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SCHOOL OF SCIENCE AND TECHNOLOGY

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COURSE TITLE: GENERAL BIOCHEMISTRY LABORATORY I

BIO 215: GENERAL BIOCHEMISTRY LABORATORY I

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UNIT 1 INTRODUCTION TO LABORATORY AND LABORATORY EQUIPMENT

CONTENTS

1.0 INTRODUCTION

Scientific research and investigations will be of little value without good field and laboratory work. These investigations are normally carried out through the active use of processes which involves laboratory or other hands-on activities. Many devices and products used in everyday life resulted from laboratory works. They include car engines, plastics, radios, televisions, synthetic fabrics, etc.

2.0 OBJECTIVES

Upon completion of studying this unit, you should be able to:

1. Define the meaning of laboratory
2. List different types of laboratories
3. Identify different types of laboratory equipment

3.0 MAIN BODY

3.1 What is a Laboratory?

A laboratory informally, lab is a facility that provides controlled conditions in which scientific research, experiments, and measurement may be performed. It is a place equipped for investigative procedures and for the preparation of reagents, therapeutic chemical materials, and so on. The title of laboratory is also used for certain other facilities where the processes or equipment used are similar to those in scientific laboratories. These notably include:

the film laboratory or darkroom

the computer lab

the medical lab

the public health lab

Scientific laboratories can be found in schools and universities, in industry, in government or military facilities, and even aboard ships and spacecraft. A laboratory might offer work space for just one to more than thirty researchers depending on its size and purpose.

Labs used for scientific research take many forms because of the differing requirements of specialists in the various fields of science. A physics lab might contain a particle accelerator or vacuum chamber while a chemist or biologist might use a wet laboratory. Scientists in other fields will use still other types of laboratories. Despite the great differences among laboratories, some features are common. The use of workbenches or countertops at which the scientist may choose to either sit or stand is a common way to ensure comfortable working conditions for the researcher, who may spend a large portion of his or her working day in the laboratory. The provision of cabinets for the storage of laboratory equipment is quite common. It is traditional for a scientist to record an experiment's progress in a laboratory notebook, but modern labs almost always contain at least one computer workstation for data collection and analysis.

3.2 Laboratory equipment

Laboratory equipment refers to the various tools and equipment used by scientists working in a laboratory. They are generally used either perform an experiment or to take measurements and gather data. Larger or more sophisticated equipment is generally called a scientific instrument. These include tools such as Bunsen burners, and microscopes as well as specialty equipment such as operant conditioning chambers, spectrophotometers and calorimeters. Another important type of laboratory equipment is Laboratory glassware such as the beaker or reagent bottle.

Laboratory glassware refers to a variety of equipment, traditionally made of glass, used for scientific experiments and other work in science, especially in chemistry, biochemistry and biology laboratories. Some of the equipment is now made of plastic for cost, ruggedness, and convenience reasons, but glass is still used for some applications because it is relatively inert, transparent, more heat-resistant than some plastics up to a point, and relatively easy to

customize. Borosilicate glasses—formerly called Pyrex—are often used because they are less subject to thermal stress and are common for reagent bottles. For some applications quartz glass is used for its ability to withstand high temperatures or its transparency in certain parts of the electromagnetic spectrum. In other applications, especially some storage bottles, darkened brown or amber (actinic) glass is used to keep out much of the UV and IR radiation so that the effect of light on the contents is minimized. Special-purpose materials are also used; for example, hydrofluoric acid is stored and used in polyethylene containers because it reacts with glass. For pressurized reaction, heavy-wall glass is used for pressure reactor.

Laboratory glassware include: Beaker, Büchner funnel, Burette, Cold finger, Condenser, Conical measure, Cuvette, Dropping funnel, Eudiometer, Gas syringe, Graduated cylinder, Pipette, Petri dish, Pycnometer, Separatory funnel, Soxhlet extractor, Watch glass.

Flasks: Büchner, Erlenmeyer, Fleaker, Florence, Retort, Round-bottom, Schlenk, Volumetric

Tubes: Boiling, Ignition, NMR, Test, Thiele, Thistle

Others: Agar plate, Aspirator, Autoclave, Biosafety cabinet, Bunsen burner, Calorimeter, Chemo stat, Colony counter, Colorimeter, Laboratory centrifuge, Crucible, Fume hood, Homogenizer, Hot air oven, Incubator, Magnetic stirrer, Microscope, Microtiter plate, Picotiter plate, Plate reader, Retort stand, Spectrophotometer, Stirring rod, Thermometer Vortex mixer, Wash bottle.

Modern biochemistry laboratory Showing a fume cupboard, heated water bath and glassware (Retrieved from "http://en.wikipedia.org/wiki/Laboratory_equipment")

Three beakers, a conical flask, a graduated cylinder and a volumetric flask (Retrieved from "http://en.wikipedia.org/wiki/Laboratory_glassware")

4.0 CONCLUSION

In conclusion the student should have learnt what a laboratory is and different type of laboratory equipment.

5.0 SUMMARY

A laboratory is a place equipped for investigative procedures and for preparation of reagent.

Laboratory equipment is the tool and equipment used by scientists working in a laboratory.

In this unit, you learnt

1 the meaning of a laboratory and there are many different types of Laboratories

2 different laboratory equipment and glassware

6.0 TUTOR-MARKED ASSIGNMENT

1. What are laboratory glasswares?

2. List three types of flasks

3. Mention five laboratory equipment, which are not made of glass.

7.0 REFERENCES/FURTHER READING

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UNIT 2 LABORATORY SAFETY

CONTENT

1.0 INTRODUCTION

Scientific research and investigations will be of little value without good field and laboratory work. These investigations are normally carried out through the active use of processes which involves laboratory or other hands-on activities. Many devices and products used in everyday life resulted from laboratory works. They include car engines, plastics, radios, televisions, synthetic fabrics, etc.

2.0 OBJECTIVES

At the end of this lesson student should be familiarized with laboratory safety and the do and don’t of the laboratory

3.0 MAIN BODY

3.1 Safety

Safety is freedom from harm or accident. Laboratory safety may appear at first sight to be a dull subject but experience has shown that our students and majority of staff and untrained technicians in the science and science-related discipline do not understand the importance of safety and safety practices in the science classroom and laboratory.

In some laboratories, the conditions are no more dangerous than in any other room while in many labs, hazards are present. Laboratory hazards are as varied as the subjects of study in laboratories, and might include poisons; infectious agents; flammable, explosive, or radioactive materials; moving machinery; extreme temperatures; lasers, strong magnetic fields or high voltage. In laboratories where dangerous conditions might exist, safety precautions are important. Rules exist to minimize the individual's risk, and safety equipment is used to protect the lab user from injury or to assist in responding to an emergency

3.2 Lab Safety Rules:

Lab safety rules are guidelines designed to help keep you safe when experimenting. Some equipment and chemicals in a biochemistry laboratory can cause serious harm.
It is always wise to follow all lab safety rules. Don't forget, the most helpful safety rule is to use plain old common sense. The following lab safety rules are a sample of the most basic rules that

should be followed when in biochemistry lab. Most biochemistry labs have the safety rules posted in the lab and your instructor will most likely go over them with you before you begin working.

3.2.1 Be Prepared:

Before you enter a biochemistry lab, you should be prepared for and knowledgeable about any lab exercises that are to be performed. That means you should read your lab manual to know exactly what you will be doing. Review your biochemistry notes and relevant sections in your biochemistry book before your lab begins. Make sure you understand all procedures and purposes, as this will help you understand the lab activities you will perform. It will also help you get your thoughts organized for when you have to write your lab report.

3.2.2 Be Neat:

When working in a biochemistry lab, make sure you keep your area neat and organized. If you happen to spill something, ask for assistance when cleaning it up. Also remember to clean your work area and wash your hands when you are finished.

3.2.3 Be Careful:

An important biochemistry lab safety rule is to be careful. You may be working with glass or sharp objects, so you don't want to handle them carelessly.

3.2.4 Wear Proper Clothing:

Accidents do happen in a biochemistry lab. Some chemicals have the potential to damage clothing. With that in mind, you want to make sure that the clothing you wear is something you could do without if it becomes damaged. As a precaution, wearing an apron or lab coat is a good

idea. Laboratory coats are not status symbol but are meant to protect the wearer from chemical splashes and infectious material. Cotton is better material for a better lab coat than nylon as it has a greater absorptive capacity and is generally resistant to chemical splashes. You will also want to wear proper shoes that can protect your feet in case something gets broken. Sandals or any type of open-toed shoes are not recommended.

3.2.5 Be Cautious With Chemicals:

The best way to remain safe when dealing with chemicals is to assume that any chemical you handle is dangerous and handled accordingly. Be sure you understand what type of chemicals you are using and how they should be properly handled. If any chemical comes in contact with your skin, wash immediately with water and inform your lab instructor. Wear protective eyewear when handling chemicals.

