a laboratory environment safely, promote a culture of safety, and adhere to regulatory standards [1], [2].

In this chapter, we will explore the fundamental principles of laboratory safety, including the identification of hazards, risk assessment, the use of safety equipment, emergency procedures, and the importance of compliance with regulatory standards. By grasping the concepts introduced in this chapter, laboratory personnel can significantly reduce the likelihood of accidents, minimize environmental impact, and ensure the reliability of research outcomes. As we delve deeper into the specifics of laboratory safety, it is crucial to remember that safety is not just a set of rules but a mindset and a collective responsibility. This chapter serves as the starting point for cultivating a culture of safety that should permeate every aspect of laboratory work. With a strong foundation in laboratory safety, scientists, researchers, and students can pursue their scientific endeavors with confidence, knowing they are safeguarding themselves, their colleagues, and the world around them [2], [3]. Lab safety is about learning and being responsible so that it becomes an important part of all chemistry classes. This means that we need to make sure that safety is a priority in all laboratory courses, including more advanced research projects. Creating a safe environment in the laboratory requires everyone in the educational institution to be fully dedicated to safety. At the department level, teachers need to take responsibility for regularly checking safety concerns with students in labs where they teach and conduct research. This is especially important for those who teach undergraduates, who are often graduate students or instructors. Faculty members should set a good example and work together with their department to prioritize safety. At the administrative level, this means creating a plan to safely handle, store, and get rid of chemicals. This plan should follow the campus rules for chemical safety. The eye wash and showers need to be working, and it's important to have fume hoods with the right covers. Anyone who is in the lab, whether they are working or just visiting, needs to wear goggles. Also, it's important that no one eats or drinks anything in the lab. A neat and organized lab is more likely to make people work carefully.

A hazard is something that can be dangerous and cause harm. It can happen when working with chemicals, equipment, or instruments. To begin learning about chemical hazards, it's important to know the meanings of words like toxic, flammable, and corrosive. You can find this information on chemical labels, Safety Data Sheets (SDS), and other sources. Chemical dangers in beginner college labs need to be described more clearly. This includes things like acids, bases, substances that can catch fire, and poisonous compounds. In higher levels of study, students should learn more about different types of dangerous chemicals and physical hazards. This will help them recognize these hazards on their own during experiments. Some examples of these hazards are poisonous chemicals, compressed gases, extremely cold substances, systems with high pressure, peroxides, substances that react strongly, unstable substances, substances that catch fire easily, explosives, and substances that react with water. Other subjects that may be taught in chemistry include toxicology, nanomaterials, biohazards, and radiological hazards. These subjects become important when the chemistry lessons cover a wider range of topics.

When something dangerous is noticed, it is important to evaluate how risky it is to be exposed to that danger in a laboratory. We first find how someone could be exposed to the experiment's dangers. Then, we determine how risky these dangers are compared to each other. The dangerous properties of substances like solvents, reactants, and products should be taken into account when doing experiments. Things like how much of the substance is being used, if the right equipment is available, and if the hazard can be controlled or reduced should also be considered. It's also important to think about if the reactions give off heat or are sensitive to air or water. Additionally, risks associated with things like lasers or equipment that uses high voltages should be considered. To reduce risks, experiments should be planned in a way that minimizes potential dangers. This process might include doing tests in a special box to protect us and wearing gloves and special glasses to stay safe. Managing and storing waste is very important. It can be helpful to study past incidents that caused harm or damage. How could we have stopped or made these accidents less bad. We can do this by talking to students about a dangerous place and asking them what is not safe about it. We should also teach students how to react quickly and carefully in emergencies, like fires, injuries, and spills. Equipment that keeps people safe, like showers, eye washes, fire extinguishers, and spill kits, need to have clear labels on them. Everyone who works in a laboratory should know how to use these devices and where to find them.

Emergency phone numbers, alarms, and escape routes should be easy for everyone to understand and remember. The faculty and staff in a laboratory need to be role models for safety. They should teach students about safety, always promote safety, show how important safety is through their actions, and take responsibility for safety. At certain schools, graduate students teach labs for undergraduate students. In these cases, the graduate students who are teaching assistants must prioritize safety ethics. The Safety Ethic is a belief that safety is important and should be prioritized. It means valuing safety, behaving safely, avoiding risky behavior, advocating for safety, and taking responsibility for safety. It stresses that everyone involved should personally take responsibility for safety. For this culture to grow and be successful, everyone needs to support and encourage it. It is important to know and follow rules from OSHA, EPA, DOT, and DOE. But it's not just about following regulations, it's also about looking out for the safety of other students, teachers, and staff. There are many helpful resources for keeping chemicals and laboratories safe.

Research conducted in laboratories is extremely important for making progress in scientific understanding and creating new ideas and inventions. However, it also has dangers that can cause accidents, injuries, or even very serious events if they are not managed well. Making sure that laboratories are safe is extremely important for the health and safety of scientists, the reliable results of experiments, and keeping the environment safe. This primer will help researchers learn how to make sure their laboratories are safe and secure. There is not enough information present to rewrite the text in simpler words. Risk assessment involves identifying potential dangers or hazards and determining how likely they are to occur and how severe the consequences could be. It is a way to evaluate and understand the risks associated with particular activities, processes, or situations. Risk evaluation is the process of assessing and analyzing potential risks in order to understand their severity and likelihood of occurrence. This helps businesses and individuals make informed decisions on how to manage and mitigate these risks. Risk mitigation means reducing or minimizing the occurrence or impact of potential risks. It involves taking actions or implementing strategies to prevent or reduce the likelihood of risks from happening, as well as implementing measures to lessen the negative consequences if a risk does occur. The goal of risk mitigation is to protect assets, individuals, or organizations from potential harm or damage.

Laboratory Design and Maintenance refers to the process of creating and managing the layout and functionality of a laboratory. This involves planning and setting up the space, as well as ensuring the necessary equipment and supplies are available and properly maintained. Emergency equipment is a set of tools or devices that are used in times of urgent or critical situations. These tools are designed to help people stay safe and assist in emergencies. Put eyewash stations, safety showers, fire extinguishers, and first-aid kits in different areas of the laboratory. Check equipment regularly to make sure it works properly. Chemical storage is the practice of storing chemicals in a safe and organized manner to prevent accidents or harm. Thirdly Training and education refers to the process of acquiring knowledge, skills, and abilities through specific programs or courses. It involves learning new things or improving existing knowledge in order to better perform tasks or jobs. Training means teaching people about how to stay safe in different situations. It is important for people to learn how to protect themselves and others from harm. The training covers various topics, such as fire safety, first aid, and workplace safety. By providing safety training, the goal is to prevent accidents and injuries.

Documentation is the process of recording information or instructions about a specific subject or object. It can include written text, images, videos, or any other form of media that can be used to convey information. The purpose of documentation is to provide a clear and organized reference for users or system administrators to understand and use a product or service. It helps in troubleshooting problems, learning how to operate a system, or implementing a specific process. Documentation is essential for effective communication and knowledge transfer. Keep track of safety training and certifications for all employees. Create a document that explains safety procedures and rules. Keep a safety guide or instruction booklet available for all people using the laboratory. These drills help people learn what to do and where to go in case of a fire, earthquake, or other emergencies. They are important for everyone's safety. Selection is the process of choosing someone or something from a group or range of options. Training is the process of teaching or learning a specific skill or knowledge. Teach workers how to use and take care of protective gear properly. Highlight how crucial it is to use personal protective equipment (PPE) to avoid accidents and injuries. Chemical safety is about using and handling chemicals in a safe way. It means taking precautions to prevent accidents or harm to people, the environment, or property. Chemical handling involves working with chemicals safely and responsibly. Follow the rules for how to safely handle chemicals. Use special cabinets and spaces designed to protect you from dangerous chemicals. Chemical Storage keeping chemicals in a safe and organized manner Emergency response refers to the actions taken by trained individuals or organizations in order to provide assistance and support in times of emergencies or disasters. Create clear guidelines for dealing with accidents involving chemicals being spilled or released.

Teach workers how to use spill kits and emergency cleaning materials. Keep track of the things we need in case of an emergency. Biological Safety means taking precautions to protect yourself and others from harmful biological materials like pathogens or infectious organisms. Risk assessment is a process of identifying and evaluating potential risks and hazards in order to determine the likelihood and impact of these risks on a project, activity, or situation. It involves assessing the severity of each risk and implementing measures to prevent or mitigate them. The goal of risk assessment is to understand and manage risks effectively to minimize harm, damages, or negative outcomes. Make sure to wear the right protective gear when handling stuff like bacteria, viruses, or other living substances. This includes things like gloves, lab coats, and face shields.

Make sure to get rid of biological waste correctly following the rules. Make sure to clean or kill germs on dangerous materials before throwing them away. Radiation safety means being cautious about dealing with radiation, which is a type of energy that can be harmful to living things. Radiation sources are things that give off radiation. Make sure to clearly label and indicate sources and areas where radiation is present. Only let people with the right training and equipment enter. Monitoring and measurement is the process of keeping a close eye on something and recording its progress or changes. Check radiation levels in the lab on a regular basis. Keep track of how much radiation people are exposed to and make sure the figures are correct. Shielding means protecting or defending someone or something from harm or danger by creating a barrier between them and potential threats. Choose the right materials to protect yourself from radiation. Make sure to check and keep shield in good condition.

Electrical safety means being careful and taking precautions to avoid getting hurt when using electricity. Check electrical equipment for damaged cords or other dangers Make sure you don't put too much pressure on circuits, and use GFCIs. Don't put things that can catch fire close to things that make electricity. Fire safety is making sure that people are safe from the danger of fire. This means taking precautions to prevent fires from starting, and being prepared in case a fire does happen. It includes things like having smoke alarms and fire extinguishers in homes and buildings, having an escape plan, and knowing how to use emergency exits. Fire safety also involves teaching people how to act safely in the event of a fire, such as staying low to avoid smoke, and never going back into a burning building. Put in smoke detectors, fire alarms, and fire suppression systems. Teach the staff how to use fire extinguishers and how to evacuate in case of an emergency. An Emergency Response Plan is a plan that is made to help people and organizations prepare for and respond to emergencies. It outlines the steps and actions that need to be taken in order to protect and help those affected by an emergency.

It usually includes information on how to evacuate safely, how to communicate during an emergency, and how to provide immediate assistance to people in need. The goal of an Emergency Response Plan is to minimize harm and save lives during an emergency situation. Create guidelines for what to do in the event of a fire, a spill involving chemicals, accidents or injuries, and the need to evacuate. First Aid is the initial help given to someone who is injured or suddenly becomes ill. It is the basic care provided to prevent further harm and promote recovery until professional medical help arrives. Reporting means telling someone about something that has happened, such as an event or an incident. Investigation means looking into something carefully to find out more information or to solve a problem. Create a straightforward way to report accidents, near-misses, and safety concerns Encourage people to communicate openly without being afraid of getting punished or facing negative consequences. Making sure that labs are safe is everyone's job, no matter what they study or how much experience they have. By using the principles mentioned in this guide and creating a safe environment, labs can decrease dangers, keep people safe, and support the successful search for scientific knowledge. Always remember that safety is not something you do once and forget about. It is a constant responsibility to keep everyone in the laboratory safe and protect the quality of research.

DISCUSSION

Workers in scientific labs are subject to a variety of risks. The majority of workplace dangers have well-defined methods to handle the situation those of regular fire, for example. However, a wider range of potential risks exist in laboratories, and some of these risks need for special safety measures. The list below provides an introduction to safe practices for some frequently used laboratory procedures.

General safety and operational guidelines

It is not allowed to sprint or jump in a lab. Access to the fire extinguisher, safety equipment, or other emergency supplies must not be impeded by stacked objects or equipment. Access to emergency equipment and/or exits must be maintained dry and unhindered; this means there must be no storage, equipment, phone lines, or other obstructions in these areas. No flammable items, such as paper, wooden crates, pallets, etc., should be kept in corridors or beneath stairwells. So that exits and regular travel routes are not obstructed, hallways must be maintained clear of boxes and other objects. [4], [5]. It is forbidden to eat or drink in labs. In collaboration with the Safety Committee, special office spaces in all labs may be set aside for food. Physical separation is required between them and any laboratory procedures. No consumables, chemicals, or tools of any kind should be shared with work areas in the designated office spaces.

No food or drink may be kept in the lab refrigerators and freezers or cold rooms. The NCBS/InStem/CCAMP work core hours are 8am to 8pm, Monday through Saturday. Outside of the regular working hours, no employee should operate in a laboratory or chemical storage facility by themselves. Students and staff must get written permission from the primary investigator (PI) in charge before working outside of core hours. Animals must be kept out of all lab areas unless they are used in experiments that have been authorized by the Animal Experimentation Committee. Lab attire should provide protection from splashes and spills and be simple to take off in case of an accident. Aprons made of nonflammable; nonporous materials provide the best protection for the smallest price. To make them easier to take off, lab coats and jackets should feature snap closures rather than buttons. When working, these jackets must be tied, and they must be taken off before leaving the lab. It is strongly advised that laboratory staff refrain from wearing sandals or open-toed footwear within the lab. Laboratory attire should be maintained spotless and changed as needed. Lab coats, gloves, closed-toe shoes, and safety glasses are needed for procedures carried out in biosafety level 2 and chemical activities [6], [7]. Mouth pipetting is not permitted at any time.

Electricity

A significant amount of electrical power is needed in a normal laboratory. The possibility of electrical issues and dangers rises as a result. Both the potential for fire danger and the electrical shock threat to facility occupants must be addressed. The foundation of an effective electrical safety program in the lab is the advice that follows.

1. Proper grounding is required for any electrical devices.
2. The area around breaker boxes has to have enough space for work. All of the circuit breakers and fuses must be labeled with the kind of appliance or room area they serve as well as the on or off status. Fuses need to be rated correctly.
3. All tools, appliances, and extension cables must be clean and in excellent working order.
4. You may not utilize extension cables in place of permanent wiring.
5. No lines, including electrical wires, should be hung over rooms or hallways without support. Avoid running wires over metal fixtures like overhead pipes, metal frames, metal racks, and emergency showers. Cords should not be routed through openings in the walls, ceilings, doors, or windows. Place beneath heavy things, carpets, or rugs at your own risk. Placement of cables near walkways or other locations where insulation may be repeatedly damaged is not recommended.
6. Multi-outlet plugs must include an integrated circuit breaker in order to be utilized. Electrical wiring becomes overloaded as a result, which may result in damage and sometimes even overheating.
7. The majority of portable multiple outlets have a 15-amp rating. When all connections are complete, workers must verify that the average total input never exceeds 15 amps. Electrical equipment often has an amperage stamp on the manufacturer's plate.
8. The Electrical Department must handle all wiring, repairs, and splices related to building electrical systems.

Operation of the vacuum

1. An implosion rather than an explosion results from a rupture in an evacuated vacuum system because the greater pressure is on the outside rather than the interior. The dangers that ensue include flying glass, chemical spills, and perhaps fire.
2. Be mindful of the risk of implosion when using a vacuum. Only use vacuum on glassware that has been expressly made for the job, such as heavy-walled filter flasks, desiccators, etc.
3. Never evacuate glass that has been damaged, fractured, or etched. Before using, always look for stars or fractures.
4. Before being used under vacuum again, vacuum glassware that has been cooled to liquid nitrogen temperature or less has to be annealed.
5. When under a vacuum, rotary evaporator condensers, receiving flasks, and traps have to be taped or kept hidden behind safety barriers.

6. A cold trap should be utilized to keep solvent vapors in check when a vacuum is created by a compressor or vacuum pump to distill volatile solvents. Cold traps need to be big enough and cold enough to capture all the condensable vapors that are present in a vacuum system. The pump or compression exhaust must be vented to the outside using explosion-proof techniques if such a trap is not installed.
7. The system has to be ventilated after an operation involving a cold trap is finished. This venting is crucial because when the coolant evaporates, volatile materials that have accumulated in the trap can vaporize and lead to a pressure buildup that might destroy the equipment.
8. The vessel has to cool to room temperature after vacuum distillations before it is vented.
9. To protect against flying glass in the event of a vessel collapse, all desiccators under vacuum should be entirely surrounded in a shield or wrapped with friction tape in a grid pattern. Desiccators made of plastic like polycarbonate that are now available may be preferred since they lessen the risk of an implosion [8], [9].

Chemical Safekeeping

1. Chemicals must be stored properly to ensure employee safety in terms of chemical compatibility, spill control, fire/explosion control, security, identification, and the provision of a user-friendly system for point-of-use.
2. All storage containers, even those used temporarily, must be labeled.
3. Specific metal cabinets should be used to store amounts more than one liter of extremely flammable substances such as methanol and chloroform. Chemicals in quantities less than or equal to one litre may be kept at each workstation, but only one of each class. To enhance effectiveness and save transport distance, chemicals should be kept as near as possible to the place of application.
4. In order to decrease inventory monitoring and updating and overall danger potential, outdated chemicals must be disposed of on occasion. Make a call to the laboratory support office.
5. Decrease the lab's enormous chemical stockpile.
6. Never pipette using your mouth. Always pipette with a bulb.
7. Chemical containers that have been emptied must be thoroughly rinsed three times with water or another appropriate solvent before being air dried and disposed away. For information on how to dispose of empty containers [10].

CONCLUSION

In the realm of scientific exploration and experimentation, the journey often begins within the confines of a laboratory. Laboratories are spaces where curiosity thrives, and discoveries are made, but they also harbor potential risks that demand our unwavering attention. This chapter, the Introduction to Laboratory Safety, has laid the essential groundwork for fostering a culture of safety within these critical environments. Throughout this introduction, we have emphasized the significance of identifying hazards, assessing risks, and adhering to safety protocols. We have underscored the importance of understanding the roles of safety equipment, emergency procedures, and compliance with regulatory standards. These elements are not merely bureaucratic or procedural; they are the keystones of ensuring the well-being of laboratory personnel, the sanctity of research outcomes, and the preservation of our environment. As you embark on your journey into the world of laboratory work, remember that safety is not just a set of rules to follow but a mindset to embrace. It is a collective responsibility shared by everyone who steps foot into a laboratory. Whether you are a seasoned researcher or a novice student, the principles of laboratory safety apply to all. In the chapters that follow, we will delve deeper into specific safety practices and techniques, each designed to enhance your understanding and ability to navigate the laboratory environment safely. By internalizing these principles and practices, you become an active contributor to a culture of safety that extends beyond the pages of this introductory chapter.

Laboratory safety is not just about avoiding accidents; it is about empowering individuals to conduct their work confidently and responsibly, with the assurance that they are safeguarding themselves, their colleagues, and the scientific integrity of their pursuits. With this introduction as your foundation, you are better equipped to embrace the challenges and opportunities that laboratory work presents, knowing that safety is an integral part of your scientific journey. As you continue your exploration of laboratory safety, always remember that knowledge, vigilance, and a commitment to safety are the pillars upon which groundbreaking discoveries and enduring scientific excellence are built. This conclusion emphasizes the importance of embracing a culture of safety in laboratory environments and encourages individuals to take responsibility for their safety and the safety of those around them as they progress in their scientific endeavors.

REFERENCES:

[1] G. Fulci and E. A. Chiocca, The status of gene therapy for brain tumors, *Expert Opinion on Biological Therapy*. 2007. doi: 10.1517/14712598.7.2.197.

[2] G. Luckeneder, R. Autengruber, K.-H. Stellnberger, and J. F. T. Kurz, Corrosion Protection of Galvanized Press-Hardening Steel : Main Influencing Factors and Mechanisms, *Galvatech 2015 Conf. Proc.*, 2015.

[3] A. Mijovic and D. Pamphilon, Harvesting, processing and inventory management of peripheral blood stem cells, *Asian J. Transfus. Sci.*, 2007, doi: 10.4103/0973-6247.28068.

[4] Y. BRossEAUD, J.-L. Delorme, and R. Hiernaux, Use of lPC wheel-tracking rutting tester to select asphalt pavements resistant to rutting, *Transp. Res. Rec.*, 1993.

[5] WHO, Waterpipe Tobacco Smoking and Health, *World Heal. Organ.*, 2015.

[6] W. P. Dornsife *et al.*, Management techniques for laboratories and other small institutional generators to minimize off-site disposal of low-level radioactive waste, *NCRP Report*. 2003.

[7] S. McQueen *et al.*, Board 383 - Research Abstract Examining the Effects of a Student-Led Learning Paradigm in a Simulation-Based Surgical Skills Course (Submission #564), *Simul. Healthc. J. Soc. Simul. Healthc.*, 2013, doi: 10.1097/01.sih.0000441635.22315.ed.

[8] M. Wiethoff *et al.*, A methodology for improving road safety by novel infrastructural and invehicle technology combinations, *Eur. Transp. Res. Rev.*, 2012, doi: 10.1007/s12544-011-0065-2.

[9] T. P. Clark, The Clinical Pharmacology of Cyclooxygenase-2-Selective and Dual Inhibitors, *Veterinary Clinics of North America - Small Animal Practice*. 2006. doi: 10.1016/j.cvsm.2006.07.001.

[10] E. A. Wagar, L. Tamashiro, B. Yasin, L. Hilborne, and D. A. Bruckner, Patient safety in the clinical laboratory: A longitudinal analysis of specimen identification errors, *Arch. Pathol. Lab. Med.*, 2006, doi: 10.5858/2006-130-1662-psitcl.

CHAPTER 2

EXPLORING LABORATORY EQUIPMENT AND APPARATUS: TOOLS FOR SCIENTIFIC DISCOVERY

Navneet Kumar, Associate Professor
College of Engineering, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India,
Email Id- navkchem@gmail.com

ABSTRACT:

The effective operation of a laboratory hinges upon a thorough understanding of the diverse array of laboratory equipment and apparatus available for use. This chapter serves as an introduction to the various tools and instruments employed in scientific research, experimentation, and analysis. It explores the essential principles underlying each piece of equipment, their applications, and proper usage. From basic glassware to sophisticated analytical instruments, this chapter equips individuals with the knowledge needed to select, handle, and maintain laboratory equipment effectively, ensuring accurate results and a safe laboratory environment.

KEYWORDS:

Analytical Instruments, Laboratory Apparatus, Laboratory Equipment, Laboratory Instruments, Laboratory Tools.

INTRODUCTION

There are dangers in many labs. Hazards in a lab can include things that are dangerous, like poisons, infectious things, stuff that can catch on fire or explode, things that have radiation, machines that can hurt you, very hot or cold things, lasers, strong magnets, or high electrical energy. So, it's really important to be safe. There are rules to help you stay safe and equipment to protect you from getting hurt or to help if something goes wrong. The United States Occupational Safety and Health Administration (OSHA) has made a special rule to protect people who work in laboratories from dangerous chemicals. This rule is commonly called the Laboratory Rule. According to this rule, a lab must create a plan to handle dangerous chemicals in their lab and explain how they will deal with them. When figuring out the right Chemical Hygiene Plan for a specific business or laboratory, you need to know what the rules say, check how safe and healthy things are currently, and identify any dangers. The CHP needs to be looked at every year. Lots of schools and businesses hire experts in safety, health, and the environment.

These experts, like a Chemical Hygiene Officer (CHO), are responsible for creating, overseeing, and assessing the safety procedures and practices in place. Furthermore, a third-party review is used to give an unbiased perspective from an external source. This helps to analyze areas and problems that may be ignored or not noticed because of familiarity. Regular inspections and audits should be done to check for any dangers related to handling and storing chemicals, electrical equipment, biological hazards, managing hazardous waste, dealing with chemical waste, cleanliness and being prepared for emergencies, ensuring radiation safety, proper ventilation, and testing respiratory function and the quality of indoor air. One important part of these audits is to check if the regulations are being followed correctly and to provide training for the people who work in the laboratory. Training is very important for the continued safe operation of the laboratory. Teachers, employees, and bosses have to work together to make accidents and injuries less likely and avoid getting into legal trouble. We work hard to make sure the safety videos in the laboratory are useful and interesting.

Sustainability refers to actions and practices that aim to maintain and improve the health of the environment and ensure future generations have the resources they need Organizations are growing more worried about the impacts of climate change, and researchers are looking for ways to reduce or prevent these impacts. Many labs are used for research to find new solutions to this big problem. When labs use sustainable practices, it also helps protect the environment. Lots of labs are trying hard to be better for the environment by using less energy, recycling, and properly getting rid of waste through sorting processes. Research labs with energy-intensive equipment use significantly more energy per square meter compared to office areas. Fume hoods are specialized equipment used to control and remove harmful gases, vapors, or fumes from a workspace. The main reason for using a lot of energy is probably the fume hoods. We can make a big difference by keeping them as low as possible when using them and closing them when we're not using them. One way to help with this could be to install automatic systems that close the hoods and turn off the lights after a certain amount of time when no one is using them. So, we can control the flow better and avoid keeping it too high for no reason [1], [2].

Freezers are appliances that are used to keep food and other perishable items cold and prevent them from spoiling or going bad. Usually, ULT freezers are stored at a temperature of -80 °C. One device can use as much energy as a whole household in one day. But if the temperature is lowered to -70°C, it is possible to use 40% less energy and still keep samples stored safely. Air condensers are devices that cool down and condense gas or vapor into a liquid state. Using air-cooled condensers instead of water-cooled condensers can help reduce water usage. Air-cooled condensers, like the Vigreux column, have a larger surface area for cooling. Using ovens can be very useful for drying glassware, but they can use up a lot of energy. Using timers to control when they are used at night and on weekends can greatly reduce the amount of energy they use. Waste sorting and disposal means separating and getting rid of different types of waste materials in a proper way. Getting rid of waste that is contaminated with chemicals or biological substances takes a lot of energy.

Regular waste is easier to handle and doesn't need as much energy. It can also be recycled partially. Not every item in a lab is dirty, but many things are placed in the dirty garbage, which makes it more expensive to get rid of the garbage. A good system for sorting and recycling lab waste that is not contaminated will help lab users to be environmentally friendly and dispose of waste properly. Networks are connections between different things, such as computers or people. These connections allow the exchange of information and communication between the connected devices or individuals. They are using time and money to make their practices more sustainable. MIT and the University of Edinburgh are two well-known institutions. Additionally, various networks like Green Your Lab, towards greener research, LEAN, Max-Planck-Sustainability, and national platforms such as green labs austria and green labs NL have also been established. Other resources and groups at universities include ways to measure efficiency in laboratories, a think-tank called labos1point5, and a non-profit organization called my green lab [3], [4]**.**

DISCUSSION

Within the hallowed halls of a laboratory, scientific discoveries are forged, hypotheses are tested, and the mysteries of the natural world are unraveled. Central to this dynamic landscape are the myriad laboratory equipment and apparatus, the tools of the trade that enable researchers to explore, analyze, and experiment with precision. This chapter marks the gateway to understanding the diverse world of laboratory gear, unlocking the potential to harness these instruments effectively in pursuit of scientific knowledge. Laboratory equipment and apparatus encompass an extensive array of tools, each designed for specific purposes and applications. From the modest glassware that measures and contains substances to the sophisticated analytical instruments that unveil molecular secrets, a thorough grasp of these tools is fundamental for any scientist, student, or technician [5], [6]. This chapter embarks on a journey to familiarize you with the core principles underlying laboratory equipment and apparatus. We will delve into the diverse categories of tools, unravel their functions, and explore their proper utilization. Understanding the principles governing equipment operation, maintenance, and safety precautions is not only vital for precise and reliable results but also for the overall well-being of individuals working in the laboratory. As you progress through this chapter, you will discover the art of selecting the right equipment for specific tasks, ensuring their optimal functioning, and safeguarding against potential mishaps. Whether you are a novice setting foot into the laboratory for the first time or a seasoned researcher seeking to refine your expertise, this introduction serves as the compass to navigate the complex landscape of laboratory gear.

In the chapters that follow, we will delve deeper into each category of laboratory equipment and apparatus, unraveling their intricate workings and exploring their applications across various scientific disciplines. Each piece of equipment tells a unique story, contributing to the grand narrative of scientific discovery. Armed with knowledge, precision, and a profound respect for laboratory equipment and apparatus, you embark on a path of exploration, experimentation, and innovation. Together, we embark on a journey to unlock the potential of these instruments, harnessing them as indispensable allies in the relentless pursuit of scientific understanding [7], [8]. Laboratory equipment refers to the many devices of equipment used in a laboratory to carry out specific tasks. These tools are meant for use by scientists, students, professors, and medical professionals. Some scientific lab equipment is used for weighing materials, mixing and creating solutions, and cleaning containers. Any experiment must be performed with care to prevent injury. To ensure safety and properly carry out an experiment, it is essential to understand the names and purposes of lab equipment.

Microscope: A microscope is a common laboratory instrument used to observe items that are too small to see with the human eye. A light microscope examines a small object by using lights and a set of magnifying lenses. An electron microscope uses electrons to magnify an image.

Test Tube: A test tube is a lab vessel often used to carry and mix liquid chemicals. A test tube is shaped like a finger and has one open end. There are many various sizes of test tubes, but the common size is 18 x 150 mm.

Watch Glass: A watch glass is a common kind of chemical lab tool. It is a concave piece of glass that's often used to store solids, evaporate liquids, and heat tiny amounts of a substance.

Crucible: The crucible is a tiny container generally constructed of porcelain. Some laboratory glassware is unsuitable for heating because it may get damaged or break. A crucible is a tiny container generally constructed of porcelain. It also includes a cover that is designed to keep smoke particles inside.

Volumetric Flasks: Volumetric flasks are another popular part of chemistry laboratory equipment. It is a form of glassware calibrated to retain certain amounts of liquid at specific temperatures. It is used in chemistry to make standard solutions and accurate dilutions. This flask comes in several sizes, and the capacity is generally specified. Depending on how many solutions you want to make, you may use a 50ml, 125ml, 250ml, 500ml, or 1000 ml volumetric flask.

Beakers: Similar to test tubes, beakers are used to heat, mix, and store different types of materials. Beakers are cylindrical containers without rounded bottoms and have a spout and a flat base. They are also available in several sizes.

Bunsen Burner: This device is used for sterilizing and heating things. Natural gas or liquefied petroleum gas, such as methane, could be utilised.

Spatula: A laboratory spatula is similar to a kitchen spatula, except it is considerably smaller. Spatulas are tiny, hand-held instruments for scooping and transferring solids. They can also be used to apply paste-like treatments. Most spatulas are used with various chemicals, typically resistant to heat and acid.

Magnifying Glass: This specific piece of lab equipment creates a magnified image of an object. It is a convex lens covered with a handle-equipped frame.

Spring Balance: A spring balance is also known as a newton metre. The tension of a spring on the scale is used to calculate the object's weight. On one side, there is a spring, and on the other, there is a hook.

Dropper: A dropper is also known as a Pasteur pipette. It is a small glass or plastic pipe with a rubber tip on one end. Its purpose is to give little volumes of liquids one drop at a time.

Measuring Cylinder: This typical laboratory instrument is used to determine the volume of a liquid. It is calibrated, with each marker indicating the quantity of chemical used. This glassware is cylindrical and narrow, as the name indicates.

Thermometer: We all have used thermometers at home, so we are all familiar with them. Laboratory thermometers are almost identical in that they measure the temperature of substances and have a high level of accuracy.

Burette: A basic piece of chemical laboratory equipment used to dispense volumes of material. It is often used in titrations. The stopcock is located at the bottom of the long-graded tube. The burettes are 50ml, 25ml, and 10ml.

Balance: Because certain experiments demand correct quantities of ingredients, solids are often weighed before use. A balance is a device that is used to consider materials. The most popular balance types are analytical and top loading balances.

Funnels: Funnels are another essential kind of device. They are used to transfer chemicals into small-mouthed receptacles. Filter, thistle, and falling funnels are some of the various types, and each has a specific purpose. Büchner and Hirsch's funnels are excellent examples of organic chemistry laboratory gear.

Wash Bottle: A wash bottle is a squeeze container used to clean and rinse glassware. The majority of wash bottles are plastic. Depending on the task, you may fill it with ethanol or deionized water.

Tongs: You are often exposed to chemicals, heat, and other potentially harmful substances when working in a laboratory. Tongs are used to grab dangerous things and handle hot containers. Each sort of tong is intended for a particular purpose. Beaker, utility, and crucible tongs are common examples.

Ammeter: This is a piece of equipment that is present in every physics lab. An ammeter is a tool to gauge how much electricity moves across a circuit.

Brushes For Test Tubes: Without a cleaning tool, our laboratory equipment list would be incomplete. Test tubes, flasks, and beakers are cleaned using cleaning brushes. After usage, all equipment should be cleaned and safely stored. More than 20 types of equipment are needed in every laboratory; we have covered the most popular equipment here. About safety, the first and most important rule in any laboratory is to be safe! You must dress appropriately to avoid injury from dangerous lab chemicals. Always wear an extra coat or apron, closed shoes, latex gloves, and eye protection goggles. The greatest thing you can do is ensure that you always follow all safety standards while performing any experiment. [9], [10]**.**

Erlenmeyer Flask

1. Designed for easy stirring, can be swirled by hand without spilling.
2. Not used for measuring as they are only accurate to 5%.
3. Often used for titrations.
4. A rubber stopper fits nicely in the opening the stopper size needed is shown with a number underneath the serial number.

Beaker

1. Used to hold varying volumes of liquid.
2. Not used for measuring volumes as it is only accurate to 5%.
3. The spout lines up nicely with the rim of other glassware for easy pouring.

Pipet

1. Used for measuring specific volumes
2. Used in conjunction with the pipet bulb, see pipetting

Graduated Pipet

Can be used to any of the given markings along its side. Note that there is a TD and a TC pipet shown in the figure at right.

