**INSTRUMENTATION**

 Soil testing laboratories world over depends for analysis of soil, water, plant, organics and fertilizer on many instruments. Some of the advance countries are using the latest instruments like ICP, Ion chromatograph, elemental analyzer etc with online services. However for routine soil and plant analysis five basic instruments are used in all soil testing laboratories. They include pH meter, Conductivity meter, Colorimeter, Flame photometer and Atomic absorption spectrometer. In this lecture the basic principles involved in these instruments, their working and the analysis part is discussed.

**NITROGEN ANALYSER – KJEDHAL DISTILLATION UNIT**

 Nitrogen determination has a long history in the area of analytical chemistry. **Johan Kjeldahl** first introduced the Kjeldahl nitrogen method in 1883 at a meeting of the Danish Chemical Society. As chairman of the chemistry department of the Carlsberg Laboratorium near Copenhagen, Kjeldahl was assigned to scientifically observe the processes involved in beer making. While studying proteins during malt production, he developed a method of determining nitrogen content that was faster and more accurate than any method available at the time. His method used simple equipment and could be performed by an inexperienced technician.

***Applications***

Since 1883, the Kjeldahl method has gained wide acceptance and is now used for a variety of applications. Kjeldahl nitrogen determinations are performed on food and beverages, meat, feed, grain, waste water, soil and many other samples. The method has been refined and tested for a wide variety of substances and approved by various scientific associations including: AOAC International (formerly the Association of Official Analytical Chemists) Association of American Cereal Chemists American Oil Chemists Society Environmental Protection Agency International Standards Organization United States Department of Agriculture

***What is the Kjeldahl method?***

The Kjeldahl method is a means of determining the nitrogen content of organic and inorganic substances. Although the technique and apparatus have been altered considerably over the past 100 years, the basic principles introduced by Johan Kjeldahl endure today. The Kjeldahl method may be broken down into three main steps:

***Digestion*** - the decomposition of nitrogen in organic samples utilizing a concentrated acid solution. This is accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The end result is an ammonium sulfate solution.

***Distillation*** - adding excess base to the acid digestion mixture to convert NH4 + to NH3, followed by boiling and condensation of the NH3 gas in a receiving solution.

***Titration*** - to quantify the amount of ammonia in the receiving solution. The amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ions in the receiving solution.

***The Digestion Process***

A general equation for the digestion of an organic sample is shown below as one basic example: Organic N + H2SO4 **→** (NH4)2SO4 + H2O + CO2 + other sample matrix by-products A number of interrelated digestion conditions determine the rate of reaction and the completeness of the breakdown of nitrogen to ammonium sulfate. Among these are heat input to the acid digestion mixture, amount of inorganic salt added to elevate the acid boiling temperature, reflux rate of H2SO4 in the neck of the digestion flask, length of digestion, and catalyst addition. Adjusting any one of these factors has an influence on the others. Proper digestion conditions for a given sample matrix are achieved through establishing a balance of these factors in a controlled and repeatable fashion. In addition, if the sample contains nitrate or nitrite nitrogen, it is possible to chemically pretreat the digest to include or exclude this nitrogen source from the analysis as desired in a particular situation.

***Acid Considerations***

Sulfuric acid has been used alone for the digestion of organic samples. (As a convenience to reduce bumping of the digestion mixture, Alundum boiling chips or pumice are often added.) The amount of acid required is influenced by sample size and relative amount of carbon and hydrogen in the sample, as well as amount of nitrogen. A very fatty sample consumes more acid. Also, heat input and digestion length influences the amount of acid loss due to vaporization during the digestion process. Remember that a Kjeldahl flask is essentially a bulb with a condenser neck off to the side for refluxing of the acid.

***Heat Input and Digestion Length***

Typically the heating elements used for Kjeldahl digestions have variable settings. Heat input is frequently specified as “that setting which brings 250 ml of water at 25° C to a rolling boil in 5 minutes.” Initially an organic sample usually chars and blackens. The reaction may at first be very vigorous depending on the matrix and the heat input. With organic decomposition the digestion mixture gradually clears as CO2 evolves. Metallic ions might tint the clear digestion mixture. Note that solution clearing itself is not an indication that all organic nitrogen has been broken down. Digestion length must be determined by recovery studies on known materials of similar matrix if a new method is being developed. Recovery. Salicylic acid followed by sodium thiosulfate has been used to pretreat the mixture to ensure complete reduction. Other reduction schemes have been devised. Or other pretreatments have been used to prevent nitrates from being reduced at all during the charring process, leaving a clear digest with no contribution from nitrate ions.

***The Distillation******Process***

The acid digestion mixture is diluted and made strongly alkaline with NaOH, liberating NH3 as follows:

***Salt Additions***

The problem with using sulfuric acid alone for digestion is very long digestion time’s result with many samples due to the slow rate of organic decomposition. The addition of an inorganic salt to the digest elevates the boiling point of the H2SO4. The solution temperature of concentrated sulfuric acid alone is about 330° C. Addition of a salt such as K2SO4 can elevate the solution temperature of the digestion mixture to 390° C or more, depending on the ratio of salt to acid. This significantly increases the rate of organic decomposition in the digestion mixture, shortening the length of time required for digestion. There are several precautions to keep in mind concerning salt addition. First, it is possible to raise the solution temperature of the digestion mixture too much. If the temperature goes much above 400° C during any phase of the digestion, volatile nitrogen compounds may be lost to the atmosphere. Remember that as acid is gradually consumed during the digestion process, for the various reasons mentioned above, the salt acid ratio of the digest gradually rises. This means that the hottest solution temperatures are attained at the end of the digestion. Heat input, consumption of acid by organic material and vaporization, salt/acid ratio, digestion length, and physical design of the Kjeldahl flask, are all interrelated. Each has an effect on the final solution temperature. A second precaution is that if the salt/acid ratio is too high, a considerable amount of material will “salt out” upon cooling of the digest. Concentrated acid pockets can be contained within the cake. These can react violently when concentrated base is added in the distillation process. A certain amount of salting out can be managed by diluting the digest with water while it is still somewhat warm, but not too hot.

***Catalyst Additions***

Several catalysts have been employed by Kjeldahl chemists over the years to increase the rate of organic breakdown during the acid digestion. Mercuric oxide has been the most effective and widely used. However, mercury forms a complex with ammonium ions during digestion. The addition of sodium thiosulfate or sodium sulfide after digestion and before distillation will break the complex and precipitate mercuric sulfide. This is also important from a safety point of view, as mercury vapor might escape to the atmosphere during the distillation process. Because of environmental concerns over the handling and disposal of mercury, other catalysts are coming more into favor. Many methods employ copper sulfate. Titanium oxide and copper sulfate in combination have been found to be more effective than copper sulfate alone. Selenium is frequently used. Commercially prepared mixtures of potassium sulfate and a catalyst are available from laboratory chemical suppliers. Bulk custom mixtures are also available.

***Nitrate and Nitrite Reduction***

Kjeldahl digestions do not always recover all forms of nitrogen in a sample. Nitrate and nitrite ions in a sample must first be reduced prior to acid digestion for quantitative

**(NH4)2SO4 + 2NaOH → 2NH3­+ Na2SO4 + 2H2O**

Ammonium sulfate heat ammonia gas. The Kjeldahl flask is attached to a water condenser and is heated to boil off the NH3 gas from the digest. The tip of the condenser is submerged in a flask of acidic receiving solution, either standard acid or boric acid solution, to again trap the distilled NH3 in receiving solution.