3.2.6 Wear Safety Goggles:

Safety goggles are not stylish and can fit awkwardly on your face, but they should always be worn when you are working with chemicals or any type of heating apparatus since workers are especially vulnerable to splashes from reagents.

3.2.7 Wear Gloves:

Heavy duty gloves must be worn when handling corrosive substances such as strong acids or alkalis. Light weight disposable gloves should be worn during weighing and handling of chemicals to avoid the risk of absorption through the skin.

3.2.8 Locate Safety Equipment:

Be sure you know where to find all safety equipment in the biochemistry lab. This includes such items as the fire extinguisher, first aid kit, broken glass receptacles, and chemical waste containers. Also be sure you know where all the emergency exits are located and which exit route to take in case of an emergency. The following symbols represent some of the safety signs used in the laboratory:


Sign for combustible material


Sign for corrosive material


Sign for toxic chemicals


Sign for environmental hazard


Sign for eyewash station


Sign for live electricity


Open Flame Prohibited Sign


Sign for fire extinguisher


Sign for nonpotable water


Sign for explosive material


Sign for materials that can be recycled


Sign for Inflammable material

(Retrieved from: http://chemistry.about.com/od/healthsafety/ig/Laboratory-Safety-Signs/)

3.3 Biochemistry Lab Don'ts:

There are several things in a biochemistry lab that you must always avoid. Here are a few major laboratory do nots.

Do Not

- eat or drink in the lab
- taste any chemicals or substances you are working with
- use your mouth for pipetting substances
- handle broken glass with bare hands
- pour chemicals down the drain without permission
- operate lab equipment without permission
- perform your own experiments unless given permission
- leave any heated materials unattended
- place flammable substances near heat
- engage in childish antics such as horseplay or pranks

3.4 Have a Good Experience:

Biochemistry lab is an important aspect of any biochemistry course. In order to have a good lab experience, make sure that you follow these lab safety rules and any instructions given to you by your lab instructor.

3.5 Laboratory Housekeeping

Housekeeping is important in any work area. A clean, well-maintained work area improves safety by preventing accidents and can enhance the overall efficiency of work performed. Keeping things clean and organized helps provide a safer laboratory. Keep drawers and cabinet doors closed and electrical cords off the floor to avoid tripping hazards. Keep aisles clear of obstacles such as boxes, chemical containers, and other storage items that might be put there even temporarily. Avoid slipping hazards by cleaning up spilled liquids promptly and keeping

the floor free of stirring rods, glass beads, stoppers, and other such items. Never block or even partially block the path to an exit or to safety equipment such as a fire extinguisher or safety shower.

Make sure that supplies and equipment on shelves provide sufficient clearance so that fire sprinkler heads operate correctly. There shall not be any storage within 18 inches of a sprinkler head.

Put ordinary wastepaper in a wastepaper basket separate from chemical wastes. Broken glass and other sharp items shall be disposed off in rigid, puncture-resistant containers to protect persons collecting the waste materials. Needles and syringes that are not contaminated may be sealed in a rigid, puncture-resistant container and placed in a regular waste receptacle. When discarding empty boxes or other containers bearing hazardous materials labels, the labels shall be defaced or removed before disposal. Contaminated boxes or containers shall not be disposed of in the regular trash. Chemical wastes and unwanted chemicals shall be disposed of promptly and not left to clutter a laboratory.

3.5.1 Washing and drying of glassware in the laboratory

Clean glassware is essential in biochemistry. The problem is that the tolerance for shmutz varies with the work you are doing, and sometimes a scientist does not know how important clean glassware is to an experiment until it has failed. When cleaning laboratory glassware, wear appropriate gloves that have been checked for tears or holes. Avoid accumulating too many articles in the cleanup area around the sink; space is usually limited, and piling up glassware

leads to breakage. Do not clean food containers in a sink that is used for cleaning contaminated glassware.

Many fingers have been badly cut by broken glass from glassware that was intact when put into the sink water. Handle glassware carefully and watch out for broken glass at the bottom of the sink. A rubber or plastic mat in the sink will help minimize breakage.

3.5.2 How to Clean Laboratory Glassware

Cleaning laboratory glassware isn't as simple as washing the dishes. How glassware is washed depends on what is in it and how it will be used, but there are some general instructions that will handle many of the situations.

3.5.3 Cleaning Basics

It's generally easier to clean glassware if you do it right away. When detergent is used, it's usually one designed for lab glassware, such as Liquinox or Alconox. These detergents are preferable to any dishwashing detergent you might use on dishes at home.

Much of the time, detergent and tap water are neither required nor desirable. You can rinse the glassware with the proper solvent, and then finish up with a couple of rinses with distilled water, followed by final rinses with deionized water.

3.5.4 How to Wash Out Common Lab Chemicals

Water Soluble Solutions: (e.g., sodium chloride or sucrose solutions) Rinse 3-4 times with deionized water then put the glassware away.

Water Insoluble Solutions: (e.g., solutions in hexane or chloroform) Rinse 2-3 times with ethanol or acetone, rinse 3-4 times with deionized water, then put the glassware away. In some situations other solvents need to be used for the initial rinse.

Strong Acids: (e.g., concentrated HCl or H_2SO_4) under the fume hood, carefully rinse the glassware with copious volumes of tap water. Rinse 3-4 times with deionized water, then put the glassware away.

Strong Bases: (e.g., 6M NaOH or concentrated NH_4OH) under the fume hood, carefully rinse the glassware with copious volumes of tap water. Rinse 3-4 times with deionized water, then put the glassware away.

Weak Acids: (e.g., acetic acid solutions or dilutions of strong acids such as 0.1M or 1M HCl or H_2SO_4) Rinse 3-4 times with deionized water before putting the glassware away.

Weak Bases: (e.g., 0.1M and 1M NaOH and NH_4OH) Rinse thoroughly with tap water to remove the base, then rinse 3-4 times with deionized water before putting the glassware away.

The deionized water rinse should form a smooth sheet when poured through clean glassware. If this sheeting action is not seen, more aggressive cleaning methods may be needed.

3.6 General Cleaning Tips

The key to cleaning is doing it a timely manner; letting dirty glassware sit for long periods of time guarantees a harder cleaning job.

•Disassemble your apparatus as soon as possible after you are finished with it. Remove all stopcocks and stoppers from addition funnels, separatory funnels and the like. Ground glass stopcocks and stoppers will freeze in place if certain reactants (e. g., bases) were used in them. Triple rinse all surfaces with an appropriate solvent to remove traces of solvents and reaction mixtures, place the rinses in the appropriate waste container.

•Separate glassware that must be quantitatively clean from that which does not. In this way you do not waste time trying to quantitatively clean items that do not need to be.

•Graduated cylinders, beakers, Erlenmeyer flasks, burettes and pipettes that were only used to dispense or store reagents generally only need to be triple rinsed with a compatible solvent followed by tap water and a final DI water rinse, if desired. Air dry on a drying rack. In some cases you may need to be more thorough, as described below.

•Büchner funnels, etc. should be rinsed with an appropriate solvent to remove substances that are clinging to them. Running solvent through them backwards using gravity (never use vacuum to speed up this process!) can help remove contamination from the inside of the funnel and from the surface of fritted funnels. Follow this by tap water and DI water rinses and air dry.

3.6.1 General Cleaning Procedure for Quantitatively Clean Glassware

The following steps should be followed for glassware for which a simple solvent rinse is not sufficient. If you need quantitatively clean glassware, these should be the first steps toward this goal, and more aggressive cleaning methods may be required (vide infra).

•Degrease your glassware's ground glass joints by wiping them with a paper towel soaked in a small amount of ether, acetone or other solvent (CAUTION! wear appropriate gloves and minimize your exposure to the vapors).

•Place the glassware in a warm concentrated aqueous solution of Alconox, or other detergent, and let it sit for several minutes.

•Scrub. Be sure that your brush is in good shape before scrubbing (not rusty, bristles are not matted down); replace it if necessary.

•Rinse thoroughly with tap water and give a final rinse with DI water.

The water will sheet cleanly off the glass, if it is quantitatively clean. If water does not sheet off the glass, and you desire the glassware to be quantitatively clean, first repeat the above soaking and scrubbing steps. If, after a second cleaning, bits of solid still adhere to the glass, or if there is clearly a greasy residue on the glass, more aggressive action must be taken.

3.6.2 Special Cases

Cuvettes: Generally, you only need to rinse a cuvette in the appropriate solvent and wipe the outside with a Kimwipe immediately after use. If something has adhered itself to a cuvette, it is best to soak the cuvette in solvent first and gently coax the solid off the side with a cotton swab. Never use a brush on a cuvette! If this fails, one of the acidic cleaning solutions mentioned above can be used (but never HF!). It is not recommended that base bath be used on cuvettes, because it tends to etch glass surfaces.