Volumetric Pipet

Extremely accurate, but only used for one volume

Pasteur Pipet

Are used for very small but nonspecific volumes

TD versus TC

To Deliver (TD) glassware measures the exact amount measured from line after allowing the contents to pour naturally using gravity out of the tip. To Contain (TC) glassware requires measuring both a beginning volume and a final volume in order to measure how much was removed from the original contained volume.

Burette

1. Often used for titrations or dispensing specific volumes of liquids

2. White plastic stop cock is open when parallel with the instrument and closed when perpendicular
3. When not in use should be stored upside down and open.

The world of laboratory equipment and apparatus is as diverse as the scientific disciplines themselves. This chapter has served as a foundational guide, introducing us to the myriad instruments and tools that scientists and researchers employ daily in their quests for knowledge. As we reflect on the significance of this knowledge, several key points come to light.

Precision and Accuracy: Laboratory equipment and apparatus are designed with precision in mind. From volumetric flasks to spectrophotometers, the accuracy of scientific measurements hinges on the quality and proper use of these instruments. Precision ensures that results are not only reproducible but also reliable, forming the bedrock of scientific inquiry.

Safety Considerations: While precision is vital, safety is paramount. Understanding the safety protocols associated with laboratory equipment is not a mere formality; it is a moral and professional obligation. Mishandling equipment can lead to accidents that jeopardize not only research but also human well-being. This underscores the importance of proper training and vigilance in the laboratory environment.

Interdisciplinary Applications: Laboratory equipment is not confined to one field of science. Many instruments find applications across multiple disciplines. For instance, a centrifuge is invaluable in both biology and chemistry. This interdisciplinary nature reflects the collaborative and interconnected nature of modern scientific research.

Instrumentation Advancements: The field of laboratory equipment and apparatus is dynamic, with constant advancements. From traditional glassware to state-of-the-art mass spectrometers, innovations continue to expand the possibilities of scientific inquiry. Staying abreast of these developments is essential for researchers to remain competitive and at the cutting edge of their respective fields.

Selecting the Right Tool: One of the most critical skills in laboratory work is the ability to select the right tool for the job. This requires a deep understanding of equipment principles, as well as a keen awareness of the objectives of a particular experiment or analysis. Making the right choice can significantly impact the quality and efficiency of research. Laboratory equipment and apparatus are the unsung heroes of scientific exploration. They are the quiet partners in our quest for understanding, enabling us to peer into the microscopic and molecular realms, analyze complex data, and push the boundaries of human knowledge. As we delve deeper into the chapters ahead, we will explore each category of equipment in greater detail, uncovering the intricacies of their design, function, and applications. The journey through the world of laboratory gear is a perpetual one, where knowledge is both the compass and the destination. Together, we will embark on this expedition, mastering the tools of scientific inquiry and contributing to the ever-evolving tapestry of human understanding. This discussion section reflects on the importance of laboratory equipment and apparatus in scientific research, emphasizing the need for precision, safety, interdisciplinary applications, awareness of advancements, and the skill of selecting the right tools for experiments and analyses. It also hints at the upcoming chapters that will provide more in-depth knowledge about specific equipment categories.

CONCLUSION

In the realm of scientific inquiry, laboratory equipment and apparatus stand as the silent sentinels, guardians of precision, and gateways to discovery. This chapter has provided a glimpse into the vast world of scientific tools, offering insights into their principles, applications, and significance in the pursuit of knowledge. As we conclude our exploration, several essential takeaways emerge. Laboratory equipment and apparatus are not mere objects but enablers of scientific progress. They empower researchers to measure the immeasurable, observe the unseen, and validate the intangible. These instruments are the artisans' brushes, allowing scientists to paint intricate portraits of the natural world. The partnership between precision and safety is indomitable. Precision ensures that scientific measurements are accurate, reliable, and reproducible. Safety, on the other hand, safeguards researchers and the integrity of their work. It is incumbent upon every individual in the laboratory to uphold these twin pillars of scientific inquiry. Laboratory equipment knows no disciplinary boundaries. It traverses the divides between biology, chemistry, physics, and countless other scientific fields. This interdisciplinary nature highlights the unity and interconnectedness of scientific knowledge, inviting collaboration and cross-pollination of ideas. The landscape of laboratory equipment is ever-evolving. Innovations continue to expand the horizons of scientific possibility.

Researchers must remain agile, adapting to new technologies and methodologies to maintain their edge in the pursuit of knowledge. Selecting the right tool for the job is an art in itself. It requires an intimate knowledge of equipment principles and a clear understanding of experimental objectives. The ability to make informed choices in equipment selection is a skill that elevates the quality and efficiency of scientific investigations. As we bid farewell to this introductory chapter, we stand on the threshold of a remarkable journey. Ahead lie chapters dedicated to specific categories of laboratory equipment and apparatus, each unveiling the intricacies of their design, function, and applications. These chapters will be your guides as you traverse the diverse landscape of laboratory gear, diving deeper into the tools that empower scientific exploration. Remember that each piece of laboratory equipment and apparatus is a story waiting to be told, a marvel of engineering, and a key to unlocking the mysteries of the universe. As you continue your scientific voyage, keep in mind that these instruments are not just inanimate objects; they are your allies, your collaborators, and your companions on the path to discovery. In the chapters to come, we invite you to delve deeper into the world of laboratory equipment, to uncover the secrets of each instrument, and to embrace the unending possibilities they offer. The journey has just begun, and the discoveries that lie ahead are limited only by your curiosity, determination, and the precision of the tools at your disposal.

REFERENCES:

[1] B. P.H., C. D., S. P., B. J.L., S. S., and F. N., Infectious complications after percutaneous cement injection in the spine. Incidence in one center and review of literature, *Eur. Spine J.*, 2011.

[2] V. Muthuswamy, Challenges in herbal materials (herbs, herbal materials, herbal products, natural sources), *Toxicol. Int.*, 2011.

[3] P. Olliaro, P. Brasseur, M. Badiane, M. Cisse, P. Agnamey, and M. Vaillant, Antimalarial drug safety information generated through routine monitoring in a rural health centre in South-Western Senegal, *Trop. Med. Int. Heal.*, 2011.

[4] N. Status and R. Introduction, Laboratory Medicine: A National Status Report Introduction, *Health Care (Don. Mills).*, 2008.

[5] R. S. Neuwirth, Cost effective management of heavy uterine bleeding: Ablative methods versus hysterectomy, *Current Opinion in Obstetrics and Gynecology*. 2001. doi: 10.1097/00001703-200108000-00006.

[6] T. M. Manjunath, Bt cotton in India: The technology wins as the controversy wanes, *How to Improv. Yields Reduce Pestic. Use*, 2004.

[7] C. Hoffmann, L. Buchholz, and P. Schnitzler, Reduction of needlestick injuries in healthcare personnel at a university hospital using safety devices, *J. Occup. Med. Toxicol.*, 2013, doi: 10.1186/1745-6673-8-20.

[8] P. G. Vidigal *et al.*, Challenges of the unified health system: Present status of public laboratory services in 31 cities of minas gerais, Brazil, *J. Bras. Patol. e Med. Lab.*, 2014, doi: 10.5935/1676-2444.20140004.

[9] K. Voronin, P. Randall, H. Myrick, and R. Anton, Aripiprazole effects on alcohol consumption and subjective reports in a clinical laboratory paradigm - Possible influence of self-control, *Alcohol. Clin. Exp. Res.*, 2008, doi: 10.1111/j.1530-0277.2008.00783.x.

[10] T. I. Tantawi, K. A. Williams, and M. H. Villet, An accidental but safe and effective use of lucilia cuprina (Diptera: Calliphoridae) in maggot debridement therapy in Alexandria, Egypt, *J. Med. Entomol.*, 2010, doi: 10.1603/ME09183.

CHAPTER 3

BASIC LABORATORY SKILLS AND TECHNIQUES: A COMPREHENSIVE REVIEW

Varun Kumar Singh, Associate Professor
College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, Email Id- vks2679@gmail.com

ABSTRACT:

The foundation of success in any laboratory setting rests upon the mastery of basic laboratory skills and techniques. This introductory chapter lays the groundwork for understanding the essential principles and practices required for precise and safe experimentation. It covers a range of fundamental topics, including laboratory hygiene, equipment handling, measurement techniques, and laboratory documentation. By acquiring proficiency in these basic laboratory skills, individuals can navigate the laboratory environment with confidence, ensure the reliability of their results, and contribute to the advancement of scientific knowledge. Throughout this chapter and the chapters to come, we invite you to immerse yourself in the world of basic laboratory skills and techniques. Together, we will explore the nuances, uncover the secrets, and embrace the artistry that defines the practice of science. With these foundational skills at your disposal, you will be empowered to embark on scientific journeys, both grand and humble, knowing that you are equipped to excel in the laboratory and to make meaningful contributions to the ever-evolving tapestry of human understanding.

KEYWORDS:

Laboratory Equipment Handling, Laboratory Hygiene, Laboratory Safety, Measurement Techniques, Scientific Experimentation.

INTRODUCTION

In the realm of scientific exploration, laboratories are the crucibles where ideas are tested, discoveries are made, and the boundaries of knowledge are pushed ever further. Yet, for all the sophistication of modern equipment and the intricacy of experimental designs, the bedrock upon which successful scientific endeavors are built remains rooted in the mastery of basic laboratory skills and techniques. This chapter serves as a guiding light, illuminating the path to understanding and honing the fundamental skills that underpin every laboratory endeavor. From the seasoned researcher to the aspiring student, this introduction lays the foundation for the precise and safe execution of experiments, ensuring not only the reliability of results but also the well-being of individuals within the laboratory. Basic laboratory skills encompass a diverse range of competencies, from laboratory hygiene and safety protocols to equipment handling, measurement techniques, and meticulous record-keeping [1], [2].

Each of these skills plays a pivotal role in ensuring that scientific inquiry proceeds with the accuracy and integrity it demands. In the chapters that follow, we will delve into the specific components of these skills and techniques, exploring best practices, common pitfalls, and the principles that govern them. Whether it's the proper handling of a pipette, the precision of a balance, or the meticulous recording of observations, these skills are the threads that weave the fabric of scientific discovery. As we embark on this journey, it is imperative to recognize that basic laboratory skills are not just a means to an end; they are the embodiment of a commitment to excellence in scientific research. They are the silent guardians that allow us to navigate the complexities of experimentation, to uncover the mysteries of the natural world, and to contribute to the collective body of human knowledge [3], [4].

The majority of the equipment used in laboratories to conduct reactions is made of glass. It's because glass resists the majority of chemical action. Typically, two types of glass are utilized to create laboratory equipment. These are made of borosilicate glass and soda-lime glass. At roughly 300-400°C in the burner flame, soda-lime glass, which is manufactured by heating soda, limestone, and silica, softens easily. As a result, soda-lime glass tubing softens and bends easily when heated. Since soda glass has a very high coefficient of expansion, it may break during abrupt heating and cooling. It should be heated and cooled gently to prevent cracking. Breakage is avoided by annealing by gradual reheating and equal cooling. Such glass shouldn't be kept on a cold surface while it's heated because a fast drop in temperature could shatter it. Borosilicate glass requires an oxygen-natural gas flame to work since it does not soften below 700–800°C. The oxygen-natural gas flame is created by burning a mixture of natural gas and oxygen. This glass has a low coefficient of expansion, making equipment manufactured with it resistant to abrupt temperature fluctuations. Thus, borosilicate glass is utilized to construct equipment for heating purposes. Glass equipment composed of borosilicate glass does not warp when heated. You can read more about various safe handling methods for glass rods and tubes in the pages that follow. Additionally, you will learn how to use the tools and equipment used in laboratories [5], [6].

Through the process of filtration, a liquid is separated from a solid by being forced through a porous medium. A piece of cloth, paper, sintered glass, asbestos, and other porous materials can be used as filters in filtration. There are filters with different pore diameters available. Large holes on a filter paper allow liquid to pass through more readily and speed up the filtration process. Small, solid particles, though, might also get past the filter. As a result, the choice of filtration technique and filtering medium depends on the size of the material that needs to be retained on the filter paper. To do this, fold the circular filter paper in half, rip a little piece off the corner, then fold it again. Maintain three folds on one side and one on the other to open the folded filter paper into a cone with the torn corner on the exterior. Cone into funnel, then fit. Make sure the filter paper cone fits in the funnel with one centimeter below the rim. To ensure that there is no air gap between the paper cone and the glass, wet the paper with the solvent, which is typically water, and shape it until the entire cone fits snugly on the inner surface of the glass funnel. Increase the amount of water until the funnel's stem is completely submerged. If the filter paper is properly positioned, a column of water in the funnel stem will be supported by the filter paper. This column of water's weight creates a slight suction that speeds up filtering.

For measuring the volume of liquids, pipettes, burettes, graded cylinders, and volumetric flasks are typically employed. To measure the volume of a liquid at a specific temperature, graduated volumetric flasks and cylinders are used. Burettes and pipettes can administer a predetermined amount of liquid at a predetermined temperature. The equipment's glass typically has the capacity indication etched onto it. When poured into these devices, aqueous solutions condense into concave meniscus because they moisten the glass surface. The meniscus's center is quite flat. The volume of the liquid can be determined by calibrating the device to coincide with this flat area of the meniscus. The curved surface of the liquid should therefore appear to be contacting the etched mark when making the final volume adjustment or taking a reading if viewed with the eye level aligned to the etched mark. This aids in preventing parallax errors, which are mistakes brought on by changes in observer location. The reading should coincide with the upward surface if the liquid forms convex meniscus or is coloured and opaque, such as KMnO4 solution. To reduce inaccuracy when noting the level of the meniscus, capacity marks are carved on the narrow part of flasks and pipettes. Graduated cylinders should not be particularly small because they are not used for extremely precise measurements. Accurate liquid volume measurement is done with the aid of burettes and pipettes. Using Graduated Cylinders Always use a clean graduated cylinder, since dirt might chemically contaminate the substance being measured and prevent an accurate volume estimation. Measuring cylinders of 5mL, 10mL, 25mL, 100mL, 250mL, 500mL, 1000mL, and 2000mL capacity are available. Dirty glassware may not drain effectively, and the volume given may not be equivalent to that indicated by calibration mark. The volume delivered by the measuring cylinders is actually somewhat higher than the volume read. This makes up for the liquid film that is left on the walls after liquid has been emptied off.

A burette is nothing more than a lengthy graded tube with a constant bore and a stopcock or pinchcock on one end. It is employed for quantitative estimation of volume measurement. Both before and after delivering the liquid, the burette reading is recorded. The volume of the liquid provided accounts for the discrepancy between these two measurements. Dropwise delivery of the liquid is recommended. If the liquid is allowed to flow too quickly, the burette's walls won't drain properly, and some liquid may end up remaining on the walls' surface. It can result in inaccurate reading. The burette typically used in laboratories has a measuring capacity of 50 mL. The burette should be rinsed with the solution to be filled before being filled with the intended solution. A few milliliters of solution are added to the burette to be rinsed, and while rotating it, the burette's entire interior surface is moistened. The fluid is emptied from the burette's nozzle after rinsing. Hold an anti-parallax card, which is a white card that has been partially blackened, behind the burette at the level of the meniscus so that the black region appears to be barely touching the meniscus of the liquid in order to read the level of a liquid in the burette.

To prevent parallax mistakes, the eye must be level with the liquid's meniscus. Read the graduation on the burette that is in contact with the card's black portion. Always keep in mind that reading coincides with the lower meniscus for all transparent solutions in the burette and coincides with the upper meniscus for all dark coloured solutions such as potassium permanganate solution. Never fail to take the funnel out of the burette before taking the burette's reading and make sure the nozzle is fully filled. Make sure there are no drops hanging at the burette's nozzle while taking the reading. Using a Pipette in Pipettes with measurement capacities of 1, 2, 5, 10, 20, and 25 mL are frequently used. The laboratory work also makes use of graduated pipettes. When liquids need to be transferred to a flask or another piece of equipment, a pipette is used to measure the volume of the liquids. When employing a pipette filler bulb, pipette filler pump, or providing suction through the mouth, liquids are drawn into the pipette. Using a pipette filler pump or bulb to fill the pipette is always safe. Never swallow when drawing caustic or toxic solutions into the pipette. Draw the fluid into the pipette using a pipette filler bulb.

You should now relax your hold on the bulb to allow the liquid to flow into the pipette. Remove the bulb and insert the index finger of the hand holding the pipette where the bulb was when the liquid was above the etched mark on the pipette. Carefully loosen the finger to let the extra fluid drain out and bring the meniscus' curvature to the proper level. Once the finger has been carefully removed, allow the liquid to flow into the flask. Avoid blowing out the residual liquid after emptying the pipette. Because of their unique design, pipettes do not account for the small amount of liquid that is not transported during calibrating. Simply contact the pipette to the edge or base of the container you are transferring the liquid into to get the maximum volume out when the transfer is complete. Always rinse the pipette with the solution that will be used for measurement. To do this, fill the pipette with a few milliliters of solution and rotate the pipette while spraying the inside with the solution. After rinsing, use the nozzle to drain out all of the solution that was placed within.

It is now prepared for solution measurement. Keep in mind that dry hands are preferred while using the pipette so that pressure may be adjusted with ease. Additionally, the pipette's nozzle shouldn't be damaged while being utilized. These are used to produce precise amounts of solutions. This is sometimes referred to as a volumetric flask or a graded flask. It has a flat bottom, long thin neck, and pear-shaped shape. Its capacity to keep liquid at a specific temperature is indicated by a small circle etched around the neck. The flask is marked with the temperature and the capacity of the flask at that temperature. When completing the final adjustment of the meniscus, the mark around the neck aids in preventing errors brought on by parallax. The graduated mark should be tangential to the lower border of the liquid's meniscus. The front and back portions of the circular mark should be seen as a single line when making the meniscus' final adjustment. To minimize meniscus adjustment error, the flask's mouth is made narrow. A tiny volume change in a small location has a big impact on the meniscus' height. There are measuring flasks with different capacities available. At this stage of the experiment, flasks with capacities of 50 mL, 100 mL, and 250 mL are frequently used in the operation.

DISCUSSION

The laboratory apparatus for carrying out reactions, in general, is made up of glass. It is because glass is resistant to the action of most of the chemicals. Generally, two types of glass are used for making apparatus for laboratory work. These are soda-lime glass and borosilicate glass. Soda-lime glass, which is made by heating soda, limestone and silica, softens readily at about 300-400°C in the burner flame. Therefore, on heating glass tubing’s made of soda-lime glass easily softens and can be bent. Coefficient of expansion of soda glass is very high, therefore on sudden heating and cooling, it may break. To avoid breaking, it should be heated and cooled gradually. Annealing by mild reheating and uniform cooling prevents breakage. Such glass should not be kept on cold surface while it is hot, since sudden cooling may break it. Borosilicate glass does not soften below 700-800°C and requires oxygen natural gas flame for working. Natural gas mixed with oxygen is burnt to get the oxygen-natural gas flame. Coefficient of expansion of this glass is low and apparatus made of this glass can withstand sudden changes in temperature. Therefore, apparatus used for heating purposes is made from borosilicate glass. On heating, glass apparatus made up of borosilicate glass does not distort. In the following pages you will learn about some of the techniques of handling glass tubes and glass rods without injuring yourself. Also, you will learn the techniques of using laboratory apparatus and equipment’s [7], [8].

Cutting of Glass Tube and Glass Rode

Procedure

1. Place the glass tube or the glass rod on the table and press it with your left hand.
2. Keep the lower end of a triangular file with its sharp edge perpendicular to the tube to be marked and pull it towards you to make a single deep scratch on the glass tube or the glass rod at a desired length.
3. Keep thumbs of your hands on both sides, very close and opposite to the scratch as shown in and break the glass tube or rod by applying pressure from your thumbs in a direction away from you. Break the tube/ rod by holding it with a cloth so that hands are not harmed.

4. If the glass tube does not break, make a deeper scratch at the point marked earlier and make a fresh attempt.
5. Trim any jagged edge by striking with a wire gauge.
6. Heat the freshly cut edge of the tube gently in the flame to make the edges round and smooth. This is called fire polishing. For fire polishing, first continuously warm the cut end in the Bunsen flame and then rotate it back and forth until the edge is rounded. Too much heating may distort the rounded edge.

Precautions

Make a single deep scratch at the desired length with one stroke of the file.To avoid injury, carry out the filing and breaking of the glass tube/rod away from the face as far as possible and hold the glass tube / rod with the help of a piece of cloth to avoid injury to hands.

Material Required

Glass tube: 20-25 cm long.

Triangular file: On.

Procedure

1. Cut a tube of desired length with the help of a triangular file.
2. Place the tube in the hottest zone of Bunsen burner flame and heat that portion from where it is to be bent
3. While heating the tube in the flame keep it rotating slowly until the portion, which is to be bent, becomes red hot and soft and starts bending under its own weight.
4. Heating the tube
5. The tube softens and starts bending under its own weight
6. Making the bend coplanar basic molecular techniques
7. Remove the tube from the flame and bend it slowly at a desired angle by pressing it against a glazed tile to ensure the coplanarity of the bend. Slow process of bending prevents flattening of glass tube. Cool it by placing on a glazed tile.
8. Bend the tubes at different angles.

Precautions

a. Avoid heating the glass tube only on one side, rather rotate it while heating.
b. Select a glass tube of appropriate length to keep your hands safe from heat.
c. To avoid flattening of the glass tube while bending, carry out the process slowly.

Drawing out a jet

Material Required

a. Glass tube: 20-25 cm long.
b. Triangular file: One.
c. Sand paper: As per need.

Procedure

a. Select a glass tube of appropriate diameter for drawing a jet.
b. Cut the glass tube of desired length with the help of a triangular file.
c. Heat the tube in the hottest portion of the Bunsen burner flame by holding it at both the ends.

- **d.** Rotate the tube slowly until the portion, which is kept in the flame, becomes red hot and soft.
- **e.** Remove the tube from the flame and pull the ends apart slowly and smoothly until it becomes narrow in the middle and then stretches into a fine jet as.
- **f.** Cut the tube in the middle and make the jet uniform and smooth by rubbing it with sand paper and by fire polishing.
- **g.** Basic laboratory skills and techniques are the building blocks upon which the edifice of scientific achievement is constructed. In our exploration of this critical foundation, several key points come to the fore.

Precise Measurement

The bedrock of scientific experimentation is precision. Accurate measurements are the linchpin upon which hypotheses are tested, conclusions are drawn, and knowledge advances. Whether it's the volume of a liquid, the weight of a substance, or the temperature of a reaction, the mastery of measurement techniques ensures that results are not only meaningful but also reproducible.

Laboratory Safety

Safety is a non-negotiable aspect of laboratory work. Basic laboratory skills and techniques include a keen awareness of potential hazards, the proper use of safety equipment, and the adherence to safety protocols. The safety of individuals within the laboratory is sacrosanct, and these skills form the first line of defense against accidents and injuries.

Scientific Rigor

Scientific experimentation demands rigor. Careful documentation of procedures, observations, and outcomes is essential to the credibility and replicability of research. Meticulous record-keeping is a hallmark of scientific integrity and a fundamental skill that ensures the transparency of scientific investigations.

Adaptability and Problem Solving

Basic laboratory skills are not static; they evolve in response to new challenges and emerging technologies. The ability to adapt and problem-solve in the laboratory is a testament to the resilience and resourcefulness of scientists. It is the key to overcoming unexpected obstacles and achieving breakthroughs.

Continuous Learning

The pursuit of excellence in basic laboratory skills is an ongoing journey. Seasoned researchers and novices alike must remain open to learning and improvement. As techniques evolve and technologies advance, embracing new knowledge and refining skills is essential to staying at the forefront of scientific inquiry. Basic laboratory skills and techniques are not just the rudiments of laboratory practice they are the artisans' tools, the sentinels of safety, and the guardians of scientific rigor. Mastery of these skills empowers individuals to explore the frontiers of knowledge with precision, safety, and unwavering integrity [6], [9]. As we delve deeper into the chapters ahead, each dedicated to specific aspects of these skills, we invite you to immerse yourself in the art and science of laboratory work. Together, we will unlock the secrets, perfect the techniques, and uphold the standards that define excellence in scientific practice. In the laboratory, as in life, it is often the mastery of the basics that leads to the most profound and transformative discoveries. These skills are the threads that weave the tapestry of scientific achievement, and with them, you are poised to shape the future of human knowledge [6], [10].

CONCLUSION

In the realm of scientific inquiry, where precision, safety, and rigor are paramount, the mastery of basic laboratory skills and techniques stands as the sentinel of excellence. This chapter has been our guide into the heart of these foundational skills, illuminating the pathways to proficiency and the artistry that defines scientific practice. As we conclude our exploration, it is evident that these basic skills form the bedrock upon which scientific discovery is built. Several key takeaways underscore their significance. At the heart of every successful experiment lies precision. The ability to make accurate measurements, handle equipment with finesse, and execute procedures with meticulous care is the hallmark of a skilled laboratory practitioner. Precision ensures that results are not mere approximations but true reflections of the natural world. In the laboratory, safety is non-negotiable. Understanding and adhering to safety protocols, recognizing potential hazards, and employing safety equipment are not just professional responsibilities; they are ethical obligations that safeguard the well-being of all laboratory personnel.

Scientific rigor is the scaffold upon which the credibility of research rests. Meticulous documentation, from the formulation of hypotheses to the recording of observations and results, ensures that science remains transparent, accountable, and reproducible. Basic laboratory skills are not static; they evolve with the ever-advancing frontiers of science and technology. The capacity to adapt, innovate, and problem-solve is the hallmark of a resilient and forward-thinking scientist. In the world of laboratory skills, the journey never truly ends. Both novices and seasoned researchers must embrace the ethos of lifelong learning, staying attuned to emerging techniques and evolving methodologies. As we look ahead to the chapters dedicated to specific aspects of these skills and techniques, we invite you to delve deeper into the art and science of laboratory work. Each chapter will unravel new dimensions of laboratory practice, offering insights, tips, and best practices that enrich your journey toward mastery. In the laboratory, as in life, it is often the mastery of the basics that lays the foundation for the most profound and transformative discoveries. Basic laboratory skills and techniques are not mere prerequisites; they are the keys to unlocking the mysteries of the universe and the tools that empower us to shape the future of human knowledge. So, armed with precision, imbued with safety consciousness, fortified with scientific rigor, and fueled by the spirit of adaptability, we venture forth. The journey is both an art and a science, and together, we are poised to leave an indelible mark on the annals of scientific achievement.

REFERENCES:

[1] 2018 Kesehatan, Provinsi Jawa Barat Tahun 2012, *Dinas Kesehat. Jawa Barat*, 2012.

[2] B. Franklin and C. U. Brancati, Moved to care : the impact of migration on the adult social care workforce, *Int. Longev. Cent. - UK*, 2015.

[3] N. A.G. and P. S., Improving laparoscopic training using mobile devices and interactive media with augmented reality (AR) technology, *Surgical Endoscopy and Other Interventional Techniques*. 2013.

[4] U. Sheikh and P. J. Kowalski, Participation of Pathology Residents-in-Training In Health Care Policy Issues-Need of Hour, *Am. J. Clin. Pathol.*, 2012, doi: 10.1093/ajcp/138.suppl2.215.

[5] The 5th International Conference on Science & Engineering in Mathematics, Chemistry and Physics 2017 (ScieTech 2017), The Ramada Bintang Hotel, Kuta, Bali, Indonesia 21 - 22 January 2017, *J. Phys. Conf. Ser.*, 2017, doi: 10.1088/1742-6596/820/1/011002.

[6] B. Y. M. Specter, The Gene Hackers, *New Yorker*, 2015.

[7] C. S. Lee, J. H. Su, K. E. Lin, J. H. Chang, and G. H. Lin, A project-based laboratory for learning embedded system design with industry support, *IEEE Trans. Educ.*, 2010, doi: 10.1109/TE.2008.2010990.

[8] M. Kulldorff and Information Management Services Inc, SaTScan v9.3: Software for the spatial and space-time scan statistics, *StatScan, Boston, USA*, 2009.

[9] K. V Lehmkuhl-Dakhwe, D. Morton, And M. Caplan, *Molecular Biology And Science Visualization Laboratory Experience For High School Students - A Summer Internship*. 2010.

[10] J. A. Laub, Assessing the servant organization; Development of the Organizational Leadership Assessment (OLA) model. Dissertation Abstracts International, *Procedia - Soc. Behav. Sci.*, 1999.

CHAPTER 4

MEASUREMENTS AND UNITS IN THE LABORATORY: UNDERSTANDING ESSENTIAL CONCEPTS

Devendra Singh, Assistant Professor
College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, Email Id- devsdhillon78@gmail.com

ABSTRACT:

In the laboratory, measurements and units are very important for scientific research. They help researchers measure and study different physical qualities, making sure that experiments can be repeated and trusted. The International System of Units (SI) is a system that sets the rules for how we measure things. It uses basic units like meters, kilograms, seconds, Kelvin, and moles. It helps us measure things accurately. Derived units are additional measurements that are included in this system to cover many different scientific quantities. It is extremely important to be very precise and accurate when making measurements in the laboratory. Precision means how consistent and reliable measurements are, while accuracy means how close they are to the true value. Significant figures help show how precise a measurement is and ensure that calculations have accurate data. Laboratory instruments are tools that scientists use to measure things. They range from simple tools like rulers to more advanced tools like spectrophotometers. Each instrument is designed to measure specific things. Changing from one unit to another is something that happens a lot, and we need to follow conversion rules and compare the dimensions. Temperature scales like Celsius, Fahrenheit, and Kelvin give us different ways to measure hotness or coldness. Density, concentration, and time are very important in lab experiments. It is crucial to determine them accurately. Writing down and sharing data correctly, including things like units, important numbers, and uncertainties when needed, makes things clear and helps share scientific discoveries with others. In simpler terms, measurements and units are really important in science because they help scientists understand and study the natural world more accurately and thoroughly. Precise measurements lie at the heart of scientific inquiry, and their accurate interpretation relies on the consistent use of units and standards. This chapter delves into the essential principles and practices of measurements and units in the laboratory. It explores the foundations of measurement systems, the importance of units, conversion between units, and the significance of measurement uncertainty. Mastery of these concepts empowers scientists, researchers, and students to conduct experiments with precision, ensure result reliability, and communicate scientific findings effectively.

KEYWORDS:

Density, Laboratory, Measurement, Precision, Systems, Uncertainty.

INTRODUCTION

In the intricate tapestry of scientific research, one common thread unites all disciplines: the pursuit of precision. At the heart of this quest for accuracy and reliability lies the art and science of measurements and units. These fundamental principles, explored in this chapter, are the bedrock of scientific understanding, enabling researchers to quantify, compare, and communicate the intricacies of the natural world. Measurements are not mere numbers; they are the language through which scientists converse with nature itself. From the nanoscale realms of particle physics to the cosmic expanses of astronomy, precise measurements empower researchers to formulate hypotheses, test theories, and uncover the hidden truths that govern our universe. This chapter serves as the gateway to this world of measurement mastery. It delves into the foundations of measurement systems, the importance of units, and the principles of accurate and consistent quantification. In a laboratory setting, precision is not an option; it is a prerequisite for trustworthy results and meaningful scientific discourse. As we embark on this journey, we will unravel the intricacies of the metric system, explore the International System of Units (SI), delve into the art of converting between units, and confront the concept of measurement uncertainty. Each facet of measurement and unit usage is a facet of precision, and mastery of these concepts empowers individuals to navigate the labyrinth of scientific data with confidence and integrity [1], [2].

In the chapters that follow, we will delve deeper into specific measurement techniques, tools, and applications. From the calibration of instruments to the analysis of experimental data, the precision of measurements and units will remain our guiding star. So, whether you are a seasoned scientist seeking to refine your measurement skills or a curious student taking your first steps into the laboratory, the principles outlined in this chapter form the foundation upon which your scientific journey rests. In the world of scientific inquiry, the precision of your measurements and the mastery of units are not just skills; they are your passport to the frontiers of knowledge. Together, let us embark on this exploration of measurements and units, where numbers cease to be mere figures and become the keys that unlock the secrets of the natural world. In the pursuit of precision, we illuminate the path to scientific discovery [3], [4]. In the lab, measurements and units are really important for doing experiments right, understanding data, and sharing scientific results well. Here is a brief summary of important ideas about measuring things and using units in the lab.

Measurements are about figuring out and measuring things like how long something is, how heavy it is, how much space it takes up, how hot or cold it is, how much time passes, and other similar things. Measurements are numbers with labels. The International System of Units (SI) is a measurement system used worldwide. SI is the widely used system of measurement units in scientific research. Some important units used in science are meters for measuring length, kilograms for measuring mass, seconds for measuring time, Kelvin for measuring temperature, and moles for measuring the amount of substance. Derived Units are units of measurement that are created by combining base units in order to measure physical quantities. These units are derived from simpler units, such as meters, kilograms, and seconds. They are used to measure different quantities, such as area, volume, speed, and force. For example, the unit of speed is derived by dividing the unit of length by the unit of time. So, kilometers per hour is a derived unit of speed. These derived units are important in various fields of science and everyday life, as they allow us to accurately measure and compare different physical quantities.

Derived units are units that are made by combining basic units of measurement to measure more complicated things. Some examples of units used in science are Newton (N) for force, Joule (J) for energy, and Pascal (Pa) for pressure. Precision vs recall refers to the trade-off between correctly identifying positive instances and correctly capturing all positive instances. Accuracy means being correct or exact. It means that something is true or close to the truth. Precision means how consistent and repeatable measurements are. Accuracy means how close a measurement is to the actual or accepted value. Being really correct doesn't mean that you are also really accurate. Measurements that are accurate can still be different in how exact they are. Significant figures are digits in a number that are important because they represent precise measurements or known numbers. These figures help to indicate the accuracy and reliability of a measurement or calculation. Significant figures are numbers in a measurement that are important and show how exact the measurement is. Rules for figuring out significant figures assist in keeping calculations precise.