***Digestion Mixture Dilution***

The acid digestion mixture is usually cooled and diluted with ammonia-free water. As mentioned above, with digestion mixtures containing high salt/acid ratios, dilution prevents or minimizes caking. Sometimes this is done while the digestion mixture is still quite warm (with caution!), and the K2SO4 has not yet salted out. With some digestion mixtures, a cake forms, but dissolves or breaks up if time is allowed following dilution. Alternatively, warming or sonicating after dilution may break up the cake. Cake material may cause low nitrogen recoveries in the distillation step. Also, entrapped acid in a cake may react violently with base during the distillation process. Dilution of the digestion mixture before making it alkaline and distilling also reduces the likelihood of bumping. Additional boiling chips added just before distillation also reduces bumping, especially towards the end of the distillation as the solution becomes more concentrated. Two or three drops of tributyl citrate may be added as an aid to reduce foaming.

***NaOH Addition***

Concentrated NaOH (usually 50% solution) is added slowly down the neck of the flask. Being heavier, it forms a layer underneath the diluted acid digestion mixture. Generally, for each 5 ml of concentrated sulfuric acid used in the digestion, 20 ml of 50% sodium hydroxide is required to make the digest strongly alkaline (pH of >11). The flask is connected to the condenser and mixed before heating and distillation begins. For samples not requiring a digestion step, such as direct ammonia determinations in water, the sample is buffered to a pH of 9.5 with a solution of sodium tetraborate and sodium hydroxide, to decrease hydrolysis of any complex organic nitrogen compounds present.

***Distillation***

The majority of the NH3 is distilled and trapped in the receiving acid solution within the first 5 or 10 minutes of boiling. But depending on the volume of the digestion mixture and the method being followed, 15 to 150 ml of condensate should be collected in the receiving flask to ensure complete recovery of nitrogen. Further extension of the distillation times and volumes collected simply results in more water being carried over to the receiving solution. Excess water does not change the titration results. Distillation times and distillate volumes collected should be standardized for all samples of a given methodology. The rate of distillation is affected by condenser cooling capacity and cooling water temperature, but primarily by heat input. Typically the heating elements used for distillation have variable temperature controllers. A distillation rate of about 7.5 ml/minute is most commonly cited in accepted methods. Connecting bulbs or expansion chambers between the digestion flask and the condenser is an important consideration to prevent carryover of the alkaline digestion mixture into the receiving flask. The slightest bit of contamination of the receiving solution can cause significant error in the titration step. When very low levels of nitrogen are being determined, it is advisable to “precondition” the distillation apparatus prior to distillation. This can be done by distilling a 1:1 mixture of ammonia-free water and 50% NaOH for 5 minutes just before sample distillation to reduce contamination from atmospheric ammonia.

***Receiving Solutions***

If the receiving solution is standardized HCl or H2SO4, it is desirable to have only a slight excess left after the NH3 is distilled and trapped in the receiving solution to minimize the back titration. Based on the anticipated amount of nitrogen in the sample, a target amount of standard acid can be calculated from the following formula:

***The Titration Process***

There are two types of titration: back titration, commonly used in Macro Kjeldahl; and direct titration. Both methods indicate the ammonia present in the distillate with a color change and allow for calculation of unknown concentrations.

***Nitrogen Determination by back titration***

The ammonia is captured by a carefully measured excess of a standardized acid solution in the receiving flask. The excess of acid in the receiving solution keeps the pH low, and the indicator does not change.

**[(% nitrogen expected in sample) x (sample aliquot used) x gram sample wt.] + 2 (normality of standard acid) x 1.4007 x digestion dilution volume**

If boric acid is used, the exact concentration is not needed because the titration directly measures the amount of ammonia in the distillate by neutralizing the 1:1 complex formed by ammonia and boric acid. Large quantities of boric acid may be added to the receiving solution so complete absorption of the ammonia is assured. Receiving solution volumes may be increased by the addition of ammonia-free water so that the tip of the delivery tube is immersed. Delivery tubes should always be allowed to drain momentarily into the receiving flask before removal from the distillation apparatus. The receiving solution should remain below 45° C during distillation to prevent loss of ammonia. Ammonia standard sulfuric acid ammonium sulfate excess sulfuric acid

**2 N H 3 + 2 H 2S O 4 → (N H 4) 2S O 4 + H 2S O4color** change)

The excess acid solution is exactly neutralized by a carefully measured standardized alkaline base solution such as sodium hydroxide. A color change is produced at the end point of the titration. Ammonia sulfate measured excess acid measured sodium hydroxide ammonium sulfate

**(NH4)2SO4 + H2SO4 + 2NaOH → (Na)2SO4 + (NH4)2SO4 + 2H2O**

***Nitrogen Determination by direct titration***

If boric acid is used as the receiving solution instead of a standardized mineral acid, the chemical reaction is: ammonia gas boric acid ammonium-borate complex excess boric acid.

**N H 3 + H 3B O 3 → N H 4+: H 2B O 3 - + H 3B O 3**

The boric acid captures the ammonia gas, forming an ammonium-borate complex. As the ammonia collects, the color of the receiving solution changes. Ammonium borate complex, sulfuric acid, ammonium sulfate, boric acid

**2NH4H 2BO3 - + H2SO4 → (NH4) 2SO4 + 2H3BO3**

in tTThe addition of sulfuric acid exactly neutralizes the ammonium borate complex, and a reverse color change is produced. The boric acid method has two advantages: only one standard solution is necessary for the determination and the solution has a long shelf life.

***Indicator Solutions***

Many different indicators have been used to provide a “sharp end point” color change. The analyst’s use of specific types of indicators can be a personal choice. The combination of methyl red and methylene blue indicators is frequently used in many methods. The color change of the indicator must fall within the equivalent point of the reaction. For standard acid/base titrations, methyl orange is usually the preferred indicator. If color change end points are difficult to detect, reference solutions made from a blank with an indicator can be very helpful.

***Calculations***

The calculations for % nitrogen or % protein must take into account which type of receiving solution was used and any dilution factors used during the distillation process. The equations given here are in long form. They are often simplified in the published standard methods. In the equations below, “N” represents normality. “ml blank” refers to the milliliters of base needed to back titrate a reagent blank if standard acid is the receiving solution, or refers to milliliters of standard acid needed to titrate a reagent blank if boric acid is the receiving solution. When standard acid is used as the receiving solution, the equation is:

***Equipment and Apparatus***

Very early Kjeldahl digestions and distillations were performed using stone fume hoods and gas mantles as a heat source. In the 1920’s these were replaced by what is now known as classical macro-Kjeldahl digestion and distillation apparatus. Macro setups use Kjeldahl flasks from 500 to 800 ml volume and handle sample sizes from 0.5 to about 5.0 g. A smaller version of this apparatus is referred to as micro-Kjeldahl equipment. The equipment consists of smaller benchtop heater units, and Kjeldahl flasks of 30 to 100 ml volume. A third more recent variation in equipment makes use of ceramic or aluminum heating blocks designed to accept a number of straight digestion tubes at once. “Block digestors” are often used in conjunction with benchtop distillation units with steam generators to shorten the distillation time. Each of these three types of apparatus is described in more detail below. In all cases, since the Kjeldahl process involves significant corrosive fumes, appropriate attention must be given to fume removal. Equipment must be suitably constructed of corrosion-resistant materials.