Fritted Funnels: These can generally be cleaned by inverting and allowing to solvent to flow by gravity through the frit in reverse (do not use vacuum to speed this process). Solvent can also be pulled through the frit (in the normal direction) under vacuum. Recalcitrant gunk can usually be removed by soaking in acid, followed by copious rinsing with water under vacuum. Because HF and the base bath solution etch glass, they should not be used on fritted funnels (a brief exposure to a base bath is not usually fatal to a frit, but prolonged soaking should be avoided).

Protein Contamination: Usually proteins can be removed scrubbing with detergent, but occasionally protein defies removal. In that event, you can proceed to the more aggressive acidic solutions, or you can prepare a peptidase solution (an enzyme that degrades proteins). The enzymatic approach is a bit slower than the forcing methods, but it is gentler and so can be used in situations that the contaminated item is incompatible with acid.

3.6.3 Washing Special Glassware:

Burettes: Wash with hot "soapy" water using the burette brush and lab detergent. Rinse with TAP water three or four times. Rinse with deionized water three or four times and put it away. If the burette does not sheet properly, contact your instructor for further washing instructions.

Pipettes and Volumetric Flasks: Pipettes and volumetric flasks are really good examples of items that should be cleaned immediately after use. They can be very difficult to clean later on. The best that can be done with a dirty pipette or volumetric flask is rinsing with "soapy" water, solvents, water, acetone or deionized water as needed. Sometimes overnight soaking is required.

Glassware used for organic reactions: If the contents are water soluble, rinse with water. If the contents are ethanol soluble, rinse with ethanol and then water. If the contents are solvent

soluble, rinse with the appropriate solvent, then rinse with ethanol and water. If it needs scrubbing, scrub with hot "soapy" water and a brush. If more is needed, ask your instructor.

3.6.4 To Dry or Not Dry Glassware:

Not Drying: Normally you do not need to dry glassware. In fact you should not dry glassware with a paper towel or compressed air because it will contaminate the glassware. If the item will not be used till next week, put it away and let it air dry. If the next chemical you are going to put in the glassware contains water, just leave the glassware wet (Assuming the water will not affect the final concentration). If the next reaction will be carried out in a solvent such as ether, rinse with ethanol or acetone to remove the water and then with the final solvent to remove the ethanol or acetone.

Rinsing With The Reagent: An exception to leaving the glassware wet is if the extra water will adversely affect the concentration of the final solution. In that case it is best to triple rinse with the final solution before filling the container. You still don't need to dry it though.

Drying: If the glassware must be used immediately, cannot have any solvent of any kind in it and must be dry, rinse it with acetone two or three times. The acetone removes the water and will dry very quickly.

Wet glassware can be dried by 1) placing it on the drying rack (or invert on a paper towel), 2) placing it in the drying oven (for items that are water-wet only, no flammable solvents) or 3) rinsing with a solvent such as acetone, methanol or ethanol and then gently blowing compressed air into the vessel until it is dry. The first method is preferred for drying quantitatively clean glassware (provided that the prongs of the drying rack are not inside the item, thus contaminating

it). Volumetric glassware and cuvettes are never to be placed in drying ovens, even if they are not quantitatively clean. The third method is acceptable only when the compressed air supply is known to be free oil and other contaminants. An alternative to blowing air into the item is to use an aspirator, or house vacuum, to pull air into the item.

4.0 CONCLUSION

With the tips on laboratory safety and housekeeping student should have been equipped with what it takes to work safely and efficiently in a biochemical laboratory.

5.0 SUMMARY

***** Laboratory safety means freedom from harm or accident when working in the laboratory

* Laboratory safety rule are guideline design to help keep one safe when experimenting.

* A clean well-maintain work area prevent accident and enhance the overall efficiency of work performed

* The way laboratory glassware is washed depend on what is in it and how it we be used.

6.0 TUTOR MARKED QUESTION

1**.** Itemize some safety precaution to be taking in a biochemistry laboratory

2. What is safety equipment? Give two examples.

3. List three general cleaning procedure for quantitatively clean glassware

7.0 REFERENCE/FURTHER READING

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Office for Research Safety Laboratory Housekeeping **researchsafety@northwestern.edu**

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UNIT 3 ACCURACY OF MEASUREMENT AND TRANSFER OF LIQUIDS AND SOLIDS

CONTENTS

1.0 INTRODUCTION

Increased usage sometimes causes definitions to become tainted. In the laboratory, two terms which are often used interchangeably are accuracy and precision, or inaccuracy and imprecision. These, in fact, are different terms with very distinct meanings. Knowing the true definition influences upon, and importance of each is critical to the development of a sound quality control program in the laboratory. A sound quality control program assures the reliability of your results allows the interchangeability of results between laboratories, reduces the likelihood of expensive repeat testing, and, in a worst case scenario, could prevent failed inspection or the questioning of vital research data.

2.0 OBJECTIVE

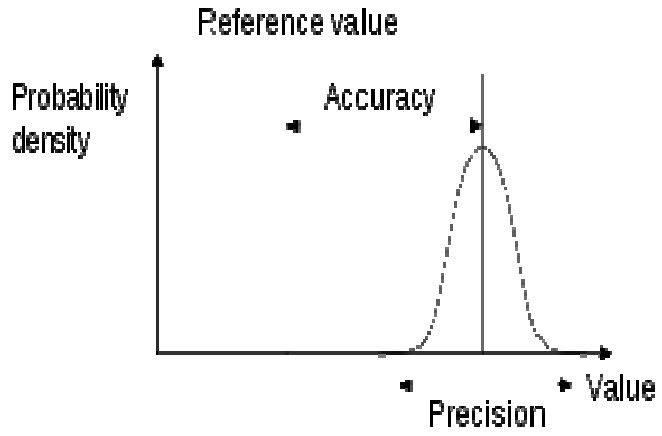
At the end of studying this unit you should be able to:

1. Explain the importance of accuracy and precision in measurement in the laboratory.
2. You should be familiar with the different ways of expressing measurement in SI unit of mass, volume, amount of substance and concentration
3. Report measured volume, mass, concentration to appropriate number of significant figure (that is appropriate to the level of precision or accuracy of the measurement)
4. You should be aware of error in all practical

3.0 MAIN BODY

3.1 Accuracy of measurement of liquid

In the fields of science, engineering, industry and statistics, the accuracy of a measurement system is the degree of closeness of measurements of a quantity to that quantity's actual (true) value. The precision of a measurement system, also called reproducibility or repeatability, is the degree to which repeated measurements under unchanged conditions show the same results. Although the two words can be synonymous in colloquial use, they are deliberately contrasted in the context of the scientific method.



Accuracy indicates proximity of measurement results to the true value, precision to the repeatability or reproducibility of the measurement.

A measurement system can be accurate but not precise, precise but not accurate, neither, or both. For example, if an experiment contains a systematic error, then increasing the sample size generally increases precision but does not improve accuracy. The end result would be a consistent yet inaccurate string of results from the flawed experiment. Eliminating the systematic error improves accuracy but does not change precision.

Therefore, the biochemists like all scientists seek to obtain accurate measurements in the laboratory and also to present an accurate account of the experiment. However, before dealing with accuracy, it is as well to remind ourselves of the units and quantities used in biochemical experiments and to consider some simple calculations.

3.1.1 SI units

The SI units (*Systeme International d'U nites*) based on metric system. These were approved in 1960 by The General Conference of Weights and Measures and are being adopted by scientific laboratories throughout the world. They are a *coherent* system of units so that if two unit

quantities are multiplied or divided, then the answer is the unit of the resultant quantity. In this way the number of multiple and submultiples of units now in use will be reduced.

3.1.2 Basic units

The basic SI units of quantity are the mole which gives the amount of substance present in say, a test tube irrespective of the present volume. It is define as the molecular weight of the substance in gram =molecular weight in gram = $6X10^{23}$ (Avogadro's number)

The term mole is also applied to other particle of define composition such as atom, ions, or free radical, as well as molecule.

1 mole of glucose (molecular weight 180) is 180g

1 mole of albumin (molecular weight 68000) is 68000g or 68Kg

There are seven basic units on which all others are based and these are set out in the table shown below:

Table 2 SI units

Physical quantity	Name	Symbol
Length	Meter	m
Mass	Kilogram	kg
Time	Second	S
Amount of substance	Mole	mol
Thermodynamic temperature	Kelvin	K
Electric current	Ampere	A
Luminous intensity	Candela	cd

3.1.3 Derived units: in addition to those above, there also a number of derived SI units obtained by appropriate combination of these basic units. For convenience, these derived units are given special names and those which are likely to be met in biochemical work are listed in Table 2.2.