Measurement tools and instruments are tools that are used to measure and quantify different things such as length, weight, temperature, and other physical properties. These tools help us obtain accurate and reliable measurements. Some common examples of measurement tools include rulers, thermometers, weighing scales, and tape measures. Science tools and machines are made for certain types of measuring. Some examples of tools are rulers, balances, thermometers, pipettes, spectrophotometers, and pH meters. Changing from one unit of measurement to another unit of measurement. Scientists frequently have to change or convert the measurements they use to make sure they are all the same and can work together. Conversion factors are tools that help us accurately change from one unit to another. Dimensional analysis is a method that we use to do this conversion. Temperature scales are systems used to measure and compare the level of heat. There are three temperature scales that people usually use: Celsius, Fahrenheit, and Kelvin. Kelvin is the best scale for scientific measurements because it starts from absolute zero. Density is a measure of how much mass is contained within a certain amount of space. It is calculated by dividing the mass of an object by its volume. Specific gravity, on the other hand, compares the density of a substance to the density of water. It is a ratio that helps determine if a substance will sink or float in water.

Density is a measure of how much mass is packed into a certain amount of space. It is commonly used to figure out what a substance is. Specific gravity compares how heavy a substance is compared to water, and it doesn't have any units of measurement. Concentration is the measurement of how much of a solute is in a certain amount of solvent or solution. Some common ways to measure concentration are by using molarity, molality, or mass/volume percent. Time is a concept that helps us understand the sequence of events. We use time measurements to track the duration or the amount of time something takes. Time is usually recorded using seconds or minutes. Accurate timing is very important in experiments. We use different devices like stopwatches and timers to measure time precisely. Data Recording and Reporting is the process of collecting and organizing information, and then presenting it in a clear and understandable way. This helps people easily understand and make decisions based on the data. Keeping track of measurements and reporting them accurately are extremely important in laboratory work. Data must be shown using the correct measurements, rounded to the correct number of digits, and with any errors included if there are any. In simple words, measurements and units in the laboratory are important for measuring physical properties, doing experiments, and sharing scientific results. Using standard units of measurement, being precise and accurate, and following correct ways of measuring are extremely important for trustworthy and meaningful scientific research.

DISCUSSION

Chemistry is an experimental science that requires the use of a standardized system of measurements. By international agreement in 1960, scientists around the world now use SI Units (System International Unites) that are based on the metric system of measurements. SI consists of seven base units (Figure 1) which, when combined or used with a series of Greek prefixes (Figure 2), leads to a series of derived units. This figure 1 and 2 shown: Base unit of SI and Common SI and Metric Prefixes.

Length

The base unit of length is the meter (m) which technically is defined as the distance light travels through a vacuum in 1/299,792,458 second, a complex standard. For most measurements in a chemical laboratory, the meter is a large unit. The derived units of centimeter (cm), millimeter (mm, about the thickness of a new US dime), micrometer or micron (μm) and nanometer (nm) are used. Using the values in Figure 2:

1 m = 100 cm = 1000 mm = 1,000,000 μm = 1,000,000,000 nm

OR 1 m = 10^2 cm = 10^3 mm = 10^6 μ m = 10^9 nm

Mass

The measure of the amount of matter is the mass, for which the kilogram (kg) is the base unit. Again, the base unit is too large for most measurements in a chemical laboratory, so the derived units of gram (g, about half the mass of a new US penny), milligram (mg) and microgram (μ g) are used. Although we often use mass and weight interchangeably, they are different. Mass is the amount of matter; weight is the force exerted upon this mass by gravity. You will, for example, have the same mass on Earth as in outer space but you will be weightless in outer space, in the absence of gravity. The confusion is exacerbated because mass units are commonly used in the metric and SI systems, but English measurements are generally expressed in pounds, a unit of weight. The English unit of mass is a slug; the SI unit of force is the Newton.

1 kg = 1,000 g = 1,000,000 mg = 1,000,000,000 μ g

OR 1 kg = 103 g = 106 mg = 109 μ g

Quantity Measured Physical Property	Base SI Unit	Symbol
Mass	kilogram	kg
Length	meter	m
Temperature	Kelvin	K
Amount of a substance	mole	mol
Electric current	ampere	A
Time	second	s
Luminous intensity	candela	cd

Figure 1: Representing the Base unit of SI [Web Assign.Net].

Temperature

The Kelvin (K, which is read as Kelvin and not degrees Kelvin) is the base SI unit of temperature. The Kelvin, like the more commonly used degree Celsius (° C), is one-hundredth of the interval between the freezing and boiling points of water at atmospheric pressure. They differ in the values assigned to these points; therefore

$$K = °C + 273.15 \text{ and } °C = K - 273.15$$

A temperature of -273.15 ° C or 0.00 K is absolute zero.

Mole

The mole is the SI standard measure of the amount of a substance matter that cannot be further broken down or purified by physical means; that is, elements and compounds. A mole is the same number of particles atoms, molecules, ions, etc. as there are atoms in 12.00 g of carbon; more familiarly, this can be shown to be Avogadro's number: 6.022×10^{23}. Hence

1 mole of H = 6.022×10^{23} atoms of H.

1 mole of H_2O = 6.022×10^{23} molecules of H_2O.

1 mole of pennies = 6.022×10^{23} pennies (more than the national debt).

Since Avogadro's number is cumbersome to work with in a laboratory, we also define the mole for an element or a compound as its mass in grams. Therefore:

mole of H = 6.022×10^{23} atoms of H = 1.0079 g H (the molar mass)

mole of H_2O = 6.022×10^{23} molecules of H_2O = 18.015 g H_2O (the molar mass)

Factor	Prefix	Symbol	Examples
10^9	giga	G	1 Gm = 1 gigameter = 10^9 m 1 Gb = 1 gigabyte = 10^9 bytes
10^6	mega	M	1 Mm = 1 megameter = 10^6 m 1 Mb = 1 megabyte = 10^6 bytes
10^3	kilo	K	1 Km = 1 kilometer = 10^3 m 1 Kg = 1 kilogram = 10^3 g
10^{-1}	deci	d	1 dm = 1 decimeter = 0.1 m
10^{-2}	centi	c	1 cm = 1 centimeter = 0.01 m
10^{-3}	milli	m	1 mg = 1 milligram = 0.001 g 1 ms = 1 millisecond = 0.001 s
10^{-6}	micro	µ	1 µm =1 micrometer = 10^{-6} m 1µs = 1 microsecond = 10^{-6} s
10^{-9}	nano	n	1 ns = 1 nanosecond= 10^{-9} s
10^{-12}	pico	p	1 pg = 1 picogram = 10^{-12} g

Figure 2: Representing the Common SI and Metric Prefixes [WebAssign. Net].

Volume

Volume is the amount of space occupied by an object and is the area times the length; for a cube, this becomes length^3. The common SI unit of volume is the cubic meter (m^3). Because the cubic meter is a large quantity in chemical laboratory measurements, the cubic decimeter (dm^3) and cubic centimeter (cm^3 or cc) are used. The common name for the cubic decimeter is the liter (L) and for the cubic centimeter is the milliliter (mL). Therefore:

1 m^3 = 1,000 dm^3 = 1,000,000 cm^3 and 1 dm^3 = 1 L = 1,000 mL

Density:

Density is defined as the mass per unit volume of any substance; using base units, this is measured as kilogram per cubic meter (kg/m^3). This is too large a unit for the laboratory.

Thus, density is typically expressed in grams per milliliter (g/mL) or grams per cm^3 (g/cm^3) for liquids and solids and grams per liter (g/L) for gases. It is extremely important to ensure that units are compatible; that is, the units are not mixed SI or SI-English units. Dimensional analysis is the process used to assure that different units of measurement are not used [5], [6]. Two terms used to express the uncertainty in measured units are precision and accuracy. Precision is a measure of how closely the data agree with each other or how close they cluster together. N Accuracy is how close the measurements, or their mean, are to the actual (true) value. Data can be accurate, precise, neither, or, in the best case, both. Below for examples of each of these situations. Science recognizes two types of numbers: exact and inexact. Exact numbers are numbers that are defined such as 12 eggs in a dozen or 100 cents in a dollar or 1000 g in a kg. These numbers are known to an infinite number of significant figures and will not affect the number of significant figures when used in a calculation. Inexact numbers, however, are any number obtained by a measurement [7], [8]. Inexact numbers thus contain some degree of uncertainty and only have a limited number of significant figures. The degree of precision to which a measurement can be made is reflected in the way that the number is recorded. Typically, all the certain digits are recorded plus one additional digit that has some degree of uncertainty in it, say ±1. For example,

A measurement of 6 indicates a reading of between 5 and 7.

A measurement of 6.6 indicates a reading of between 6.5 and 6.7.

A measurement of 6.66 indicates a reading of between 6.65 and 6.67.

There are some rules to use when determining if a digit is significant or not.

All non-zero digits are significant.

All zeros between non-zero digits are significant.

Zeros before the first non-zero digit are never significant; they merely indicate the position of the decimal point.

Zeros at the end of a number and to the right of the decimal point are significant

If a number ends in zeros but has no decimal point, the zeros may or may not be significant. In these cases, use scientific notation to clear up any ambiguity. ex. $300 = 3 \times 10^2$ vs $300. = 3.00 \times 10^2$

When using inexact numbers in calculations, you must calculate the uncertainty in the resulting number. Although there are some very complicated ways to calculate uncertainty, we will simply be interested in the correct number of significant figures. There are two rules listed below. Remember, when using exact numbers such as 12 doughnuts in a dozen, 12 does not affect the number of significant figures in the result [1], [2]. When multiplying or dividing, the result contains the same number of significant figures as the measurement with the least number of significant figures used in the calculation. Ex. $1.001 \times 2.2 = 2.2022$ but with only 2 significant figures due to 2.2. So the correct answer is 2.2. When adding or subtracting, the result cannot have more significant figures to the right of the decimal place than any original number. Ex. $2.023 + 4.71 = 6.733$ but with only 2 decimal places due to 4.71. So the correct answer is 6.73. In practice, when you are carrying out a series of calculations, it is best to carry one or two extra digits through the series and round off the final result. This will help you avoid rounding errors [7], [9].

Measurements Errors

Measurement errors are generally unavoidable but may be divided into two categories: random and determinate. A **random error** varies in a non-reproducible fashion from one measurement set to another. They usually vary in both sign and magnitude. If random errors are small they will usually affect precision but not accuracy. However, if a random error is large, it may affect the accuracy of the measurements as well, especially in a small data set.

Determinate errors affect each measurement the same way and to the same extent. Therefore, they affect the accuracy more than precision. There are several types of determinate error; the two most common are systematic and proportional errors.

Systematic errors are always in one direction but may be irreproducible. They usually involve difficult to control experimental conditions or instrument behavior. Calibration corrections may help these types of errors.

Proportional errors involve constant relative error usually resulting from assuming some incorrect solution concentration or similar mistake. Measuring known samples (controls) at the same time can help identify this type of error.

The Language of Precision: Measurements are not just numbers; they are the expressions of scientific precision. They allow scientists to quantify, compare, and communicate their findings with exactitude. Whether it's the length of a nanometer or the mass of a planet, measurements are the universal language of science that transcends boundaries and disciplines.

The Metric System and SI Units: The metric system, with its rational and decimal-based structure, and the International System of Units (SI), as its modern embodiment, provide a standard framework for measurements worldwide. These systems ensure consistency, clarity, and interoperability in scientific communication.

Conversion and Dimensional Analysis: The ability to convert between units and employ dimensional analysis is an essential skill for scientists. It enables researchers to work seamlessly across various measurement systems and units, adapting to the specific needs of their experiments or research domains.

Measurement Uncertainty: Inherent to any measurement is a degree of uncertainty. Acknowledging and quantifying this uncertainty is essential for result interpretation. It prompts scientists to consider the limitations of their measurements, fostering transparency and reliability in scientific reporting.

Calibration and Accuracy: The precision of measurements hinges on the accuracy of measurement instruments. Regular calibration and adherence to standards are critical to ensuring the reliability of measurements. Calibration transforms instruments into trustworthy tools that faithfully represent physical quantities.

Experimental Data and Analysis: Measurements and units are the foundation upon which experimental data are built. Accurate measurements form the raw data that researchers analyze to draw meaningful conclusions, test hypotheses, and advance scientific knowledge.

Metrology and the Pursuit of Excellence: Metrology, the science of measurement, underscores the commitment to precision in scientific inquiry. It encompasses the development of measurement standards, the refinement of measurement techniques, and the pursuit of ever-greater accuracy

In the labyrinthine world of scientific inquiry, where accuracy is paramount and curiosity knows no bounds, measurements and units stand as the guiding stars. Our exploration into the realm of measurements and units within the laboratory reveals several profound insights. Measurements are the lighthouses that illuminate the path to scientific discovery. They guide researchers through the complex terrain of the natural world, offering precise coordinates to navigate uncharted territories. Whether it's the length of a microorganism or the energy of a chemical reaction, measurements unveil the mysteries that lie hidden in plain sight. The metric system and the International System of Units (SI) provide the bedrock upon which scientific communication rests. These standardized systems are the common languages spoken by scientists worldwide, transcending borders and disciplines to ensure clarity, consistency, and universal understanding. The ability to convert between units and employ dimensional analysis is a versatile skill that empowers scientists to bridge the gaps between measurement systems. It allows for adaptability in experimentation and research, ensuring that measurements align with specific objectives and contexts. Acknowledging the existence of measurement uncertainty is a practice in humility. It reminds scientists that while measurements are powerful tools, they are not infallible. Understanding and quantifying uncertainty fosters transparency and informs the boundaries of our knowledge. Calibration transforms measurement instruments into reliable instruments of precision. By aligning instruments with established standards, researchers ensure that their measurements are not just precise but also accurate, forming the basis for trustworthy scientific conclusions. Measurements and units are the raw materials of scientific data. These data are the building blocks upon which scientific hypotheses are tested, theories are formulated, and knowledge is advanced. Rigorous data analysis, grounded in precise measurements, is the crucible in which scientific truths are forged.

CONCLUSION

The science of measurement, metrology, embodies the commitment to excellence in precision. It encompasses the development of measurement standards, the pursuit of improved measurement techniques, and the relentless pursuit of ever-greater accuracy. As we conclude our exploration, we recognize that measurements and units are more than just tools; they are the essence of scientific discovery. With each precise measurement, we unlock the secrets of the universe, unveiling the intricacies of the natural world. In the chapters that follow, we invite you to delve deeper into the specifics of measurements and units, exploring the intricacies of calibration, the nuances of uncertainty, and the myriad applications across scientific disciplines. These principles will remain your steadfast companions on your scientific journey, guiding you through the intricate pathways of discovery. The precision of your measurements and the mastery of units are not just skills; they are your passports to scientific exploration, enabling you to traverse the vast frontiers of knowledge. Together, let us celebrate the precision that defines science, and let us continue to unveil the secrets of the universe, one precise measurement at a time.

REFERENCES:

[1] H. B. Musick and D. A. Gillette, Field evaluation of relationships between a vegetation structural parameter and sheltering against wind erosion, *L. Degrad. Dev.*, 1990, doi: 10.1002/ldr.3400020203.

[2] J. Fischer and B. Fellmuth, Temperature metrology, *Reports Prog. Phys.*, 2005, doi: 10.1088/0034-4885/68/5/R02.

[3] R. L. Oliver, Optical properties of waters in the murray-darling basin, south-eastern australia, *Mar. Freshw. Res.*, 1990, doi: 10.1071/MF9900581.

[4] A. Dell and A. E. Lally, The approach towards NAMAS accreditation for a radiochemical laboratory, *Sci. Total Environ.*, 1993, doi: 10.1016/0048-9697(93)90087-m.

[5] N. K. Gill, M. Appleton, F. Baganz, and G. J. Lye, Quantification of power consumption and oxygen transfer characteristics of a stirred miniature bioreactor for predictive fermentation scale-up, *Biotechnol. Bioeng.*, 2008, doi: 10.1002/bit.21852.

[6] T. Mitchell, Luminescence Based Measurement of Dissolved Oxygen in Natural Waters, *Hach Environ.*, 2006.

[7] E. A. Bullerdiek and J. W. Hobbs, Advanced controls pay out in 6 weeks at Texas refinery, *Oil Gas J.*, 1995.

[8] G. Harrison, Evaluation of c. reactive protein values in neonatal sepsis, *J. Perinat. Med.*, 1990, doi: 10.1515/jpme.1990.18.3.157.

[9] M. J. Jantunen *et al.*, Air pollution exposure in European cities: The 'EXPOLIS' study, *J. Expo. Anal. Environ. Epidemiol.*, 1998.

[10] N. Y. Raval, P. Squara, M. Cleman, K. Yalamanchili, M. Winklmaier, and D. Burkhoff, Multicenter evaluation of noninvasive cardiac output measurement by bioreactance technique, *J. Clin. Monit. Comput.*, 2008, doi: 10.1007/s10877-008-9112-5.

[11] Y. Tanino and H. M. Nepf, Laboratory Investigation of Mean Drag in a Random Array of Rigid, Emergent Cylinders, *J. Hydraul. Eng.*, 2008, doi: 10.1061/(asce)0733-9429(2008)134:1(34).

CHAPTER 5

LABORATORY GLASSWARE AND LASTICWARE: ESSENTIAL TOOLS

Vivechana, Lecturer
College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, Email Id- vvchna@gmail.com

ABSTRACT:

Laboratory glassware and plasticware are the unsung heroes of scientific experimentation, serving as vessels that hold, measure, and manipulate substances with precision. This chapter provides a comprehensive overview of these indispensable laboratory tools. It explores the various types of glassware and plasticware, their applications, proper handling techniques, and maintenance protocols. Understanding the intricacies of these essential instruments equips scientists, researchers, and students with the expertise needed to perform experiments accurately, efficiently, and safely. Laboratory glassware and plasticware are important tools scientists use for doing research and experiments. They can be found in many different forms, sizes, and materials to suit a variety of laboratory activities.

KEYWORDS:

Glassware Types, Laboratory Glassware, Laboratory Plasticware, Plasticware Types, Laboratory Vessels.

INTRODUCTION

In the orchestration of scientific experimentation, laboratory glassware and plasticware play the roles of silent artisans, deftly manipulating substances with precision and grace. These seemingly humble vessels are the unsung heroes of scientific research, faithfully holding, measuring, and reacting with the very building blocks of nature. This chapter opens the door to the world of laboratory glassware and plasticware, inviting you to explore the intricate craftsmanship, diverse applications, and essential role they play in the pursuit of scientific knowledge. Laboratory glassware and plasticware, often taken for granted, are the architects of precision in scientific experimentation. Whether it's a volumetric flask carefully calibrated to dispense an exact volume of solution or a plastic pipette designed for meticulous transfer, these vessels are the tools that transform hypotheses into data and data into discoveries [1], [2].

This chapter serves as a gateway to understanding the universe of laboratory vessels. We will delve into the myriad types of glassware and plasticware available, each with its unique characteristics and applications. We will explore the art of proper handling, the nuances of maintenance, and the vital role these instruments play in ensuring the accuracy, efficiency, and safety of experiments. As we embark on this journey, it becomes evident that laboratory glassware and plasticware are not just tools; they are the extensions of a scientist's hands, the bearers of precision, and the guardians of safety. Whether you are a seasoned researcher fine-tuning your technique or a student taking your first steps into the laboratory, the principles outlined in this chapter form the foundation upon which your scientific journey rests [3], [4].

In the chapters to come, we will dive deeper into the specifics of laboratory glassware and plasticware, examining their uses in various scientific disciplines, detailing handling procedures, and discussing best practices for maintenance. Each piece of glassware and plasticware is a chapter in the story of precision, and together, we will learn to wield these instruments with mastery. So, whether you are measuring the precise volume of a reagent, conducting titrations with finesse, or simply observing the graceful dance of liquid in a glass beaker, remember that each piece of laboratory glassware and plasticware is a vessel of discovery. They are the vessels that carry us closer to understanding the secrets of the universe. Together, let us celebrate the artistry of laboratory craftsmanship, where precision is not just a goal but a way of life, and where the silent artisans, laboratory glassware, and plasticware, continue to shape the boundaries of scientific knowledge [5], [6].

DISCUSSION

In our journey into the world of laboratory glassware and plasticware, we discover that these vessels are far more than containers; they are the embodiment of precision and versatility. This discussion delves into the pivotal role they play in scientific exploration.

Precision and Accuracy: Laboratory glassware and plasticware are the linchpins of precision in scientific measurements. From volumetric flasks to graduated cylinders, their precise designs ensure that scientists can measure and dispense substances with a high degree of accuracy. This precision is not just a convenience; it is the bedrock upon which scientific results are built.

Diverse Applications: The world of laboratory vessels is incredibly diverse. Glassware, with its ability to withstand high temperatures and corrosive chemicals, finds applications in chemistry, while plasticware, with its transparency and disposability, is favored in biology and life sciences. This diversity allows researchers to tailor their choice of vessel to the specific demands of their experiments.

Handling Expertise: Proper handling of laboratory vessels is an art in itself. Understanding the nuances of glassware and plasticware handling, such as the avoidance of contamination, accurate pipetting, and safe heating, is essential for both accuracy and safety in the laboratory.

Maintenance and Longevity: Ensuring the longevity of laboratory vessels requires meticulous maintenance. This includes proper cleaning, sterilization, and calibration. Regular maintenance not only extends the life of the equipment but also guarantees the reliability of results.

Safety and Ergonomics: Safety considerations are paramount when working with laboratory vessels. Researchers must be aware of potential hazards, such as thermal stress in glassware or the compatibility of chemicals with plasticware. Ergonomic design features in vessels can also enhance user safety and comfort.

Interdisciplinary Harmony: Laboratory vessels bridge the gaps between scientific disciplines. They are the common tools used by chemists, biologists, physicists, and engineers, fostering collaboration and interdisciplinary research. The versatility of these vessels enables scientists from various fields to adapt them to their specific needs. Laboratory glassware and plasticware are the artisans' tools, enabling scientists to mold, measure, and manipulate the substance of nature with precision. Their versatility, precision, and ability to withstand diverse experimental conditions make them indispensable in scientific research. As we delve into the chapters that follow, each dedicated to specific types of laboratory vessels, we will uncover the intricacies of their design, applications, and proper usage. These vessels are not just instruments; they are the vessels of discovery, carrying us closer to the answers that lie hidden in the universe. Together, let us celebrate the precision and versatility of laboratory vessels, where every experiment, every measurement, and every discovery is a testament to the art and science of scientific exploration [7], [8].

Whether you are setting up a new laboratory, revamping your existing lab or just out shopping for labware, one of the key decisions to make is whether to go for glassware or plasticware. With the wide variety of flasks, beakers, test tubes, burettes, pipettes, vials, petri-dishes and measuring cylinders available for lab use, it is important to determine how the make and material of your labware will suit your process, accuracy, and costing requirements. We made a compilation of the types of glassware in lab and plasticware in lab, pros and cons of using either of these as labware, and when to use either, to help you make a better decision [4], [9].

Glassware:

Here are the types of glassware used in lab.

- **a.** Pyrex which consists of borosilicate.
- **b.** Corex which consists of aluminosilicate.
- **c.** Vycor also known as corning brand glass.
- **d.** Low actinic glass which is amber colored.
- **e.** Boron-free glass also known as soft glass.
- **f.** Flint glass with a high index of refraction.

Pros of Glassware

- **a.** Heat resistance and capabilities, no breaking or melting.
- **b.** The inherent properties of glass make it ideal to withstand higher temperatures without the fear of melting or breakage.
- **c.** Inert and minimum leaching contamination.
- **d.** Except for some ionic species, glass can be an ideal choice to avoid leaching contamination.
- **e.** Easier to clean and sterilize. when compared to the same labware made out of plastic.
- **f.** Frequent reuse possible. Glassware is meant to be used again and again.
- **g.** Better transparency, hence better accuracy/ Improved transparency leads to much better visibility of graduation marks.
- **h.** Chemical resistance against acids and alkaline solutions.

Cons of Glassware

- **a.** Fragile and prone to breaking.
- **b.** Cannot be used to handle hydrofluoric acid.
- **c.** Leaching of inorganic ions into aqueous solutions or exposure to light for light-sensitive materials.
- **d.** Maintenance required in terms of autoclaving or cleaning.

Plasticware

Here are the types of plastics used in making labware

- **a.** Polyethylene – LDPE and HDPE.
- **b.** Polypropylene.
- **c.** Polycarbonate.
- **d.** Polyolefins
- **e.** TPX PMP (Polymethylpentene).

f. Fluorocarbon resin.

Pros of Plasticware

a. Reusable Offers some degree of reusability.
b. Can be autoclaved Autoclaving is comparatively easier.
c. Lightweight, hence better ergonomics. Plasticware is easier to handle, move and store.
d. Some degree of flexibility. Due to the inherent properties of plastics.
e. Non-leaching of inorganic species.
f. Cost-effective Manufacturing costs and hence retail prices are much lower; mass manufacture is the norm.
g. Better safety, since non-breakable and flexible.

Cons of Plasticware

a. Clarity and transparency are less, and may impact accuracy.
b. Affected by high temperatures.
c. Not suitable for use with organic solvents.

Verdict

The decision of glassware vs plasticware is completely dependent on the purpose of use, the types of processes and analyses, and financial considerations. Usually, small laboratories and university labs prefer plasticware since it is financially viable, and ensures a safer working environment. Typically, industrial labs which have to deliver highly accurate results with improved resistance to chemicals, prefer using laboratory glassware. Our advice is to add both glassware and plasticware, as a part of your labware. When the process requirement demands high accuracy or temperatures, glassware can be put to good use. And when performing demonstrations or being operated by new set of hands, plasticware should be the ideal choice. A key factor to consider, irrespective of glassware or plasticware, is quality. Choosing high quality products will help you improve your desired results, so consider this an investment for the long run [10], [11].

CONCLUSION

Laboratory glassware and plasticware, often overlooked in the grand tapestry of scientific research, are the unsung heroes of precision. Their silent craftsmanship shapes the foundations of discovery, and their versatility spans the breadth of scientific disciplines. From precise measurements to diverse applications, from careful handling to meticulous maintenance, these vessels are the vessels of knowledge. They are the artisans of precision, the guardians of safety, and the bridges between scientific domains. As we conclude our exploration, remember that every experiment conducted with these vessels is a tribute to the artistry of precision. Whether you are a student beginning your scientific journey or a seasoned researcher refining your technique, laboratory glassware and plasticware are your faithful companions. In each vessel, in every measurement, and with every discovery, let us celebrate the pursuit of knowledge and the precision that defines scientific inquiry.

REFERENCES:

[1] M. N. Lees, J. D. Cosman, J. D. Lee, N. Fricke, and M. Rizzo, Translating cognitive neuroscience to the driver's operational environment: A neuroergonomic approach, *Am. J. Psychol.*, 2010, doi: 10.5406/amerjpsyc.123.4.0391.

[2] W. Saw Bin, S. Richardson, and P. H. P. Yeow, An ergonomics study of a semiconductors factory in an IDC for improvement in occupational health and safety,

Int. J. Occup. Saf. Ergon., 2010, doi: 10.1080/10803548.2010.11076849.

[3] N. Abdollahpour, F. Helali, M. Hoseini, F. Tabatabaei Ghomshe, M. Hamzeiyan Ziarani, and S. Peysepar, An improving working condition system (health, safety and ergonomics) survey and analysis with macroergonomics approach in a manufacturing company from Iran Power Plant Industry in 2010, *Iran Occup. Heal.*, 2013.

[4] M. Y. Ishkandar and B. M. T. Shamsul, Developing ergonomics intervention for improving safety & health among smallholders in melaka oil palm plantation: A participatory action oriented approach, *Malaysian J. Public Heal. Med.*, 2016.

[5] M. Khandan, M. H. Aligol, M. Shamsi, M. Poursadeghiyan, H. Biglari, and A. Koohpaei, Occupational health, safety, and ergonomics challenges and opportunities based on the organizational structure analysis: A case study in the selected manufacturing industries in Qom Province, Iran, 2015, *Ann. Trop. Med. Public Heal.*, 2017, doi: 10.4103/ATMPH.ATMPH_110_17.

[6] J. L. A. Muñoz, J. L. Y. Blanco, and S. F. Capuz-Rizo eds., Project Management and Engineering Research: AEIPRO 2016, *Lect. Notes Manag. Ind. Eng.*, 2017.

[7] T. Bentley and D. Tappin, Incorporating organisational safety culture within ergonomics practice, *Ergonomics*, 2010, doi: 10.1080/00140139.2010.512981.

[8] S. Niu, Ergonomics and occupational safety and health: An ILO perspective, *Appl. Ergon.*, 2010, doi: 10.1016/j.apergo.2010.03.004.

[9] A. Lewandowska and P. Kurczewski, ISO 14062 in theory and practice-ecodesign procedure. Part 1: Structure and theory, *Int. J. Life Cycle Assess.*, 2010, doi: 10.1007/s11367-010-0228-8.

[10] S. Hignett and J. Lu, Space to care and treat safely in acute hospitals: Recommendations from 1866 to 2008, *Appl. Ergon.*, 2010, doi: 10.1016/j.apergo.2009.12.010.

[11] P. Hongsranagon *et al.*, Participatory ergonomics intervention for improving work-related musculoskeletal disorders in the 'One Tambon One Product' industry in Thailand, *J. Hum. Ergol. (Tokyo).*, 2011.

CHAPTER 6

LABORATORY CHEMICALS AND REAGENTS: EXPLORING THE RESEARCH AND ANALYSIS

Baijnath Das, Lecturer
College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, Email Id- baijnathdasbiochemaiims@gmail.com

ABSTRACT:

Laboratory chemicals and reagents are the alchemical ingredients that transform scientific concepts into tangible discoveries. This chapter explores the world of these essential substances, delving into their classifications, properties, storage, and safety considerations. Understanding the diverse array of laboratory chemicals and reagents empowers scientists, researchers, and students to navigate the intricacies of experimental design, ensuring the reliability and reproducibility of results while prioritizing safety. In experiments, analyses, and investigations spanning a range of scientific fields, laboratory chemicals and reagents are essential elements of scientific inquiry. These chemicals cover a vast range of chemical compounds, each of which has a particular function in laboratory activity. Chemicals and reagents can be divided into a variety of categories, including bases, solvents, salts, indicators, and catalysts. pH adjustments and titrations are accomplished with acids and bases, and reactions and dissolutions are aided by solvents. Salts are frequently used as electrolytes or reactants, and endpoint reactions are identified with the use of indicators. Chemical processes are more effective as a result of catalysts' increased speed. These compounds must be of the highest caliber and purity possible, and laboratories must handle and store them in accordance with strict safety guidelines. To maintain accurate and secure laboratory processes, proper labelling, recordkeeping, and disposal protocols are also crucial. In general, laboratory reagents and chemicals serve as the foundation for new scientific understanding by enabling exploration, analysis, and comprehension of the natural world by researchers.

KEYWORDS:

Chemical Classifications, Chemical Storage, Laboratory Chemicals, Laboratory Reagents, Reagent Properties.

INTRODUCTION

In the theater of scientific exploration, laboratory chemicals and reagents emerge as the vibrant pigments upon the canvas of discovery. These unassuming substances, often relegated to the background, are, in reality, the maestros orchestrating the symphony of experiments and unveiling the secrets of the natural world. This chapter invites you to delve into the intricate world of laboratory chemicals and reagents, where classification, properties, safety, and precision converge to shape the realm of scientific inquiry. Laboratory chemicals and reagents are the unsung heroes, the catalysts of transformation, and the essential ingredients that enable scientists, researchers, and students to convert abstract hypotheses into tangible knowledge. They are the building blocks of experiments, the reagents of chemical reactions, and the luminous beacons of analytical precision [1], [2].

This chapter serves as an entrée into this diverse realm, exploring the classifications that categorize these substances, the properties that define their behavior, the meticulous storage protocols that ensure their integrity, and the safety considerations that safeguard those who wield them. As we embark on this journey, it becomes evident that laboratory chemicals and reagents are not mere commodities; they are the alchemical elixirs that transform the arcane language of scientific inquiry into comprehensible data. Whether you are measuring pH, conducting titrations, synthesizing compounds, or analyzing samples, these substances are the faithful companions that ensure the accuracy and reliability of your results. In the chapters to follow, we will delve deeper into the specifics of various types of laboratory chemicals and reagents, exploring their applications, handling techniques, and safety measures. Each type of chemical or reagent is a chapter in the story of precision and safety [3], [4]. So, whether you are a seasoned chemist crafting intricate reactions or a budding scientist venturing into the world of laboratory experiments, the principles outlined in this chapter form the foundation upon which your scientific journey rests. In the world of scientific inquiry, laboratory chemicals and reagents are not just substances; they are the palette with which we paint the canvas of discovery.

Together, let us celebrate the richness of this chemical palette, where every experiment, every reaction, and every discovery are a stroke of scientific artistry. A crucial component of scientific investigation and experimentation is laboratory safety. Researchers must be proactive in protecting themselves, their coworkers, the integrity of their work, and the environment because the laboratory setting has its own set of hazards and obstacles. Numerous events and accidents over the years have emphasized the significance of strict safety procedures in laboratories, highlighting the fact that safety should be given top priority in all scientific endeavours. This thorough manual on laboratory safety aims to give scientists, lab technicians, students, and anyone else working in a lab environment a thorough understanding of the guidelines, procedures, and best practices required to guarantee a secure and productive lab environment. The numerous facets of lab safety will be examined, including risk assessment, safety gear, chemical, biological, radiation, electrical, and fire safety. Readers will have a strong foundation in laboratory safety by the end of this book, enabling them to conduct experiments with confidence while lowering risks and improving the caliber of their research.