***Classical Macro-Kjeldahl Apparatus***

Many governmental and regulating agencies have developed methodologies that specify the classical macro-Kjeldahl apparatus. For example, standard methods for low level nitrogen determinations in water (0-10 mg/1) require a sample size of 250 to 500 ml, and therefore large Kjeldahl flasks. Methodologies in the agricultural industry often require larger sample sizes and involve matrices which can significantly foam. Equipment engineered to accommodate macro methods can be substantial. Apparatus designed to process 2 to 12 samples simultaneously is typical, and involves a bank of heaters and a fume manifold for digestion, and a second bank of heaters with condensers for distillation. The apparatus is available freestanding or contained within an integral hood. Freestanding installations require additional laboratory room modifications to handle fumes and heat. In either case the equipment is large, expensive, involves significant installation considerations, and ongoing utility and reagent costs. The digestion apparatus has separate heating mantles to retain each flask. Typically each mantle has an individual temperature controller. The long necks of the Kjeldahl flasks are positioned to release digestion fumes into a common manifold. Manifold aspiration can be provided either by a mechanical blower with exhaust through ductwork to the outside, or by a large water aspirator that provides water spray to dilute the fumes for disposal down a drain. Like the digestion apparatus, the distillation apparatus has separate heating mantles to retain each flask. With the flask in place on the mantle, the neck of each flask is attached to a “connecting bulb” or an expansion chamber that acts as a trap to keep any of the concentrated liquid digest from mechanically carrying over through the condensers and into the receiving solution.

**[(ml standard acid x N of acid) - (ml blank x N of base)] - (ml std base x N of base) x 1.4007** **weight of sample in grams**

If the sample weight is in milligrams, the molecular weight of nitrogen should be changed to 1400.67. When boric acid is used as the receiving solution the equation is:

**%=( ml standard acid - ml blank) x N of acid x 1.4007** **weight of sample in grams**

If it is desired to determine % protein instead of % nitrogen, the calculated % N is multiplied by a factor, the magnitude of the factor depending on the sample matrix. Many protein factors have been developed for use with various types of samples. The list below represents just a few of the factors described in the standard methods of analysis published by the American Association of Cereal Chemists (AACC) and AOAC International. 6.38 milk and dairy 6.25 other grains 5.95 rice 5.70 wheat flour

Downstream from the connecting bulb is the condenser, which is a tube of stainless steel surrounded by a second water jacket tube. Typically multiple condenser/jacket units are ganged together in a single assembly. A glass delivery tube is attached to the end of the condenser. The delivery tube has a ball-shaped tip with small holes to help disperse large bubbles and eliminate pressure fluctuations during distillation. The tip is submerged in the flask of receiving solution to ensure that the distilling ammonia is completely captured.

***Micro-Kjeldahl Apparatus***

These are miniature versions of the macro-digestion apparatus which are movable and intended for use in a laboratory hood. They are designed to digest small samples in 30or 100 ml digestion flasks. Individually controlled heaters allow multiple flasks to be handled simultaneously. Some micro-digestors include a one-piece glass manifold for fume removal. The glass manifold is connected to a standard water aspirator to dilute and remove fumes that do not reflux in the flask necks. Whether or not a glass manifold is used, the digestion apparatus should be operated inside a laboratory hood. Digested samples can be diluted and an aliquot taken for distillation on a micro steam distillation unit. Micro steam distillation units are available to accept digested samples of up to 4 ml concentrated acid and volumes of about 55 ml. An electric immersion heater produces steam heat that causes the sample to boil and release ammonia gas. The vapors pass into a condenser where contact with water cooled glass condenses the vapors, which drip into the receiving solution via a delivery tube. Distillation time is approximately 5 minutes. Methodologies have been specifically developed for micro-Kjeldahl apparatus. These most often are methods that involve homogeneous samples, relatively high nitrogen levels in the sample, and small sample sizes normally less than 0.25 g. The installation considerations, initial expense, space requirements, and ongoing utility and reagent cost are much less with micro equipment.

***Block Digestors***

Block digestors are high temperature ceramic or aluminum blocks with wells to accept straight walled digestion tubes. They can accommodate from 2 to 25 tubes at once. Block digestors are heavy but movable self-contained units designed to be used in a laboratory hood. Digestion tubes range up to 300 ml capacity, suitable for sample sizes up to about 2 g or 75 ml. Since narrow, straight-walled digestion tubes are used instead of traditional Kjeldahl flasks with a bulb and neck, and since a number of tubes are grouped closely together in a vertical configuration in the block, acid reflux parameters are different. This means salt/acid ratios and heat inputs appropriate for macro-Kjeldahl methods do not exactly transfer to digestion in a block. Methods have been developed specifically for block digestors similar, but not exactly identical, to macro-Kjeldahl methods.

Typically block digestors have one controller that adjusts the temperature of the entire block. The controller can be automated, allowing for timed ramping and multiple temperature settings during the course of a digestion. Most often block digestors are used together with fume removal devices, even though they are operated in a hood. One such system consists of a glass manifold with individual bulb eductors in a rack designed to rest on top of a set of digestion tubes in the block. Another system uses individual exhaust caps with flexible tubing. In either case the manifold is connected to a water aspirator. Or, instead of a water aspirator, there are alternative units that employ mechanical aspiration and include bubbling the acid fumes through a neutralizing base solution. Block digestion followed by steam distillation is sometimes referred to as “rapid Kjeldahl” partly because a number of block digestion methodologies are shorter than classical macro-Kjeldahl digestions and benchtop steam distillation units in use are faster than classical distillations.

***Rapid Steam Distillation Apparatus***

The development of block digestors as an alternative to macro-Kjeldahl digestion has been paralleled by the development of benchtop steam distillation units. Basically, this equipment consists of a steam generator to inject steam into the alkaline digestion mixture and an evaporator-type condenser, where contact with water-cooled glass coils causes the vapors to condense and drip into the receiving solution via a delivery tube. Usually the unit is designed to accept straight digestion tubes from block digestors, though there is no reason in theory why any digestion techniques could not be combined with any distillation technique. Steam distillation is much more rapid than classical macro-distillation, typically taking from 3 to 8 minutes. On the other hand, only one distillation at a time can be done. Rapid steam distillation units are available with different features, ranging from relatively manual models to highly automated models. Basic models dispense dilution water and base under control of a push button. Some models include a timer to control the length of distillation, shutting down automatically. Some units automate the entire distillation process once the digestion flask is in place, and others automatically titrate to end point after distillation, and calculate and display the results in a printed report.

***Titrators***

Of course, receiving solutions may be individually hand titrated using an indicator solution and burette, but several models of benchtop automatic titrator instruments are also available. Some units will titrate one receiving solution at a time to a set end point. Others will automatically titrate a number of receiving flasks sequentially. Still other models provide a printout of results along with digital readout. **(Fig – 40)**

# SCRUBBER

**Scrubber** systems are a diverse group of air pollution control devices that can be used to remove some particulates and/or gases from industrial exhaust streams. Traditionally, the term "scrubber" has referred to pollution control devices that used liquid to "scrub" unwanted pollutants from a gas stream. Recently, the term is also used to describe systems that inject a dry reagent or slurry into a dirty exhaust stream to "scrub out" acid gases. Scrubbers are one of the primary devices that control gaseous emissions, especially acid gases.

## Removal and neutralization

The exhaust gases of combustion may at times contain substances considered harmful to the environment, and it is the job of the scrubber to either remove those substances from the exhaust gas stream, or to neutralize those substances so that they cannot do any harm once emitted into the environment as part of the exhaust gas stream...

###  *Wet scrubbing*

A wet scrubber is used to clean air or other gases of various pollutants and dust particles. Wet scrubbing works via the contact of target compounds or particulate matter with the scrubbing solution. Solutions may simply be water (for dust) or complex solutions of reagents that specifically target certain compounds.