Table 2.2: The special names and symbol of some derived SI units

Physical quantity	Name	Symbol	Units
Frequency	Hertz	Hz	s^{-1}
Force	Newton	N	kg m s^{-2}
Pressure	Pascal	Pa	N m^{-2}
Energy or work	Joule	J	N m
Power	Watt	W	J s^{-1}
Electric charge	Coulomb	C	A s
Electric capacitance	Farad	F	A s V^{-1}
Potential difference	Volt	V	W A^{-1}
Resistance	Ohm	Ω	V A^{-1}
Conductance	Siemens	S	Ω
Radioactivity	Becquerel	Bq	S^{-1}
Absorbed dose of irradiation	Gray	Gy	J kg^{-1}
Customary temperature	Degree Celsius	^0C	$^0C = K - 273.15$

3.1.4 Units in Conjunction with SI units

There are some units used in biochemical work which are likely to be replaced altogether because of their convenience and it is probable that they will continue to be used in conjunction with SI units for some time. They include: Litre (l), microlitre (µl), millilitre (ml); gram (g), microgram (µg), milligram (mg) and minute, hour year.

3.2 Accurate Measurement of Solid

During the course of experimental exercise which involves weighing and taking measurement, any of these (volume, mass and length) and other measurement cannot be carried out with absolute precision or accuracy, there are inevitable error or uncertainties. These error which arise as a result of imperfection (not faulty apparatus) in apparatus and/or inevitability of experimenter, may be minimized by appropriate choice of apparatus and the exercise of care on your part. This is to; at least, increase your awareness of error in all practical exercise.

3.2.1 Sources of Error

No measurement taken in the laboratory is exact; all measurement are of limited accuracy and are liable to error so the potential source of error should be appreciated.

An error is the amount of deviation from the true value and not a mistake on the part of the worker. Errors arise from statistical variation and have to be lived with although they can be minimized by careful working. Mistakes on the other hand are instances of human incompetence which can and must be eliminated.

Human mistake These can arise from a badly designed experiment where insufficient control is exercised. For example, in many biological experiments the temperature and illumination of the environment may have a profound effect on the system under investigation and should therefore be carefully controlled. Experiment should be planned in such a way that only one variable is introduce at a time and all other factor effecting the experiment is kept constant.

A common source of human error arises from the careless reading of scale or meniscus when the problem of parallax is not appreciated. For this reason many instrument incorporate a mirror

behind the pointer so the true reading is obtained when the pointer and its reflection is superimposed. Some manufacturer overcomes this problem by giving a digital readout on their instrument rather than a deflection on the scale. Probably one of the greatest source of human error has been the careless reading of the meniscus on a pipette but this has now been eliminated by the introduction of automatic or digital pipette.

Limitation of Apparatus The limit set by accuracy of a particular piece of equipment is usually known and is allowed for. These error are also known and can be taken into account

Standards and Blanks To obtain as accurate a value as possible from an estimation, error must be reduce to the minimum and this can be done by careful working and the use of standard solution. Standard solution of a substance to be estimated should be included in any test, even when calibrated equipment and standard reagent are used.vavled obtained for the test solution should fall within the range of the standard curve and the value of the test can be read. Usually only one standard is included for volumetric estimations or when a standard curve has previously been constructed.

Blank solution should be included in any measurement. The same volume of distill water replaces the substance to be estimated and the blank is then treated in exactly the same way as the test and standard. Any value obtain for the blank is, of course, subtracted from the value of the test and standard in the final calculations, since the blank value is due to the reagent used and not the substance under investigation. The practical used of blanks and standards is well illustrated in the numerous colometric estimation in this volume.

Random Error: Finally there are random errors which are individually unpredictable. These are seen when one individual carried out a number of determinations under identical conditions and obtains a slightly different result each tie. This random error can be considerable reduce by taking a large number of measurements and calculating the average value. For many purposes, duplicate estimation are sufficiently provided there is a good agreement between them, and this is usually the situation for reading obtain in, say, the construction of a calibration curve. The degree of agreement between replicate experiments is term the precision. Precision does not mean accuracy because an experiment may be precise but inaccurate due to faulty instrument or technique.

3.3 EXPERIMENT

3.3.1 The calibration of laboratory pipette

The volume delivered by the pipette is determine by weighing the amount delivered and dividing this by the density of the water at room temperature

Materials

1. Balance
2. Micropipette
3. Graduated pipette (5ml)
4. Distilled water

Method

(a) Fill the graduated pipette to the 5ml mark, carefully wipe the end and allow the content to drain into a weighed bottle. Carefully note the temperature of the liquid and calculate the volume

delivered from the weight and density of water at that temperature. Repeat this 10 times and tabulate your reading.

(B) Set the micropipette to 100µL, draw distill water up to the tip and pipette into a small weighed beaker. Calculate the volume actually delivered as given above and repeat the experiment with the same tip 10 times.

Accuracy and tolerant limit Determine the mean volume delivered and calculate the standard deviation of the result. What value will you use as 'tolerant limit' for the pipette and why?

Low temperature Many biochemical experiment involve pipetting of ice-cold solution, so use your calibrated pipette to deliver distill water at 0 ^{o}c. calculate the auctual volume delivered as before, compare this to the calibrated volume and calculate the error involve in pipette ice-cold solution.

4.0 Conclusion

This study of accuracy of measurement must have giving the student the student the ability to:

i. explain the important of accuracy and precision in measurement

ii. Report and express measurement in SI unit.

5.0 Summary

* Accuracy is the degree of conformity to the true value.

* The SI unit is the system of unit based on metric system.

* There are both basic and derive SI units.

* No measurement is taken in the laboratory is exact; all measurement are of limited accuracy.

6.0 Tutor marked assignment

1. Give the SI unit of the following quantities: time, energy, force, length and electric current.

2. List the sources of error taking in the laboratory

3. Mention two apparatus needed in the calibration of laboratory pipette.

7.0 Reference/further reading

Plummer, T. D.(1987) An introduction to Practical Biochemistry.3rd Edition, McGraw-Hill Book Company (UK) Limited. England.

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UNIT 4 INTRODUCTION TO PHOTOMETRY AND COLORIMETRY

CONTENT

1.0 INTRODUCTION

Spectrophotometry is a technique involving the determination of the amount of light that is transmitted or absorbed by a substance at a given wavelength. The ability of the molecule to scatter or absorb light incident on it depend on the properties. Colorimetry is the science and technology to quantify and describe by the help of mathematical models the human colour percepts. As colour perception is a psychophysical phenomenon, to understand colorimetric methods it is right to overview first the physiological basis of colour perception. From the point of view of photometry one of the most important aspects of colorimetry is the quantification of the colour rendering properties of light sources. Colour measurement is performed by spectrophotometric, spectroradiometric and tristimulus measurements.

2.0 OBJECTIVE

At the end of this unit student should be able to:

1. Define Spetrophotometry
2. Explain Colorimetry
3. Explain Photometry

3.0 MAIN BODY

3.1 Photometry

Photometry concerns itself with visible light. Visible means as perceived by human eye so is tied to the response of the human eye and its perception of this portion of the electromagnetic spectrum. Photometry is the science concerned with measuring human visual response to light. Because the eye is a highly complex organ, this is by no means a simple task. It involves the meeting of many disciplines: psychology, physiology, and physics among them.

Photometry can be said to have become a modern science in 1924, when the Commission Internationale de l'Eclairage (CIE) met to define the response of the average human eye. The Commission measured the light-adapted eyes of a sizable sample group, and compiled the data into the photopic curve. Simply stated, the curve reveals that people respond strongest to the color green, and are less sensitive to the spectral extremes, red and violet.

The eye has an altogether different response in the dark-adapted state, wherein it also has difficulty determining color. This gave rise to a second set of measurements, and the scotopic curve.

3.2 Photometric Applications

Photometry involves the measurement of electromagnetic radiation in terms of its capacity to stimulate the human visual system, and to be perceived as light. Different applications require different types of measurements, and therefore, different types of photometric instruments (photometers).

3.3 Introduction to Colorimetry

Many assays in biochemistry depend on measuring the light absorbed by a substance in the visible or ultraviolet region of the electromagnetic spectrum. The estimation is based on comparison of the light absorbed by substances in solution of unknown concentration with the light absorbed by the same substance in solution of known concentration. This method can be used in estimation of coloured substance; and substances, which absorb only in the UV, such as protein and nucleotides; and colourless substances such as sugars, which can undergo a series of chemical reactions to give a coloured derivative. Probably the most widely used method for determining the concentration of biochemical compounds is colorimetry, which makes use of the property that when white light passes through a coloured solution, some wavelengths are absorbed more than others; and the resultant colour is due to the light, which is transmitted. Maximum absorption of coloured solutions occurs in the regional opposite colour to that of the solution. Many compounds are not themselves coloured, but can be made to absorb light in the visible region by reaction with suitable reagents. These reactions are often fairly specific and in most cases very sensitive, so that quantities of material in the region of millimole per litre concentrations can be measured.