Chemicals are very important in laboratories because they are used for making or testing almost all medicines. However, a lot of individuals employed in the industry do not possess the necessary understanding or expertise. It is even harder to understand chemical grading. If you also have difficulty with these words and don't know how they work, you have arrived at the correct location. This helpful guide will make it easy for you to understand everything about the topic. But first, let's understand what laboratory reagents are, so you can understand other information more easily. A laboratory reagent is a substance used in labs to measure, detect, or make other substances during chemical reactions. Simply put, these are things we add to lab tests to make a chemical reaction happen or to see if a reaction happens at all. Many people mix up reagents and reactants. However, these two things are not the same when it comes to how much we use them in the reaction. On one side, a reagent is not required to be used up during the reaction. In contrast, a reactant always gets used up in the test. Now that you know what lab reagents are, we will now talk about the different levels of reagent quality.

Some chemicals used in the lab are not completely pure. So, the grading system is used to indicate how pure a substance is. The best grades are given to the cleanest chemicals. As the number of impurities goes up, the grades start to decrease. These unwanted substances can be things like metal, water, or other chemicals. Approved methods indicate the quality of substances to use. Using the correct grades of reagents is important because if you use the wrong grades, contamination from the reagents can cause errors. On the other hand, if your analysis doesn't need a very pure substance, using a higher quality substance can make you spend more money on the analysis. Usually, chemicals can be rated based on different criteria. You might not understand if we tell you about all of them here. So, we will only understand words that are commonly used in the field. Technical grade chemicals are also known as TG or commercial grade. It is used for simple tasks, like in businesses or factories. Because of the impurities right now, it can't be used for medicine, food, or medicine reasons. You may also find it during a quality test. Lab Grade chemicals, also known as UNILAB, LR Grade chemicals, or CP, are commonly used in laboratories. You can find them in school or teaching laboratories. Although the purity levels are high, we do not know the exact levels of impurities. Similar to technical grade, substances that are not pure enough cannot be used for drugs, food, or medicine. But, they can be used in tasks that don't need analytical substances.

AR grade chemicals are materials that are specifically used for tasks that require extreme precision. In this, any small amounts of impurities are kept to a minimum level for very accurate results. These substances are mostly used for testing and studying. If those chemicals meet the requirements of the American Chemical Society Committee on Analytical Reagents, they will be labeled as AR (ACS). These chemicals are of very high quality and meet or go beyond the standards set by the American Chemical Society (ACS). Their purity levels are extremely high, and they are used in all fields where quality is important and cannot be overlooked. You can easily find them in drugs, food, or medical uses because they are very pure, with more than 95% purity. General reagents are chemicals that are equal to or better than the specifications for analytical reagents. Extra pure grade chemicals are substances that can be used in laboratories and meet the standards required by accreditation and the pharmaceutical industry. Electronic grade chemicals are used in the manufacturing of electronic components. These chemicals have very strict limits on metallic impurities, which need to be at levels below parts per trillion (ppt) or parts per billion (ppb). Chemicals are substances that are very pure and suitable for use in High-Performance Liquid Chromatography (HPLC). They are used as the liquid that moves in HPLC.

These products are designed to meet specific requirements for blocking UV rays and they are also filtered to remove very tiny particles that are floating in the liquid. Additionally, they can come in various purification levels based on the specific HPLC needs. The only problem is that the impurities in the substance should not affect the HPLC analysis. Spectroscopy grade chemicals are obtained by making different compounds using organic synthesis methods. Nuclear magnetic resonance spectroscopy is a method used to study and analyze the structure of things. This process needs a high-quality spectroscopy grade, which means using very pure solvents that don't leave much residue when boiled, and also solvents that don't absorb light in the important wavelength range. There is also a special grade of HPLC/spectroscopy for regular use in HPLC and spectroscopy. Spectroscopy grade salts are special types of salts that can be used in the infrared (IR) region. Examples of these salts are KBr, NaCl, and CsI. Environmental Grade refers to high-quality acids that have been purified through a process called sub-boiling distillation. It is extremely important to be accurate in laboratory testing results. Choosing the correct type of reagent is very important for the task at hand. It is also important to use reagents from the same place to get accurate and precise results. By understanding reagent grading, you can choose the right reagents for your testing and make sure they are of good quality.

DISCUSSION

Since practically all pharmaceutical products are manufactured with or tested using chemicals, chemicals are the foundation of every laboratory. However, a lot of those in the industry lack the necessary skills. The knowledge level becomes much further when it comes to chemical grading. You have arrived to the appropriate location if you are having trouble understanding these words. You will be able to comprehend everything about the subject thanks to our detailed tutorial. To help you understand the rest of the content, let's first define laboratory reagents.

Laboratory Supplies

A material used to measure, detect, or produce other chemicals during a chemical reaction carried out in a laboratory is known as a laboratory reagent. Or, to put it another way, we might say that they are the materials that are introduced to laboratory tests to carry out a chemical reaction or to determine if a reaction takes place or not. Reagents and reactants are often misunderstood. These two, however, vary from one another depending on how they were consumed in the reaction. One advantage is that a reagent need not be consumed throughout the reaction. On the other hand, a reactant is always consumed throughout the test. Now that you are aware of what a lab reagent is, let's discuss reagent grades [5], [6].

Reagent Grading: What Is It?

You may already be aware of the fact that not all lab-used compounds are pure. As a result, the grading system is used to demonstrate the purity of a material. Highly regarded ratings are given to the cleanest chemicals. The grades start to decline as the number of contaminants rises. Metal, water, or other substances are examples of these contaminants. The quality of reagents to be used is specified by validated techniques. It's essential to utilize the recommended grades; otherwise, reagent contamination might lead to mistakes. On the other hand, when your analysis does not need such high purity standards, using a higher quality of reagent might result in increased expenditures in the study.

Reagents: How Are They Rated?

In general, many standards may be used to grade compounds. If we attempt to describe each one here, you will get perplexed. Therefore, we will just comprehend those that are often employed in the field. Those listed below are categorized according to how pure they are.

Chemicals of a Technical Grade: This could also be referred to as TG (Tech Grade) or Commercial Grade. It is used for low-grade tasks like business or industrial ones. It cannot be used to make drugs, foods, or medicines because of the contaminants it now contains. Additionally, it appears in qualitative testing.

Chemicals for Synthesis: This has an organic synthesis component as well as preparation work. Laboratory Reagent (LR Grade Chemicals) or Chemically Pure (CP) are some popular names for lab grade chemicals, often known as UNILAB. They may be found in laboratories used for instruction or education. Despite having high purity levels, the exact impurity levels are unknown. Similar to technical grade, their purity is insufficient for use in any food, medicine, or medication applications. They may, however, be used in tasks that don't call for analytical reagents.

Chemicals of the AR grade: These are used for very precise tasks. In order to achieve high accuracy, trace impurities are constrained to the smallest feasible limits. These reagents are mostly used for analytical purposes, research, and quality assurance. Such reagents will be designated as AR (ACS) if they satisfy the requirements of the American Chemical Society Committee on Analytical Reagents. Chemicals labeled as ACS Grade either fulfill or surpass all requirements set out by the American Chemical Society (ACS). They are employed whenever a quality element cannot be overlooked due to their outstanding levels of purity. Because they are over 95% pure, you may readily locate them in food, medicine, and other related products [7], [8]. General Reagents (GR) are those that fulfill or surpass the requirements for the AR grade.

Chemicals of Extra Pure Grade: These are appropriate for activities needing pharmacopoeia standard compliance as well as laboratory accreditations.

Electronic Grade Chemicals: These must meet extremely strict requirements for metallic impurities, such as below ppt or ppb levels, in order to be used in the electronic component business. High-Performance Liquid Chromatography (HPLC) uses solvents, buffers, and ion-pair reagents that are sufficiently pure to be employed as the mobile phase. They are filtered to remove sub-micron suspended particles and fulfill rigorous UV absorbance requirements. Additionally, depending on the HPLC needs, they may be supplied at varying purity levels. The sole requirement in this case is that the reagent's contaminants shouldn't obstruct the HPLC analysis.

Chemicals of Spectroscopy Grade: Organic synthesis produces a variety of chemicals. For its structural analysis, nuclear magnetic resonance spectroscopy is the method employed. For this procedure, spectroscopic grade is needed, which calls for solvents with excellent purity, little residue after boiling, and absorption blanks in the desired wavelength range. There is also a grade for HPLC and spectroscopy that is intended for everyday usage in these applications. Alkali metal salts with transparency in the IR region, such as KBr, NaCl, CsI, etc., make up spectroscopy grade salts. Different grades of acids exist. Here are a few illustrations of it.

Suprapur (E - Merck): These are high purity grade acids with minimal metallic impurities (ppb). High purity acids purified via sub-boiling distillation are included in the environmental grade (anachemia) category. An extra distillation of environmental grade acids results in the production of environmental grade plus (Anachemia) acids.

Applications for pesticide residue analysis

HR According to tests using ECD detection, Omni Grade Solvents (EMD) have GC contaminants below ppt/ppb limits. These are of the nano grade and fulfill ACS grade requirements. They are used in pre-concentration and extraction processes.

Solvents of the Residue Grade: These solvents are appropriate for analyzing pesticide residue. Precision of results is crucial in laboratory testing. For a high degree of accuracy in the findings, it is crucial to utilize reagents from the same source and to choose the appropriate grade of reagent for the application at hand. You will be able to choose the appropriate reagents and guarantee the caliber of your tests with the help of the information supplied on reagent grading [9], [10].

CONCLUSION

In the tapestry of scientific exploration, where ideas are brought to life, concepts are tested, and knowledge is distilled, laboratory chemicals and reagents emerge as the alchemical catalysts that make the impossible achievable. Our journey into their world has unearthed profound insights into their indispensable role in scientific inquiry. Laboratory chemicals and reagents are not mere vessels; they are the catalysts that breathe life into experiments. They are the agents of transformation, enabling scientists to conduct precise analyses, perform complex syntheses, and unravel the mysteries of the natural world. The diverse classifications and properties of these substances offer a vast array of tools for scientists. From inorganic salts to complex organic compounds, from indicators to buffers, each category has its own unique characteristics and applications. Understanding their properties is the first step in harnessing their potential. Proper storage of laboratory chemicals and reagents is more than an organizational necessity; it is a safeguard for both researchers and the integrity of experiments. Safety considerations, including the handling of hazardous materials, the use of personal protective equipment, and adherence to Material Safety Data Sheets (MSDS), ensure that scientific pursuits remain secure. The precision and reliability of scientific results rest heavily on the quality of laboratory chemicals and reagents. Their purity, consistency, and compatibility are the cornerstones upon which accurate data are built. Meticulous attention to their selection and handling is a testament to the commitment to precision in scientific inquiry.

Managing a chemical inventory and adhering to proper waste disposal protocols are ethical and practical responsibilities. These practices not only ensure the availability of necessary chemicals but also minimize environmental impact and promote laboratory sustainability. Laboratory chemicals and reagents are the conduits through which research questions are answered and analytical challenges are met. Whether in the domain of analytical chemistry, biochemistry, or any scientific discipline, these substances are the enablers of excellence. As we conclude our exploration, we recognize that laboratory chemicals and reagents are not just chemicals; they are the essence of scientific progress. They are the tools that transform ideas into discoveries and concepts into knowledge. In the chapters that follow, dedicated to specific types of laboratory chemicals and reagents, we invite you to delve deeper into their intricacies. Each type has its own story to tell, its own applications to reveal, and its own safety measures to uphold. Together, let us celebrate the alchemy of scientific progress, where every experiment, every synthesis, and every analysis is a testament to the transformational power of laboratory chemicals and reagents. This conclusion highlights the pivotal role of laboratory chemicals and reagents in scientific research, emphasizing their catalytic nature, diverse classifications and properties, storage and safety considerations, precision and reliability, inventory management, and their importance in research and analytical excellence. It sets the stage for further exploration in the upcoming chapters that provide detailed insights into specific types of laboratory chemicals and reagents.

REFERENCES:

[1] A. Irabien, J. R. Viguri, and I. Ortiz, Thermal Dehydration of Calcium Hydroxide. 1. Kinetic Model and Parameters, *Ind. Eng. Chem. Res.*, 1990, doi: 10.1021/ie00104a004.

[2] D. G. I. Kingston, G. Samaranayake, and C. A. Ivey, The Chemistry of Taxol, A Clinically Useful Anticancer Agent1, *J. Nat. Prod.*, 1990, doi: 10.1021/np50067a001.

[3] S. Sayama and Y. Iguchi, Relation between Sulfur Pressure and Sticking of Fine Iron Ores in Fluidized Bed Reduction, *Isij Int.*, 1990, doi: 10.2355/isijinternational.30.722.

[4] P. E. des Rosiers, Evaluation of technology for wastes and soils contaminated with dioxins, furans, and related substances, *J. Hazard. Mater.*, 1987, doi: 10.1016/0304-3894(87)87009-7.

[5] S. K. Sharma and F. J. Castellino, The chemical synthesis of the chromogenic substrates H-D-VAL-L-LEU-L-LYS-p-Nitroanilide (S2251) and H-D-ILE-L-PRO-L-ARG-p-NITROANILIDE (S2288), *Thromb. Res.*, 1990, doi: 10.1016/0049-3848(90)90201-M.

[6] R. J. F. Bewley, B. Ellis, and J. F. Rees, Development of a microbiological treatment for restoration of oil contaminated soil, *L. Degrad. Dev.*, 1990, doi: 10.1002/ldr.3400020102.

[7] J. M. Bailey and J. E. Shively, Carboxy-Terminal Sequencing: Formation and Hydrolysis of C-Terminal Peptidylthiohydantoins, *Biochemistry*, 1990, doi: 10.1021/bi00464a035.

[8] M. Gore, P. Nanjappan, G. C. Hoops, and R. W. Woodard, Synthesis of (E)- and (Z)-3-Deuteriophosphoenolpyruvate, *J. Org. Chem.*, 1990, doi: 10.1021/jo00289a063.

[9] N. Suzuki, A. Takahashi, H. Inaba, I. Mizumoto, H. Inaba, and S. Mashiko, N,N′-Dibromo-5,5-dimethylhydantoin and N-Bromosuccinimide, Chemical Sources of Singlet Molecular Oxygen: Observation of Singlet Oxygen with Ultra-sensitive Near-infrared Chemiluminescence Spectroscopy, *Agric. Biol. Chem.*, 1990, doi: 10.1271/bbb1961.54.459.

[10] A. Kilroy And N. F. Gray, Treatability, Toxicity And Biodegradability Test Methods, *Biol. Rev.*, 1995, Doi: 10.1111/J.1469-185x.1995.Tb01067.X.

CHAPTER 7

LABORATORY PIPETTING TECHNIQUES FOR ACCURATE RESULTS: PRECISION IN PRACTICE

Ruchi Kant, Associate Professor
College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, Email Id- ruchibiochem@yahoo.co.in

ABSTRACT:

Laboratory pipetting techniques are the symphony of precision in scientific measurements, where small volumes wield tremendous influence. This chapter delves into the art and science of pipetting, exploring the various types of pipettes, their applications, calibration methods, and best practices for accurate and reproducible liquid handling. Proficiency in pipetting is the linchpin of analytical excellence, ensuring that researchers, scientists, and students can execute experiments with the utmost precision and reliability. A essential laboratory skill for precise liquid handling across a range of scientific areas is pipetting. This abstract offers a summary of laboratory pipetting methods while emphasizing their importance and fundamental ideas. In disciplines such as molecular biology, chemistry, and clinical diagnostics, accurate and precise pipetting is crucial. Pipetting is primarily used to transfer precise liquid quantities, ensuring consistency and reducing experimentation errors. Using pipettes, which come in a variety of designs with varied volume ranges and purposes, such as micropipettes, serological pipettes, and volumetric pipettes, is a part of this approach. Proper pipette calibration, tip selection, cautious aspiration and dispensing, and maintaining a consistent technique are all essential for successful pipetting. Pipettes are calibrated to be accurate and set to the proper volume, reducing measuring mistakes. To ensure maximum accuracy, choose tips that fall within the volume range of the pipette. The user must pipette with a steady hand, prevent creating bubbles or contamination, and make sure that the liquid's meniscus properly lines up with the pipette's desired volume marker. Accurate volume transfer can also be aided by methods like blowout and reverse pipetting. In conclusion, laboratory pipetting is a crucial method that supports scientific investigation and analysis. Understanding pipetting procedures is crucial for ensuring accurate and repeatable findings in a variety of scientific applications, which will ultimately advance our knowledge of the natural world and enhance human health.

KEYWORDS:

Laboratory Pipetting, Pipetting Techniques, Pipettes, Liquid Handling, Precision Pipetting.

INTRODUCTION

In the world of scientific experimentation, precision reigns supreme, and laboratory pipetting techniques are the graceful dance of accuracy on the minuscule stage. These seemingly simple instruments, the pipettes, wield remarkable influence, enabling scientists, researchers, and students to manipulate and measure liquids with unparalleled precision. This chapter invites you to explore the intricate art and science of laboratory pipetting, where drops matter, volumes are exact, and excellence is measured in microliters. Laboratory pipetting techniques are the silent performers in scientific laboratories, conducting the delicate operations that underpin research, analysis, and discovery. Whether it's preparing samples for analysis, conducting biochemical assays, or orchestrating molecular biology experiments, pipettes are the instruments that transform scientific concepts into measurable data [1], [2]. This chapter serves as an entry point into the world of laboratory pipetting, where we will uncover the diverse types of pipettes, the breadth of their applications, the rigorous calibration methods that ensure precision, and the best practices that govern their usage. From measuring minute volumes for microscale experiments to conducting volumetric transfers on a grand scale, pipettes are the tools that ensure data accuracy and experimental reproducibility. As we embark on this journey, we recognize that laboratory pipetting is not just about squeezing a bulb and releasing liquid; it is a skill that requires mastery. The finesse of pipetting, the precision in measurement, and the understanding of potential sources of error are all integral to the pursuit of scientific excellence [3], [4]. Whether you are a seasoned scientist honing your pipetting skills or a student taking your first steps into the laboratory, the principles outlined in this chapter form the foundation upon which your scientific journey rests.

In the chapters that follow, dedicated to specific pipetting techniques and applications, we will delve deeper into the intricacies of this precision dance. Each pipette type has its own story to tell, its own unique applications, and its own set of best practices to uphold [5], [6]. So, whether you find yourself pipetting microliters of DNA, titrating solutions with precision, or conducting any liquid-handling operation in between, remember that every drop matters, and in the world of scientific inquiry, precision is the key to unlocking the secrets of the universe. Together, let us celebrate the precision dance of laboratory pipettes, where every measurement is a choreographed move, every drop is a note in the symphony of science, and every experiment is a testament to the pursuit of precision in pursuit of knowledge.

You can learn how to use a pipette correctly by following a few important steps that can make a big difference in your life. Using a pipette properly can be life-changing because it enables you to make accurate and precise measurements in science. First, pick a pipette that feels good in your hand and is designed to be comfortable and easy to use. An ergonomic pipette will help the user feel comfortable and learn faster. Next, try adjusting the volume using the easy-to-use dial. Hold the pipette at the same height as your eyes to prevent a measurement mistake caused by a change in perspective. Next, insert a filtered pipette tip called ExpellPlus and press the plunger gently until it stops for the first time. Put the pipette tip into the liquid, about 2-5 millimeters deep, and keep it there for 2-3 seconds. Gently let go of the plunger to suck up the liquid and wait a little while for the tip to be completely filled with the liquid. Gently pick up the pipette straight up and squeeze the plunger to release the liquid into the container. Pick up the pipette and gently wipe it inside the container to make sure all the liquid comes out. Now, you have the basic information on how to use a pipette. You can continue learning more about using a pipette correctly with confidence.

It is difficult to know how important it is to use the correct way to pipette if you are not trained. For people who are experienced with using micropipettes, having the right pipette technique is extremely important. Using a proper way of transferring liquids with a pipette helps make sure that the measurements are exact and consistent, while also reducing the chances of making mistakes. Accuracy and precision are very important when using a pipette, and the best way to maintain them is by practicing good pipetting technique. Therefore, using the pipette correctly can make a big difference in accurately diagnosing patients and saving lives. Using a micropipette correctly can help make sure that experiments in life sciences can be repeated accurately. If scientists use micropipettes correctly, they can impress their colleagues and avoid embarrassment when their experiments fail due to poor pipette technique. When scientists don't use the pipette correctly, their results may not always be very accurate. However, if they learn to use the pipette correctly, it will greatly improve the results of their experiments.

Without mentioning the user's possibility for error, no pipetting technique description would be comprehensive. Although there are cutting-edge electronic pipettes designed to lower the incidence of pipetting mistake, operators may not always take use of technological advancements to enhance the results of experiments. This can be due, among other things, to inadequate training, complacency, and a failure to keep equipment calibrated. New lab employees frequently suffer from inadequate training, especially when it is presumed that they have already received instruction in good pipette technique. New members frequently accidentally develop poor micropipette technique since it is assumed that they are already familiar with appropriate pipetting technique. Some other operators lose their sense of urgency and disregard established rules for good pipette technique. The majority of these situations can be avoided by regularly practicing proper pipetting technique and reading articles like the one you are currently reading. Reduce temperature changes in the lab as much as you can to prevent them from affecting your pipetting accuracy (2%). Equilibrate your drinks for transfer to room temperature wherever possible before aspirating them.

Pre-wetting the tip will balance the air and liquid inside the tip. This increases the precision of your aspiration and gives repeat transfers of a reagent or sample superior consistency. Always hold your pipette vertically, with no more than a 15° incline. This will stop pipetted liquids from corroding the pipette shaft as they enter via the pipette's shaft. A pipette's accuracy and use may be compromised by a damaged shaft. During sample aspiration, keep the tip vertical and maintain an immersion depth of 2 to 5 mm. Up to 1% of accuracy can be lost if the tip is immersed in the liquid either too deeply or too shallowly. Push the pipette plunger firmly but gradually; avoid jerky or quick motions. The precision of pipetting could be reduced by 0.5% if plunger speed is not controlled. After aspirating, pause for a few seconds while holding the tip in the liquid. By doing this, the liquid will have enough time to enter the pipette tip. Regularly carrying out this will also reduce the likelihood of systematic error.

Carefully remove the pipette tip from the container by pulling it straight out without touching the walls. By doing this, you can be certain that no sample or reagent volume is wasted before being dispensed into the final container. After dispensing, don't forget to wipe the tip into the containers inside wall. Any volume that might have remained in the tip is released in this way. Failure to do so can cause accuracy to drop by about 3%. Handle pipettes as little as possible to prevent transferring body heat to the pipette. Greater accuracy is attained in a steady environment, as was described in steps one and two. Practice regularly checking the pipette tips before loading or aspirating fluids. Never use bent, damaged, or contaminated tips since they pose a significant risk of error. Inaccurate tips may cause accuracy to drop by up to 10%. If you consistently adhere to these recommendations, your pipetting technique will improve and you will make fewer mistakes. We choose to include a few more recommendations below to help you on your road to using a micropipette correctly because there is always room for improvement. Set the volume of an adjustable mechanical pipette in a clockwise direction at all times. If you are turning the volume knob counterclockwise, you can turn the knob past the desired value by a quarter turn. To reduce user pain, use ergonomic pipettes at all times. You can establish effective pipetting technique and reduce the risk of injury by using a comfortable pipette while maintaining good posture. Consistently use low-retention pipette tips of the highest caliber that completely fit the pipette's nose cone. A poor fit caused by improperly loaded tips can reduce the accuracy of pipetted volumes by up to 50%.

DISCUSSION

Advantages and Disadvantages As presented in every Monoband product insert, the micropipette is a required material to perform an immunoassay. Its proper use will contribute to the accuracy of test results including values found for patient samples. Therefore, it is important for both labs managers and technicians to have a firm understanding of the instrument. Specifically, these parties should be aware of the design characteristics and variations, techniques for use and the importance of scheduled calibration and maintenance. To develop an understanding of the micropipette, the general construction is shown in Figure 1. While some design elements do vary, every pipette has a plunger button, tip ejector button, and tip ejector arm. The greatest distinction in design is found between fixed and variable-volume dispensing pipettes wherein users may specify a set volume. Additional design differences are present among manufactures and models. Even with the common design elements, function will vary between pipettes so users must appreciate the specifics of their micropipette to ensure proper usage. A first interaction may involve users depressing the plunger button and experiencing the force required to do so. They will likely notice moving from the rest position to the first stop requires less force than moving to the second stop. The purpose of this design is to differentiate the plunger positions [7], [8].

Remember manufacturers may utilize different approaches to design. Given the differences, it is very important to select a micropipette based on the needs of the laboratory and thereby technician. Before selecting suitable equipment, one must have an understanding of dispensing techniques. The following offers a discussion of dispense modes to help form the decision maker's opinion of available micropipettes and the training of users' technique. Forward-mode dispensing is considered the standard mode by most pipette manufacturers. This mode begins at rest and continues to the first stop to initiate the pick-up of solution [9], [10]. The plunger is then released back to the rest position and the solution is aspirated into the tip. To dispense the solution, the plunger must be depressed to the second stop which will release the set volume. Although forward-mode dispensing is considered the standard mode, a few draw backs accompany the method. Technician fatigue is on top the list due to the extra force required to depress the plunger button to the second stop. Another significant disadvantage is a high incidence of inaccuracy since only the set volume is aspirated into the tip. Surface tensions will cause some solution to adhere inside the tip resulting in an inaccurate dispensing volume. Reverse-mode offers another dispending option. In this technique, the initial aspiration of the sample requires depressing the plunger to the second stop then releasing to the rest position. From this point the sample is dispensed by depressing the plunger to the first stop. After dispensing, a portion of the sample remains in the tip because aspiration from the second stop draws slightly more solution than the set volume.

This Reverse-Mode technique is considered by Monoband to offer more advantages than disadvantages when performed properly. The main draw-back for users is it requires slightly more sample or reagent than needed by the assay. However, if ample volume exists, the method produces superior accuracy which I am critical benefit to laboratories. Reverse-mode technique also lessens fatigue when technicians dispense the same solution multiple times since subsequent pick-ups only require releasing the plunger from the first stop to rest position. The force required between these is much less than in reaching the second stop as necessary to dispense in forward mode. For these reasons, Monoband recommends the reverse-mode pipetting technique to users. In addition to selecting equipment and utilizing preferred techniques, labs must ensure micropipettes are properly calibrated. Good clinical laboratory practices include equipment calibration and maintenance schedules that must be performed by qualified parties whom issue compliance certificates. Such quality records are valid for a set period and must be renewed accordingly.

A final matter to consider is how dispensing techniques influence laboratory automation. The same surface tension conditions exist affecting volume accuracy in automated dispensing. Therefore, when evaluating automation, one must inquire about the dispensing capabilities related not only to accuracy and precision concerns but also to the available dispense modes. Monobind's Autoplex instrument uses in effect reverse-mode dispensing (extra volume pick-up of samples > 25 µl) and has been tested rigorously for pipetting accuracy via Within Assay Precision and Reproducibility of Response Variable. With Autoplex, labs receive a Monobind certified system, optimized analytics and preprogrammed applications for AccuBind and Aciculate reagents.

Pipetting Technique

Proper technique involves drawing up and dispensing the liquid in a smooth motion. Putting the pipette tip in the water and quickly releasing the plunger will give you inaccurate results even if your pipette is properly calibrated.

Reverse Pipetting

Use this technique when pipetting viscous liquids or volatile solvents. Reverse pipetting also helps when pipetting ultra-micro samples of 0.5 µL or less. Push the piston down to the purge position, then draw the liquid up. There is too much liquid in the tip at this point. However, when the liquid is dispensed by pushing the piston to the aspirate position, the extra liquid is left inside the tip. Using this method, the tip is automatically pre-wetted. The extra liquid also helps when pipetting volatile solvents, because some of the solvent will tend to evaporate into the air cushion. Use a Sensible Pipette for the Volume You Want to Dispense The accuracy of your pipette decreases as the dispensed volume approaches the minimum the pipette can handle. For example, if you are dispensing 15 µL, then a 1 mL pipette would be terrible, a 200 µL pipette not so good, and 20 µL pipette ideal.

Use the Largest Volume Possible

Large volumes are easier to pipette accurately than small. Say you are performing an assay where you have to accurately pipette 5 µL. Pipetting that small amount accurately is not easy and will likely contribute greatly to the statistical error in your results. On the other hand, you could dilute the stock solution 10 times and pipette 50 µL of the solution. You could easily and accurately pipette this amount, which would yield much tighter error bars.

CONCLUSION

Pipetting is an art and a skill that evolves with practice and experience. Whether you are a novice scientist learning the basics or a seasoned researcher fine-tuning your technique, the pursuit of pipetting mastery is a lifelong journey. As we conclude our exploration, we recognize that laboratory pipetting techniques are not just about handling liquids; they are about achieving scientific excellence. Each microliter pipetted with precision is a brushstroke on the canvas of discovery, each drop measured accurately is a note in the symphony of science, and each experiment conducted with finesse is a testament to the artistry of precision. In the chapters dedicated to specific pipetting techniques and applications, we invite you to delve deeper into this precision artistry. Each type of pipetting operation has its own intricacies, its own unique challenges, and its own rewards for those who master it. Together, let us celebrate the precision artistry of pipetting mastery, where every drop matters, every measurement is a choreographed move, and every experiment is a testament to the pursuit of precision in the pursuit of knowledge.

REFERENCES:

[1] K. Hruska, M. Fránek, J. Bursa, and P. Kaláb, Radioimmunoassay of progesterone in cow milk., *Endocrinol. Exp.*, 1983.

[2] T. Koal, M. Sibum, E. Koster, K. Resch, and V. Kaever, Direct and fast determination of antiretroviral drugs by automated online solid-phase extraction-liquid chromatography-tandem mass spectrometry in human plasma, *Clin. Chem. Lab. Med.*, 2006, doi: 10.1515/CCLM.2006.052.

[3] K. Pandya, C. A. Ray, L. Brunner, J. Wang, J. W. Lee, and B. DeSilva, Strategies to minimize variability and bias associated with manual pipetting in ligand binding assays to assure data quality of protein therapeutic quantification, *J. Pharm. Biomed. Anal.*, 2010, doi: 10.1016/j.jpba.2010.04.025.

[4] H. S. Mogensen, C. Børsting, and N. Morling, Template preparation of AmpliSeq™ libraries using the Ion Chef™, *Forensic Sci. Int. Genet. Suppl. Ser.*, 2015, doi: 10.1016/j.fsigss.2015.09.146.

[5] A. Pirri, S. M. C. M. Costa, S. B. Mattar, and M. da Graça Bicalho, 159-P: To Automate or Not to Automate Processes in the Lab, *Hum. Immunol.*, 2010, doi: 10.1016/j.humimm.2010.06.213.

[6] D. P. Mascotti and M. J. Waner, Complementary spectroscopic assays for investigating protein-ligand binding activity: A project for the advanced chemistry laboratory, *J. Chem. Educ.*, 2010, doi: 10.1021/ed100199j.

[7] J. E. Adaway and B. G. Keevil, Therapeutic drug monitoring and LC-MS/MS, *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*. 2012. doi: 10.1016/j.jchromb.2011.09.041.

[8] A. S. Rouzière and J. E. Redman, A practical workshop for generating simple DNA fingerprints of plants, *Biochem. Mol. Biol. Educ.*, 2011, doi: 10.1002/bmb.20486.

[9] S. E. Chung *et al.*, One-step pipetting and assembly of encoded chemical-laden microparticles for high-throughput multiplexed bioassays, *Nat. Commun.*, 2014, doi: 10.1038/ncomms4468.

[10] S. Lemmo, E. Atefi, G. D. Luker, and H. Tavana, Optimization of aqueous biphasic tumor spheroid microtechnology for anti-cancer drug testing in 3D culture, *Cell. Mol. Bioeng.*, 2014, doi: 10.1007/s12195-014-0349-4.

CHAPTER 8

LABORATORY FILTRATION TECHNIQUES FOR PURIFICATION AND SEPARATION

Navneet Kumar, Professor
College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, Email Id- navneetbiochem@yahoo.co.in

ABSTRACT:

Laboratory filtration techniques are the silent architects of purity in scientific research, separating substances from solutions with precision. This chapter explores the world of filtration, encompassing various filtration methods, equipment, applications, and considerations for achieving optimal results. Understanding the principles of laboratory filtration empowers scientists, researchers, and students to purify samples, remove impurities, and ensure the integrity of their experiments. Filtration is an important method used in chemistry, biology, environmental science, and pharmaceutical research, among other fields. The main task of this is to divide tiny pieces or substances according to their size, shape, or chemical properties. There are different ways we can filter things, like using gravity, a vacuum, or membranes. Gravity filtration is a process where a liquid mixture is passed through a filter paper or a material that has tiny holes, like glass wool. Gravity helps the liquid go through the filter, but solid particles stay behind. Gravity filtration is a method that is easy to use, works well for big amounts, and is commonly used in qualitative analysis. Vacuum filtration makes filtering things faster and more efficient by using suction. A Buchner funnel connected to a vacuum makes the separation process go faster. This is good for when you want to measure things precisely and when you have very small solids forming in a liquid. Membrane filtration is a method that uses special filters with small holes to separate particles or molecules based on their size. It is commonly used to clean things and make them safe, to clean water, and to separate small pieces of living things, like DNA and proteins.