Removal efficiency of pollutants is improved by increasing residence time in the scrubber or by the increase of surface area of the scrubber solution by the use of a spray nozzle, packed towers or an aspirator. Wet scrubbers will often significantly increase the proportion of water in waste gases of industrial processes which can be seen in a stack plume. Compliance agencies typically place minimum DP thresholds on wet scrubber.

###  *Dry scrubbing*

A dry or semi-dry scrubbing system, unlike the wet scrubber, does not saturate the flue gas stream that is being treated with moisture. In some cases no moisture is added; while in other designs only the amount of moisture that can be evaporated in the flue gas without condensing is added. Therefore, dry scrubbers do not have a stack steam plume or wastewater handling/disposal requirements. Dry scrubbing systems are used to remove acid gases (such as SO2 and HCl) primarily from combustion sources.

There are a number of dry types scrubbing system designs. However, all consist of two main sections or devices: a device to introduce the acid gas sorbent material into the gas stream and a particulate matter control device to remove reaction products, excess sorbent material as well as any particulate matter already in the flue gas.

Dry scrubbing systems can be categorized as dry sorbent injectors (DSIs) or as spray dryer absorbers (SDAs). Spray dryer absorbers are also called semi-dry scrubbers or spray dryers.

***Dry sorbent injection*** involves the addition of an alkaline material (usually hydrated lime or soda ash) into the gas stream to react with the acid gases. The sorbent can be injected directly into several different locations: the combustion process, the flue gas duct (ahead of the particulate control device), or an open reaction chamber (if one exists). The acid gases react with the alkaline sorbets to form solid salts which are removed in the particulate control device. These simple systems can achieve only limited acid gas (SO2 and HCl) removal efficiencies. Higher collection efficiencies can be achieved by increasing the flue gas humidity (i.e., cooling using water spray). These devices have been used on medical waste incinerators and a few municipal waste combustors.

In **spray dryer absorbers**, the flue gases are introduced into an absorbing tower (dryer) where the gases are contacted with finely atomized alkaline slurry. Acid gases are absorbed by the slurry mixture and react to form solid salts which are removed by the particulate control device. The heat of the flue gas is used to evaporate all the water droplets, leaving a non-saturated flue gas to exit the absorber tower. Spray dryers are capable of achieving high (80 + %) acid gas removal efficiencies. These devices have been used on industrial and utility boilers and municipal waste combustors.

### *Mercury removal*

Mercury has no known beneficial uses in nature, but it is a common substance found in coal that must also be removed. Wet scrubbers are only effective for mercury removal under certain conditions. Mercury vapor in its elemental form, Hg0, is insoluble in the scrubber slurry and not removed. Oxidized mercury, Hg2+, compounds are more soluble in the scrubber slurry and can be captured. The type of coal burned as well as the presence of a selective catalytic reduction unit both affect the ratio of elemental to oxidized mercury in the flue gas and thus the degree to which the mercury is removed.

## Scrubber waste products

One side effect of scrubbing is that the process only moves the unwanted substance from the exhaust gases into a solid paste or powder form. If there is no useful purpose for this solid waste, it must be either contained or buried to prevent environmental contamination. Limestone-based scrubbers can produce a synthetic gypsum of sufficient quality that can be used to manufacture drywall and other industrial products.

Mercury removal results in a waste product that either needs further processing to extract the raw mercury, or must be buried in a special hazardous wastes landfill that prevents the mercury from seeping out into the environment.

##  Bacteria spread

Until recently, scrubbers have not been associated with health risks involving bacteria spread as a result of inadequate cleaning, unlike other devices such as cooling towers. However, a 2005 outbreak of Legionnaires' disease in Norway was proven to emanate from a scrubber, causing ten deaths and more than fifty cases of infection as it spread the bacteria through the air during a period of only two scrubbers being the source of such bacteria. **(Fig – 41)**

**SPECTROPHOTOMETER**

In physics, **spectrophotometry** is the quantitative study of electromagnetic spectra. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared. Also, the term does not cover time-resolved spectroscopic techniques.

Spectrophotometry involves the use of a spectrophotometer. A **spectrophotometer** is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light. There are many kinds of spectrophotometers. Among the most important distinctions used to classify them are the wavelengths they work with, the measurement techniques they use, how they acquire a spectrum, and the sources of intensity variation they are designed to measure. Other important features of spectrophotometers include the spectral bandwidth and linear range.

Perhaps the most common application of spectrophotometers is the measurement of light absorption, but they can be designed to measure diffuse or specular reflectance. Strictly, even the emission half of a luminescence instrument is a kind of spectrophotometer.

There are two major classes of spectrophotometers; single beam and double beam. A double beam spectrophotometer measures the ratio of the light intensity on two different light paths, and a single beam spectrophotometer measures the absolute light intensity. Although ratio measurements are easier, and generally stabler, single beam instruments have advantages; for instance, they can have a larger dynamic range.

## Visible-region spectrophotometers

Visible region 400-700nm spectrophotometry is used extensively in colorimetry science. Ink manufacturers, printing companies, textiles vendors, and many more, need the data provided through colorimetry. They usually take readings every 20 nanometers along the visible region, and produce a spectral reflectance curve. These curves can be used to test a new batch of colorant to check if it makes a match to specifications. Traditional visual region spectrophotometers cannot detect if a colorant has fluorescence. This can make it impossible to manage color issues if one or more of the printing inks are fluorescent. Where a colorant contains fluorescence, a bi-spectral fluorescent spectrophotometer is used. There are two major setups for visual spectrum spectrophotometers, d/8 (spherical) and 0/45. The names are due to the geometry of the light source, observer and interior of the measurement chamber.

**PARTS OF SPECTROPHOTOMETER**

**(Fig – 42)**

**LENS**

**SLIT**

**LENS**

**FILTER**

**TEST SOLUTION**

**PHOTO TUBE**

**FILTER**

**EXIT SLIT**

**ADJUSTABLE LIGHT**

**COMPLEX**

**LIGHT SOURCE (TUNGSTEN LAMP)**

Scientists use this machine to measure the amount of compounds in a sample. If the compound is more concentrated more light will be absorbed by the sample.

Components: 1. The light source shines through the sample. 2. The sample absorbs light. 3. The detector detects how much light the sample has absorbed. 4. The detector then converts how much light the sample absorbed into a number.

## Spectroradiometers

**Spectroradiometers**, which operate almost like the visible region spectrophotometers, are designed to measure the spectral density of illuminants in order to evaluate and categorize lighting for sales by the manufacturer, or for the customers to confirm the lamp they decided to purchase is within their specifications.

Components:

 1. The light source shines onto or through the sample.

2. The sample transmits or reflects light.

 3. The detector detects how much light was reflected from or transmitted through the sample.

 4. The detector then converts how much light the sample transmitted or reflected into a number.

## UV and IR spectrophotometers

The most common spectrophotometers are used in the UV and visible regions of the spectrum and some of these instruments also operate into the near-infrared region as well. **Spectrophotometers** designed for the main infrared region are quite different because of the technical requirements of measurement in that region. One major factor is the type of photosensors that are available for different spectral regions, but infrared measurement is also challenging because virtually **everything** emits IR light as thermal radiation, especially at wavelengths beyond about 5 μm.

Historically, spectrophotometers use a monochromator to analyze the spectrum, but there are also spectrophotometers that use arrays of photosensors and, especially in the IR, there are spectrophotometers that use a Fourier transform technique to acquire the spectral information in a technique called Fourier Transform Infrared.