The following types of colorimeter are in use in laboratories:

1. The Eel spectra. The scale (0-100) it is approximately absorbance X 100 so divide your reading by 100 to get approximate absorbance.
2. The spectronic 20. The scale reading 0-1 is the absorbance scale.
3. The Beckman 200. The scale reading 0-1 is the absorbance scale.
4. Sp101 spectrophotometer. The scale reading 0-2

The amount of light absorbed by a solution is governed by the factors of path length through which the light travels and the concentration of the solution. These properties are formalized in two laws namely Beer's and Lambert's law.

3.3.1 Beer-Lambert' law

When a ray of monochromatic light of initial intensity I_o passes through a solution in a transparent vessel, some of the light is absorbed so that the intensity of the transmitted light I is less than I_o. There is some loss of light intensity from scattering by particles in the solution and reflection at the interfaces, but mainly from absorption by the solution. The relationship between I and I_o depends on the path length of the absorbing medium, I, and the concentration of the absorbing solution, c. these factors are related in the laws of Lambert and Beer.

Beer's law: This states that the absorption of a monochromatic light (uniform wavelength) passing through an absorbing medium is directly proportional to the concentration of the absorbing molecules for a constant path length (the transmittance decreases exponentially with the number of absorbing molecules); mathematically represented as

$I = I_o e^{-kc}$ where k = constant for the wavelength

Lambert's law states that when a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as the length of the absorbing medium increases arithmetically and the light absorbed is independent of the source of light. For example, if the incident light is 100% and 50% of the incident light is absorbed per unit layer, and then the intensity of light will decrease as follows: 50%, 25%, 12.5% etc.

This is represented mathematically as

$$I = I_o e^{-kl}$$

Where I = path length

Beer-lambert's law: this state that when monochromatic light passes through a solution, the amount of light transmitted decreases exponentially with the increase in the concentration of the solution and with the increase in the thickness of the layer of the solution through which the light passes. This is obviously the combination of beer's and Lambert's laws.

This is mathematically represented as

$$\text{Log } I_o/I_i = \varepsilon ct = A = \text{Log} 1/T$$

Where ε= Extinction coefficient or molar absorptivity

t= thickness of the absorbing medium of path length

c= concentration in mol/$dm^3$of absorbing species

The part of the light absorbed is referred to as the "absorbance" while the part transmitted is referred to as the "transmittance". Transmittance (T) is the ratio of intensities of the transmitted light, I and the incident light I_o.

Thus, Transmittance is given by

$$T = I/I_o$$

Transmittance is measured in percentage and varies from 0 – 100%.

The absorbance is given by the negative logarithm of the transmittance:

$$A = -\text{Log } T = \log 1/T = I_o/I$$

The expression $\log I_o/I$ is known as extinction (ε) or absorbance (A). The extinction is sometimes referred to as the optical density (OD). Therefore,

$$\varepsilon = kcl$$

If the beer-Lambert law is obeyed and l is kept constant, then a plot of extinction against concentration gives a straight line passing through the origin.

Some colorimeters and spectrophotometers have two scales, a linear one of percent transmittance and a logarithmic one of extinction. It is the latter scale that is related to concentration and is the one use in the construction of a standard curve. With the aid of such a standard curve the concentration of an unknown solution can easily be determine from its extinction.

3.3.2 Application of Beer-Lambert's law

The law is applied to quantitative measurement in the entire region of the electromagnetic spectrum although it is more extensively used in ultraviolet-visible spectroscopy. The law is demonstrated by preparing a standard solution of a sample, making serial dilutions of it and measuring their respective absorbance. A calibration curve is obtained by plotting a graph of this absorbance against their concentrations. This graph should be a straight line graph passing through the origin.

The plot of % absorbance against concentration gives a negative exponential curve (graph 4.1a) thus this is the plot of absorbance against concentration (4.2b) that is used in the determination of the concentration of an unknown solution. The slope of the calibration graph is equal to the extinction coefficient multiply by the path length (ε x l).

It should be noted that coloured solution have their own characteristic absorption spectra and careful selection of the wavelength where maximum absorption occurs enable the mixture of two coloured substance to be analysed .

3.3.3 Deviation from beer-Lambert's law

Deviation from this law occurs if:

1. The concentration of the absorbing species is too concentrated 10^{-2}M
2. The absorbing species are involved in ionization, association, dissolution or solvation with concentration or time.
3. If the wavelength of light used was not at the absorption maximum of the solution and
4. When unabsorbed stray light passes through the optical system.

When beer-Lambert's law is not obeyed a plot of absorbance against concentration gives a non-linear graph.

3.4 Experiment

The colorimetric estimation of inorganic phosphate

Principle

Most biochemical substance are colourless and can only be analysed colometricaly after reacting them with a specific chemical reagent to give a colour product. The point is illustrated in the

measurement of inorganic phosphate, this is probably one of the commonest determination carried out in a biochemical laboratory, and the production of a standard curve now prove useful in feature experiments.

Inorganic phosphate react with ammonium molybdate in an acid solution to form phosphomolybdic acid. Addition of a reducing agent reduces the molybdenium in the phosphomolybdate to give blue colour, but does not affect the uncombined molybdic acid. In this method the reducing agent used is *p*-methylaminophenol sulphate. The presence of copper in the buffer solution increases the rate at which the colour developed.

Material

1. Ammonium molybdate (50g/litre)
2. Copper acetate buffer pH 4.0 (dissolve 2.5g of copper sulphate ($CuS0_4.5H_2O$) and 46g of sodium acetate ($CH_3COONa.3H_2O$) in 1 litre of 2 mol/litre acetic acid. Check the pH and adjust the 4.0 if required).
3. reducing agent. (dissolve 20g of *p*-methylaminophenol sulphate in a 100g/litre solution of sodium sulphite ($Na_2SO_3.7H_2O$) and make up to 1 litre). Store in a dark bottle until required.
4. Trichloroacetic acid (100g/litre).
5. Stock phosphate solution containing 100 mg phosphorous/100 ml. (dissolve 438 mg of Potassium dihydrogen phosphate in water and make up to 100 ml). Store in the refrigerator
6. Working phosphate solution containing 1 mg phosphorous/100 ml.(dilute the stock solution 1 in 100ml with 50g/litre TCA)

7. Colorimeter.

Method

Pipette 0.1-1 ml of the standard of phosphate into a test tube, and, where necessary, add water to bring the final volume to 1 ml. then add 3 ml of copper acetate buffer, 0.5 ml of ammonium molybdate, and 0.5 ml of reducing agent mixing thoroughly after each addition. Allow to stand for 10 min and read the extinction at 880 nm. Set up a blank by replacing the phosphate with 1 ml of 50g/litre TCA. Prepare a graph of the extinction against the concentration of phosphate.

When measuring the concentration of phosphate in a solution containing protein, the test solution is mixed with an equal volume of 20 g/litre TCA, the precipitate centrifuged, and an aliquot of the supernatant treated as above.

4.0 Conclusion

The student by now should be able to explain what spectrophometry is all about.

5.0 Summary

***** Spectrophometer is a technique involving the determination of the amount of light that is transmitted or absorbed by a substance at a giving wavelength

* Photometry is the science involving or concern with measuring human visual response to light

* Beer-lambert's law mathematically = $\text{Log } I_o/I_i = \varepsilon ct = A = \text{Log} 1/T$

6.0 Tutor marked assignment

1. What is spectrophometry ?

2. List the factor that affect the amount of light absorb by a solution

3. State Beer-Lambert's law and state its mathematical expression.

7.0 Reference/further study

Plummer, T. D.(1987) An introduction to Practical Biochemistry.3rd Edition, McGraw-Hill Book Company (UK) Limited. England.

Amadi, B.A., Agomuo, E.N. and Ibegbulem, C.O.(2004). Research Methods in Biochemistry

http://www.udtinstruments.com/products/photometric/photometry.shtml

UNIT 5 STANDARD CURVE IN ABSORPTION SPECTRA

CONTENT

1.0 Introduction

Several Biochemical assays employ photometric quantitative techniques to measure compound that otherwise would not absorb light in ultraviolet visible or near infrared region. Such assays usually involve reaction of the type:

Colourless compound + colour forming → photometric colour proportion to
Being assays for reagent (s) amount of colourless compound

2.0 OBJECTIVES

At the end of this study student should be able to:

1. Explain the meaning of a standard curve.
2. Prepare a standard curve.
3. Know how to use a standard curve.

3.0 MAIN BODY

A standard curve is a quantitative research tool, a method of plotting assay data that is used to determine the concentration of a substance, particularly proteins and DNA. It can be used in many biological experiments.

The assay is first performed with various known concentrations of a substance similar to that being measured. For example a standard curve for protein concentration is often created using known concentrations of bovine serum albumin. The assay procedure may measure absorbance, optical density, luminescence, fluorescence, radioactivity, or something else.

Example: One assay for protein is called the Bradford assay; it is a colorimetric assay. The reagent Coomassie Brilliant Blue turns blue when it binds to arginine and aromatic amino acids present in protein. The intensity of the colour is best measured at 595 nm, which is the maximum absorbance (Amax) frequency of the blue dye, using a spectrophotometer. In this case the greater the absorbance, the higher the protein concentration.