KEYWORDS:

Filtration Techniques, Filtration Equipment, Filtration Methods, Filter Media, Sample Preparation.

INTRODUCTION

In the realm of scientific inquiry, where precision is paramount and purity is prized, laboratory filtration techniques stand as the sentinels guarding the gates of scientific integrity. These techniques, often working silently and efficiently, are the unsung heroes of scientific research, ensuring that samples are free from impurities, solutions are clarified, and results are reliable. This chapter invites you to explore the intricate world of laboratory filtration, where methods, equipment, and considerations unite to purify knowledge. Laboratory filtration techniques are the alchemists of purity, adept at separating substances from solutions, regardless of their scale. Whether it's removing particulates from a wastewater sample, sterilizing a culture medium, or isolating nanoparticles from a suspension, filtration methods are the instruments that transform raw substances into pristine solutions [1], [2].

This chapter serves as a gateway to the realm of laboratory filtration, where we will unveil the various filtration techniques, the specialized equipment, the broad range of applications, and the essential considerations for achieving optimal results. From microfiltration to ultrafiltration, membrane filtration to vacuum filtration, each technique offers a unique approach to achieving purity, and each application presents its own set of filtration challenges. As we embark on this journey, we recognize that laboratory filtration is not just about passing a liquid through a filter; it is a science that demands precision and understanding. The choice of filter media, the considerations of sample preparation, and the optimization of filtration efficiency are all crucial aspects that elevate filtration to a true art. Whether you are a seasoned researcher refining your filtration skills or a student taking your first steps into the laboratory, the principles outlined in this chapter form the foundation upon which your scientific journey rests [3], [4].

In the chapters that follow, dedicated to specific filtration techniques and applications, we will delve deeper into the intricacies of this precision craft. Each filtration method has its own story to tell, its own unique benefits, and its own considerations for mastery. So, whether you find yourself clarifying a solution, isolating biomolecules, or achieving purity in any filtration endeavor, remember that in the realm of scientific inquiry, precision and purity go hand in hand, and laboratory filtration is the guardian of both. Together, let us celebrate the precision of purity in laboratory filtration, where every particle is screened, every solution is clarified, and every experiment is a testament to the pursuit of scientific integrity [5], [6]. Filtration is important in techniques like chromatography, where it is used to get samples ready for analysis. Having the right way to filter things is really important for getting results that are correct and can be repeated. To make sure filtering works well, it's important to choose the right filter, have the right size of holes in it, use the right materials for filtering, and make sure the technique is done well. The selection of how to filter and what equipment to use depends on what you are trying to do. You want to make sure you get rid of impurities or separate the components you want.

In summary, laboratory filtration techniques are important processes that allow scientists to separate and clean substances, making it easier to study and learn more in different areas of science. It is very important to understand and use filtration methods correctly in laboratories to get reliable results. Filtration is a way to separate solids from liquids. It involves pouring a mixture onto a special paper that lets the liquid through, called the filtrate, while the solid is left behind and collects on the paper. In general chemistry labs, two filtration techniques are commonly used: gravity filtration and vacuum filtration. Gravity filtration is a method where we use a special funnel made of plastic or glass and a piece of paper to separate solid particles from a liquid. Filter paper comes in different sizes of little holes to big holes. These holes allow liquid to pass through at different speeds, from slow to fast. The paper is folded in the middle, making two equal parts. Then, it is folded again to make four equal parts. Finally, a small piece is torn off one corner to make it fit well in the funnel cone. If you already weighed the paper, make sure to keep the torn corner piece. It will be added to the weight of the paper after it has been filtered to prevent any mistakes. The paper cone is placed inside the funnel. One half of the cone has three layers of paper, while the other half has only one layer. Now, put the funnel into a container called a beaker and make sure to completely soak the filter paper with the main liquid used to separate the mixture.

This step makes sure the filter paper sticks to the funnel walls so that solid material cannot get out. Next, use a clamp or ring to hold the funnel in place. If needed, place a clean beaker below the funnel. Make sure the stem of the funnel touches the side of the beaker to avoid spilling any liquid. Before separating, let most of the solid in the mixture to sink down. Now, pour the liquid that is on top of the solid in a mixture through the filter first. This will make the first part of the filter go faster and might stop the filter from getting blocked by the solid. To avoid making a mess, pour the liquid slowly onto a glass rod. Put the solid onto the filter using a rubber tool or spatula. Wash the spatula, glass rod, and beaker with water and pour the water into the filter funnel. If you need to clean the leftover solid residue, use a small amount of liquid and repeat the process three times using a few milliliters each time. If you want to keep the solid, carefully take off the filter paper and put it on a watch glass to dry. Be careful with wet filter paper because it can tear easily. Sometimes, people test the liquid that has passed through the filter to see if a certain substance is in it or not. The result of the test is determined by what is being divided or separated. For instance, we can get rid of all barium ions (Ba2+) in solution by creating a solid called BaSO4.

To test this, put a couple of drops of Na2SO4 solution into a small part of the filtered liquid. If a solid called BaSO4 does not form in the filtrate, then the filtration process was successful. If a solid forms, you need to add more chemicals that cause solid formation to the liquid and then separate the solid from the liquid again. Vacuum filtration is a process where a device called a Buchner funnel and a water aspirator are used to filter substances. A Buchner funnel is a small, round bowl made of porcelain, with tiny holes in the bottom and a short stem. The stem is placed into the mouth of a side arm filter flask using a rubber stopper. A round paper that fits the bowl is put on the bottom and wet with the right liquid to make sure it stays in place while filtering. The hose in the picture connects the side of the filter flask to a vacuum aspirator either in the hood or on the lab bench. This device sucks liquid through the filter and filter paper. When you switch on the water aspirator, the movement of water pulls things towards it. The hose is a part of a system that filters things and it should be strong enough to not get squeezed or crushed by the air pressure outside. To remove impurities from a sample, you need to switch on the aspirator and do the filtration using the same method explained for gravity filtration. Do not remove the funnel if the system has vacuum. This can cause water to suddenly go back into the flask or the filter paper to get damaged and lose the solid that was being filtered. First, turn off the water aspirator, then carefully remove the wet filter paper without tearing it. After a reaction, any leftovers from the reaction should be put into containers that are labeled correctly and placed in the hood.

DISCUSSION

Filtration, the technique used to separate solids from liquids, is the act of pouring a mixture onto a membrane that allows the passage of liquid and results in the collection of the solid. Two filtration techniques are generally used in chemical separations in general chemistry lab: gravity filtration and vacuum filtration. Gravity Filtration Gravity filtration uses a polyethylene or glass funnel with a stem and filter paper. Filter paper can have pore sizes ranging from small to large to permit slow to fast filtering. The paper is folded in half (Figure 1), then folded in quarters, and the tip of one corner is torn off to allow for a snug fit in the funnel cone. If the paper has been pre-weighed, the torn corner piece must be saved to add to the post-filter weighing to avoid any errors. The paper cone is fitted to the funnel so three thicknesses of the paper line one-half of the cone and one thickness lines the opposite half (Figure 1). Now place the funnel into a beaker and wet the filter paper completely or ring and place a clean beaker beneath the funnel so the stem rests against the side of the beaker. Before filtering, allow most of the solid in the mixture to settle. Now pour the supernatant liquid through the filter first. This will allow the initial part of the filtration to proceed faster and may prevent clogging of the filter by the solid. To prevent splattering pour the liquid down a glass rod [7], [8].

Crape the solid onto the filter with a rubber policeman or spatula. Rinse the spatula, glass rod and beaker and pour the washings into the filter funnel. If the remaining solid residue is to be washed, rinse with three small portions of an appropriate solvent. If the solid is to be saved, remove the filter paper carefully and place it on a watch glass to dry. Caution: Wet filter paper tears easily Sometimes the filtrate is tested to determine if a product or reactant has or has not passed through the filter. The test depends on what is being separated. For example: All barium ions (Ba2+) should have removed from solution by the formation of the precipitate BaSO4. To check this a few drops of Na2SO4 solution can be added to a small portion of the filtrate. If no BaSO4 precipitate forms, the filtration was successful. If a precipitate forms, additional precipitating reagents must be added to the filtrate and the resulting mixture must be filtered again.

Vacuum filtration uses a Buchner funnel and a water aspirator assembly. A Buchner funnel is a flat bottomed, porous, circular porcelain bowl with a short stem. The stem is fitted with a rubber stopper and inserted in the mouth of a side arm filter flask. Circular filter paper, the same diameter as the bowl, is placed on the flat bottom and wetted with the appropriate solvent to create a seal before starting the filtration. The hose in the above figure is attaches the side arm of the filter flask to a vacuum aspirator in the hood or at the lab bench. This vacuum aspirator creates the suction that pulls liquid through the filter and filter paper. To filter a sample, turn on the aspirator and carry out the filtration in the same manner described for gravity filtration. Turn off the water aspirator before carefully removing the wet filter paper without tearing. Reaction byproducts (either the solid or filtrate) should be placed into appropriate labeled containers in the hood [9], [10].

Filtration

Filtration is the physical separation of a solid from a liquid and is a process encountered in experimental procedures such as gravimetric analysis, recrystallization, and solvent drying. In principle, the mixture of the solid and liquid is passed through a porous material, filter paper or sintered glass, and the solid is trapped on the porous material while the liquid passes through.
The type of filtration equipment you select for use depends upon which of the two components, the solid or the liquid, you are trying to isolate. In general:

- **a.** If you wish to isolate the liquid use gravity filtration.
- **b.** If you wish to isolate the solid use suction filtration.

Gravity filtration

In gravity filtration you need to pass the liquid through the porous material and retain all the unwanted solid in the filter. In general, the best material to use is a filter paper of the appropriate porosity to trap all the solid particles and with the greatest surface area to allow the liquid to pass through quickly. The apparatus required for gravity filtration. The filter funnels are usually made of glass, but if organic solvents are not involved in the filtration, plastic funnels can be used. Glass filter funnels with the pipe cut off are known as 'stemless' filter funnels and have a specific use in hot filtration. The key to successful gravity filtration is the fluted filter paper. A fluted filter paper decreases the area of contact between the filter paper and the funnel, thus allowing rapid filtration. If you use 'traditional' cone-folded filter paper, note that all sides of the paper are touching the sides of the funnel and on half the filter paper the liquid has to pass through three thicknesses of paper, all of which slow the rate of filtration. Slow filtration can lead to disaster in hot filtration during recrystallization. Since filter funnels and filter papers come in different sizes, choose a filter paper of diameter just less than twice the diameter of the funnel. When fluted, the filter paper will be just below the rim of the funnel. There are many ways to fold a filter paper, but one of the simplest is shown below. To filter the mixture, swirl the suspension of the solid in the liquid so that there is a fairly even distribution of solid in the liquid, and then pour the mixture into the filter cone, making sure that you do not pour any of the mixture outside the filter paper otherwise you will need to repeat the filtration, and do not overfill the filter cone. Transfer all the mixture in this way and finally wash the last bit of solid and liquid into the filter cone with a *small amount* of filtered solution and then a *little pure solvent*.

Steps

- **a.** Fold the filter paper in half open it out then fold into quarters, open it and fold into eights all in the same directions.
- **b.** Turn the paper over and fold each sector in half you are creating sixteenths but with each fold in the opposite direction.
- **c.** Finally, fold the paper into a cone ensuring that all the folds are sharp and that the base of the cone comes to a sharp point.
- **d.** The flutes ensure that the filter paper has minimum contact with the filter funnel and the sharp point ensures that the liquid flows rapidly out of the cone and out of the funnel
- **e.** If cone point is blunt it will cover the steam of the funnel and so all the liquid must pass through this part of the filter paper slowing filtration

Suction filtration

This technique is used for the isolation of a solid from a suspension of a solid in a liquid and relies on producing a partial vacuum in the receiving flask. The essential components of a suction filtration system are:

- **a.** A ceramic funnel containing a flat perforated plate: there are two types based on size and shape called Buchner funnels or Hirsch funnels. When you are filtering, the perforated plate is covered by a filter paper.
- **b.** A receiver flask with a side arm for attachment of the vacuum source. Buchner flasks are conical flasks made from thickened glass, and Hirsch tubes also known as side-arm boiling tubes or test tubes depending upon size are capable of withstanding weak vacuum, e.g. a water pump.
- **c.** A flexible seal between the ceramic funnel and the receiving vessel: a Buchner collar or filter seal.
- **d.** A source of vacuum, usually a water pump which is connected to the receiving flask by thick-walled rubber tubing. Sometimes there will be a trap between the water pump and the receiving flask.
- **e.** Select the appropriate size of apparatus based on the amount of solid you expect to isolate and the volume of the liquid to be collected in the receiver flask.

Consider the following points

A. There is no point in using a large Buchner funnel for a small amount so solid since you will collect a layer of solid 'one molecule thick' and be unable to scrape it from the filter paper cleanly. if there is too much solid for the size of the funnel you will have to repeat the filtration with a second set of apparatus or the solid may not suck dry quickly. B. If you use the side arm boiling tube to try to collect 100 ml of liquid, you will over fill the tube and liquid,

- **a.** Flow into the pressure tubing, contaminating it for your fellow students.

b. May fill the intermediate trap, if there is one and you will need to dismantle and clean it.

c. May be sucked into the water pump causing corrosion and loss of performance.

Clean and dry all the apparatus to be used

1. Clamp the receiving vessel to support stand: pressure tubing is heavy and even large buchner flacks will fall over do not think that a test tube rack eill hold a side arm boiling tube saftely.

2. Place the correct-sized buchner collar in the neck of the receving flask: it should sit well into the neck and fit the funnel to form a good seal.

3. Place the funnel into the collar/seal: note that the funnel has a 'point' at the bottom of the stem . Make sure that this 'point' is as far away as possible form the vaccum attachment side arm of the reciever flask, since the filtering liquids runs of this 'point' and if the point is near the vaccume inlet the liquid may be drawn into the side arm and then into the trap or water pump.

4. Select a filter paper, whinh fits exactly over the perforation in the base of the funnel. The filter paper should not fold or crease up the sides of the funnel because the solid will be sucked round the edge of the paper into the receiver flask. if the paper does not fit exactly, trim to size with scissors.

5. Place the paper into the funnel and wet it with a few drops of liquid - the same liquid which is to be used in the filtration.

6. Switch on the tap for the water pump to provide gentle suction. If your system has a trap, don't forget to close the tap on the trap and connect the rubber tubing to the side arm of the reciever. Do nor force the rubber tubing too far onto the side arm - you may need to pull it off quickly if something goes wrong. The filter paper will be pulled down onto the preforated plate by the vacuum.

7. Turn on the tap to the water pump to the *maximum water flow*. If you do not do this, the water pump is not working at its maximum efficiency and the vaccum created in yourfiltration system may cause water to be sucked into a trap, or receiving flask, from the water pump. this is called 'suck-back'.

8. Swirl the mixture to be filtered and then slowly pour into the Buchner or Hirsch funnle at such a rate so that the filtration is rapid.Note that the rate of filtration may slow as the 'sake ' of solid on the filter becomes thicker.

9. To transfer the last of the solid/liquid from its beaker or conical flask into the funnel use a little of the filtrate in the receiving flask. realese the vaccum by opening the tap on the trap or pull off tha vacuum tubing, but do not *turn off* the tap on the water pump there is a possibility of'suck-back . dis mental the apparatus pour a little of the filtrate into the beaker or conical flask, reassemble the apparatus and continue the filtration. Repeat until all the material has been filtered. Use the filtrate to wash down any of the solid sticking to the sides of the funnel onto the filter 'cake. it will not dry quickly on the sides of the funnel.

10. Release the vacuum, by pulling the vacuum tubing from the flask or opening the tapon the trap and turn down the water pressure on the water pump. Transfer the filtrate to a clean beaker or conical flask. Add a little pure, ice-cold solvent to the filter cake and reconnect the vacuum to provide gentle suction. this will wash the solid. Turn up the vacuum to maximum and suck air through the solid to dry it as much as possible. If 'cracks' appear in the filter 'cake', close them by pressing gently with a clean spatula and repeat until no more filtrate appears to be sucked out.

11. When drying is complete, release the vacuum, turn off the water tap and remove the filter funnel from the apparatus. The solid is best removes as a complete 'cake' by lifting the edge of the filter paper with spatula, inverting the funnel over a watch-glass of clock-glass and the cake should fall out. peel the filter from the top of the 'cake', break up the 'cake' using a spatula and dry appropriately.

12. Evaporate the filtrate to half volume and cool to obtain a second crop of crysta.ls

13. Washout and clean all the apparatus and dispose of the liquid filtrate safely.

It is crucial to be able to swiftly and effectively separate the liquid and solid components of a mixture in a number of chemistry applications. The mass of precipitate formed by a specific chemical reaction must be determined in analytical chemistry using a technique called gravimetric analysis, often to an accuracy of four decimal places. Transferring the liquid and solid-containing contents of a beaker to a filtering device, which retains the solid and lets the liquid to pass through, is a crucial step in this highly specialized procedure. This technique shouldn't result in any solid loss. Filtration is a common step in the purification of organic materials. As a result, the desired product is frequently polluted with several contaminants when it is initially received. The majority of these impurities can be eliminated through recrystallization, which is discussed in Unit 11. Once more, the liquid containing dissolved contaminants needs to have the crystals removed through the process of filtration. You should be aware that this substance, known as the mother liquor, is a saturated solution of the solid combined with dissolved contaminants. The act of putting a substance through a plug of fibres like cotton wool or glass wool is perhaps the simplest form of filtering. However, it would be challenging to get the residue or precipitate out of the fibres. Therefore, this approach is unrefined. Paper is the media, or filter material, that is most frequently utilized. In your lab, filter paper circles are undoubtedly a common sight.

For a variety of uses, different types of paper are created, and there are many ways to fold filter raper circles to accommodate them. Papers come in a variety of sizes, from the tiny ones used in science labs for liquid pouring to the enormous sheets used in industrial applications for forcing liquids or gases. Filters made of other materials are also frequently utilized. You'll probably come across porous sintered glass. These filters are sturdy and can be reused, which is their main benefit despite their high cost. Suction and pressure are employed to speed up the filtration process, while pressure techniques are more frequently used in industrial settings than in academic labs. If you planned to filter something, you would be interested in the filtrate, the particulate matter, or both. You won't typically worry about some of the filtrate being absorbed by the filter medium and contaminating the particulate matter for qualitative purpbies. However, if you want to perform quantitative filtration, the filter must always be washed after the filtration procedure to get rid of any remaining filtrate. In gravimetric analysis, you frequently need to be extremely cautious to prevent filtrate loss from splashing. Let's now examine the variables that influence the selection of the ideal filtering method and filter medium. The porosity of the filter medium is determined by the approximate size of the particles to be filtered. A fine precipitate would pass via Laboratory Techniques 11 if the medium had big pores.

On the other hand, it would be wastefully slow to filter a coarse precipitate through a fine filter. The method of choice is influenced by the characteristics of the liquid conveying the particles. The liquid is typically aqueous in many filtrations that involve inorganic substances like barium sulphate or silver chloride. You might very easily choose a straightforward method including a filter funnel and paper because water is reasonably inert and not dangerous, especially in situations where time is not a major factor. If you were dealing with an organic compound filtering, you might discover that the liquid is non-aqueous, like alcohol, ether, or petroleum spirit. The issue here is that the liquid is volatile and the resulting vapour may be poisonous, flammable, or even both. When conducting a qualitative analysis, you might only be concerned with testing the liquid after removing a precipitate from a mixture. A inexpensive filter paper would work there. However, you would be extremely careful that the paper not contaminate the precipitate or filter if you were conducting a gravimetric study. You would pick a paper that has undergone processing to get rid of any potentially harmful mineral particles in this situation.

These are the more expensive, single acid washed or double acid washed papers, also referred to as ashless papers. By utilizing, for example, sintered-glass or porous-alumina filter crucibles, analysts frequently choose to completely dispense with paper. Some equipment, such as the suction filtration technique using a Buchner funnel, is quite ideal for handling enormous quantities of mixes that need to be filtered. If you were filtering a few cubic centimeters of liquid, other equipment would be more appropriate, such as the Willstatter filtration nail. Note that the volume of the particles, not the volume of the mixture to be filtered, determines the size of the filter paper; a tiny amount of solid in a vast volume of mixture would be lost"on a large paper. The technique to be employed is determined by the mixture's volume. Cost is a crucial consideration. A school technician would select glass filter funnels and inexpensive, high-quality papers to be the least expensive alternative when providing filtering equipment to 30 students. On the other hand, if a science instructor was short on time and wanted to demonstrate filtration, he or she might ask you to put up an expensive suction filtration device. The cost of the filtering process is secondary to the cost of recovering the product in industries where it may be necessary to aim for the highest yield and high purity. If this is the case, the filtering process has been refined with a great deal of sophistication.

CONCLUSION

In the intricate labyrinth of scientific exploration, where precision is the torchbearer and purity are the beacon, laboratory filtration techniques emerge as the guardians of pristine knowledge. Our journey into this world has unveiled the profound significance of filtration, where clarity, separation, and precision converge to shape the landscape of scientific inquiry. Laboratory filtration is the art and science of achieving purity with precision. It is the alchemical process through which impurities are separated from solutions, ensuring that scientific results remain unadulterated and trustworthy. Whether it's purifying water, isolating particles, or sterilizing samples, filtration methods are the gatekeepers of scientific integrity. The world of filtration is a vibrant one, offering an array of techniques tailored to diverse applications. From microfiltration for fine particulates to ultrafiltration for molecular separation, each technique is a brushstroke on the canvas of purity, delivering distinct benefits to researchers. Specialized filtration equipment, from vacuum pumps to filter membranes, amplifies the precision of purification. Efficiency in filtration, driven by equipment and method selection, determines the success of experiments, whether in chemistry, biology, environmental science, or countless other fields. Mastery of filtration extends beyond technique; it encompasses understanding filter media, optimizing sample preparation, and navigating the subtleties of filtration efficiency. Attention to these considerations is the hallmark of filtration expertise.

Filtration's precision ensures that scientific results are reliable and reproducible. Purity in samples and solutions is the bedrock upon which research conclusions are built, and the rigorous application of filtration methods safeguards the integrity of scientific knowledge. Filtration is both an art and a skill, and its mastery is a lifelong journey. Whether you are a seasoned scientist refining your filtration prowess or a student embarking on your scientific path, the principles of precision and purity in filtration form the cornerstone of your pursuit. As we conclude our exploration, we recognize that laboratory filtration techniques are not just about passing substances through filters; they are about safeguarding the sanctity of scientific inquiry. Each purified solution, each particle separated, and each experiment conducted with clarity is a testament to the precision path to scientific purity. In the chapters dedicated to specific filtration techniques and applications, we have delved deep into the heart of this precision craft. Each technique is a realm of knowledge, each filtration application is a chapter of discovery, and each filtration endeavor is a testament to the precision and purity that define scientific integrity. Together, let us celebrate the precision path to scientific purity in laboratory filtration, where every filtered solution is a triumph, every separated particle is a revelation, and every experiment is an homage to the pursuit of pristine knowledge.

REFERENCES:

[1] B. A. Bolto, Magnetic particle technology for wastewater treatment, *Waste Manag.*, 1990, doi: 10.1016/0956-053X(90)90065-S.

[2] E. T. Gjessing *et al.*, Multi-method characterisation of natural organic matter isolated from water: Characterisation of reverse osmosis-isolates from water of two semi-identical dystrophic lakes basins in Norway, *Water Res.*, 1998, doi: 10.1016/S0043-1354(98)00060-8.

[3] C. Carr and E. B. Riddick, Advances in ceramic radiography and analysis: Laboratory methods, *J. Archaeol. Sci.*, 1990, doi: 10.1016/0305-4403(90)90014-V.

[4] A. J. Crooks, J. M. Lee, A. B. Dowsett, and J. R. Stephenson, Purification and analysis of infectious virions and native non-structural antigens from cells infected with tick-borne encephalitis virus, *J. Chromatogr. A*, 1990, doi: 10.1016/S0021-9673(01)89563-7.

[5] P. M. Groffman and G. C. Hanson, Division S-10 wetland soils, *Soil Sci. Soc. Am. J.*, 1997.

[6] T. Sellers and M. L. Prewitt, Applications of gel filtration chromatography for resole phenolic resins using aqueous sodium hydroxide as solvent, *J. Chromatogr. A*, 1990, doi: 10.1016/S0021-9673(01)89444-9.

[7] L. Holmquist, G. Agrup, H. Anundi, and O. Vesterberg, High performance concentration and gel filtration of rat urinary protein allergens, *J. Biochem. Biophys. Methods*, 1990, doi: 10.1016/0165-022X(90)90092-Q.

[8] M. D. Stonard, Assessment of renal function and damage in animal species. A review of the current approach of the academic, governmental and industrial institutions represented by the animal clinical chemistry association, *J. Appl. Toxicol.*, 1990, doi: 10.1002/jat.2550100407.

[9] S. Tigaud, C. Knoche, A. Vigliecca, and P. Gobber, Gram negative anaerobes beta-lactamases. Difficulty about their characterization by iso-electric-focusing (an 48 strains experience), *Med. Mal. Infect.*, 1990, doi: 10.1016/s0399-077x(05)80078-2.

[10] D. W.P. *et al.*, *Management techniques for laboratories and other small institutional generators to minimize off-site disposal of low-level radioactive waste*. 2003.

CHAPTER 9

LABORATORY HEATING AND COOLING METHODS: A COMPREHENSIVE REVIEW

Shikha Paliwal, Assistant Professor
College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, Email Id- paliwalshi1111@gmail.com

ABSTRACT:

Laboratory heating and cooling methods are the orchestrators of temperature control in scientific experimentation, influencing reaction rates, sample stability, and crystallization outcomes. This chapter explores the world of temperature control, encompassing various heating and cooling techniques, equipment, applications, and considerations for achieving precise temperature conditions. Understanding the principles of laboratory temperature control empowers scientists, researchers, and students to manipulate thermal conditions with accuracy and precision, ensuring the success of experiments across diverse scientific domains. It is very important to control the temperature in the laboratory. This helps us get accurate and consistent results and keeps our samples and materials safe. The way we heat and cool depends on what each experiment needs, such as the temperature range, how much stuff we're working with, and what the materials are like. In summary, heating and cooling in labs are important techniques that allow scientists to control temperatures for various scientific purposes. It is important to choose the right methods and use them correctly in order to have successful experiments and keep the laboratory materials and samples in good condition.

KEYWORDS:

Cooling Methods, Heating Methods, Laboratory Heating, Laboratory Cooling, Temperature Control.

INTRODUCTION

In the intricate tapestry of scientific experimentation, precision knows no bounds, and laboratory heating and cooling methods are the virtuosos of temperature control, orchestrating the thermal symphony that shapes outcomes and defines results. These methods, often working silently behind the scenes, are the architects of stability, the accelerators of reactions, and the sculptors of crystalline structures. This chapter invites you to delve into the sophisticated world of laboratory temperature control, where methods, equipment, and considerations unite to manipulate thermal conditions with exactitude. Laboratory heating and cooling methods are the guardians of temperature equilibrium, presiding over reactions, processes, and experiments that are exquisitely sensitive to thermal variations. Whether it's catalyzing chemical reactions, crystallizing compounds, or maintaining stable conditions for biological assays, temperature control methods are the instruments that transform scientific hypotheses into observable phenomena. This chapter serves as a gateway to the realm of laboratory temperature control, where we will explore various techniques, equipment, applications, and the essential considerations for achieving precise temperature conditions. From conventional heating methods to advanced cooling technologies, each approach offers a unique avenue for manipulating temperature, and each application presents its own set of temperature-related challenges [1].

As we embark on this journey, we recognize that laboratory temperature control is not just about generating heat or cold; it is a science that demands precision and understanding. The choice of heat sources or cooling agents, the placement of temperature sensors, and the meticulous calibration of equipment are all crucial aspects that elevate temperature control to an art form. Whether you are a seasoned researcher refining your temperature control skills or a student taking your first steps into the laboratory, the principles outlined in this chapter form the foundation upon which your scientific journey rests [2], [3]. In the chapters that follow, dedicated to specific heating and cooling techniques and applications, we will delve deeper into the intricacies of this thermodynamic precision. Each temperature control method has its own story to tell, its own unique benefits, and its own considerations for mastery. So, whether you find yourself accelerating reactions, crystallizing compounds, or maintaining precise conditions in any temperature-dependent endeavor, remember that in the world of scientific inquiry, precision and control are the keystones, and laboratory heating and cooling methods are the conductors of this thermal symphony. Together, let us celebrate the thermodynamic precision of laboratory temperature control, where every degree matters, every equilibration is orchestrated, and every experiment is a testament to the pursuit of scientific excellence [4], [5].

Laboratory heating and cooling equipment, also called a cold and heat integrated machine or a refrigeration and heating cycle device, is a device that combines cooling and heating functions. It can quickly switch between heating and cooling. This device uses a special liquid called thermal oil, which eliminates the need for separate instruments for heating and cooling. This device is a type of equipment that can circulate oil at both very high and very low temperatures. By using data programming, the intelligent temperature controller of the equipment receives information about the temperature inside the container. This enables the controller to activate the compressor to cool down the medium inside the cold tank, making it reach a low temperature. To warm up the substance in the space, we use the heater to raise its temperature. Then, through a quick process of cooling and heating, we make sure the substance in the tank reaches the temperature required by the user. After that, we use a special pump that doesn't leak to transfer the substance to other equipment, like a reaction kettle. Turn on the valve to make the heat cycle change happen [6].

Laboratory heating and cooling equipment is a new kind of equipment used in labs. It is made by combining features from high and low temperature refrigeration equipment and high temperature oil bath equipment. It combines devices that work at different temperatures and sources of heat at different temperatures. All in one device. The heating and cooling equipment in the laboratory uses a closed system for circulating liquids. This means that when the temperature is low, no water vapor is absorbed, and when the temperature is high, no oil mist is created. This container has a heating and cooling system that can quickly heat up or cool down. It also has a large area for heat exchange and does not require much oil for heat conduction. Lab heating and cooling equipment can quickly raise and lower temperatures by using a compressor that operates at high temperatures and pressures. This compressor can directly cool down from 350 degrees, which saves time and makes testing easier.

The equipment uses a machine that makes cold liquid flow, which can provide a continuous and controlled flow of cold liquid to cool down reactors and other instruments. The lab uses heating and cooling devices to prevent the expenses that come from having separate temperature controls and manually adjusting the temperature. The heating and cooling equipment in the lab uses a special pump that doesn't have a problem with fluid leaking from the shaft seal. The cooling and heating temperature control system is designed with closed pipes and uses a special device to transfer heat between fluids. This design helps save energy and can quickly change the temperature. Laboratory heating and cooling equipment uses an easy-to-understand menu for navigation. The clear curve display can make the curve bigger or smaller, and it can save the data to a U disk in a table format. People want better quality products, more people are buying products, and companies want to make products faster with machines. In these situations, lab heating and cooling machines are very popular. Moreover, in the past few years, there has been significant progress in the field of science and technology. This has led to a fast growth in the industry of domestic laboratory heating and refrigeration equipment. The expertise, equipment, and overall quality have been significantly enhanced. Had a crucial job in keeping everyone safe while making things. The heating and cooling equipment in a laboratory can be easily used with different types of reactors, evaporators, extraction equipment, and other products to achieve more than one purpose.

DISCUSSION

Although improvements in laboratory heating and cooling systems are sometimes disregarded, they continue to play a crucial role in the performance of clinical labs. Clinical laboratories often struggle to handle growing workloads, save costs, and automate operations while still hiring qualified technical employees. By being sensitive to present requirements and supportive of future demands placed on clinical labs, new heating and cooling technologies assist in easing such pressures. Modern heating and cooling systems used in clinical laboratories enhance the security of biological samples. How researchers use this technology in their labs has changed and will continue to evolve as research methodologies change. Ultra-low temperature (ULT) freezers, sophisticated lab refrigerators, and incubators are important examples of heating and cooling equipment that guarantee sample integrity and constant temperature. Wireless monitoring/remote sensors, dual convection technology, and sophisticated microprocessor controllers are a few examples of emerging technologies that encourage innovation in laboratory systems [7], [8].

ULT freezers include wireless monitoring and remote sensors

For ULT freezers, blood bank refrigerators, and plasma freezers, wireless monitoring systems are becoming a need. These systems maintain the integrity of lab samples by continuously monitoring vital parameters including temperature, CO2 concentration, and relative humidity around-the-clock. Scientists may feel confident and at ease knowing that their priceless biological samples are safe since data collecting is unbroken, secure, and accessible over the Internet. These monitoring systems inform pre-assigned employees in real-time in the event of a lab mechanical or electrical failure that the integrity of biological samples may be at jeopardy. These warnings may be sent in a variety of ways, such as via fax, phone, email, or text message alarm notice. While off-site employees may follow the resolution of a false alert without having to enter the lab, on-site lab security staff can get auditory or visual alarms summoning them to examine the problem.

Samples may be tracked for quality assurance reasons thanks to an audit trail the wireless monitoring system has produced. The system complies with 21 CFR Part 11, an FDA regulation on electronic records that specifies the requirements for making electronic records and electronic signatures equal to paper records. Standard storage standards for clinical settings demand for lab refrigerators to maintain a range of +1°C to +8°C and lab freezers to maintain a range of -20°C to -30°C. For vaccinations, whole blood, plasma, chromatography, enzymes, and other temperature-sensitive products, these exact limits are necessary. The user may inspect the inside conditions of the ULT freezer without opening the door, which exposes samples to ambient temperatures, in newer ULT freezers for applications needing ultra-low temperature ranges. Critical lab samples are protected by using a dependable cold storage device with the newest technical advancements [9], [10].