The spectrophotometer measures quantitatively the fraction of light that passes through a given solution. In a spectrophotometer, a light from a lamp in a near-IR/VIS/UV spectrophotometer (typically a deuterium gas discharge lamp) is guided through a monochromator, which picks light of one particular wavelength out of the continuous spectrum. This light passes through the sample that is being measured. After the sample, the intensity of the remaining light is measured with a photodiode or other light sensor, and the transmittance for this wavelength is then calculated.

**FLAME PHOTOMETER**

**PRINCIPLE:** The atoms or ions present in the solution gets energy in a flame, they get exited and results in the emission of srectrum. The energy absorbed by electrons, shifts them to positions more distant from the atomic nucleus. As the electrons regain their state, the previously absorbed energy is remitted as electromagnetic radiations. The wavwlengths of which correspond to the quantity of energy involved in the respective electron shifts and the quantity of radiation is directly proportional to the amount of the element emitting the rays. This is then measured with a galvanometer connected in a suitable circuit. The emission spectrophotometric analysis is most useful where a large number of soil or plant samples have to be analysed on a routine basis.

 Flame photometer has the following components and is schematically shown in the figure.

1. ***Atomizer – Burner Assembly:*** It consists of
	* A suction capillary through which the sample is sucked.
	* An air pipe which helps to spray the sample into the flame and is attached to the air – compreser.
	* A fuel gas tube attached to the gas cylinder (Acetylene or LPG)
	* A drain tube through which heavy particles of the samples are drained out.
	* A burner in which the fuel gas burns and the flame is produced.. in this flame, the element which has been sprayed is exited.
2. ***Filter:*** It selects the radiation of the required wavelength.
3. ***Galvanometer:*** It measures the light energy that has been converted to eledtrical energy.

**Steps to be followed in the use of Flame photometer:**

1. Connect the instrument to the mains after ensuring correct voltage.
2. Set the air – compressor in operation and check the pressure gauge so that the air pressure is adjusted to 0.4 to 0.6 kgcm-2 .
3. Starts gently the gas supply from the gas line fitted to the gas cylinder and light the burner carefully through the inspection window.
4. Again adjust the air and the gas pressure to give a sooth free blue flame and about 4cm in height.

**A SCHEMATIC DIAGRAM OF FLAME PHOTOMETER**

**METER**

**AMPLIFIER**

**COLLIMATING MIRROR**

 **BURNER**

**PRISM**

**SLIT**

**PHOTO**

**DETECTOR**

**(Fig - 43)**

1. Close the inspection window and set the galvanometer reading to zero by feeding distilled water into the atomizer.
2. Feed the standard solution of maximum concentration and adjust the galvanometer to read 100, feed other standard solutions and note down the reading.
3. Feed the test sample and note down the reading.
4. Turn off the gas tap first, while switching off the instrument.

**Procedure:**

* 1. Weigh 5g of soil into a 250ml conical flask.
	2. Add 25ml of normal Ammonium acetate solution.
	3. Shake the contents of the flask on an electrical shaker for 5 minutes and filter.
	4. Feed the filtrate into the atomizer of the flame photometer which has been adjusted to 100 with 40 ppm (in case of Systronics Flame Photometer) or 10 ppm (in case of Elico Flame Photometer) standard solution of Potassium and note down the reading.
	5. Locate this reading on the standard curve and calculate the amount of Potassium in the soil as shown below.

**Precautions:**

* + 1. The filtrate should be clear to avoid clogging the capillary.
		2. The air – pressure should be with in 0.4 – 0.6 kgcm-2. It should not deviate too much.
		3. The gas inlet should be opened after opening the air inlet and closed before shutting off the air supply.
		4. The flame should be soot free and blue.
		5. The instrument should be allowed to warm up for 10 – 15 minutes before using it.
		6. The light filter should be of the same element, which we want to determine.
		7. For soils having less than 2000 ppm Potassium, calibrate the instrument with 0 – 10 ppm standards of Potassium content in the range of 200 – 500 ppm, calibrate using 1 – 20 ppm range. If the Potassium content is more than 500 ppm, the soil to solution ratio can be widened to 1 : 40 to 1 : 50.
		8. When exchangeable Potassium is relatively high, it is preferable to estimate Potassium (K) on field moisture samples and then calculate on air dry basis as Potassium is likely to be fixed during drying.

**pH METER**

The pH meter of a soil is one of the most important chemical properties of soils. It influences the availability of nutrients, microbial property, physical property and suitability of a soil for a crop. It also throws light on the need for gypsum or lime application. The term pH is defined as “the negative logarithm of the activity of hydrogen ion in soil”.

**pH = - log H+**

The pH scale ranges from 0 to 14. the mid point scale i.e. 7.0 is neutral. pH > 7.0 indicates alkalinity and the pH < 7.0 indicates acidity. The pH of a solution can be measured by colorimetric indicator method or by potentiometric method. In soil testing laboratories potentiometric method is used to determine the pH of soil using pH meter.

A pH meter is an instrument, which measures the voltage developed by the combination of glass electrode and a reference electrode. The scale is graduated in terms of both pH and mill volts (mV).

***Glass Electrode:***

 It consists of a thin walled bulb made of chemically pure and soft soda glass. A suitable electrolyte, usually 0.1 N HCl is present in the bulb and a silver wire coated with AgCl, is dipping in the acid present inside the bulb. The potential developed at the glass membrane due to the difference in the connection of hydrogen of the outside and inside solutions is measured by connecting it to a reference (Calomel) electrode. The potential developed is measured in the terms of volts (generally in mV as the potential developed is very small).

An ordinary voltmeter cannot be used for measuring the voltage of the glass electrode – reference of electrode assembly for the reason that the glass electrode being a non – conductor allow an extremely small current which would not activate a galvanometer. In a pH meter, this small current is amplified through electronic tubes so that it can be measured easily.

**PARTS OF GLASS ELECTRODE** **pH METER**

**GALVANOMETER**

**ELECTRODES**

**POTENTIOMETER**

**SOLUTION**

**VALVE**

**(Fig – 44)**

***Measurement of Soil pH:***

 pH meter with glass – calomel combined electrode is most commonly used.

***Reagents:***

1. ***pH 4.0 buffer solution:*** Dissolve 10.21g of Potassium hydrogen phthalate 90.05 M in water and dilute it to one liter. This solution should not be used after 5 – 6 weeks or earlier if fungal growth is seen.
2. ***pH 7.0 buffer solution:*** Dissolve 0.948g of KH204 and 1.57g of Na2HPO4 in water and dilute it to one liter.
3. ***pH 9.2 buffer solution (0.01M Borax):*** Dissolve 3.81g of Na2B4O7.10H2O in carbon dioxide free water and dilute to one liter. This solution should be protected from atmospheric carbon dioxide and should be placed once in a month.

The buffer tablets of powder are available commercially for pH 4.0, 7.0 and 9.2. these buffer solutions can be prepared by dissolving the powder / tablet in required quantity of boiled cooled distilled water (100 ml).

***Procedure (for Elico pH meter):***

* Take 20g of soil in a 50ml beaker and add 25ml of distilled water and stir at least five times over a 30 minutes period to allow for soil and water to each equilibrium.
* In the meantime switch on the instrument , set temperature knob to room temperature and set range selector to zero.
* Set the galvanometer to zero with set zero knob after 15 minutes.
* Dip the electrodes into a buffer of known pH, turn the range selector and adjust the pH meter to pH of the buffer solution by set buffer knob.
* Turn the selector to zero, press the key stand by hand and take out the electrode. Wash the electrode with distilled water and dry it with a piece of filter paper.
* Shake the soil suspension and insert the electrodes into it. Turn the selector in the proper pH range and read the pH on the dial of the pH meter. Turn back the selector to zero and take out the electrode.
* Rinse the electrode with distilled water and lower them back into a beaker containing distilled water.