These data are used to make the standard curve, plotting concentration on the X axis, and assay measurement on the Y axis. The same assay is then performed with samples of unknown concentration. To analyze the data, one locates the measurement on the Y-axis that corresponds to the assay measurement of the unknown substance and follows a line to intersect the standard curve. The corresponding value on the X-axis is the concentration of substance in the unknown sample.

Generally, standard curve are derived from colorimetric assays involving the preparation of series of reaction tubes of varying concentration of the compound being assayed for and a blank

tube (Containing all the compound expect for the compounds being assayed for). In addition, a wide range of aliquot sizes are considered in colorimetric assays. However the linearity of the standard curve derivable from such assays is only perfect for dilute solution (for which Beer's law holds) of the compound being assayed for. Standard curve plot involve two variable the magnitude of one of the variable (the dependent variable) is assumed to be determined by (i.e. is a function of) the magnitude of the second (the independent) variable, whereas the reverse is not true. For example, the relationship between absorbance at 260 nm and concentration of DNA in a solution, absorbance may be considered the dependent variable and concentration the independent variable. The independent variable is plotted up the abscissa (x-axis) and the dependent variable on the ordinate (y-axis).

Such dependent relationships that follow straight lines are term linear regression. Raw data for the construction of standard curves are usually subjected to statistical regression analysis to deduce line of best fit and eliminate errors. To further ensure reliability, reaction leading to the data for construction of standard curves are carried out on the same day to eliminate error due to delay of sensitive reagent on storage standard curves are useful in quantifying compound whose initial concentration is unknown. Similar treatment is normally given to the compound then the concentration value is extrapolated from the standard curve

The procedure outlined below would help you understand the preparation and uses of standard curve for example:

Proteins which consist only of amino acids linked together by peptide bond do not absorb in the visible region. In an alkaline solution of cupric ions (biuret reagent) proteins give a purple colouration. The reagent is named after the simplest compound, biuret, which will give this colour reaction.

Procedure

Prepare the dilution of the standard protein solution (10 mg/ml) making up to final volume of 1ml with distill water. Prepare serial dilutions (1/2, 1/4, 1/8, 1/16) of the protein solution x of unknown concentration and take 1ml portions of these dilutions for development with the reagent. Prepare reagent blank using 1ml of distill water.

Add 4.0ml of biuret reagent to the dilution of the standard and unknown and to the 1ml of distill water (reagent blank). Mix the solutions thoroughly and allow them to develop at room temperature for 30 min. Measure the absorbance of the developed solution at the wavelength of maximum absorbance for this reagent i.e. 540 nm, zeroing the spectrophotometer with the reagent blank. Develop the standard curve using data obtained and determine the concentration of the unknown.

4.0 Conclusion

Standard curves are used to determine the concentration of unknown substance.

5.0 Summary

***** An example of a quantitative research tool is the Bradford

* In developing a standard curve assay measurement is plotted against concentration

* The concentration of the unknown is determined by extrapolation using the assay measured on the standard curve.

6.0 Tutor marked question

1. What do you understand by absorbance optical density?

2. What is the significant of the slope of a standard curve

3. In the world of absorption spectroscopy, what is a "blank" and why is it necessary?

4. If you perform four two-fold serial dilutions, what will be the concentration of the final (most dilute) sample relative to that of your starting sample? Show your work.

7.0 REFERENCE /FURTHER STUDY

Plummer, T. D.(1987) An introduction to Practical Biochemistry.3rd Edition, McGraw-Hill Book Company (UK) Limited. England.

Layne, E. Spectrophotometric and Turbidimetric Methods for Measuring Proteins. *Methods in Enzymology 3:* 447-455. 1957.

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"Bradford Protein Assay". *Bio-Rad Quick Start™ Bradford Protein Assay Instructional Manual*. http://www.bio-rad.com/cmc_upload/Literature/55685/4110065A.pdf.

UNIT 6 pH AND BUFFER SYSTEM

CONTENT

1.0 Introduction

pH and Buffer measurement are commonly used in biochemical assays . The pH is used to measure the acidity or alkalinity of a solution. pH is not an absolute value but within a narrow range.

2.0 Objective

At the end of this study student should be able to:

1. Define pH and Buffer solution.
2. Know the relevance and uses of Buffer system.

3. How to calculate pH.

4. How to prepare a Buffer solution.

3.0 Main body

3.1 Definition

pH is strictly as the negative logarithm of the hydrogen ion activity, but in practice the hydrogen ion concentration is usually taken and this is virtually the same as the activity except in strongly acidic solution. Hydrogen ion concentrations are routinely express as pH value.

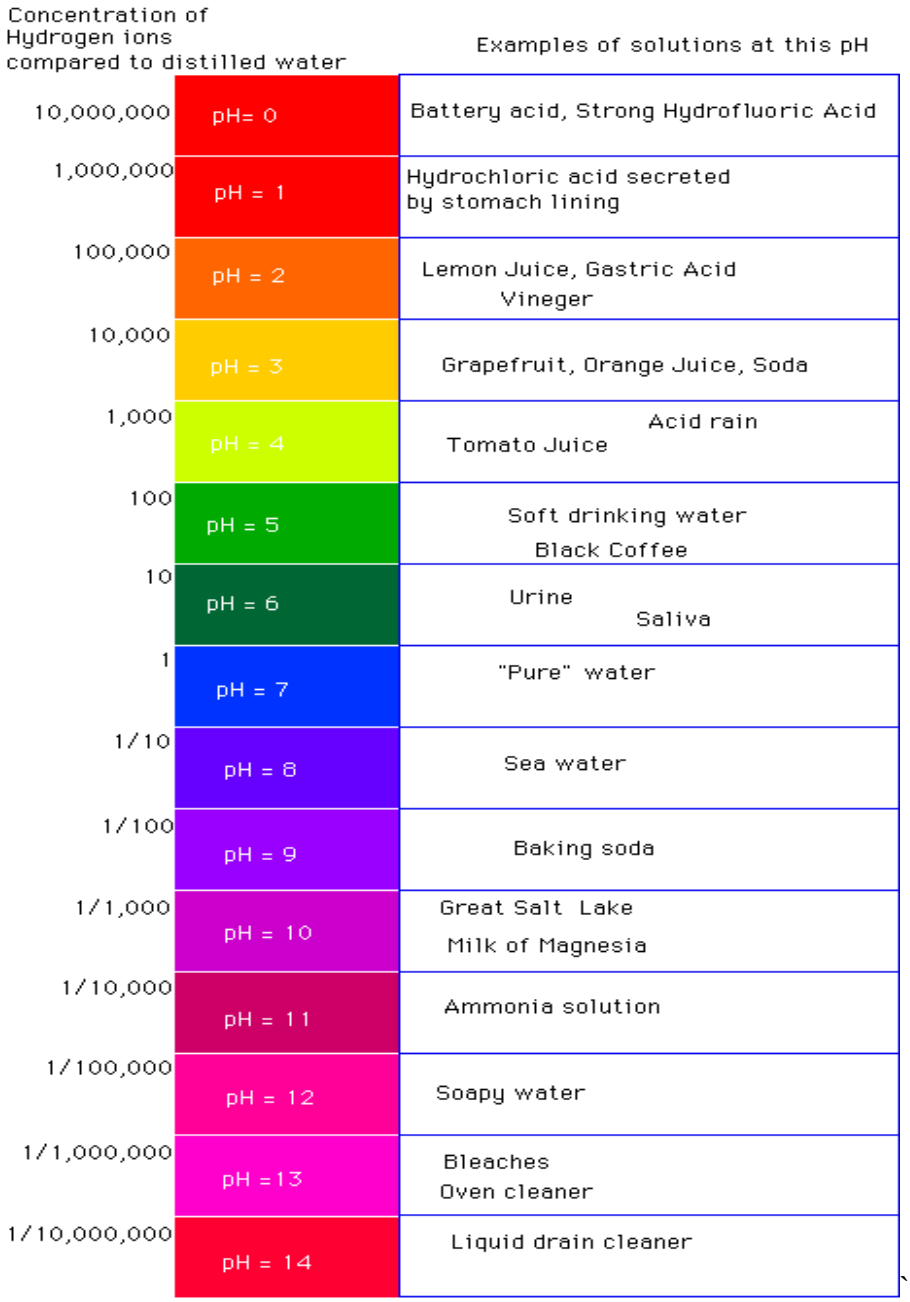
$$pH = \log 1/[H^+] = -\log [H^+]$$

Although pH is an expression of hydrogen ion and activity (as measured by pH meters), no distinction is usually made between ion activity and concentration. pH value offer a convenient means of stating widely varying $[H^+]$ in small positive number. The pH scale of 0-14 to accommodates H^+ concentrations of 1 M to 1 x 10^{-14} M. A pH value of 7 ($[H^+]$ of pure water) is considered the neutral pH. Increase in $[H^+]$ (pH values smaller than 7) result in acidic conditions. The value of using pH can be seen in the case of human blood which has an extremely low hydrogen ion concentration:

Plasma $H^+ = 0.398 \times 10^{-7}$ mol/litre

Plasma pH = $-\log (0.398 \times 10^{-7}) = 7.4$

In the use of pH values, it is important to remember that the numbers represent a logarithmic function and that a decrease or increase of one pH unit, e.g pH 7→ pH 6, represents a tenfold difference in $[H^+]$. Study the pH chart given below carefully. Note that each decrease in pH by one pH unit means a tenfold increase in the concentration of hydrogen ions.