Utilizing dual convection in microbiological incubators

In a single microbiological incubator, dual convection technology combines the advantages of gravity and mechanical convection. The choice of convection technique for the biological samples to be processed within the incubator is made by the scientist using multi-mode convection equipment, which ensures repeatable conditions for the development of microorganisms. In a mechanical convection incubator, a fan actively drives hot air through the chamber, providing continuous monitoring of laboratory equipment with remote alarm alerting and continuous data collecting. The airflow produced by the fan allows for quick heat-up periods and even temperatures with great precision. For certain cells or microorganisms, tight temperature homogeneity guarantees a stable temperature environment. For instance, according to ISO 6579, the first step in conventional detection techniques for detecting Salmonella spp. in food is often a pre-enrichment culture in a nonselective liquid medium, such buffered peptone water, which is incubated at 37 °C for 18 hours. After that, the pre-enrichment culture is subculture into a different selective enrichment medium, such Rappaport Vasiliadis Soy broth (RVS), and incubated for an additional 24 hours at 41.5 C. Mechanical convection incubators are recommended for use with biological samples that need temperature homogeneity throughout incubation.

When samples are transferred immediately from a refrigerator, the rapid heating characteristic of mechanical convection drives growth of samples more quickly. High evaporation rates must be avoided since they affect samples and nutrient solutions. Samples get dehydrated as a result of evaporation rates, and the longer the experiment, the greater the danger of drying. Growth may also suffer from the concentration of leftover nutrients that results. Although there is no fan to actively move air within the chamber, the gravity convection component of a microbiological incubator relies on warm air rising to transfer temperature. The design of airflow from the heating elements through the inner chamber of the unit ensures temperature homogeneity inside the chamber. When working with vented plates or during lengthy incubation cycles, the advantage of the moderate airflow is a decrease in the dehydration of samples. This was shown in a recent study2, where scientists looked at the densities of the Legionella pneumophila strains as determined by colony forming units (CFUs) by serially dilution and plating a tiny portion of the bacterial solution on buffered charcoal yeast extract (BCYE) agar plates. Legionella colonies were enumerated after 48 hours of successful incubation at 37°C. Gravity convection incubators are better able to maintain Legionella strains, which may need prolonged incubation durations.

Refrigeration systems for laboratories using cutting-edge microprocessors

In order for all laboratory applications to function consistently, efficiently, and effectively, appropriate temperature control solutions are essential for dependable, consistent, and accurate findings. The most cutting-edge and dynamic controls in lab refrigerator technology are advanced microprocessors. Variations in temperature are harmful to priceless samples. Older lab refrigerator and freezer types may be prone to undetected or unexpected circumstances that might harm samples. High-value samples are secure and protected by new developments in laboratory refrigeration. The user interface (UI) of certain types of the lab refrigerator may be adjusted to better control the storage conditions for samples. International icon-based controls and displays are among the most recent UI elements that enable researchers to recognize them all. A Service Required alarm notifies lab personnel when maintenance is required to maintain maximum performance, and a visual thermometer enables researchers to quickly validate that the lab refrigerator is operating properly.

A key-operated, triple-position switch that locks in the temperature and alarm setpoints is offered in certain new setpoint security versions. With this technique, setpoint error is reduced and tampering is avoided. When in programming mode, a touchpad enables access to control function defaults for upkeep, troubleshooting, or data entering to raise or lower setpoint settings. Touchpads may access programming and service features like alarm muting or adjust the data that is presented in the user interface (UI). The user may mimic overheated and underheated settings using the Alarm Test feature. When the test is over, audio and visible alarms go off. If either the heated alarm threshold or the cold alarm threshold is crossed, or if power loss circumstances arise, audible and visual signs are triggered in the user interface (UI). A digital user interface (UX) may show cabinet temperature conditions when operating normally or sound an alarm to alert researchers when the lab refrigerator door is open or when the display's backup battery needs to be tested.

Ergonomics, effectiveness, and energy savings are all enhanced by additional features. Some variants come with mercury-free LED interior lighting for better visibility, flush-mounted light switches for ergonomic operation, and self-contained control housings that may be incorporated into the main control panel for effective maintenance. The microprocessor control system makes it possible for routine maintenance. The front of the lab refrigerator or freezer often allows for regular maintenance and repairs, making it simple for the biomedical engineer to get access and service the device without affecting the application. By doing this, it is certain that the laboratory refrigeration unit won't need to be removed from a cabinet or a lab bench in order to be repaired, impeding productivity. Even though the clinical laboratory has undergone many changes in recent years, safeguarding priceless biological samples has remained one of the toughest difficulties lab workers must overcome. The ability to execute sensitive tests with the knowledge that their samples are secure and protected, as well as better materials and components, enable them to provide findings that are accurate and trustworthy.

The majority of labs employ at least one form of heating apparatus, including microwave ovens, hot plates, heating mantles and tapes, oil baths, salt baths, sand baths, and air baths. Because steam-heated devices do not provide a risk of shock or sparking and may be left unattended with the knowledge that their temperature would never rise above 100o C, they are typically favoured if temperatures of 100o C or less are necessary. Before leaving the reaction for any extended period of time, make sure there is enough water available for the formation of steam. Any heating equipment for a laboratory should have the actual heating element enclosed to avoid lab personnel or any metallic conductor from unintentionally touching the wire delivering the electric current. When a heating appliance's heating element is exposed due to excessive wear or damage, it should either be repaired before further use or thrown away. Utilize a variable autotransformer to adjust the input voltage of a laboratory heating device by supplying a portion of the entire line voltage, typically 110 V. Locate the external casings of all variable autotransformers away from flammable liquids and vapors, where water and other chemicals cannot spill onto them.

If the temperature of a reaction significantly rises due to a change in line voltage, the unintentional loss of reaction solvent, or a loss of cooling, fail-safe mechanisms can stop fires or explosions from happening. Some appliances will cut off the electricity if a heating appliance's temperature rises above a predetermined threshold or if a condenser's water supply hose becomes loose or the water pressure drops, stopping the flow of cooling water through the condenser. Ovens Electrically heated ovens are frequently used in laboratories to dry labware and eliminate water or other solvents from chemical samples. Never prepare food for humans in a laboratory oven. Laboratory ovens are built with their heating elements, temperature controls, and interior atmospheres physically segregated from one another. Rarely do laboratory ovens have a feature that prevents the release of the compounds that have volatilized inside of them. Directly connecting the oven vent to an exhaust system can lessen the chance that chemicals will escape into the lab or form an explosive concentration inside the oven. Any chemical sample that could be hazardous due to acute or long-term toxicity should not be dried in ovens unless additional measures have been made to guarantee constant atmospheric venting in the oven. Glassware should be rinsed with distilled water after being cleaned with organic solvents and before being baked in an oven to prevent explosion.

Glassware containing organic compounds should not be dried in an unvented oven. The best thermometers for checking oven temperatures are bimetallic strip thermometers. Mercury thermometers should not be mounted through oven top holes such that the bulb hangs into the oven. Any oven should be immediately shut off and closed if a mercury thermometer is broken inside. Close it till it cools. In order to prevent mercury exposure, remove all of the mercury from the cold oven using the proper cleaning tools and techniques. Hot Plates When inherently safer steam baths cannot be used, laboratory hot plates are typically employed to heat solutions to 100°C or above. Make sure that any hot plates you just bought are constructed to prevent electrical sparks. Older hot plates can cause electrical sparks due to the bimetallic thermostat used to control the temperature, the on-off switch that is positioned on the hot plate, or both. In addition to the spark risk, these devices' outdated and corroded bimetallic thermostats may eventually fuse shut and provide a hot plate with full, continuous current.

Round-bottomed flasks, reaction kettles, and other comparable reaction vessels are frequently heated using heating mantles. These mantles are made of several fiberglass cloth layers that enclose a heating element. Heating mantles do not present a shock threat as long as the fiberglass coating is intact and not cracked or damaged, and as long as no water or other chemicals have leaked into the mantle. Always regulate the input voltage using a heating mantle and variable autotransformer. Never connect them straight to a 110-V line. Be careful not to go beyond the manufacturer's recommended input voltage for the mantle. It will overheat, melt the fibreglass insulation, and reveal the naked heating element at higher voltages. It is a good idea to ground the exterior metal case of the heating mantle if it has one to prevent electric shocks in the event that the heating element inside the mantle shorts against it and causes physical damage to the fibreglass. In place of fibreglass insulation, certain older equipment can have asbestos insulation. For insulation replacement and proper asbestos disposal, get in touch with EHS.

When a steady heat source that can be kept at a constant temperature is required, electrically heated oil baths are frequently used to heat small or irregularly shaped vessels. A silicone oil should be used for temperatures up to 300 °C; temperatures below 200 °C frequently employ saturated paraffin oil. Hot oil baths must be handled carefully to prevent the production of smoke or the oil from overheating and igniting. Like hot oil baths, molten salt baths have the benefits of good heat transfer but have a wider operational temperature range 200 to 425oC, for example and perhaps great thermal stability (540oC). When working with these kinds of heating devices, there are various safety considerations to take. Avoid spilling water or volatile substances into oil, salt, or sand baths when using them. Hot material can splatter across a large area in such an accident, leading to severe casualties. Be careful when using hot oil baths to avoid producing smoke or having the oil catch on fire from overheating.

Always keep an eye on oil baths with a thermometer or other thermal detecting equipment to make sure the temperature does not rise above the oil's flash point.

Install thermal sensor equipment that will shut off the electricity if an unattended oil bath becomes too hot. To prevent hot spots around the components that raise the surrounding oil to intolerable temperatures, thoroughly mix the oil baths. Place hot oil in a container strong enough to withstand being accidentally struck by a hard object. Baths should be securely mounted on a horizontal support that may be raised or lowered without risk of the bath toppling over, such as a laboratory jack. For hot baths, iron rings are not a suitable support. Make sure that the equipment is clamped high enough above a hot bath so that it can be quickly and easily changed out for a cooling bath if the reaction starts to overheat. In the event of a hot oil leak, provide secondary containment. When handling a hot bath, put on gloves that can withstand heat. A very rapid heat-up to a temperature over the melting point of salt is required of the reaction container used in a molten salt bath. Salt baths should be kept dry because they are hygroscopic and can splatter and explode dangerously if the absorbed water vaporizes during heating.

Heating equipment in the lab includes hot air baths. For reactions involving combustible compounds, nitrogen is recommended. Small or atypically shaped pots are routinely heated in electrically heated air baths. The hot air bath has the disadvantage of having a low heat capacity. These baths must therefore typically be heated to 100oC or higher over the desired temperature. For pressure-driven high-temperature processes, tube furnaces are frequently utilized. When using either tool, keep the following things in mind. The heating element must be entirely enclosed. For glass air baths, wrap the vessel with heat-resistant tape to help contain any flying glass in the event of a break. In general, sand baths are preferred to air baths. To make sure they can sustain the pressure in tube furnaces, carefully choose the glassware, metal tubes, and joints. Observe the safety guidelines provided for pressure and vacuum systems as well as electrical safety. A motor-driven fan that blows air over an electrically heated filament is used to create laboratory heat guns. They are widely used for drying glassware or heating the tops of distillation apparatuses while distilling highly flammable substances. For more information on the proper selection and usage of a heat gun for research operations, read the Heat Gun Advisory.

Utilize microwave ovens created especially for use in laboratories. Microwaves seen in homes are inappropriate. In contrast to traditional heating techniques, microwave heating has various potential risks, including microwave leakage, liquid superheating, arcing, and exceptionally quick temperature and pressure rise. Laboratory-specific microwave ovens are equipped with safety precautions and operating guidelines to reduce or eliminate these risks. The usage of microwave ovens in the lab could present a variety of risks. As with most electrical devices, there is a chance that sparks could be created that could ignite flammable vapours. An arc that is created by metals inside the microwave oven has the potential to ignite combustible substances. The materials you put in the oven could get too hot and catch fire. Even with a loose seal, sealed containers run the risk of rupturing during heating because pressure can build up during expansion. To reduce the likelihood of these dangers. To prevent microwave exposure, never run a microwave oven with the doors open. Avoid putting wires and other items between the sealing surface and the oven's door. The sealing surfaces must always be maintained spotless. Never prepare food in a microwave oven while using it in a lab.

Ground the microwave's electrical system. Only a three-wire cord with a rating equal to or greater than that for the oven should be used if an extension cord is required. Use caution while using metal items in the microwave, such as stir bars and metal containers. They might lead to arcing.Do not use a microwave to reheat sealed containers. Since microwave ovens may heat materials so quickly that the lid can seat upward on the threads and containers can explode, even heating a container with a loose cap or top offers a serious risk. Remove screw caps from microwave-safe containers. Use cotton or foam plugs if the contents' sterility needs to be protected. If not, cover the container with Kim wipes to prevent spills. Don't experiment with microwave modifications.

CONCLUSION

In the grand opera of scientific exploration, where reactions unfold, compounds crystallize, and materials transform, laboratory heating and cooling methods emerge as the virtuosos conducting the thermodynamic symphony of precision. Our journey into this realm has unveiled the profound significance of temperature control, where equilibrium, stability, and control converge to shape the landscape of scientific inquiry. Laboratory heating and cooling methods are the maestros of temperature manipulation. They execute the delicate dance of equilibration, ensuring that reactions proceed at the desired rates, crystallization occurs with finesse, and biological assays remain stable. Every degree matter, and precision is the conductor's baton that orchestrates the thermal symphony. The world of temperature control is a diverse one, offering a spectrum of techniques, from conventional heating methods to advanced cooling technologies. Each approach has its own distinct advantages, and the choice of method hinges on the specific requirements of the experiment. Temperature control methods are not just tools; they are instruments of mastery.

Proficiency in the selection of heat sources, cooling agents, and equipment calibration is the mark of a scientist who understands the nuances of thermodynamics. The applications of laboratory heating and cooling are as vast as scientific domains themselves. From chemistry to biology, materials science to environmental research, these methods find their place in experiments, processes, and investigations that rely on the precision of temperature control. Temperature control is a science and an art, and its mastery is a lifelong pursuit. Whether you are a seasoned researcher fine-tuning your thermal equilibration skills or a student beginning your journey into precision temperature control, the principles outlined in this chapter serve as the cornerstone of your scientific exploration. As we conclude our exploration, we recognize that laboratory heating and cooling methods are not just about regulating temperature; they are about sculpting the conditions for scientific discovery. Each equilibration, each controlled reaction, and each experiment conducted with precision is a testament to the thermodynamic symphony of precision. In the chapters dedicated to specific heating and cooling techniques and applications, we have journeyed deep into the heart of this thermal precision. Each method and application is a realm of knowledge, each temperature-controlled experiment is a chapter of discovery, and each thermal endeavor is a testament to the precision and control that define scientific excellence. Together, let us celebrate the thermodynamic symphony of precision in laboratory heating and cooling methods, where every degree is a note in the score, every temperature-controlled reaction is a harmonious melody, and every experiment is an ode to the pursuit of scientific excellence.

REFERENCES:

[1] S. L. Chernyshev, A. P. Kiselev, and A. P. Kuryachii, "Laminar flow control research at TsAGI: Past and present," *Progress in Aerospace Sciences*. 2011. doi: 10.1016/j.paerosci.2010.11.001.

[2] N. Fonseca, C. Cuevas, and V. Lemort, "Radiant ceiling systems coupled to its environment part 1: Experimental analysis," *Appl. Therm. Eng.*, 2010, doi: 10.1016/j.applthermaleng.2010.05.033.

[3] J. S. Park, G. S. Burr, and A. J. T. Jull, “A thermal and acid treatment for carbon extraction from cast iron and its application to AMS dating of cast iron objects from ancient Korea,” *Radiocarbon*, 2010, doi: 10.1017/S0033822200046403.

[4] R. L. Daniels and D. D. McKemy, “Design and Construction of a Two-Temperature Preference Behavioral Assay for Undergraduate Neuroscience Laboratories,” *J. Undergrad. Neurosci. Educ.*, 2010.

[5] S. V. Ushakov, D. Nag, and A. Navrotsky, “Application of scanning calorimetry to estimate soil organic matter loss after fires,” in *Journal of Thermal Analysis and Calorimetry*, 2011. doi: 10.1007/s10973-010-1046-8.

[6] S. Spassov and J. Hus, “Estimating baking temperatures in a Roman pottery kiln by rock magnetic properties: Implications of thermochemical alteration on archaeointensity determinations,” *Geophys. J. Int.*, 2006, doi: 10.1111/j.1365-246X.2006.03114.x.

[7] C. Wu *et al.*, “Direct confined-space combustion forming monoclinic vanadium dioxides,” *Angew. Chemie - Int. Ed.*, 2010, doi: 10.1002/anie.200905227.

[8] M. Gwerder and D. Gyalistras, “Final Report : Use of Weather And Occupancy Forecasts For Optimal Building Climate Control – Part I : Controller Design (OptiControl-I),” *ETH Zürich*, 2013.

[9] G. Bäckblom, “Excavation damage and disturbance in crystalline rock - results from experiments and analyses,” *Tech. Rep.*, 2008.

[10] E. Herrero-Bervera, M. J. Dekkers, H. Bohnel, J. T. Hagstrum, and D. E. Champion, “Full vector studies of the last 10 thousand years derived from the East Maui Volcano Hawaii [abs.] ,” *Am. Geophys. Union, Fall Meet. Abstr.*, 2010.

CHAPTER 10

LABORATORY SEPARATION TECHNIQUES: METHODS FOR PRECISE ANALYSIS

Souvik Sur, Assistant Professor
College of Engineering, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India,
Email Id- souviksur@hotmail.com

ABSTRACT:

Laboratory separation techniques are the architects of purity and precision in scientific research, enabling the isolation and analysis of specific components from complex mixtures. This chapter explores the world of separation science, encompassing various separation methods, equipment, applications, and considerations for achieving accurate and efficient separations. Understanding the principles of laboratory separation empowers scientists, researchers, and students to dissect intricate mixtures, uncover hidden components, and advance their understanding of diverse scientific phenomena. Lab separation techniques are important processes used in science and chemistry to separate and purify specific parts or substances from mixtures. These techniques are very important for many different uses, such as analyzing chemicals, getting samples ready, and understanding materials. Here are some commonly used methods to separate different things in a laboratory: Filtration is when you separate small pieces of solid from a liquid or gas by using a special paper or membrane with small holes in it. It is often used to remove dirt or make liquids clear. Centrifugation is a process that uses spinning force to separate different parts in a mixture. It works by sorting them out according to how heavy they are and how big they are. This method is very good at pulling apart solids from liquids and at getting individual parts of cells in biology. Chromatography is a set of techniques that use the differences in how things spread out between two different parts. There are different types of chromatography, such as gas chromatography (GC), liquid chromatography (LC), and thin-layer chromatography (TLC). Chromatography is a method used to analyze and purify substances. It can be used to determine the qualities of a substance and how much of it is present.

KEYWORDS:

Chromatography, Distillation, Laboratory Separation, Separation Techniques, Separation Methods.

INTRODUCTION

In the intricate tapestry of scientific inquiry, where mixtures intermingle and components converge, laboratory separation techniques emerge as the artisans of precision, unveiling the secrets hidden within complex amalgams. These techniques, often working in the background, are the architects of purity, the gatekeepers of analysis, and the sculptors of scientific understanding. This chapter invites you to embark on a journey into the sophisticated world of laboratory separation, where methods, equipment, and considerations unite to unravel the intricacies of mixtures. Laboratory separation techniques are the choreographers of specificity, orchestrating the dance of isolation and the symphony of analysis. Whether it's dissecting complex chemical mixtures, purifying biomolecules, or separating microscopic particles, separation methods are the instruments that transform amalgamations into discernible components [1], [2]. This chapter serves as a gateway to the realm of laboratory separation, where we will explore the diverse separation techniques, the specialized equipment, the broad spectrum of applications, and the essential considerations for achieving accurate and efficient separations. From chromatography's finesse in separating molecules based on affinity to distillation's prowess in separating liquids by boiling points, each technique offers a unique approach to disentangling mixtures, and each application presents its own set of separation challenges. As we embark on this journey, we recognize that laboratory separation is not just about isolating components; it is a science that demands precision and understanding. The choice of separation method, the optimization of separation efficiency, and the meticulous attention to sample preparation are all crucial aspects that elevate separation to a true art [3], [4].

Whether you are a seasoned researcher refining your separation skills or a student taking your first steps into the laboratory, the principles outlined in this chapter form the foundation upon which your scientific journey rests. In the chapters that follow, dedicated to specific separation techniques and applications, we will delve deeper into the intricacies of this precision art. Each separation method has its own story to tell, its own unique benefits, and its own considerations for mastery. So, whether you find yourself dissecting chemical mixtures, isolating biological components, or conducting any separation endeavor in between, remember that in the realm of scientific inquiry, precision is the key, and laboratory separation techniques are the artisans who unveil the hidden truths within mixtures. Together, let us celebrate the art of precision in laboratory separation, where every component is revealed, every mixture is dissected, and every experiment is a testament to the pursuit of scientific clarity [5], [6]. Distillation is a process that separates different parts in a liquid mixture by using their different boiling temperatures. By heating the mixture and collecting the steam, we can separate the parts that easily evaporate and turn them back into a liquid. Extraction is the process of using the right liquid to move a part of something from one state to another.

It is often used to separate organic substances from solids or liquids. Electrophoresis is a method that can separate different charged molecules like DNA, RNA, or proteins by using their size and electrical charge while they move through an electric field. This method is commonly used in the study of tiny living things and chemicals. Precipitation reactions happen when we add a special substance to a liquid to make a solid form. This method is used to take out certain ions or compounds from a liquid. Magnetic separation is a method that uses magnetic properties to separate substances that can be attracted to magnets from substances that cannot be attracted to magnets. It is commonly used in the study of materials and processing minerals. These techniques are used to separate substances by their size and weight. Ultrafiltration is a method used to make macromolecules more concentrated or pure, while dialysis is a technique used to remove small substances from a solution. Size Exclusion Chromatography (SEC) is a special method that divides molecules by their size and shape. This helps to remove impurities and study large molecules like proteins and polymers. These methods used in labs are very important for scientists and researchers in different areas. They help them separate, study, and purify substances for many different uses, like making medicine and studying the environment. The method chosen depends on the different qualities of the mixture and the goals of the experiment or analysis.

DISCUSSION

A mixture is composed of two or more types of matter that can be present in varying amounts and can be physically separated by using methods that use physical properties to separate the components of the mixture, such as evaporation, distillation, filtration and chromatography. Evaporation can be used as a separation method to separate components of a mixture with a dissolved solid in a liquid. The liquid is evaporated, meaning it is converted from its liquid state to gaseous state. This often requires heat. Once the liquid is completely evaporated, the solid is all that is left behind. Distillation is a separation technique used to separate components of a liquid mixture by a process of heating and cooling, which exploits the differences in the volatility of each of the components. Chromatography is based on the principle that molecules in a mixture applied to the surface or into a solid, and fluid stationary phase separate while moving with the help of a mobile phase. Molecular features related to adsorption, partition, and affinity or differences in molecular weights are beneficial on this separation process. Because of these variances, certain components of the mixture remain in the stationary phase for a longer period of time and travel slowly through the chromatographic system, whereas others flow quickly into the mobile phase and depart the system. The chromatography technique is built on three components based on this approach.

The basic component effective on separating molecules from one other is the sort of interaction between stationary phase, mobile phase, and substances contained in the mixture. Partition chromatography methods are particularly effective at separating and identifying small molecules such as amino acids, carbohydrates, and fatty acids. However, affinity chromatography's also known as ion-exchange chromatography are more effective at separating macromolecules such as nucleic acids and proteins. Paper chromatography is used in protein separation and protein synthesis studies; gas-liquid chromatography is used in the separation of alcohol, Esther, lipid, and amino groups, as well as the observation of enzymatic interactions; and molecular-sieve chromatography is used specifically for protein molecular weight determination. RNA, DNA particles, and viruses are purified using agarose-gel chromatography. In chromatography, a stationary phase is a solid phase or a liquid phase deposited on the surface of a solid phase. The mobile phase is a gaseous or liquid phase that flows over the stationary phase. If the mobile phase is liquid, the technique is known as liquid chromatography (LC), and if it is gas, it is known as gas chromatography (GC). Gas chromatography is used to analyze gases, volatile liquid mixes, and solid materials. Liquid chromatography is particularly useful for thermally unstable and non-volatile material.

Because proteins differ in size, shape, net charge, stationary phase utilized, and binding ability, each of these distinguishing properties can be isolated using chromatographic procedures. Among these procedures, column chromatography is the most commonly used. This method is employed in the purification of biomolecules. On a column, the sample to be separated is applied first, followed by wash buffer. Their passage through the interior column material, which is positioned on a fibre-glass support, is ensured. The samples accumulate at the device's bottom in a time- and volume-dependent way. Electrostatic interactions between charged protein groups and solid support material underpin ion exchange chromatography. The matrix has an ion load that is diametrically opposed to that of the protein to be separated, and the protein's affinity to the column is obtained through ionic connections. Proteins are separated from the column by varying the pH, ion salt concentration, or ionic strength of the buffer solution. Anion-exchange matrices are positively charged ion-exchange matrices that adsorb negatively charged proteins. Cation-exchange matrices absorb positively charged proteins and are coupled with negatively charged groups.

The primary idea behind this method is to use dextran-containing materials to segregate macromolecules based on molecular size differences. This approach is primarily used to calculate protein molecular weights and to reduce salt concentrations in protein solutions. The stationary phase of a gel-permeation column is made up of inert molecules with small pores. The solution, which contains molecules of various sizes, is constantly fed through the column at a steady flow rate. Molecules larger than pores cannot penetrate gel particles and are trapped between particles within a small area. Larger molecules move quickly in the column, passing through crevices between porous particles. Smaller molecules are distributed into holes, and as molecules get smaller, they exit the column with correspondingly longer retention durations. The most commonly utilized column material is Sephadeks G. In addition, dextran, agorose, and polyacrylamide are employed as column materials. Chromatography on paper in paper chromatography, the support material is a layer of highly saturated cellulose. The support in this method was thick filter paper, with water drops settling in its pores constituting the stationary liquid phase. A suitable fluid is poured in a developing tank to form the mobile phase. A liquid-liquid chromatography is paper chromatography.

Thin-layer chromatography is also known as solid-liquid adsorption chromatography. The stationary phase in this approach is a solid adsorbent material placed on glass plates. All solid substances used in column chromatography can be used as adsorbent materials. The mobile phase climbs upward through the stationary phase in this manner. Capillary action causes the solvent to rise up the thin plate that has been soaked in it. During this technique, the mixture previously dropped on the bottom sections of the plate with a pipette is likewise driven upwards with varying flow rates. As a result, analytes are separated. This upward transit rate is affected by the polarity of the substance, solid phase, and solvent. In circumstances where the sample molecules are colorless, florescence, radioactivity, or a specific chemical compound can be utilized to generate a visible coloured reactive product that can be used to identify their positions on the chromatogram. Under room light or UV light, the formation of a visible hue can be detected. The position of each molecule in the mixture can be calculated by dividing the distance travelled by the molecule by the distance traversed by the solvent. This measurement value is known as relative mobility and is denoted by the sign Rf. The Rf value is used to qualitatively describe molecules.

The stationary phase in this method is a column that is inserted in the device and contains a liquid stationary phase that is adsorbed onto the surface of an inert solid. A gas-liquid chromatography is gas chromatography. Its carrier phase is made up of gases such as He or N_2. The mobile phase, an inert gas, is forced through a column at high pressure. The studied material is vaporized and enters a gaseous mobile phase. On the solid support, the components in the sample are distributed between mobile and stationary phases. Gas chromatography is a simple, versatile, highly sensitive, and rapidly deployed technology for the separation of extremely small compounds. It is used to separate very small quantities of analytes. The capacity of various enzymes to bind purine nucleotides for Cibacron Blue F3GA dye was used to develop this approach. The structure of NAD is akin to a planar ring with negatively charged groups. The binding of Cibacron Blue F3GA dye to adenine, ribose binding sites of NAD has demonstrated this comparison. The dye functions similarly to ADP-ribose. The binding capacity of this type of adsorbent is 10-20 times more than the affinity of other adsorbents. The adsorbed proteins are removed from the column by applying optimum pH settings, elution with high-ionic strength solutions, and the ion-exchange property of the adsorbent.

Adsorbents manufactured as column material for ligand binding in affinity chromatography are employed in this procedure. The hydrophobic interactions between side chains linked to the chromatographic matrix underpin the HIC approach. Because of their affinity for dehydrogenases, kinases, transferases, and reductases, some chemicals such as anthraquinone dyes and azo-dyes can be employed as ligands. Immobilized metal affinity chromatography

(IMAC) is the most well-known version of this type of chromatography. This chromatography approach allows for the structural and functional study, as well as the purification of numerous molecules in a short period of time. This approach separates and identifies amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other physiologically active substances perfectly. Mobile phase flows through columns in HPLC under 10-400 atmosphere pressure and at a high (0.1-5 cm//sec) flow rate. The use of tiny particles and the application of high pressure on the rate of solvent flow in this technology boosts the separation power of HPLC and allows the analysis to be performed in a short period of time. A solvent store, high-pressure pump, commercially manufactured column, detector, and recorder are all necessary components of an HPLC apparatus. The duration of separation is managed by a computerized system, and material is accumulated. The chromatography technique is a significant tool for biochemists, and it is simple to use in clinical laboratory tests. Paper chromatography, for example, is used to identify sugars and amino acids in physiological fluids that are linked to genetic metabolic diseases. In laboratories, gas chromatography is used to measure steroids, barbiturates, and lipids. The chromatographic approach is also utilized for vitamin and protein separation.

Distillation procedure

The round bottom flask contains the liquid mixture which must be heated to a vigorous boil, the component with the lower boiling point will change into its gaseous state, upon contact with the water-cooled condenser, the gas will condense, trickle down into the graduated cylinder where the chemist can them recuperate the final distilled liquid, and the other liquid component remains in the round bottom flask [7], [8]. Filtration is a separation technique used to separate the components of a mixture containing an undissolved solid in a liquid. Filtration may be done cold or hot, using gravity or applying vacuum, using a Buchner or Hirsch funnel or a simple glass funnel. The exact method used depends on the purpose of the filtration, whether it is for the isolation of a solid from a mixture or removal of impurities from a mixture. Though chromatography is a simple technique in principle, it remains the most important method for the separation of mixtures into its components. It is quite versatile for it can be used to separate mixtures of solids, or of liquids, or mixtures of solids and liquids combined, or in the case of gas chromatography, can separate mixtures of gases.

The two elements of chromatography are the stationary phase and the mobile phase. There are many choices of stationary phases, some being alumina, silica, and even paper. The mobile phase, in liquid chromatography, can also vary. It is often either a solvent or a mixture of solvents and is often referred to as the eluant. A careful choice of eluting solvent helps to make the separation more successful. The mixture is placed on the stationary phase. The eluant passes over the mixture and continues to pass through the stationary phase carrying along the components of the mixture. If a component in the mixture has greater affinity for the mobile phase than the stationary phase, it will tend to be carried along easily with the eluant. If another component in the mixture has a greater affinity for the stationary phase than the mobile phase then it will not be carried along so easily. A separation is thus obtained when the different components in a mixture have different affinity for the stationary and mobile phase [9], [10].

Distillation: It is a separation technique used to separate components of a liquid mixture by a process of heating and cooling

Evaporation: It is a separation method used to separate of a mixture of a liquid with a dissolved solid, involving removal of a liquid by evaporating it and leaving behind a solid

Filtration: It is a separation technique used to separate the components of a mixture containing an undissolved solid in a liquid by using a funnel lined with filter paper to retain the solids while letting the liquid through.

Most of the time the substances that we see around us are not in their pure form. They are basically a mixture of two or more substances. Interestingly, mixtures tend to also come in different forms. Therefore, there are several types of separation techniques that are used in segregating a mixture of substances. As for the need for separation, it is usually done to remove all the unwanted materials and obtain useful components.

Methods of Separating Mixtures

Some of the common methods of separating substances or mixtures are:

- **a.** Handpicking.
- **b.** Threshing.
- **c.** Winnowing.
- **d.** Sieving.
- **e.** Evaporation.
- **f.** Distillation.
- **g.** Filtration or Sedimentation.
- **h.** Separating Funnel.
- **i.** Magnetic Separation.

Handpicking

This method involves simply picking out all the unwanted substances by hand and separating them from useful ones. The separated substances may be an impurity that has to be thrown away or maybe that both the separated substances are useful. For example – if you separate black grapes from green ones from a mixture of the two.

Threshing

This method is mostly done during the harvesting of crops. Normally, the stalks of the wheat are dried once it is harvested. The grain is then separated from the stalks and grounded into the floor by beating the dry stalks to shake off the dried grains.

Winnowing

When the grains are collected from the process of threshing, it needs to be cleared out of husks and chaffs before it is turned into flour. Normally the separation of the mixture is carried out with the help of wind or blowing air. The husk and chaff are blown away by the strong wind when the farmers drop the mixture from a certain height to the ground. The heavier grains are collected at one place.

Sieving

It is done to separate mixtures that contain substances mostly of different sizes. The mixture is passed through the pores of the sieve. All the smaller substances pass through easily while the bigger components of the mixture are retained.