The pH value is erratic and unreliable when determinations are made to lower soil moisture due to

* 1. Poor contact between soil and the glass electrode
	2. The high mechanical resistance of the soil

On the other hand, too high a soil – water ratio increases the pH value owing to dilution. In general, more dilute the soil suspension, the higher the soil pH values found, whether the soil is acidic or alkaline. The rise in the soil pH by 0.2 to 0.5 units as moisture content increases from the sticky point. For uniform results ratio of 1: 2.5 of soil to water is used.

***Precautions in the use of pH meter:***

1. The electrodes should not be allowed to remain in suspension or test solution longer than necessary.
2. The electrode should be washed with a gentle stream of distilled water immediately.
3. The electrodes should be suspended in distilled water when it is not in use. The drying of the electrodes should be avoided.
4. If soil has more soluble salts, soil suspension may be prepared, using either 0.1M CaCl2 of KCl solution instead of water to overcome the effect of soluble salts on pH.

***Interpretation:***

**Table - 6**

|  |  |  |
| --- | --- | --- |
| **pH** | **Interpretation** | **Management required** |
| < 6.5 | Acidic | Requires liming |
| 6.5 – 8.5 | Neutral | No amendments, optimum for crops |
| > 8.5 | Alkaline | Requires Gypsum for reclamation |

**CONDUCTIVITY METER**

Electrical Conductivity (EC) is an important soil chemical parameter. It tells us about the quality of dissolved salts present in the soil. This property affects the ability of a crop to grown in a given soil. Soil containing excess salts is saline and need to be reclaimed for growing normal crops.

***Principle:***

Solutions offer resistance to the passage of electricity through them depending on the concentration of soluble salts. Higher the salt content, lower the resistance to the flow of current. The conductivity is the reciprocal of resistance and it depends on:

1. Length of the solution (1) or distance between the electrodes.
2. Area of the electrodes (a).
3. Nature of the salts present and
4. Temperature of the solution.

Conductance (C) = a /1 = (K.a) / 1

 (C X 1)

 Hence, K = ----------

 A

***Apparatus:*** Conductivity meter

***Reagent used:*** 0.01 M KCl.

***Procedure: Determination of Cell Constant (K)***

The cell constant is the ratio of the distance (1) between the electrodes to the area (a) of the electrode. Cell constant of a given cell is determined by measuring the conductivity of 0.01M KCl solution whose specific conductivity (1.412 dSm-1 at 25ºC) is taken as a reference standard.

**THE ARRANGEMENT OF THE APPARATUS FOR THE DETERMINATION OF THE CONDUCTIVITY OF SOIL SUSPENSIONS**

**R2**

**R1**

 **R3**

**B2**

**B1**

**G2**

**G1**

 **X2**

**To Conductivity cell**

**Valve Oscillator**

 **Calculated Conductance of KCl 1.412**

**Cell Constant = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ X \_\_\_\_\_\_\_\_**

 **Observed Conductance of KCl X**

**(Fig – 45)**

***Determination of Electric Conductivity:***

***Principle:***The EC of a soil is measured with a conductivity meter known as ‘Solute Bridge’. It is based on the principle of Wheat – Stone Bridge in which an alternating current is used instead of direct current in order to eliminate the polarization of electrodes. The balance point is indicated by a ‘magic eye’ of a telephone. The schematic diagram of a soluble bridge is given in the figure below.

 A conductivity cell consists of two platinum electrodes in the form of rectangular pieces fused on glass on one side and covered with black spongy platium on the other. Two wire heads connect it to proper terminals on the Soluble Brodge. A figure known as cell constant is marked on the cell.

***Procedure: ( for Elco Soluble – Bridge):***

1. Take 10g of soil into a beaker and add 25ml of distilled water.
2. Stir the suspension intermittently and leave it overnight in order to obtain a clear supernatant solution.
3. Switch on the instruments (by setting it at room temperature for 15 minutes before taking reading for stabilization.
4. Dip the conductivity ell into the supernatent solution.
5. Move the pointer on the dial ti get maximum area in shade in the magic eye.
6. Note the reading on the Solute Bridge and Calculate EC as given below.

***Calculation and Observation:***

Cell constant of the Conductivity Cell = X

 Solute Bridge reading = Y m. mhos.

**EC = X x Ym.mhos cm-1**

***Interpretation:***

**Table - 7**

|  |  |
| --- | --- |
| **Electrical Conductivity****(m. mhos cm-1 )** | **Nature of Soil** |
| < 0.8 | Normal |
| 0.8 – 1.6 | Critical for tolerant crops |
| 1.6 – 2.5  | Critical for salt tolerant crops |
| > 2.5 | Injurious for many crops |

***Precautions:***

1. Keep the soil – water ratio in the suspension at 1: 2: 5 (EC decteases with dilution).
2. Allow the soil suspension to stand for a sufficient time to obtain clear supernatant.
3. Adjust the temperature of the Solute Bridge while measuring EC.
4. Dip the electrodes of the cell completely into the solution to get accurate reading.
5. Keep the electrodes dipped in distilled water when not in use( to get more accurate reading).

**ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)**

***Principle:***  The absorption energy by ground state atoms in the gaseous state forms the basis of Atomic Absorption Spectroscopy (AAS). When a solution containing metallic species is introduced into a flame, the vapour of mettallic species will be obtained. Some of the metal atoms may be raised to an energy level ssufficiently high to emit the characteristics radiation of the metal – a phenomenon that is utilized in the familiar technique of emission lame photometry. But a large percentage of metal atoms will remain in the non – emitting ground state. These ground state atoms of a particular element are receptive of light radiation of their own specific resonance wavelength (in general the same wavwlength as they would emit if they are exited). Thus, when a light of this wavelength is alloowed to pass through the flame, having atoms of metallic species, a part of that light will be absorbed and the adsorption will be proportional to the density of the atoms in the flame. Thus, in AAS, one determines the amount of light absorbed. Once the value of absorption is known the concentration of metallic element can be known because of the absorption is proportional to the density of the atoms in the flame.

**Instrumentation:**

Light of certain wavelength ( produced by a special kind of lamp), which is able to emit the spectral lines corresponding to the energy required for an electronic transition from the ground state to the exited state is allowed to pass through the flame. Meanwhile, the sample solution is aspirated into the flame. Before it enters the flame, the solution gets dispersed into a mist of a very small droplets which evaporate in the flame to give the dry salt and the vapoir of the salt. At least, a part of this vapour will be dissociated into atoms of the element to be measured, thus, the flame processes free un – exited atoms which are capable for absorbing radiation. Froman external source when the radiation corresponds exactly to the energy required for transition element from the ground electronic state to an upper exited level. Then the unabsorbed radiation from the flame is allowed to passa through a monochromator which isolates the exited spectral lines of light is then registered by a photodetector, the output of which is amplified and measured on a recorder. Absorption is measured by the differences in the transmitted signal in the presence and absence of the test element.

***Components of Atomic Absorption Spectrophotometer (AAS):***

1. **Radiation source*:*** The radiation source of AAS should emit stable intense radiation of the elemeny to be determined.