(Source: Paul Decelles (2006) staff.jccc.net/pdecell/chemistry/phscale.html)

Figure 6.1: the pH of some aqueous fluids

3.2 pH measurement

The most convenient and reliable method for measuring is by the use of a pH meter which measures the e.m.f (electromotive force) of a concentration cell formed from a reference electrode, the test solution, and glass electrode sensitive to hydrogen ion.

The pH measurement take place in two specific steps: first is the standardization of the pH meter followed by the actual measurement. The pH meter is standardized by dipping its glass electrode into buffer solution of pH 4 and pH 8 respectively. This is followed by rinsing it with deionized water (distill water can be used) and drying of the glass electrode with clean wood or tissue paper. The actual measurement is then done by dipping the glass electrode into the test solution and reading off the value.

Two types of pH meter (Retrieved from http://:en.wikipedia.org/wiki/PH_meter; www.microscopesblog.com/2009/06/ph-meter.html respectively)

3.3 Buffer system

A buffer is a solution of a particular pH that can resist pH changes on the addition of acid or alkali. Such solution is use in many biochemical experiments where the pH needs to be accurately controlled. Buffers are in fact mixtures of weak acid or bases and their salt. It is the extent of dissociation that determines whether an acid/base is strong or weak. A weak acid is a better buffer because it does not dissociate completely but the strong acid dissociate completely. Some common buffer mixture use in biochemical experiment include, acetic acid-sodium acetate (buffering range 3.5-5.5), mono- and disodium phosphate (buffering range 6-8), sodium bicarbonate –carbonate (buffering range 7-9) and tris (hydroxymethyl) aminomethane or tris (buffering range 7-9). In man and most other mammals, the plasma pH lies within the very narrow limit of 7.35-7.45 at normal body temperature if this pH value for some pathological reasons fall to 7.0 or below, death assuredly would result from acidotic coma and at pH 7.8 or above death occur as a result of acute tetany. The ability of a buffer to resist change in pH is referred to as buffer capacity.

3.4 Uses of pH and buffer solution

Most cells can actually function between very narrow limit of pH and require buffer system to resist to the changes in pH that would otherwise occur in metabolism. The three main buffer systems in living material are protein, bicarbonate and phosphate and the relative importance of each depends on the type of cell and organism**.**

3.5 Principles of buffer preparation

There are basically two different approaches to the preparation of any buffer:

1. A situation in which both component of the conjugate acid /base pair are accurately weighed out separately to give the desired ratio in the Henderson-Hasselbalch equation and then dissolve in the ionize water.

2. A situation in which both components are obtained from a predetermine amount (by prior calculation) of only one component from which the second component is formed by addition of an appropriate amount of strong acid or strong base. Sample calculation of method one is shown below.

Question: To prepare 250 ml of 0.2M sodium phosphate buffer of pH 6.4, the following chemicals are available: dibasic sodium phosphate, monobasic sodium phosphate and 0.129M solution of NaOH. Assume that the pKa_1, pKa_2, and pKa_3 of phosphoric acid are 2.12, 6.8 and 12.3 respectively. Molecular weights of dibasic and monobasic sodium phosphate salts are 142 and 138 respectively.

Calculation:

Dibasic sodium phosphate = Na_2HPO_4

Monobasic sodium phosphate = NaH_2PO_4

Since the pH of the buffer solution will are require to prepare is 6.4 will shall be interested only in the second dissociation of phosphoric acid described by the following equilibrium

$H_2PO_4 = HPO + H$

Conjugate Acid — Conjugate base

From the Henderson-Hasselbalch

$$pH = + pKa_2 + \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

Substituting the value of the pH and pKa2 into this equation will give:

$$6.4 = 6.8 + \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

$$\text{i.e } \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = -0.40 = (-1 + 0.6000)$$

$$\text{Hence } \frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = \text{antilog } 0.6000 = 0.399$$

Since $\log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$ is a negative number, the ratio $\log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$ must be

$$\frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = \frac{0.398}{1}$$ not 3.98 or 39.8 etc.

Therefore

$$[HPO_4^{2-}] = \frac{0.398}{1} \times 0.2 \text{ mole/litre}$$

Similarly

$$[H_2PO_4] = \frac{1}{1.398} \times 0.2 \text{ mole/litre}$$

Since each of the anionic species HPO_4^{2-} is introduce into the solution in the form of a salt. To obtain the actual weight of each required to prepare the buffer. We must make use of the given molecular weight of the two sodium salt.

$$\text{Weight of } Na_2HPO_4 \text{ required} = \frac{0.0398}{1.398} \times 0.2 \text{ moles/litre of buffer}$$

$$\frac{0.0398}{1.398} \times \frac{0.2}{1} \times \frac{142}{4} \text{ g/250 moles/litre of buffer solution} = 2.02$$

$$\text{Weight of } Na_2HPO_4 = \frac{1}{1.398} \times 0.2 \times \frac{138}{4} \text{ g/250mole/litre of buffer} = 4.29\text{g.}$$

Therefore to prepare this buffer, weigh out 2.02g of Na_2HPO4 and 4.29g of NaH_2PO_4. Mix both salt in 250ml volumetric flask dissolve with about 100ml 0f deionize water, and make up the volume with water. Check the pH of the resulting solution and adjust with acid and base if necessary.

4.0 Conclusion

- The pH meter should be standardized with a buffer solution before use.
- Increase or decrease in pH can be very dangerous as found in the case of plasma

5.0 Summary

*****A buffer solution a particular pH usually resists pH changes

*Acute tetany and acidotic coma are death occurrences as a result of fluctuation of the pH of Plasma

*A pH scale is usually divided into 3 regions; acidic, neutral and basic

6.0 Tutor marked assignment

1. Mention the 3 main buffer system in a living material.

2. What is the hydrogen ion concentration of a solution with a pH of:

a. 3.25 b. 7.28 c. 13.3

3. What is the pH of a solution with the following $[H^+]$:

a. 0.025M b. 0.15M c. 0.2M

7.0 REFERENCE/FURTHER READING

Plummer, T. D.(1987) An introduction to Practical Biochemistry.3rd Edition, McGraw-Hill Book Company (UK) Limited. England.

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UNIT 7 QUALITATIVE AND QUANTITATIVE TESTS FOR AMINO ACIDS AND PROTEINS

CONTENT

1.0 Introduction

This lab presents the student with selected chemical reactions of the proteins and some of the amino acids which make up proteins. These compounds are essential components of living organisms, including the human body, and the range of tests available shows how much time scientists have devoted to this branch of biological chemistry.

2.0 Objectives

At the end of this study the student should be able to

1. Define proteins and amino acids
2. Know the qualitative and quantitative tests for proteins and amino acids

3. To get familiar with amino acids as the building blocks of proteins in the body.
4. To understand how twenty α-amino acids can produce proteins with very marked differences in their physical and chemical properties.

3.0 Main Body

3.1 Definition of Proteins and amino acids

Proteins are biological macromolecules of high molecular weight occurring abundantly in living systems. Proteins contain elements like carbon, nitrogen, hydrogen and oxygen with some uncommon ones like phosphorus and sulphur. A single protein molecule is made of a large number of amino acid molecules linked by peptide bonds by the removal of water molecules. A complex array of protein is built from 20 amino acids and the constituent amino acids that make up a given protein molecule can be found from the products of its decomposition.

Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures, nutritional attributes and physiochemical properties. Amino acids on the other hand are organic compounds that contain amino and carboxyl groups, and therefore possess both acidic and basic properties.

Proteins are important constituents of foods for a number of different reasons. They are a major source of *energy*, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Proteins are also the major structural components of many natural foods, often determining their overall texture, *e.g.*, tenderness of meat or fish products.

3.2 Qualitative Analysis of Proteins and Amino Acids

Experiment 1: Ninhydrin Test

Background: This test is widely used in biochemistry and in food science. Although compounds other than proteins and amino acids also give positive reactions, standard procedures used in analysis can make the reaction a positive test for amino acids and proteins.

Principle: Amino acids or proteins containing α-amino groups when heated with ninhydrin give a characteristic lilac, violet, deep blue or purple colour while amino acids containing imino (-NH) group (proline and hydroxyproline) give a pale yellow colour with ninhydrin.