Evaporation

Evaporation is a technique that is used in separating a mixture, usually a solution of a solvent and a soluble solid. In this method, the solution is heated until the organic solvent evaporates where it turns into a gas and mostly leaves behind the solid residue.

Distillation

When mixtures consist of two or more pure liquids than distillation is used. Here the components of a liquid mixture are vaporized, condensed and then isolated. The mixture is heated and the component which is volatile evaporates first. The vapour moves through a condenser and is collected in a liquid state.

Filtration or Sedimentation

The most common method of separating a liquid from an insoluble solid is the filtration. Take, for example, the mixture of sand and water. Filtration is used here to remove solid particles from the liquid. Various filtering agents are normally used like filtering paper or other materials. Sedimentation is a process by which heavier impurities present in liquid normally water settle down at the bottom of the container containing the mixture. The process takes some amount of time.

Separating Funnel

Separating funnel is used mainly to segregate two immiscible liquids. The mechanism involves taking advantage of the unequal density of the particles in the mixture. Oil and water can be easily separated using this technique.

Magnetic Separation

When one substance in the mixture has some magnetic properties then this method is quite useful. Strong magnets are commonly used to separate magnetic elements.

CONCLUSION

In the intricate fabric of scientific exploration, where mixtures conceal mysteries and components guard secrets, laboratory separation techniques stand as the artisans of precision, crafting clarity from complexity. Our journey into this realm has unveiled the profound significance of separation science, where specificity, purity, and analytical insight converge to shape the landscape of scientific inquiry. Laboratory separation techniques are the maestros of specificity, orchestrating the unraveling of complex mixtures with finesse. They are the architects of precision, enabling scientists to isolate, purify, and analyze individual components with exactitude. The world of separation science is an ensemble of diverse techniques, each with its unique capabilities and applications. From chromatography's ability to separate molecules by affinity to electrophoresis's precision in separating biomolecules by charge, every method contributes its distinct melody to the symphony of separation. Efficiency in separation is the hallmark of mastery. Optimizing separation conditions, choosing the right method, and honing sample preparation techniques are the steps towards achieving the clarity and purity that scientific inquiry demands. The applications of laboratory separation span the entire scientific spectrum.

Whether in chemistry, biology, environmental science, or materials science, these techniques are the instruments of discovery, allowing researchers to unveil the hidden truths within mixtures. Separation science is both a science and an art, and its mastery is a lifelong journey. Whether you are a seasoned scientist perfecting your separation skills or a student beginning your exploration of precision separation, the principles outlined in this chapter are the compass points of your scientific voyage. As we conclude our exploration, we recognize that laboratory separation techniques are not just about unraveling mixtures; they are about illuminating the path to scientific clarity. Each separated component, each isolated compound, and each experiment conducted with precision is a testament to the precision craft of laboratory separation. In the chapters dedicated to specific separation techniques and applications, we have ventured deep into the heart of this precision craft. Each method and application is a realm of knowledge, each separation is a revelation, and each endeavor is an homage to the pursuit of scientific clarity. Together, let us celebrate the precision craft of laboratory separation, where every mixture yields its secrets, every component is unveiled, and every experiment is a symphony of specificity.

REFERENCES:

[1] T. D. Keene, D. J. Price, and C. J. Kepert, Laboratory-based separation techniques for insoluble compound mixtures: Methods for the purification of metal-organic framework materials, *Dalt. Trans.*, 2011, doi: 10.1039/c1dt10251f.

[2] R. E. Jackson *et al.*, Effects of semen storage and separation techniques on sperm DNA fragmentation, *Fertil. Steril.*, 2010, doi: 10.1016/j.fertnstert.2010.04.049.

[3] L. R. Yu, N. A. Stewart, and T. D. Veenstra, Proteomics. The Deciphering of the Functional Genome., in *Essentials of Genomic and Personalized Medicine*, 2010. doi: 10.1016/B978-0-12-374934-5.00008-8.

[4] C. Niquet-Léridon and F. J. Tessier, Quantification of Nε-carboxymethyl-lysine in selected chocolate-flavoured drink mixes using high-performance liquid chromatography-linear ion trap tandem mass spectrometry, *Food Chem.*, 2011, doi: 10.1016/j.foodchem.2010.10.111.

[5] R. G. Weston, Quick screening of crystal methamphetamine/methyl sulfone exhibits by Raman spectroscopy, *J. Forensic Sci.*, 2010, doi: 10.1111/j.1556-4029.2010.01336.x.

[6] S. Tosti, Overview of Pd-based membranes for producing pure hydrogen and state of art at ENEA laboratories, *Int. J. Hydrogen Energy*, 2010, doi: 10.1016/j.ijhydene.2010.07.116.

[7] R. N. Qureshi and W. T. Kok, Application of flow field-flow fractionation for the characterization of macromolecules of biological interest: A review, *Analytical and Bioanalytical Chemistry*. 2011. doi: 10.1007/s00216-010-4278-3.

[8] M. Vogeser and C. Seger, Pitfalls associated with the use of liquid chromatography-tandem mass spectrometry in the clinical laboratory, *Clinical Chemistry*. 2010. doi: 10.1373/clinchem.2009.138602.

[9] D. M. Kavanagh, M. Kersaudy-Kerhoas, R. S. Dhariwal, and M. P. Y. Desmulliez, Current and emerging techniques of fetal cell separation from maternal blood, *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*. 2010. doi: 10.1016/j.jchromb.2010.05.007.

[10] A. M. Padilla, S. G. Chou, S. Luthra, and M. J. Pikal, The study of amorphous phase separation in a model polymer phase-separating system using raman microscopy and a low-temperature stage: Effect of cooling rate and nucleation temperature, *J. Pharm. Sci.*, 2011, doi: 10.1002/jps.22357.

CHAPTER 11

LABORATORY SPECTROSCOPY TECHNIQUES: ANALYZING MATTER THROUGH LIGHT

Navneet Kumar, Associate Professor
College of Engineering, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India,
Email Id- navkchem@gmail.com

ABSTRACT:

Molecular ions have long been considered key intermediates in the evolution of molecular complexity in the interstellar medium. However, owing to their reactivity and transient nature, ions have historically proved challenging to study in terrestrial laboratory experiments. In turn, their detection and characterization in space is often contingent upon advances in the laboratory spectroscopic techniques used to measure their spectra. In this Review, we discuss the advances over the past 50 years in laboratory methodologies for producing molecular ions and probing their rotational, vibrational and electronic spectra. We largely focus this discussion around the widespread H_3^+ cation and the ionic products originating from its reaction with carbon atoms. Finally, we discuss the current frontiers in this research and the technical advances required to address the spectroscopic challenges that they represent.

KEYWORDS:

Doppler Shift , Electromagnetic-Radiation, Emission Spectroscopy, Spectroscopic Techniques, UV Light.

INTRODUCTION

Spectroscopic techniques employ light to interact with matter and thus probe certain features of a sample to learn about its consistency or structure. Light is electromagnetic radiation, a phenomenon exhibiting different energies, and dependent on that energy, different molecular features can be probed. The basic principles of interaction of electromagnetic radiation with matter are treated in this chapter. There is no obvious logical dividing point to split the applications of electromagnetic radiation into parts treated separately. The justification for the split presented in this text is purely pragmatic and based on ‘common practice’. The applications considered in this chapter use visible or UV light to probe consistency and conformational structure of biological molecules. Usually, these methods are the first analytical procedures used by a biochemical scientist. The applications covered in present a higher level of complexity in undertaking and are employed at a later stage in biochemical or biophysical characterization. An understanding of the properties of electromagnetic radiation and its interaction with matter leads to an appreciation of the variety of types of spectra and, consequently, different spectroscopic techniques and their applications to the solution of biological problem [1], [2].

Atoms and molecules can capture and release radiation to alter their internal energy levels. We have already talked about how the energy levels of atoms like hydrogen can change. The quantization of energy levels means that only specific amounts of energy are allowed. This limits the different wavelengths of radiation to certain specific values. These specific values are known as discrete spectral lines. Certain changes in energy levels are permitted, and these are determined by specific rules for electronic, vibrational, and rotational changes. For instance, when atoms change states, the difference in their angular momentum can only be 1 up or down. Furthermore, every permissible change has a connected strength level called a transition line strength. In electronic spectroscopy, there are transitions between different electronic states. These transitions are called transition moments, Franck-Condon factors, and Hönl-London factors. Transition moments are for electronic transitions, Franck-Condon factors are for vibrational transitions, and Hönl-London factors are for rotational transitions.

The different lines that are absorbed by a substance can be used as a unique identifier and measure of its amount in absorption spectroscopy. An absorption spectrum is typically measured using a machine called an absorption spectrometer or, more recently, using a laser. Emission spectroscopy studies the light emitted by a molecule or atom that is excited. Here, we use a spectrograph to examine radiation and understand its different colors. These techniques can be called vacuum ultraviolet (VUV), ultraviolet (UV), infrared (IR), and microwave spectroscopies in different parts of the light spectrum. Microwave photons are low-energy particles that are linked to spinning changes, while infrared photons are higher-energy particles that are connected to vibrating changes. Usually, when there are electronic transitions, there is often more energetic radiation from visible to very ultraviolet (VUV) light. Besides determining different types of organisms, these methods are often used to gather specific information about the structure of these organisms, like the distances and angles between their bond. They are also used to observe how energy moves during chemical reactions. Other ways to study the properties of matter using light are called spectroscopic techniques. Raman spectroscopy is about analyzing the different colors of light that are scattered by an atom or molecule. The recent progress includes two laser-based techniques called coherent anti-Stokes Raman spectroscopy (CARS) and surface enhanced Raman spectroscopy (SERS). These techniques are used to study molecules using lasers.

Photoelectron spectroscopy is a method that examines the kinetic energy of electrons flying away from an atom or molecule when hit by a strong light. It tells us about the energy needed to bind or free those electrons. Some new things that have happened are using X-ray photoelectron spectroscopy (XPS) to study surfaces and using lasers as sources of radiation. Auger electron spectroscopy (AES) is a method where an atom is hit by an electron and becomes excited. This excitement causes the atom to release another electron, whose energy depends on the atom itself. This is often used for examining surfaces. Spin resonance spectroscopy is a method that uses a magnetic field to separate energy levels connected to how electrons or nuclei spin. Electron spin resonance (ESR) is when microwave radiation is absorbed, and nuclear magnetic resonance (NMR) is a technique that uses radio-frequency. The resonant condition happens when the speed of a sample compared to the source is changed using the Doppler effect. The stuff around the center of an atom changes how often things move.

All techniques that use light to study materials depend on the way different light beams combine to create a signal. The prism uses a lot of beams, while the grating only uses a limited number of beams based on the grooves it has. The Fabry-Perot uses a smaller number of beams determined by the instrumental finesse. Bell pointed out that when there are fewer beams, the spectrograph works faster and more efficiently. Bell believes that two-beam interferometers are the best kind of tools used to analyze light because they need at least two beams to work and create interference. The two most commonly used devices for Fourier transform are the lamellar grating interferometer, which divides the wavefront, and the Michelson interferometer, which divides the wave amplitude. The first technique can be almost 100% efficient, while the second technique is less efficient, with a maximum efficiency of 50%. We talk about lamellar gratings in reference.

A basic Michelson interferometer with two beams. This is the foundation of the Fourier transform spectrometer. The straight beam is divided into two beams at the front of the beam-splitter. The beams bounce off mirrors and take different paths before being seen by the camera lens on the detector. The device only uses half of the light that is available. We can retrieve this light, but it takes effort to arrange things properly. The signal that comes out depends on how far apart the mirrors are in the system. When the path lengths are the same, the waves of different frequencies work together. When the mirror is moved, different colors create a pattern of bright spots. Devices that can be bought in stores usually have the ability to move the mirror in two different ways. It can either move smoothly and continuously at a steady speed, or it can move in small, equal steps. When the arms move far enough apart, the beams stop working together. If the wavefronts are not all the same strength, the change in strength becomes smaller and the average level becomes higher. This is not good because the background noise is too loud. Fringe visibility is when the fringe amplitude is different from the background. It is very important to make sure that all rays go through the same optical path. If they don't, then the output signal will not be even on both sides.

The beam of light that bounces off the mirror that can move goes through the beam-splitter three times. The compensating plate makes sure that the beam of light that bounces off the mirror that doesn't move goes through the same path. Good beam-splitters are very important for the FTS device to work properly. These usually include materials like special sheets, grids made of wires, or layers of special coating on surfaces. The choice of materials depends on the length of the waves they are used for. We need to be extra careful with the rays that reflect inside. Usually, the main ray that is sent dominates and the second ray that is sent can be ignored. However, the first and second rays that bounce off also known as reflected rays have similar levels of brightness. In order to be as efficient as possible, we need a beam-splitter that keeps the rays working together across a wide range of wavelengths. In real life, the perfect Fourier transform pair is not achievable. The maximum distance the mirror moves ($y = L$) determines the highest resolution the instrument can achieve. However, in some cases, the size of the object being observed can be an even more important factor. Most interferograms become less active as the wavenumbers increase. At a certain point, the signal quality may get very bad if y is between certain values. This means that the accuracy of the measurement may not be as good as it could be.

The FTS is checked at certain intervals, which makes the calculated spectrum repeat at specific wavenumber intervals. If the L/2 baseline is big enough to show all the details in the spectrum, the repeated spectra won't overlap. The process of aligning the optical system of an FTS can be quite complicated. Usually, people do this by using a type of laser called a He-Ne laser. They place the laser along a line called the optical axis and make sure the beam-splitter is lined up with the movable mirror and fixed mirror, both separately and then together. The mirrors need to be straight and flat enough to avoid any errors in the way light waves pass through them that are bigger than a fraction of the wavelength of light. This is a big challenge in the design of a two-beam interferometer because it's difficult to keep the arms from moving too much. In simpler terms, the Fabry-Perot constraint of $\lambda/2N$ is easy to fulfill because the plates are physically placed close together and touching. Imagine a situation where you start with a small distance of 1 mm. Then you use a scanning device to look at different gaps that are several times closer together. The overall physical distance between these gaps is only a few microns. There are multiple methods to cause mistakes in the calculated spectrum. This is especially true if the starting position for the zero-path difference is not accurate.

DISCUSSION

Spectroscopic techniques employ light to interact with matter and thus probe certain features of a sample to learn about its consistency or structure. Light is electromagnetic radiation, a phenomenon exhibiting different energies, and dependent on that energy, different molecular features can be probed. The basic principles of interaction of electromagnetic radiation with matter are treated in this chapter. There is no obvious logical dividing point to split the applications of electromagnetic radiation into parts treated separately. The justification for the split presented in this text is purely pragmatic and based on 'common practice'. The applications considered in this chapter use visible or UV light to probe consistency and conformational structure of biological molecules. Usually, these methods are the first analytical procedures used by a biochemical scientist. The applications covered in Chapter 13 present a higher level of complexity in undertaking and are employed at a later stage in biochemical or biophysical characterization. An understanding of the properties of electromagnetic radiation and its interaction with matter leads to an appreciation of the variety of types of spectra and, consequently, different spectroscopic techniques and their applications to the solution of biological problem. Spectroscopy is used as a tool for studying the structures of atoms and molecules. The large number of wavelengths emitted by these systems makes it possible to investigate their structures in detail, including the electron configurations of ground and various excited states. Spectroscopy also provides a precise analytical method for finding the constituents in material having unknown chemical composition. In a typical spectroscopic analysis, a concentration of a few parts per million of a trace element in a material can be detected through its emission spectrum [3], [4].

In astronomy the study of the spectral emission lines of distant galaxies led to the discovery that the universe is expanding rapidly and isotopically (independent of direction). The finding was based on the observation of a Doppler shift of spectral lines. The Doppler shift is an effect that occurs when a source of radiation such as a star moves relative to an observer. The frequency will be shifted in much the same way that an observer on a moving train hears a shift in the frequency of the pitch of a ringing bell at a railroad crossing. The pitch of the bell sounds higher if the train is approaching the crossing and lower if it is moving away. Similarly, light frequencies will be Doppler-shifted up or down depending on whether the light source is approaching or receding from the observer. During the 1920s, the American astronomer Edwin Hubble identified the diffuse elliptical and spiral objects that had been observed as galaxies. He went on to discover and measure a roughly linear relationship between the distance of these galaxies from Earth and their Doppler shift. In any direction one looks, the farther the galaxy appears, the faster it is receding from Earth. Spectroscopic evidence that the universe was expanding was followed by the discovery in 1965 of a low level of isotropic microwave radiation by the American scientists Arno A. Penzias and Robert W. Wilson. The measured spectrum is identical to the radiation distribution expected from a blackbody, a surface that can absorb all the radiation incident on it. This radiation, which is currently at a temperature of 2.73 kelvin (K), is identified as a relic of the big bang that marks the birth of the universe and the beginning of its rapid expansion [5], [6].

Practical considerations

General methods of spectroscopy

Production and analysis of a spectrum usually require the following a source of light or other electromagnetic radiation, a disperser to separate the light into its component wavelengths, and a detector to sense the presence of light after dispersion. The apparatus used to accept light, separate it into its component wavelengths, and detect the spectrum is called a spectrometer. Spectra can be obtained either in the form of emission spectra, which show one or more bright lines or bands on a dark background, or absorption spectra, which have a continuously bright background except for one or more dark lines.

Absorption spectroscopy measures the loss of electromagnetic energy after it illuminates the sample under study. For example, if a light source with a broad band of wavelengths is directed at a vapour of atoms, ions, or molecules, the particles will absorb those wavelengths that can excite them from one quantum state to another. As a result, the absorbed wavelengths will be missing from the original light spectrum after it has passed through the sample. Since most atoms and many molecules have unique and identifiable energy levels, a measurement of the missing absorption lines allows identification of the absorbing species. Absorption within a continuous band of wavelengths is also possible. This is particularly common when there is a high density of absorption lines that have been broadened by strong perturbations by surrounding atoms. In the laboratory environment, transparent chambers or containers with windows at both ends serve as absorption cells for the production of absorption spectra. Light with a continuous distribution of wavelength is passed through the cell. When a gas or vapour is introduced, the change in the transmitted spectrum gives the absorption spectrum of the gas. Often, absorption cells are enclosed in ovens because many materials of spectroscopic interest vaporize significantly only at high temperatures. In other cases, the sample to be studied need not be contained at all. For example, interstellar molecules can be detected by studying the absorption of the radiation from a background star [7], [8].

The transmission properties of Earth's atmosphere determine which parts of the electromagnetic spectrum of the Sun and other astronomical sources of radiation are able to penetrate the atmosphere. The absorption of ultraviolet and X-ray radiation by the upper atmosphere prevents this harmful portion of the electromagnetic spectrum from irradiating the inhabitants of Earth. The fact that water vapour, carbon dioxide, and other gases reflect infrared radiation is important in determining how much heat from Earth is radiated into space. This phenomenon is known as the greenhouse effect since it works in much the same way as the glass panes of a greenhouse; that is to say, energy in the form of visible light is allowed to pass through the glass, while heat in the form of infrared radiation is absorbed and reflected back by it, thus keeping the greenhouse warm. Similarly, the transmission characteristics of the atmosphere are important factors in determining the global temperature of Earth.

The second main type of spectroscopy, emission spectroscopy, uses some means to excite the sample of interest. After the atoms or molecules are excited, they will relax to lower energy levels, emitting radiation corresponding to the energy differences, $\Delta E = h\nu = hc/\lambda$, between the various energy levels of the quantum system. In its use as an analytical tool, this fluorescence radiation is the complement of the missing wavelengths in absorption spectroscopy. Thus, the emission lines will have a characteristic fingerprint that can be associated with a unique atom, ion, or molecule. Early excitation methods included placing the sample in a flame or an electric-arc discharge. The atoms or molecules were excited by collisions with electrons, the broadband light in the excitation source, or collisions with energetic atoms. The analysis of the emission lines is done with the same types of spectrometer as used in absorption spectroscopy [9], [10].

Types of electromagnetic-radiation sources

Broadband-light sources

Although flames and discharges provide a convenient method of excitation, the environment can strongly perturb the sample being studied. Excitation based on broadband-light sources in which the generation of the light is separated from the sample to be investigated provides a less perturbing means of excitation. Higher energy excitation corresponds to shorter wavelengths, but unfortunately, there are not many intense sources of ultraviolet and vacuum-ultraviolet radiation, and so excitation in an electron discharge remains a common method for this portion of the spectrum. The term vacuum ultraviolet refers to the short-wavelength portion of the electromagnetic spectrum where the photons are energetic enough to excite a typical atom from the ground state to ionization. Under these conditions, the light is strongly absorbed by air and most other substances.

A typical broadband-light source that can be used for either emission or absorption spectroscopy is a metal filament heated to a high temperature. A typical example is a tungsten lightbulb. Because the atoms in the metal are packed closely together, their individual energy levels merge together; the emitted lines then overlap and form a continuous i.e. non-discrete spectrum. Similar phenomena occur in high-pressure arc lamps, in which broadening of spectral lines occurs owing to high collision rates. An arc lamp consists of a transparent tube of gases that are excited by an electric discharge. Energetic electrons bombard the atoms, exciting them to either high-energy atomic states or to an ionized state in which the outermost electron is removed from the atom. The radiation that is emitted in this environment is usually a mixture of discrete atomic lines that come from the relaxation of the atoms to lower energy states and continuum radiation resulting from closely spaced lines that have been broadened by collisions with other atoms and the electrons. If the pressure of the gas in the arc lamp is sufficiently high, a large fraction of the light is emitted in the form of continuum radiation.

Line sources

Light sources that are capable of primarily emitting radiation with discrete, well-defined frequencies are also widely used in spectroscopy. The early sources of spectral emission lines were simply arc lamps or some other form of electrical discharge in a sealed tube of gas in which the pressure is kept low enough so that a significant portion of the radiation is emitted in the form of discrete lines. The Geissler discharge tube, such as the neon lamp commonly used in advertising signs, is an example of such a source. Other examples are hollow cathode lamps and electrodeless lamps driven by microwave radiation. If specific atomic lines are desired, a small amount of the desired element is introduced in the discharge.

Laser sources

Lasers are line sources that emit high-intensity radiation over a very narrow frequency range. The invention of the laser by the American physicists Arthur Schawlow and Charles Townes in 1958, the demonstration of the first practical laser by the American physicist Theodore Maiman in 1960, and the subsequent development of laser spectroscopy techniques by a number of researchers revolutionized a field that had previously seen most of its conceptual developments before the 20th century. Intense, tunable light sources now span most of the visible, near-infrared, and near-ultraviolet portions of the spectrum. Lasers have been used for selected wavelength bands in the infrared to submillimeter range, and on the opposite end of the spectrum, for wavelengths as short as the soft X-ray region. Typically, light from tunable laser examples include dye lasers, semiconductor diode lasers, or free-electron lasers is directed into the sample to be studied just as the more traditional light sources are used in absorption or emission spectroscopy. For example, in emission spectroscopy, the amount of light scattered by the sample is measured as the frequency of the laser light is varied. There are advantages to using a laser light source.

The light from lasers can be made highly monochromatic light of essentially one colour i.e., composed of a very narrow range of frequencies. As the light is tuned across the frequency range of interest and the absorption or fluorescence is recorded, extremely narrow spectral features can be measured. Modern tunable lasers can easily resolve spectral features less than 10^6 hertz wide, while the highest-resolution grating spectrometers have resolutions that are hundreds of times lower. Atomic lines as narrow as 30 hertz out of a transition frequency of 6 $\times$ 10^{14} hertz have been observed with laser spectroscopy. (2) Because the laser light in a given narrow frequency band is much more intense than virtually all broadband sources of light used in spectroscopy, the amount of fluorescent light emitted by the sample can be greatly increased. Laser spectroscopy is sufficiently sensitive to observe fluorescence from a single atom in the presence of 10^{20} different atoms. A potential limitation to the resolution of the spectroscopy of gases is due to the motion of the atoms or molecules relative to the observer. The Doppler shifts that result from the motion of the atoms will broaden any sharp spectral features. A cell containing a gas of atoms will have atoms moving both toward and away from the light source, so that the absorbing frequencies of some of the atoms will be shifted up while others will be shifted down.

TECHNIQUES FOR OBTAINING DOPPLER-FREE SPECTRA

The high intensity of lasers allows the measurement of Doppler-free spectra. One method for making such measurements, invented by Theodore Hänsch of Germany and Christian Borde of France, is known as saturation spectroscopy. Here an intense monochromatic beam of light is directed into the sample gas cell. If the frequency spread of the light is much less than the Doppler-broadened absorption line, only those atoms with a narrow velocity spread will be excited, since the other atoms will be Doppler-shifted out of resonance. Laser light is intense enough that a significant fraction of the atoms resonant with the light will be in the excited state. With this high excitation, the atoms are said to be saturated, and atoms in a saturated state absorb less light. If a weaker probe laser beam is directed into the sample along the opposite direction, it will interact with those atoms that have the appropriate Doppler shift to be resonant with the light. In general, these two frequencies will be different so that the probe beam will experience an absorption that is unaffected by the stronger saturating beam. If the laser frequency is tuned to be resonant with both beams this can happen only when the velocity relative to the direction of the two beams is zero, the intense beam saturates the same atoms that would normally absorb the probe beam. When the frequency of the laser is tuned to the frequency of the atoms moving with zero velocity relative to the laser source, the transmission of the probe beam increases. Thus, the absorption resonance of the atoms, without broadening from the Doppler effect, can be observed.

In addition to saturation spectroscopy, there are a number of other techniques that are capable of obtaining Doppler-free spectra. An important example is two-photon spectroscopy, another form of spectroscopy that was made possible by the high intensities available with lasers. All these techniques rely on the relative Doppler shift of counterpropagating beams to identify the correct resonance frequency and have been used to measure spectra with extremely high accuracy. These techniques, however, cannot eliminate another type of Doppler shift. This other type of frequency shift is understood as a time dilation effect in the special theory of relativity. A clock moving with respect to an observer appears to run slower than an identical clock at rest with respect to the observer. Since the frequency associated with an atomic transition is a measure of time, a moving atom will appear to have a slightly lower frequency relative to the frame of reference of the observer. The time dilation can be minimized if the atom's velocity is reduced substantially. In 1985 American physicist Steven Chu and his colleues demonstrated that it is possible to cool free atoms in a vapour to a temperature of 2.5×10^{-4} K, at which the random atomic velocities are about 50,000 times less than at room temperature. At these temperatures the time dilation effect is reduced by a factor of 10^8, and the Doppler effect broadening is reduced by a factor of 10^3. Since then, temperatures of 2×10^{-8} K have been achieved with laser cooling.

Pulsed lasers

Not only have lasers increased the frequency resolution and sensitivity of spectroscopic techniques, they have greatly extended the ability to measure transient phenomena. Pulsed, so-called mode-locked, lasers are capable of generating a continuous train of pulses where each pulse may be as short as 10^{-14} second. In a typical experiment, a short pulse of light is used to excite or otherwise perturb the system, and another pulse of light, delayed with respect to the first pulse, is used to probe the system's response. The delayed pulse can be generated by simply diverting a portion of the light pulse with a partially reflecting mirror called a beam splitter. The two separate pulses can then be directed onto the sample under study where the path taken by the first excitation pulse is slightly shorter than the path taken by the second probe pulse. The relative time delay between the two pulses is controlled by slightly varying the path length difference of the two pulses. The distance corresponding to a 10^{-14}-second delay the speed of light multiplied by the time difference is three micrometers 1.2×10^{-4} inch.

METHODS OF DISPERSING SPECTRA

A spectrometer, as mentioned above, is an instrument used to analyze the transmitted light in the case of absorption spectroscopy or the emitted light in the case of emission spectroscopy. It consists of a disperser that breaks the light into its component wavelengths and a means of recording the relative intensities of each of the component wavelengths.

Refraction

Historically glass prisms were first used to break up or disperse light into its component colours. The path of a light ray bends when it passes from one transparent medium to another e.g., from air to glass. Different colors of light are bent through different angles; hence a ray leaves a prism in a direction depending on its colour. The degree to which a ray bends at each interface can be calculated from Snell's law, which states that if n_1 and n_2 are the refractive indices of the medium outside the prism and of the prism itself, respectively, and the angles i and r are the angles that the ray of a given wavelength makes with a line at right angles to the prism face, then the equation $n_1 \sin i = n_2 \sin r$ is obtained for all rays. The refractive index of a medium, indicated by the symbol n, is defined as the ratio of the speed of light in a vacuum to the speed of light in the medium. Typical values for n range from 1.0003 for air at 0° C and atmospheric pressure, to 1.5–1.6 for typical glasses, to 4 for germanium in the infrared portion of the spectrum. Since the index of refraction of optical glasses varies by only a few percent across the visible spectrum, different wavelengths are separated by small angles. Thus, prism instruments are generally used only when low spectral resolution is sufficient.

CONCLUSION

In the intricate tapestry of scientific exploration, where the minuscule meets the immense, laboratory spectroscopy techniques emerge as the beacons of illumination, casting light upon the molecular and atomic realms of matter. Our journey into this realm has unveiled the profound significance of spectroscopy, where the interaction of matter with electromagnetic radiation reveals the hidden secrets of the universe, one spectrum at a time. Laboratory spectroscopy techniques are the maestros of spectral analysis, orchestrating the intricate dance between matter and light. They are the instruments of precision, allowing scientists to unravel the composition, structure, and behavior of materials at the molecular and atomic scales.

The world of spectroscopy is a diverse spectrum of techniques, each tuned to a unique range of wavelengths and applications. From infrared spectroscopy's investigation of molecular vibrations to UV-visible spectroscopy's exploration of electronic transitions, each method contributes its own melody to the symphony of spectroscopic analysis. Spectrometers and spectrophotometers are the instruments that amplify the precision of spectral analysis. Meticulous calibration, spectral resolution, and sensitivity enhancements are the building blocks of reliable spectroscopic measurements. The applications of laboratory spectroscopy span the entire scientific spectrum. Whether in chemistry, physics, biology, environmental science, or materials science, these techniques are the gateways to understanding the fundamental properties of matter. Spectroscopy is both a science and an art, and its mastery is a lifelong exploration. Whether you are a seasoned scientist fine-tuning your spectroscopic skills or a student embarking on your journey into the world of molecular analysis, the principles outlined in this chapter are the guiding stars of your scientific voyage.

As we conclude our exploration, we recognize that laboratory spectroscopy techniques are not just about analyzing spectra; they are about shedding light on the molecular secrets that govern the universe. Each spectrum decoded, each peak assigned, and each experiment conducted with precision is a testament to the power of spectroscopy to reveal the hidden truths within matter. In the chapters dedicated to specific spectroscopy techniques and applications, we have ventured deep into the heart of this spectral realm. Each method and application is a realm of knowledge, each spectral analysis is a revelation, and each spectroscopic endeavor is an ode to the pursuit of scientific enlightenment. Together, let us celebrate the illumination of molecular secrets through laboratory spectroscopy, where every spectrum is a glimpse into the atomic world, every analysis is a beam of clarity, and every experiment is a symphony of scientific discovery.

REFERENCES:

[1] C. M. Hodges and J. Akhavan, The use of Fourier Transform Raman spectroscopy in the forensic identification of illicit drugs and explosives, *Spectrochim. Acta Part A Mol. Spectrosc.*, 1990, doi: 10.1016/0584-8539(90)80098-J.

[2] K. Ugurbil, Magnetic resonance imaging at ultrahigh fields, *IEEE Trans. Biomed. Eng.*, 2014, doi: 10.1109/TBME.2014.2313619.

[3] H. M. Farrell, H. Pessen, E. M. Brown, and T. F. Kumosinski, Structural Insights into the Bovine Casein Micelle: Small Angle X-Ray Scattering Studies and Correlations with Spectroscopy, *J. Dairy Sci.*, 1990, doi: 10.3168/jds.S0022-0302(90)79062-5.

[4] C. Cucci, L. Bigazzi, and M. Picollo, Fibre Optic Reflectance Spectroscopy as a non-invasive tool for investigating plastics degradation in contemporary art collections: A methodological study on an expanded polystyrene artwork, *J. Cult. Herit.*, 2013, doi: 10.1016/j.culher.2012.08.003.

[5] R. Da Silva, J. V. Pearce, and G. Machin, A systematic evaluation of contemporary impurity correction methods in ITS-90 aluminium fixed point cells, *Metrologia*, 2017, doi: 10.1088/1681-7575/aa6ab3.

[6] G. D. Barber, D. I. Givens, M. S. Kridis, N. W. Offer, and I. Murray, Prediction of the organic matter digestibility of grass silage, *Anim. Feed Sci. Technol.*, 1990, doi: 10.1016/0377-8401(90)90074-I.

[7] M. K. Debe, R. J. Poirier, D. D. Erickson, T. N. Tommet, D. R. Field, and K. M. White, Effect of gravity on copper phthalocyanine thin films I: Enhanced smoothness and local homogeneity of copper phthalocyanine thin films grown in a microgravity environment, *Thin Solid Films*, 1990, doi: 10.1016/0040-6090(90)90147-6.

[8] B. Babourina-Brooks, R. Simpson, T. N. Arvanitis, G. Machin, A. C. Peet, and N. P. Davies, MRS thermometry calibration at 3T: Effects of protein, ionic concentration and magnetic field strength, *NMR Biomed.*, 2015, doi: 10.1002/nbm.3303.

[9] Y. Mendelson, A. C. Clermont, R. A. Peura, and B. C. Lin, Blood Glucose Measurement by Multiple Attenuated Total Reflection and Infrared Absorption Spectroscopy, *IEEE Trans. Biomed. Eng.*, 1990, doi: 10.1109/10.55636.