***Hollow Cathode Lamp:***

The hollow cathode lamp consists of anode and cathode (which is terminated in the lamp as a hollow cup). The anode is generally a tungsten wire and the cathode cup is made up of such a element whole spectrum is desired or it may be constructed from an inert element into which the desired element or the salt of the desired element is kept. The hollow cathode lamp is generally filled with neon gas at low pressure. When a voltage of 300 – 500 is put across the anode and the cathode, the atom of the filter gas undergo ionozation at the anode and are rapidly attracted by the cathode and physically displaces the surface metal atoms of the cathode into neon (or argon gas). Further collision of the vapourised metal atoms from the cathode atoms which emit characteristic spectrum of the metal used to construct the cathode. Thus the emission spectrum produced by the hollow cathode lamp is the sharp linef spectrum of the cathode material and the filled gas. The neon or helium gas filled in the hollow cathode lamp performs three functions:

* + It is the main souce of the current carrying capacity in the hollow cathode lamp.
	+ It dislodges the atoms from the surface of the cathode.
	+ It is primarly responsible for the exitation of the ground metal atoms.
1. **Chopper:**

A rotation wheel is interposed between the hollow cathode lamp and the flame. This rotation wheel is known as chopper and is interposed to break the steady light from the lamps into an intermittent or pulsating light. This gives a pulsating current into the photocell and only the pulsating or alternating current is amplified and recorded and thus, the absorption of light will be measured witjout interference from the light emmited by flame itself.

1. **Production of Atomic Vapour:**

 In order to achieve absorption by atoms, it becomes necessary to reduce sample to the atomic state. The most common way is to use a flame which is used for converting the liquid sample to a gaseous state and also for cinversion of the molecule entities into an atomic vapour. This is achieved by use of total consumption burner of pre – mixed burmer.

1. **Nebulisation of the Liquid sample:**

Before the liquid sample enter the burner, it is first converted into small droplets.This method of formation of small drops from the liquid sample is called nebulisation. A common method method of nebulisation is by use of gas moving at high velocity called pneumatic nebulization.

* 1. **Monochromators:**

In AAS the most common monochromator is prism and gratings. The function of monochromator is to select the fiven absorption lines emmited from the hollow cathode lamp.**(Fig – 46)**

* 1. **Detectors:**

For AAS the Photo – multiplier tube (PMT) is most suitable. PMY is an evacuated envelopewhich contains a photo – cathode, a series of electrodes called dynodes and an anode. As soon as the photon strikes the photo – cathode, an electron is dislodged and the proton is accelerated to anode to 1, resulting in the liberation of two or more electrons fromthis dynode. Similarly the electron from this dynode 1 is accelerated to dynode 2 and resulting in the liberation of more electron. Thus, the current multiplied at each dynode and the resultant electron current is received by the anode to produce EMF across RL which goes to the external ampliflier and read – out system.

* 1. **Ampliflier:**

 The electric current from the PMT is fed to the ampliflier which ampliflies the electronic current many times. Generally a “Lock in” amplifiers are preferred which provide a narrow frequency and band pass and help to achieve an excellent signal noise ratio.

* 1. **Read – out system:**

The results are fed directly to the computer terminal which can be either seen on the screen or stored in the memory.

**COOLING CENTRIFUGE**

A **centrifuge** is a piece of equipment, generally driven by a motor that puts an object in rotation around a fixed axis, applying force perpendicular to the axis. The centrifuge works using the sedimentation principle, where the centripetal acceleration is used to separate substances of greater and lesser density. There are many different kinds of centrifuges, including those for very specialized purposes.

## Theory

Protocols for centrifugation typically specify the amount of acceleration to be applied to the sample, rather than specifying a rotational speed such as revolutions per minute. The acceleration is often quoted in multiples of g, the acceleration due to gravity at the Earth's surface. This distinction is important because two rotors with different diameters running at the same rotational speed will subject samples to different accelerations. The acceleration can be calculated as the product of the radius and the square of the angular velocity.

## History and predecessors

**English military engineer Laval (1707-1751)** invented a whirling arm apparatus to determine drag, and **Antonin Prandl** invented the first centrifuge in order to separate cream from milk to make churning butter much easier.

## Types and uses

There are basically four types of centrifuge:

* Tabletop/clinical/desktop centrifuge or micro centrifuge
* High-speed centrifuge
* Cooling centrifuge
* Ultra-centrifuge

### *Biology and biochemistry*

Simple centrifuges are used in biology and biochemistry for isolating and separating biomolecules, cell organelles, or whole cells. They vary widely in speed and capacity. They usually comprise a rotor containing two, four, six, or many more numbered wells within which centrifuge tips may be placed.

The rotor is covered by a plastic cover. The cover is usually interlocked to prevent the motor from turning the rotor when it is open, and from allowing the cover to be opened before the rotor stops for several minutes. The cover protects the user from being injured by touching a rapidly spinning rotor. It also protects the user from fragments in case the rotor fails catastrophically.

To ensure that the rotor does not turn unbalanced, it must be balanced by placing samples or blanks of equal mass opposite each other. Since most of the mass is derived from the solvent, it is usually sufficient to place blanks or other samples of equal volume. As a safety feature, some centrifuges may stop turning when wobbling is detected.

The centrifuge tips are usually made of plastic or glass; they may vary in capacity from tens of milliliters, to much smaller capacities used in micro centrifuges used extensively in molecular biology laboratories. These micro centrifuges typically accommodate micro centrifuge tubes with capacities from 250 μl to 2.0 ml.

The ultracentrifuge was invented in 1925 by **Theodor Svedberg**, which by use of very high acceleration, and allowing the observation of sedimentation rates for macromolecules, allowed for the determination of their approximate molecular weights. Svedberg's 1926 Nobel Prize in Chemistry was earned for work made possible using his ultracentrifuge. Other centrifuges, the first being the Zippe-type centrifuge, are used to separate isotopes, and these kinds of centrifuges are in use in nuclear power and nuclear weapon programs.

Gas centrifuges are used in uranium enrichment. The heavier isotope of Uranium (Uranium-238) in the uranium hexafluoride gas tend to concentrate at the walls of the centrifuge as it spins, while the desired Uranium-235 isotope is extracted and concentrated with a scoop selectively placed inside the centrifuge. It takes many thousands of centrifuges to enrich uranium enough for use in a nuclear reactor (around 3.5% enrichment), and many thousands more to enrich it to atomic bomb-grade (around 90% enrichment).

### *Commercial applications*

* Standalone centrifuges for drying (hand-washed) clothes - usually with a water outlet.
* Centrifuges are used in the attraction Mission: SPACE, located at Epcot in Walt Disney World, which propels riders using a combination of a centrifuge and a motion simulator to simulate the feeling of going into space.
* In soil mechanics, centrifuges utilize centrifugal acceleration to match soil stresses in a scale model to those found in reality.
* Large industrial centrifuges are commonly used in water and wastewater treatment to dry sludge. The resulting dry product is often termed **cake**, and the water leaving a centrifuge after most of the solids have been removed is called **centrate**.

## Use and safety

The load in a laboratory centrifuge must be carefully balanced. Small differences in mass of the load can result in a large force imbalance when the rotor is at high speed. This force imbalance strains the spindle and may result in damage to centrifuge or personal injury.

Centrifuge rotors should never be touched while moving, because a spinning rotor can cause serious injury. Modern centrifuges generally have features that prevent accidental contact with a moving rotor.Because of the kinetic energy stored in the rotor head during high speed rotation, those who have experienced the loss of a rotor inside of an ultracentrifuge compare the experience to having a bomb explode nearby.

## Calculating relative centrifugal force (RCF)

## Relative centrifugal force is the measurement of the force applied to a sample within a centrifuge. This can be calculated from the speed (RPM) and the rotational radius (cm) using the following calculation.