Materials/Reagents

1. Amino acids (0.1 mmol/litre aspartic acid, arginine, leucine, and proline)
2. Acetate buffer (4 mol/litre, pH 5.5)
3. Ninhydrin reagent
4. Acetone

Procedure

Dissolve 3g of ninhydrin in 100ml of acetone to form the ninhydrin solution. Add 0.2ml of ninhydrin to 2ml of the test protein solution in a test tube and boil in a water bath for 10 minutes. The presence of lilac (blue or purple) colour confirms the presence of a protein with amino group.

Experiment 2: Biuret Test for Protein

Background: Biuret is a compound obtained when a protein is heated to 108 ^{0}C. It reacts with copper sulphate in alkaline solution to give a violet color with an absorption maximum at 550 nm. This compound has given its name to the color reaction which was also found to occur with other compounds-those having two or more amide groups or peptide bonds joined directly together, or through a single atom of carbon or nitrogen.

Principle: The biuret test is a chemical test used for detecting the presence of peptide bonds. In the presence of peptides, a copper (II) ion forms a violet-colored (purple) complex in an alkaline solution. The violet coloured complex formed between the amide group (-CONH) in proteins and the copper II ions in $CuSO_4$ form the basis of this test.

Materials/Reagents

Protein sample

Sodium or potassium hydroxide

Aqueous copper (II) sulfate

Procedure: An aqueous sample is treated with an equal volume of 1% strong base (sodium or potassium hydroxide most often) followed by a few drops of aqueous copper (II) sulfate. If the solution turns purple, protein is present.

Xanthoprotein reaction

Principle: This reaction is specific for cyclic amino acids such as phenylalanine, tyrosine, tryptophane and histidine. Aromatic amino acids react with HNO_3 yielding a yellow nitrocompound, which changes color to orange in alkaline medium owing to the formation of

salt. Reaction of concentrated nitric acid with some substituted aromatic rings gives a yellow color (Xanthos = yellow in Greek).

Materials

Amino acid (tryptophan, glycine, tyrosine)

Concentrated nitric acid (HNO_3)

Sodium hydroxide (NaOH)

Procedure

Set up test tubes as follows: In one tube put 2 drops of tryptophan solution, in another 2 drops of unknown compound, in another 2 drops of glycine solution, and in another about 5 mg tyrosine powder. Then with great care, add 1.0 mL concentrated nitric acid (HNO_3) to each test tube. Hold the tubes so that they do not point at you or anyone else. Heat the tubes gently in a water bath until they boil. Cool the tubes slowly and add the 4% NaOH, drop by drop, until the solutions are alkaline. Did any color change take place before the alkali was added? What was the final color of each of the solutions?

3.1 Quantitative Analysis of Proteins and Amino Acids

Experiment 1: Ninhydrin Test

Principle: Ninhydrin (triketohydrindene hydrate) reacts with α-amino acids between pH 4 and 8 to give a purple-coloured compound. Not all amino acids give exactly the same intensity of colour and this must be allowed for in any calculation. The amino acids proline and hydroxyproline give a yellow colour, so these are read at 440 nm.

Materials

1. Amino acids (0.1 mmol/litre aspartic acid, arginine, leucine, and proline)
2. Acetate buffer (4 mol/litre, pH 5.5)
3. Ninhydrin reagent
4. Ethanol
5. Spectrophotometer

Procedure: pipette 2 ml of the amino acid solution into a test tube and add 2ml of the buffered ninhydrin reagent. Shake vigorously for 30 seconds to oxidize excess hydration. Heat in a boiling water bath for 15 minutes. Cool to room temperature, add 3 ml of 50% ethanol, and read the absorbance (extinction) at 570 nm after 10 min. set up the appropriate blanks and compare the colour equivalence of the amino acids investigated.

Experiment 2: Biuret test

Principle: The Biuret reaction can be used to assay the concentration of proteins because peptide bonds occur with the same frequency per amino acid in the peptide. The intensity of the color, and hence the absorption at 540 nm, is directly proportional to the protein concentration, according to the Beer-Lambert law.

$$2NH_2.CO.NH_2 \rightarrow NH_2.CO.NH.CO.NH_2 + NH_3$$

In spite of its name, the reagent does not in fact contain biuret ($(H_2N\text{-}CO\text{-})_2NH$). The test is so named because it also gives a positive reaction to the peptide bonds in the biuret molecule.

Material

1. Protein standard (%mg albumin/ml). prepare fresh

2. Biuret reagent. (Dissolve 3 g of copper sulphate ($CuSO_4.5H_2O$) and 9 g of sodium tartrate in 500 ml of 0.2 mol/litre sodium hydroxide; add 5 g of potassium iodide and make up to 1 litre with 0.2 mol/litre sodium)
3. Water bath at 37^0C

Procedure: make a serial dilution of the standard protein solution (e.g. BSA) in the range of 0-10 mg/ml. this is done by measuring the BSA in the range of 0.0, 0.1mg, 0.2mg, 0.3mg, etc. and with 0.2N of NaOH making them up to 1ml. add 3ml of Biuret reagent into each of the test tubes, mix and warm for 15 minutes at 37^oC and then cool. Measure the absorbance of each tube at 540 nm. Add 3ml of Biuret reagent into 1ml of each unknown protein solution. Use the method above for the standard on the protein solution. Measure the absorbance at the same wavelength (540 nm) and extrapolate form a prepared standard curve the concentration of each unknown protein solution.

Spectrophotometric Protein Assays

This exercise introduces students to four methods of determining protein concentrations: absorbance at 280 nm, and the biuret, Lowry, and Bradford assays. (I haven't yet incorporated the BCA assay; I'm trying to balance thoroughness with available time.) The goals of the exercise are to let students see and evaluate these commonly-used assay methods. I use just two proteins, bovine serum albumin (BSA) and gelatin. BSA is fairly expensive, but 100 g (over $100) lasts many years. Egg albumin would work, probably, but it seems if I just stare at it too long, it coagulates, whereas BSA forms a clear, stable solution. I prepare solutions of BSA and gelatin at 0.1, 0.2, 0.3, 2, 4, and 6 mg/mL in water. The latter three are for the biuret assay and the former are for the rest. The assay procedures are as follows:

Biuret: Mix 0.50 mL of protein with 2.50 mL of biuret reagent and measure the absorbance at 540 nm.

A_{280}: Measure the absorbance of 1 mL at 280 nm.

BIURET REAGENT: stirring, 300 mL of 10% (w/v) NaOH to 500 mL of a solution containing 0.3% copper sulfate pentahydrate and 1.2% sodium potassium tartrate, then dilute to one liter. The reagent is stable for a few months but not a year. Adding one gram of potassium iodide per liter and storing in the dark makes it stable indefinitely.

The reagent can be used either qualitatively or quantitatively. In a typical reaction, one volume of sample is mixed with two to five volumes of reagent; the optimal ratio depends on the maximum protein concentrations you want to be able to resolve. The presence of protein gives a violet color with maximum absorbance around 550-555 nm; we typically read absorbances at

Lowry: Mix 0.25 mL of protein with 2.5 mL of Lowry reagent 1. After 10 minutes, add 0.25 mL of Lowry reagent 2 and mix well immediately. After 30 minutes, measure the absorbance at 750 nm (if you're using a Spectronic 20 with a normal phototube, 750 may be too long; 600 nm gives lower absorbances but works okay).

Bradford: Mix 0.25 mL of protein with 2.5 mL of Bradford reagent and measure the absorbance at 595 nm.

All series should include a zero protein (water) tube (reagent blank). The students should zero

their instruments with the reagent blanks. This is especially important with the Bradford assay, for which the reagent blank has quite a high absorbance (>0.5). Two other practical concerns with the Bradford assay are its great sensitivity to detergent (tubes must be rinsed very well) and the fact that it stains the cuvettes blue.

After plotting their data on separate graphs for each assay, students are asked to evaluate each method by the criteria of convenience (how easy is it to do?), sensitivity (how well does it detect small amounts of protein; for example, what mass of protein is needed to give an absorbance of 0.1?), generality (how consistent are the results among the two different proteins?), and linearity (does it give a straight line plot of absorbance vs. protein?).

4.0 Conclusion

- The pH meter should be standardized with a buffer solution before use.
- Increase or decrease in pH can be very dangerous as found in the case of plasma

5.0 Summary

*****A buffer solution a particular pH usually resists pH changes

*Acute retany and acidotic coma are death occurrences as a result of fluctuation of the pH of plasma

*A pH scale is usually divided into 3 regions; acidic, neutral and basic

6.0 Tutor marked assignment

1. Mention the 3 main buffer system in a living material.

2. What is the hydrogen ion concentration of a solution with a pH of:

a. 3.25 b. 7.28 c. 13.3

3. What is the pH of a solution with the following [H^+]:

a. 0.025M b. 0.15M c. 0.2M

7.0 REFERENCES

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