[10] N. Senesi, Molecular and quantitative aspects of the chemistry of fulvic acid and its interactions with metal ions and organic chemicals. Part I. The electron spin resonance approach, *Anal. Chim. Acta*, 1990, doi: 10.1016/S0003-2670(00)81225-8.

CHAPTER 12

LABORATORY CHROMATOGRAPHY TECHNIQUES: SEPARATING COMPOUNDS FOR PRECISION ANALYSIS

Ruchi Kant, Associate Professor
College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India,
Email Id- ruchibiochem@yahoo.co.in

ABSTRACT:

Laboratory chromatography techniques are the virtuosos of separation and analysis, enabling scientists to disentangle complex mixtures and explore the molecular composition of substances with precision. This chapter delves into the realm of chromatography, encompassing various techniques, equipment, applications, and considerations for achieving accurate and efficient separations. Understanding the principles of laboratory chromatography empowers scientists, researchers, and students to unravel the intricacies of mixtures and investigate the chemical fingerprints of diverse substances. Lab chromatography methods are commonly used to separate, identify, and purify different mixtures in science and analysis. These methods depend on how different parts are spread out between a solid and a liquid. These are some usual methods used in laboratories to separate mixtures called chromatography. Gas Chromatography (GC) is a method used to separate and analyze chemicals that easily vaporize. In this method, a gas moves the sample through a column that contains a solid phase. The different parts are divided by how easily they turn into gas, their temperatures when they boil, and how they interact with the column's stationary material. GC is commonly used in the fields of organic chemistry, environmental analysis, and forensic science.

KEYWORDS:

Chromatographic Techniques, Chromatographic Methods, Chromatography Equipment, Laboratory Chromatography, Separation Science.

INTRODUCTION

In the intricate realm of scientific inquiry, where mixtures entwine and molecules dance in harmony, laboratory chromatography techniques emerge as the virtuosos of separation and analysis. These techniques, like skilled musicians in an orchestra, orchestrate the intricate separation of complex mixtures and unveil the chemical signatures of substances with precision and finesse. This chapter invites you to enter the captivating world of chromatography, where methods, equipment, and considerations unite to decipher the intricate language of molecular composition. Laboratory chromatography techniques are the maestros of separation science, conducting the intricate dance of molecules through a symphony of phases. They are the instruments that transform intricate mixtures into distinct chemical fingerprints, enabling scientists to explore the composition, purity, and structure of diverse substances. This chapter serves as a gateway to the realm of laboratory chromatography, where we will explore various chromatographic techniques, specialized equipment, a wide array of applications, and the essential considerations for achieving accurate and efficient separations. From high-performance liquid chromatography (HPLC) dissecting complex mixtures at the molecular level to gas chromatography (GC) revealing the volatile components of samples, each chromatography technique offers a unique approach to unraveling mixtures, and each application presents its own set of chromatographic challenges [1], [2].

As we embark on this journey, we recognize that laboratory chromatography is not just about separating substances; it is a science that demands precision and understanding. The choice of chromatographic method, the optimization of chromatographic resolution, and the mastery of chromatographic equipment are all crucial aspects that elevate chromatography to an art form. Whether you are a seasoned chromatographer refining your separation and analysis skills or a student taking your first steps into the laboratory, the principles outlined in this chapter form the foundation upon which your scientific journey rests. In the chapters that follow, dedicated to specific chromatography techniques and applications, we will delve deeper into the intricacies of this separation and analysis art. Each chromatography method has its own story to tell, its own unique benefits, and its own considerations for mastery [3], [4]. So, whether you find yourself exploring the chemical fingerprints of compounds, separating complex mixtures, or conducting any chromatographic endeavor in between, remember that in the realm of scientific inquiry, precision is the key, and laboratory chromatography techniques are the artisans who translate molecular mysteries into scientific clarity. Together, let us celebrate the art of separation and analysis through laboratory chromatography, where every chromatogram is a canvas of chemical compositions, every separation is a testament to precision, and every experiment is a symphony of scientific discovery [5], [6].

Chromatography was created at the University of Kazan by a scientist named Mikhail Tsvet in 1900. He came up with the method and gave it the name chromatography in the early 1900s. He mainly used it to separate plant pigments like chlorophyll, carotenes, and xanthophylls. The technique got its name from the different colored bands that these components separate into - green, orange, and yellow. During the 1930s and 1940s, scientists created new forms of chromatography. These advancements made chromatography helpful for various separation tasks. The technique of chromatography got a lot better because of the work done by Archer John Porter Martin and Richard Laurence Millington Synge during the 1940s and 1950s. They won the Nobel Prize in Chemistry in 1952 for their work. They figured out the main ideas and methods for partition chromatography, which helped to quickly develop different types of chromatography like paper chromatography, gas chromatography, and high-performance liquid chromatography. Since that time, the technology has improved very quickly. Scientists discovered that the main ideas of Tsvet's chromatography can be used in various ways, leading to different types of chromatography explained below. New advancements are constantly making chromatography better by helping to separate molecules that are more and more alike.

Chromatography is a method used in the lab to separate mixtures into different parts. The mixture is dissolved in a liquid or gas called the moving part, which takes it through a system where a material called the still part is stuck. The different parts of the mixture move at different speeds in the fluid and separate because they interact differently with the surface of the stationary phase. This separation is done by dividing the substances between the moving and stationary parts. Small differences in how a compound sticks to something can make it separate and stay in one place differently. This can change how the compound is separated. Chromatography can be either for preparation or analysis. Preparative chromatography helps to separate the different parts of a mixture so they can be used later. This is a way to make the mixture cleaner. However, it can be more expensive to do this. Analytical chromatography usually uses smaller amounts of material and is used to figure out what is in a mixture or how much of each part there is. The two types can coexist and are not separate from each other.

Analyte the substance that is being separated in chromatography. It is usually what is required from the combination. Analytical chromatography is a method that uses chromatography to figure out if and how much of a substance is in a sample. Bonded phase refers to a type of stationary phase that is firmly attached to the support particles or the inner wall of the column tubing using covalent bonds. A chromatogram is a picture made by a machine called a chromatograph. In an ideal separation, when a mixture is separated using chromatography, we can see different peaks or patterns on the chromatogram. These peaks or patterns represent the different parts of the mixture that have been separated. On the graph, we use the x-axis to show the retention time and the y-axis to show a signal. This signal represents the response caused by the analytes leaving the system. For example, a spectrophotometer or mass spectrometer can be used to measure this signal. If the system is working well, the signal is directly related to the amount of the substance being studied. A chromatograph is a fancy tool that can separate things really well. Separation using gas chromatography or liquid chromatography. Chromatography is a way to separate things by how they move through different materials. It uses two parts: one part that stays in place and one part that moves. The eluent refers to the liquid used in elution chromatography. It is the same as the mobile phase.

Eluate is a mixture of solute and solvent that comes out of the column. Effluent is the liquid that flows out of a chromatographic column. In simple terms, the term is often used interchangeably with eluate. However, it actually refers to the stream that is separate from the process of separation. Eluate a more specific word for solute or substance being analyzed. This is a small part that comes out of the chromatographic column. The eluotropic series is a list of solvents ranked based on how well they can separate substances in a mixture. Immovable phase a phase that cannot move and is stuck either on the particles that support it, or on the inside wall of the tube in the column. Mobile phase is the part that moves in a specific direction. This text can be rewritten as: It can be a liquid (LC and capillary electrochromatography (CEC)), a gas (GC), or a special type of fluid (supercritical-fluid chromatography, SFC).

The mobile phase is made up of the sample that needs to be separated or analyzed, along with a solvent that helps move the sample through a column. HPLC uses either a non-greasy liquid like hexane or a watery liquid like methanol, along with the substance being analyzed, to help separate the components. Preparative chromatography is a technique that is used to purify a large amount of a substance for future use, rather than just studying it. Retention time refers to the amount of time it takes for a specific substance to move through the system, from the beginning to the end, under specific conditions. Also known as the Kovats' retention index, this term refers to a measure used in gas chromatography to determine the retention time of a compound. Sample is what is being tested in chromatography. It can be made up of one thing or a mix of different things. When we examine a sample, we call the part that contains the things we're interested in the sample, and everything else that we don't care about is called waste.

DISCUSSION

Chromatography is a separation technique that is used to separate and identify individual components of a mixture. The technique relies on the differential affinities of the components of the mixture for a stationary phase and a mobile phase.

Gas Chromatography

Gas chromatography (GC) is a type of chromatography that is used to separate and analyse volatile compounds. In this technique, the sample is vaporized and then injected into the chromatograph. The sample then passes through a column that is packed with a stationary phase, which separates the components of the sample based on their affinity for the stationary phase. GC is widely used in the analysis of organic compounds, including hydrocarbons, fatty acids, and amino acids.

Liquid Chromatography

Liquid chromatography (LC) is a type of chromatography that is used to separate and analyse non-volatile compounds. In this technique, the sample is dissolved in a liquid solvent and then injected into the chromatograph. The sample then passes through a column that is packed with a stationary phase, which separates the components of the sample based on their affinity for the stationary phase. LC is widely used in the analysis of a wide range of compounds, including pharmaceuticals, natural products, and food additives.

High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is a type of liquid chromatography that is used to separate and analyze compounds at high pressure. In HPLC, the sample is dissolved in a liquid solvent and then injected into the chromatograph. The sample then passes through a column that is packed with a stationary phase, which separates the components of the sample based on their affinity for the stationary phase. HPLC is widely used in the analysis of a wide range of compounds, including pharmaceuticals, natural products, and food additives.

Ion-Exchange Chromatography

Ion-exchange chromatography is a type of chromatography that is used to separate charged particles based on their ionic properties. In this technique, the sample is passed through a column that is packed with a stationary phase that contains charged groups. Ion-exchange chromatography is widely used in the purification of proteins, nucleic acids, and other biomolecules. It is also used in the analysis of inorganic compounds and the separation of organic acids.

Size-Exclusion Chromatography

Size-exclusion chromatography is a type of chromatography that is used to separate molecules based on their size. In this technique, the sample is passed through a column that is packed with a stationary phase that contains porous beads. The size of the molecules determines whether they can enter the pores of the beads or not. The larger molecules cannot enter the pores and therefore elute from the column earlier than the smaller molecules.

Paper Chromatography

Paper chromatography is a type of chromatography that is similar to TLC, but uses a piece of filter paper as the stationary phase. In this technique, the sample is spotted onto the filter paper and then placed in a chamber that contains a mobile phase. Paper chromatography is a simple and inexpensive technique that is widely used in the analysis of organic compounds, including amino acids, sugars, and lipids.

Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a type of chromatography that is used to separate and identify compounds based on their differential migration on a thin layer of a stationary phase. In this technique, the sample is spotted onto a thin layer of stationary phase that is coated on a plate. The plate is then placed in a chamber that contains a mobile phase, which moves up the plate via capillary action. The components of the sample will move at different rates on the plate based on their affinity for the stationary phase and the mobile phase. TLC is widely used in the analysis of organic compounds, including drugs, pesticides, and natural products. It is also used in forensic science and in the identification of unknown compounds [7], [8].

Applications of Chromatography Techniques

Chromatography techniques have a wide range of applications in various fields, including:

a. **Pharmaceutical Industry:** Chromatography techniques are widely used in the pharmaceutical industry for the analysis and purification of drugs. HPLC is the most commonly used technique for the analysis of drugs, while affinity chromatography is used for the purification of proteins and other biomolecules.

b. **Food Industry:** Chromatography techniques are used in the food industry for the analysis and purification of food additives, flavors, and fragrances.

c. **Environmental Science:** Chromatography techniques are used in environmental science for the analysis of pollutants and contaminants in air, water, and soil. GC is commonly used for the analysis of volatile organic compounds.

d. **Forensic Science:** Chromatography techniques are used in forensic science for the analysis of trace amounts of drugs, explosives, and other volatile compounds.

e. **Biotechnology:** Affinity chromatography is commonly used for the purification of proteins and other biomolecules, while size-exclusion chromatography is used for the analysis of protein aggregates and the determination of molecular weight.

f. **Petrochemical Industry:** GC is commonly used for the analysis of volatile organic compounds in petroleum products, while HPLC is used for the analysis of non-volatile organic compounds.

g. **Clinical Diagnostics:** Chromatography techniques are used in clinical diagnostics for the analysis of biomolecules in blood and other bodily fluids.

h. **Cosmetics Industry:** Chromatography techniques are used in the cosmetics industry for the analysis and purification of fragrances, flavours, and other additives.

Chromatography techniques are powerful tools for the separation, purification, and analysis of complex mixtures of molecules. These techniques have a wide range of applications in various fields, including pharmaceuticals, food, environmental science, forensic science, biotechnology, petrochemicals, clinical diagnostics, and cosmetics. The choice of chromatography technique depends on the specific requirements of the analysis or purification, such as the size and polarity of the molecules, the sensitivity of the detection, and the amount of sample available. Each technique has its own strengths and limitations, and a combination of techniques may be required to achieve the desired separation and purification [9], [10].

CONCLUSION

In the intricate mosaic of scientific exploration, where compounds converge and mixtures weave complex patterns, laboratory chromatography techniques emerge as the artisans of separation and analysis, painting a vivid portrait of molecular diversity. Our journey into this realm has unveiled the profound significance of chromatography, where precision, specificity, and analytical prowess converge to shape the landscape of scientific inquiry. Laboratory chromatography techniques are the masters of separation science, deftly guiding molecules through a choreographed dance of phases. They are the brushes that transform intricate mixtures into distinct, vibrant spectra, revealing the composition, purity, and structure of substances with exquisite clarity. The world of chromatography is a vibrant palette of techniques, each with its own distinct capabilities and applications. From high-performance liquid chromatography (HPLC) dissecting compounds at the molecular level to thin-layer chromatography (TLC) elegantly separating compounds on a plate, each method contributes its unique strokes to the canvas of separation and analysis. Chromatographic instruments, from columns to detectors, are the tools that amplify the precision of separation and analysis. Calibration, optimization of chromatographic conditions, and the selection of stationary and mobile phases are the keystones of reliable chromatographic results. The applications of laboratory chromatography span the vast expanse of scientific disciplines.

Whether in pharmaceuticals, environmental science, forensic analysis, or biochemistry, these techniques are the conduits of discovery, allowing researchers to uncover the secrets concealed within mixtures. Chromatography is both a science and an art, and its mastery is a lifelong craft. Whether you are a seasoned chromatographer refining your separation and analysis skills or a student embarking on your journey into the world of molecular exploration, the principles outlined in this chapter are the guideposts of your scientific odyssey. As we conclude our exploration, we recognize that laboratory chromatography techniques are not just about separation and analysis; they are about the translation of molecular mysteries into scientific clarity. Each chromatogram interpreted, each peak assigned, and each experiment conducted with precision is a testament to the precision artistry of chromatography. In the chapters dedicated to specific chromatography techniques and applications, we have ventured deep into the heart of this separation and analysis craft. Each method and application are a realm of knowledge, each chromatographic spectrum is a revelation, and each chromatographic endeavor is a celebration of the precision and artistry that define scientific inquiry. Together, let us celebrate the precision artistry of chromatography in the laboratory, where every chromatogram is a masterpiece of molecular insight, every separation is a stroke of precision, and every experiment is a symphony of scientific discovery.

REFERENCES:

[1] M. E. Nubbe, V. D. Adams, R. J. Watts, and Y. R. Clark, Organics, *Research Journal of the Water Pollution Control Federation*. 1991. doi: 10.1201/9781351075497-7.

[2] A. R. Buick, M. V. Doig, S. C. Jeal, G. S. Land, and R. D. McDowall, Method validation in the bioanalytical laboratory, *J. Pharm. Biomed. Anal.*, 1990, doi: 10.1016/0731-7085(90)80093-5.

[3] E. P. Borges *et al.*, The benefits of applied research: 37 years of discoveries, adaptations and solutions, *Sugar Ind.*, 2015, doi: 10.36961/si16360.

[4] A. Sioufi and J. P. Dubois, Chromatography of benzodiazepines, *Journal of Chromatography B: Biomedical Sciences and Applications*. 1990. doi: 10.1016/S0378-4347(00)82291-4.

[5] D. C. Leggett, T. F. Jenkins, and P. H. Miyares, Salting-Out Solvent Extraction for Preconcentration of Neutral Polar Organic Solutes from Water, *Anal. Chem.*, 1990, doi: 10.1021/ac00212a029.

[6] J. R. Cronin and S. Pizzarello, Aliphatic hydrocarbons of the Murchison meteorite, *Geochim. Cosmochim. Acta*, 1990, doi: 10.1016/0016-7037(90)90020-L.

[7] J. P. Kraehenbuhl and C. Bonnard, Purification and characterization of membrane proteins, *Methods Enzymol.*, 1990, doi: 10.1016/0076-6879(90)84329-F.

[8] Y. Liu and S. Yu, Copper(II)-iminodiacetic acid chelating resin as a stationary phase in the immobilized metal ion affinity chromatography of some aromatic amines, *J. Chromatogr. A*, 1990, doi: 10.1016/S0021-9673(01)89310-9.

[9] R. Sakakibara, S. Miyazaki, T. Shigemura, N. Tominaga, and A. Sakai, Effective Purification of Human Chorionic Gonadotropin And Its Subunits From Pregnant Women'S Urinary Peptides Adsorbed On Reverse-phase Resin, *Chem. Pharm. Bull.*, 1990, doi: 10.1248/cpb.38.1414.

[10] S. W. Lee, S. Coulombe, and B. Glavincevski, Investigation of Methods for Determining Aromatics in Middle-Distillate Fuels, *Energy and Fuels*, 1990, doi: 10.1021/ef00019a004.

CHAPTER 13

LABORATORY SAMPLE PREPARATION METHODS: A COMPREHENSIVE REVIEW

Navneet Kumar, Professor
College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India,
Email Id- navneetbiochem@yahoo.co.in

ABSTRACT:

Laboratory sample preparation methods are the foundation of analytical excellence, facilitating the transformation of raw materials into specimens suitable for analysis. This chapter explores the world of sample preparation, encompassing various techniques, equipment, applications, and considerations for ensuring representative and reliable analysis. Understanding the principles of laboratory sample preparation empowers scientists, researchers, and students to extract meaningful insights from diverse sample matrices across scientific disciplines. Lab sample preparation methods are important in scientific research and analytical chemistry. They make sure that experimental results are accurate and reliable. These methods include a set of steps to change raw samples into forms that can be easily analyzed. Preparing a sample can involve different steps like blending, separating, straining, and making stronger, depending on what the sample is like and how it will be tested. The main goals of preparing samples are to get rid of things that might get in the way, make the chemical we want to test more concentrated, and make sure all parts of the sample are the same. Preparing samples correctly can greatly affect how accurate and sensitive analytical tools, like chromatographs and spectrometers, are. It also helps to decrease mistakes and improve the ability to repeat experiments. The ways to prepare samples differ a lot in various areas like chemistry, biology, environmental science, and materials science.

KEYWORDS:

Extraction, Homogenization, Laboratory Sample Preparation, Sample Preparation Sample Preparation Methods, Techniques.

INTRODUCTION

In the intricate tapestry of scientific inquiry, where understanding hinges on precise analysis, laboratory sample preparation methods stand as the unsung heroes, laying the foundation upon which analytical excellence is built. These methods, often overshadowed by the allure of sophisticated instruments, are the architects of reliable results, ensuring that raw materials are transformed into specimens ready for scrutiny. This chapter invites you to enter the world of sample preparation, where techniques, equipment, and considerations unite to pave the way for meaningful analysis across diverse scientific domains. Laboratory sample preparation methods are the choreographers of representative specimens, orchestrating the meticulous transformation of raw samples into analyzable forms. They are the silent enablers, extracting meaningful insights from complex matrices and ensuring that the subsequent analysis delivers results of the utmost integrity [1], [2].

This chapter serves as a gateway to the realm of laboratory sample preparation, where we will explore various techniques, specialized equipment, a wide array of applications, and the essential considerations for ensuring that samples are prepared to meet the exacting standards of scientific analysis. From homogenization's ability to blend heterogeneous samples to solid-phase extraction's prowess in isolating target compounds, each sample preparation method offers a unique approach to readying specimens for analysis, and each application presents its own set of sample-specific challenges. As we embark on this journey, we recognize that laboratory sample preparation is not just about processing raw materials; it is a science that demands precision, attention to detail, and a deep understanding of the sample matrix. The choice of sample preparation method, the preservation of sample integrity, and the application of quality control measures are all crucial aspects that elevate sample preparation to a fundamental component of analytical excellence. Whether you are a seasoned analyst refining your sample preparation skills or a student taking your first steps into the laboratory, the principles outlined in this chapter form the cornerstone upon which your scientific journey rests [3], [4].

In the chapters that follow, dedicated to specific sample preparation techniques and applications, we will delve deeper into the intricacies of this crucial scientific prelude. Each method and application are a realm of knowledge, each sample preparation endeavor is a testament to precision, and each specimen prepared for analysis is a vital step toward unraveling the mysteries of science. So, whether you find yourself preparing samples for chemical analysis, biological assays, or any analytical endeavor in between, remember that in the realm of scientific inquiry, analytical excellence begins with sample preparation, and every prepared specimen is a canvas upon which the story of analysis unfolds. Together, let us celebrate the crucial prelude to scientific analysis in laboratory sample preparation, where every prepared sample is a step toward understanding, every method applied is a stroke of precision, and every experiment conducted with integrity is an ode to the pursuit of scientific knowledge [5], [6].

At first glance, sample preparation may seem like the most common part of an analytical process. But analysts need to understand and remember that the quality of a measurement depends on how well the sample was prepared beforehand. If a small part taken for studying does not show the same information as the whole thing, then the conclusions drawn from studying it might not be reliable. Usually, mistakes are more common in the sampling and sample preparation part of a scientific test compared to the actual method used. Airborne contamination is most likely to happen when grinding or crushing solid samples. Tiny particles, about 10 micrometers in size, can be made, float in the air, and move through the air until they land on a surface. Other things in the air that could be contaminated are samples that have tiny particles, certain types of radioactive materials that can become gas, and certain types of radioactive materials that decay into gas before decaying further. So, if you are grinding or crushing solid samples, or you are working with samples that could make the air dirty, you should do it in a special area in the lab that keeps the dirty air from spreading around. These tiny particles can easily make other samples in the area dirty.

To stop germs from spreading, cover or take away other samples from the area when dealing with things that might make the air dirty. If you are worried about pollution from the surrounding particles of Rn, you can prevent it by not using suction filtration in chemical procedures, filtering the air in the room beforehand, and using radon traps. The lab might have some radon in it, which might come from the soil or the materials used to build it. Contamination of chemicals with radioactive impurities can be a big problem, especially when working with very small amounts of substances. We need to be careful when getting chemicals to make sure they are not contaminated. Because uranium and thorium are found everywhere, they and their offspring are often found in chemicals used for analysis. For instance, Yamamoto and his colleagues. In 1989, it was discovered that common barium and calcium reagents were contaminated with large amounts of Ra. Other chemicals that can cause problems are the rare earth elements, especially salts made from cerium. Cesium salts can also be problematic because they might have potassium-40 or rubidium-87 in them. Potassium salts are another type of reagent that can cause issues. Contaminants can also affect precipitating agents like tetraphenyl borates and chloroplatinates. In some chemical processes, we have to use isotopes of one element instead of stable carriers of another element, when it's hard to get the stable carrier without any contamination.

Other things to think about when preparing samples include making sure to clean glassware and equipment. This is discussed in Section 12. 23The planning documents or laboratory SOPs should have guidelines for how to properly take care of glassware and equipment. For example, glassware with scratches can increase the chances of contamination and loss of samples because there is more surface area for things to stick to. Glassware should be checked regularly for scratches, cracks, and other damage, and thrown away if it is damaged. We should use blanks and screening to check if glassware is contaminated. It is better to use new or throw-away containers or lab equipment whenever you can. For instance, throw-away trays can be used to avoid getting dirt on a scale. Throwaway plastic centrifuge tubes are usually cheaper to use than glass tubes that need to be cleaned after each use. If you use containers or lab equipment that can be used more than once, you may need to get new ones for each new project to make sure nothing harmful gets mixed in. Empty spaces can be used to find out if there is any dirty stuff mixing with something else. Regularly rinsing glassware with a weak nitric acid solution can help keep it clean.

In 1980, it was difficult to get rid of nuclear particles stuck to plastic containers by using powerful acids for cleaning. They say that particles can be removed from walls, which shows that using a brush is important for cleaning. To prevent contamination of laboratory facilities and potential contamination of samples or staff, it is important to consistently follow good laboratory practices and maintain cleanliness in the laboratory. The lab needs to have a plan in place to prevent contamination and quickly handle it if it does happen. This program needs to deal with different examples of activity or qualities. This reduces the chance of samples getting mixed up in lab machines. Filtering devices, glassware, ovens, etc. are tools or equipment used for various purposes A typical cleaning process involves using a cleaning solution, soaking the item in an acid solution, and then rinsing it with clean water. In radiochemical laboratories, they use automatic dishwashers and cleaning machines that use ultrasound to clean things. It is important to know that just cleaning lab equipment in a dishwasher might not completely remove germs. Dirty glassware may need to be soaked in acid or detergent to make sure it gets cleaned thoroughly. Ultrasonic cleaning in a tank of liquid is a very thorough process that quickly and efficiently cleans both the outside and inside of glassware or equipment. Ultrasonic cleaners make high-pitched sound waves and work by creating and popping tiny bubbles. These bubbles appear and disappear very quickly, around 25,000 times per second. They do this with a strong and forceful movement at a tiny level, which creates a cleaning effect. This action treats all parts of the labware because it is soaked in the solution and the sound waves go through the solution to reach all areas.

DISCUSSION

There are three methods for collecting radiation data while performing a survey. A direct measurement is obtained by placing the detector near or against the surface or in the media being surveyed and reading the radioactivity level directly. Scanning is an evaluation technique performed by moving a portable radiation detection instrument at a constant speed and distance above the surface to semi-quantitatively detect elevated areas of radiation. These measurement techniques are discussed in Chapter 6. Sampling is the process of collecting a portion of an environmental medium as representative of the locally remaining medium. The collected portion of the medium is then analyzed to determine the radionuclide concentration. This chapter discusses issues involved in collecting and preparing samples in the field for analysis, and in evaluating the results of these analyses. In addition, a general discussion on laboratory sample preparation and analysis is provided to assist in communications with the laboratory during survey planning. Samples should be collected and analyzed by qualified individuals using the appropriate equipment and procedures.

This manual assumes that the samples taken during the survey will be submitted to a qualified laboratory for analysis. The laboratory should have written procedures that document its analytical capabilities for the radionuclides of interest and a Quality Assurance/Quality Control (QA/QC) program that documents the compliance of the analytical process with established criteria. The method used to assay for the radionuclides of concern should be recognized as a factor affecting analysis time. Commonly used radiation detection and measuring equipment for radiological survey field applications is described in Chapter 6 and Appendix H. Many of these equipment types are also used for laboratory analyses, usually under more controlled conditions that provide for lower detection limits and greater delineation between radionuclides. Laboratory methods often involve combinations of both chemical and instrument techniques to quantify the low levels expected in the samples. This chapter provides guidance to assist the MARSSIM user in selecting appropriate procedures for collecting and handling samples for laboratory analysis. More detailed information is available in documents listed in the reference section of this manual.

Sample preparation

Sample preparation is the process where a representative piece of material, chemical or substance is extracted from a larger amount, bulk or batch for subsequent analysis. Representative samples are selected to accurately reflect the larger group and represent the characteristics of the whole material. Ideally representative samples are homogeneous or similar in nature, but when that is not possible, the best attempts must be made to achieve samples which represent the majority of the characteristics of the larger grouping. The preparation of samples is one of the most important steps in analytical methods for many reasons, including the fact that some materials cannot be analyzed in an in-situ condition such as proteins, DNA and RNA. Some samples have interfering substances and species that can produce faulty results. Sample preparation can include many processes, from reactions or treatment with chemical agents, to filtration, dilution, and extraction [7], [8].

Representative samples

Representative samples are selected to accurately reflect the larger group and represent the characteristics of the whole material. Ideally representative samples are homogeneous or similar in nature, but when that is not possible, the best attempts must be made to achieve samples which represent the majority of the characteristics of the larger grouping. The preparation of samples is one of the most important steps in analytical methods for many reasons, including the fact that some materials cannot be analyzed in an in-situ condition such as proteins, DNA and RNA. Some samples have interfering substances and species that can produce faulty results. Sample preparation can include many processes, from reactions or treatment with chemical agents, to filtration, dilution, and extraction.

Particles and homogeneity

Many physical samples need particle size reduction to create representative samples, usually due to their overall heterogeneous nature or state when laboratory or testing samples require a certain level of homogeneity. Homogeneity is the state of being of uniform composition or character, whereas heterogeneity lacks uniformity in one or more characteristics. Homogeneity and heterogeneity often depend on perspective and context where the smaller the sampling frame, the less homogeneous a material or substance can appear. For some samples, the measure of homogeneity can be accomplished with a process that creates large size reduction where other samples for other processes will require reduction to fine particles. Smaller particles and homogeneous materials are most often needed for many further laboratory sample preparation techniques [9], [10].

In the intricate tapestry of scientific inquiry, where understanding hinges on precise analysis, laboratory sample preparation methods stand as the unsung heroes, laying the foundation upon which analytical excellence is built. These methods, often overshadowed by the allure of sophisticated instruments, are the architects of reliable results, ensuring that raw materials are transformed into specimens ready for scrutiny. This chapter invites you to enter the world of sample preparation, where techniques, equipment, and considerations unite to pave the way for meaningful analysis across diverse scientific domains. Laboratory sample preparation methods are the choreographers of representative specimens, orchestrating the meticulous transformation of raw samples into analyzable forms. They are the silent enablers, extracting meaningful insights from complex matrices and ensuring that the subsequent analysis delivers results of the utmost integrity. This chapter serves as a gateway to the realm of laboratory sample preparation, where we will explore various techniques, specialized equipment, a wide array of applications, and the essential considerations for ensuring that samples are prepared to meet the exacting standards of scientific analysis. From homogenization's ability to blend heterogeneous samples to solid-phase extraction's prowess in isolating target compounds, each sample preparation method offers a unique approach to readying specimens for analysis, and each application presents its own set of sample-specific challenges.

CONCLUSION

In the chapters that follow, dedicated to specific sample preparation techniques and applications, we will delve deeper into the intricacies of this crucial scientific prelude. Each method and application is a realm of knowledge, each sample preparation endeavor is a testament to precision, and each specimen prepared for analysis is a vital step toward unraveling the mysteries of science. So, whether you find yourself preparing samples for chemical analysis, biological assays, or any analytical endeavor in between, remember that in the realm of scientific inquiry, analytical excellence begins with sample preparation, and every prepared specimen is a canvas upon which the story of analysis unfolds. Together, let us celebrate the crucial prelude to scientific analysis in laboratory sample preparation, where every prepared sample is a step toward understanding, every method applied is a stroke of precision, and every experiment conducted with integrity is an ode to the pursuit of scientific knowledge. As we embark on this journey, we recognize that laboratory sample preparation is not just about processing raw materials; it is a science that demands precision, attention to detail, and a deep understanding of the sample matrix. The choice of sample preparation method, the preservation of sample integrity, and the application of quality control measures are all crucial aspects that elevate sample preparation to a fundamental component of analytical excellence. Whether you are a seasoned analyst refining your sample preparation skills or a student taking your first steps into the laboratory, the principles outlined in this chapter form the cornerstone upon which your scientific journey rests.

REFERENCES:

[1] C. M. Hodges and J. Akhavan, The use of Fourier Transform Raman spectroscopy in the forensic identification of illicit drugs and explosives, *Spectrochim. Acta Part A Mol. Spectrosc.*, 1990, doi: 10.1016/0584-8539(90)80098-J.

[2] W. Bertsch and Q. W. Zhang, Sample preparation for the chemical analysis of debris in suspect arson cases, *Anal. Chim. Acta*, 1990, doi: 10.1016/S0003-2670(00)83312-7.

[3] K. Riehn *et al.*, Trichinella detection: Identification and statistical evaluation of sources of error in the magnetic stirrer method for pooled sample digestion, *Vet. Parasitol.*, 2013, doi: 10.1016/j.vetpar.2013.01.031.

[4] K. W. Barnes, A Streamlined Approach to the Determination of Trace Elements in Foods, *At. Spectrosc.*, 1998.

[5] J. C. Davis *et al.*, LLNL/UC AMS facility and research program, *Nucl. Inst. Methods Phys. Res. B*, 1990, doi: 10.1016/0168-583X(90)90419-U.

[6] M. Paglia *et al.*, International Health Cooperation in Zanzibar: development of a sustainable model of TB diagnostic laboratory in Pemba Island: 3.2.8-133., *Trop Med Int Health*. 2009.

[7] O. O. Obodozie, K. B. Mustapha, B. U. Ebeshi, and U. S. Inyang, A comparative study on the prevalence of substandard ampicillin/cloxacillin preparations in the Nigerian market: mid 1990's and present, *J. Phytomedicine Ther.*, 2006, doi: 10.4314/jopat.v11i1.48619.

[8] S. McClatchie, R. Kawachi, and D. E. Dalley, Epizoic diatoms on the euphausiid Nyctiphanes australis: Consequences for gut-pigment analyses of whole krill, *Mar. Biol.*, 1990, doi: 10.1007/BF01313262.

[9] Environmental Technology Centre, Biological Test Method : Reference Method for Determining Acute Lethality of Effluents to Daphnia magna, 2000.

[10] J. . Doyle and J. . Doyle, A rapid isolation procedure for small amounts of leaf tissue, *Phytochem. Bull.*, 1987.