**g = RCF = 0.00001118 × *r* × *N*2**

Where,

*g* = Relative centrifuge force

*r* = rotational radius (centimeter, cm)

*N* = rotating speed (revolutions per minute, r/min).

 **(Fig – 47)**

**HOT AIR OVEN**

 **Hot air ovens** are electrical devices used in sterilization. The oven utilizes dry heat to sterilize articles. Generally, they can be operated between 50 deg. C to 250/300 deg. There is a thermostat controlling the temperature. These are digitally controlled to maintain the temperature. Their double walled insulation keeps the heat in and conserves energy, the inner layer being a poor conductor and outer layer being metallic. There is also an air filled space in between to aid insulation. An air circulating fan helps in uniform distribution of the heat. These are fitted with the adjustable wire mesh plated trays or aluminium trays and may have an on/off rocker switch, as well as indicators and controls for temperature and holding time. The capacities of these ovens vary. Power supply needs vary from country to country, depending on the voltage and frequency (Hertz) used. Temperature sensitive tapes or other devices like those utilizing bacterial spores can be used to work as controls, to test for the efficacy of the device in every cycle.

***Advantages and disadvantages***

They do not require water and there is not much pressure build up within the oven, unlike an autoclave, making them safer to work with. This also makes them more suitable to be used in a laboratory environment. They are much smaller than autoclaves but still as efficacious. They can be more rapid than an autoclave and higher temperatures can be reached compared to other means. As they use dry heat instead of moist heat, some organisms like prions, may not be killed by them every time.

***Usage***

A complete cycle involves heating the oven to the required temperature, maintaining that temperature for the proper time interval for that temperature, turning the machine off and cooling the articles in the closed oven till they reach room temperature. The standard settings for a hot air oven are:

* 1.5 to 2 hours at 160 °C (320 °F)
* 6 to 12 mins at 190 °C (374 °F)

plus the time required to preheat the chamber before beginning the sterilization cycle. If the door is opened before time, heat escapes and the process becomes incomplete. Thus the cycle must be properly repeated all over. These are widely used to sterilize articles that can withstand high temperatures and not get burnt, like glassware, powders, paraffin. Linen gets burnt and surgical sharps lose their sharpness.

***Construction:*** It is a double walled steel chamber with a slout door with insulation om other side. The interior contains one or more perforated metal shelves or shelves made up of wire mesh and the oven is fitted with a thermoregulator and a thermometer. The top or side contains a ventilator which is left open during sterilization to disperse any medium, moisture and volatile matter. Hot air passes between the wall and is usually heated by electricity through some order models are heated by gas. The electric heaters are placed at the bottom between the two walls.

Place the articleds to be sterilized tubes, flasks, pipettes wrapped in paper or placed in lens on the shelves. Make sure all articles are perfectly dried. Close the doors firm and turn on the source of heat. Take the time for oven reaches the required temperature. Note the time that heat was turned on. The time of sterilization should be 1 hour at 167ºC or 1.5 hours at 150ºC. turn off heat and allow the ovwn to become quite cold before opening the door. Otherwise glass articles may crack on sudden exposure to the cooler room air.

**(Fig – 48)**

**COLORIMETER**

 Colorimetry is based on the principle that colored solutions preferentially absorb some wavelengths and the color seen is charactristic of the wavelength not abvsorbed. The colour on the wavelength band of the incident light used for a colorimetric determination is selected to coincide with that most absorbed by the test solution and this is done by the passage of light through light filter.

 According to Beer’s law, equal successive increments of concentration dC of a true solution containing a coloured constituent absorb equal fractions, di/l of the incident light.

 Mathematically,

 **dt**

 **T = \_\_\_\_\_ X 100**

 **Lº**

If the colorimeteer gives transmittance readings, these can be converted to absorbance by the following relationship:

**Absorbance = 2 – log (% T)**

**(Fig – 15)**

**CYCLOMIXER**

A variable speed mixer to eliminate time consuming hand mixing. Holding tube against vibrating rubber cup does rapid mixing of contents. Speed regulator controls the degree of vibration. A unique touch feature operates the unit when tube is pressed on the rubber cup.

 (**Fig – 14)**

**MICROSCOPE**

Microbiology is the branch of biology which deals with the microscopic oganisms which are too small to be seen with naked eye. The word microscope is derived from two greek words micro – small and scope – view.A microscope is a instrument use dfor the visual examination of small objects which cannot be examined properly with naked eyes. A simple microscope condists of a biconvex lens along with a stage to keep the specimen for examination and a light reflecting mirror. The compound microscope employs two separate lens system objective lens and an occular lens or an eye piece in order to achieve greater magnification. The objective lens focuses the light rays from the specimen to form a real image with in the body tube of the microscope. The real image is further magnified by the occular lens system which is sutuated at the top of the draw tube.The microscope consist of a mechanical portion whose function is to hold the two separate lens system in position and to fold the object being examined. An optical portion serves to magnify the objective, the mechanical portion consists of a ‘U’ shaped base stand from which arises a short vertical pillar at the upper end of which arises a ‘c’ shaped side arm which is joined to the pillar by an inclination joined which allows the arms to be tilted at a convinient angle at the top of pillar is the horizontal platform or stage with an opening called the stage aperature which can be brought into line with the long axis of the body tube. There are two stage clips which help to hold the microscopic slides carrying the object for examination.At the top of the arm is attached to a body tube which carries the objective lens at the lower end an occular lens at the upper end. The body tube so can be raised and lowered by means of the coarse adjustment screw. The fine adjustment screw is to bring the object exactly into focus the body tube sometimes contains within it a closely fitting under inner tube is called the draw tube which can be drawn out and that increases the distance between the two lens systems. There is a revolving nose piece to which the special objective can be screwed low power objective with high power objecive (Dry objective and oil immersion).

**OPTICAL PORTIONS:**

***Low power objective*:** it ususlly has a total distance of 16mm. It is sometimes called 10mm objective. It gives a magnification of 10 times and is marked as 10X.

**HIGH POWER DRY OBJECTIVE:**

Usually it has a focal distance of 4mm objective it gives a magnification of 40 times and is marked as 40X.

**OIL IMMERSION:**

This objective is so named because it is necessary to place a drop of oil between the lower end of the objective and the objective being examined in order to get good resolution and a clear image. The focal distance is about 1.5mm and it is called as the 1.8mm objective. The magnification is usually 90 – 100 and the objective is marked as 90X, 95X and 100X. these objectives are served into the revolving nose piece and can be brought into use one by one until the particular objective lens has its long axis in line with the long axis of the body tube. The objectives are the most important and most expensive parts of microscope.

**OCCULAR EYEPIECE:**

If the microscope has only one eye piece. It is called mono – locular microscope. If it has two occular.then it is called as Bilocular microscope. A few microscopes have a third occular and is called tri – locular microscope to which is attached to a camera for taking photograph. The eye piece consists of two lenses at either ends of a smsll tube. The magnification given by the eye piece will depend on the lenses and the distance between the two lenses. The most commomly used eye piece magnifies the object sometimes and is called as 10X. Devices for containing light to get the best image an optimum amount of light is required and the microscopes have certain devices to regulate the light they are

***Condensor:***

This consists of one or more lenses which helps to focus the light rays to the object being examined on the stage. The condensor can be raised or lowered and the change of position of the condensor will change the light getting through it.

***Iris Diaphragm:***

This can be opened or shut to allow the full light to pass or to restrict the amount of light.

***Source of light:***

Some microscopes especially bilocular models have an electric bulb immediately under the condensor, the mirror has two surfaces from the source of light are parallel. The concave surface is used when the rays from the light source are divertred which occur when light bulb is the source.

**(Fig – 49)**