STABILITY INDICATING POTENTIOMETRIC METHOD FOR DETERMINATION OF ANTI-TUBERCULAR DRUGS USING SENSOR

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ABSTRACT

Tuberculosis (TB) is a disease caused by infection that the bacteria *Mycobacterium tuberculosis*, it produces several million deaths annually. There is a need to study the stability of the tubercular drugs. This work deals with the stability indicating potentiometric method for the determination of anti-tubercular drugs using two different sensors. Isoniazid+rifampicin, ethambutol and combination of isoniazid+rifampicin, ethambutol. The drugs were subjected to oxidative degradation by refluxing with hydrogen peroxide. The stability response in concentration, pH and temperature were studied. Among the compounds (Ia, IIa, IIIa, Ia1, IIa2, IIIa3) IIIa3 have shown more stable response. The method involved the use of ion-selective electrodes (ISEs) for the detection of drug molecules in solution. The ISEs were fabricated by incorporating the drug specific ionophore in a polymeric membrane, which was then attached to a glass electrode. The proposed method was validated according to international counselling for harmonisation guidelines for specificity, linearity, accuracy, recision and robustness. The results demonstrated that the method was specific and able to differentiate between the drug and its degradation products under diiferent stress conditions. The developed stability-indicating potentiometric method using sensors can be used for the reliable determination of anti-tubercular drugs and can be a useful to for the quality control of these drugs in pharmaceutical industry. The method is rapid, easy to use, and requires minimal sample oreoaration, making it a promising alternative to existing analytical method for the determination of anti-tubercular drugs.

INTRODUCTION

PHARMACEUTICAL ANALYSIS:

The pharmaceutical analysis is a branch of chemistry, which involves the series of process for the identification, determination, quantitation, and purification. This is mainly used for the separation of the components from the mixture and for the determination of the structure of the compounds. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, microorganisms, minerals and various synthetic products.

The sample to be analysed is called as analyse and on the basis of size of sample, they can be classified as macro (0.1 g or more), semi micro (0.01 g to 0.1 g), micro (0.001 g to 0.01 g), sub micro (0.0001 g to 0.001 g), ultra micro (below 10-4 g), trace analysis (100 to 10000 ppm).

Based upon the determination type, there are mainly two types of analytical methods.

They are as follows:

- Qualitative analysis (identification)
- Quantitative analysis (estimation)

1. Qualitative analysis: Qualitative analysis is a method to analyse a certain subject using non-numeric plus non-quantifiable indicators, behaviour, and characteristics to determine its overall situation, quality, value, or any other parameter. It lies at the core of making business decisions and recognizing investment opportunities by investors and companies.

Various types of qualitative analysis

a. Chemical methods

b. Electrical methods

c. Instrumental methods

d. Biological or Microbiological methods

2. Quantitative analysis: Quantitative analysis is the process of collecting and evaluating measurable and verifiable data such as revenues, market share, and wages in order to understand the behaviour and performance of a business. with data technology, quantitative analysis is now considered a better approach to making informed decisions.

a. Data analysis

b. Factor analysis

c. Analysis of variance

d. Descriptive statistics

e. Regression analysis

f. Data mining

1. POTENTIOMETRIC METHOD

In analytical chemistry, **potentiometric titration** is a technique similar to direct titration of a redox reaction. It is a useful means of characterizing an acid. No indicator is used; instead the electric potential is measured across the analyte, typically an electrolyte solution. To do this, two electrodes are used, an indicator electrode (the glass electrode and metal ion indicator electrode) and a reference electrode. Reference electrodes generally used are hydrogen electrodes, calomel electrodes, and silver chloride electrodes. The indicator electrode forms an electrochemical half-cell with the interested ions in the test solution. The reference electrode forms the other half-cell.

The overall electric potential is calculated as

$E_{cell} = E_{ind} - E_{ref} + E_{sol}$.

E_{sol} is the potential drop over the test solution between the two electrodes. E_{cell} is recorded at intervals as the titrant is added. A graph of potential against volume added can be drawn and the end point of the reaction is halfway between the jump in voltage. E_{cell} depends on the concentration of the interested ions with which the indicator electrode is in contact.

Potentiometric titration is a type of volumetric analysis that is used to determine the concentration of an analyte in a sample. The analyte is typically a strong acid or base, and the titration is performed using a titrant of known concentration. The concentration of the analyte is determined by measuring the potential of the solution at various points during the titration.

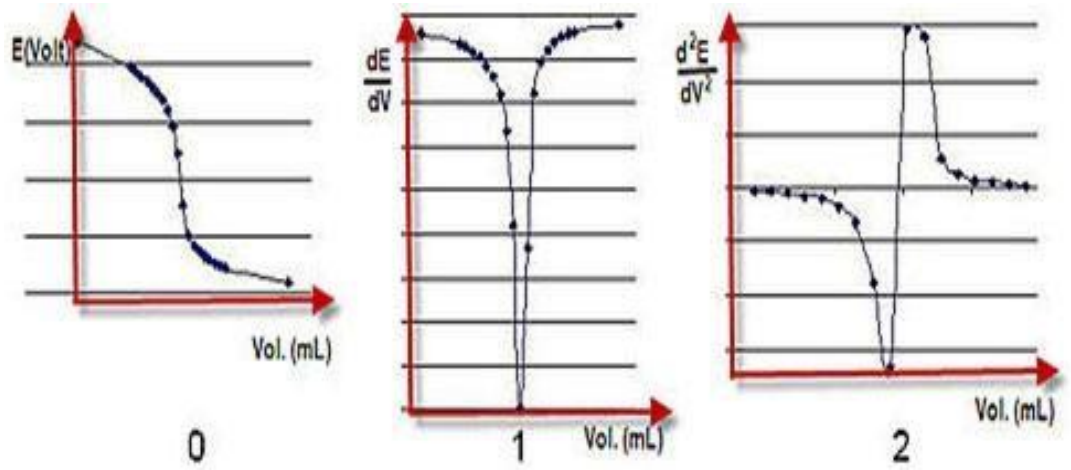
In potentiometry, we measure the potential of an electrochemical cell under static conditions. Because no current or only a negligible current flows through the electrochemical cell, its composition remains unchanged. For this reason, potentiometry is a useful quantitative method. The first quantitative potentiometric applications appeared soon after the formulation, in 1889, of the Nernst equation, which relates an electrochemical cell's potential to the concentration of electroactive species in the cell.

Potentiometry initially was restricted to redox equilibria at metallic electrodes, limiting its application to a few ions. In 1906, Cremer discovered that the potential difference across a thin glass membrane is a function of pH when opposite sides of the membrane are in contact with solutions containing different concentrations of $H3O^+$. This discovery led to the development of the glass pH electrode in 1909. Other types of membranes also yield useful potentials. For example, in 1937 Kolthoff and Sanders showed that a pellet of AgCl can be used to determine the concentration of Ag^+. Electrodes based on membrane potentials are called ion-selective electrodes, and their continued development extends potentiometry to a diverse array of analytes.

The first potentiometric titration was carried out in 1893 by Robert Behrend at Ostwald's Institute in Leipzig. He titrated mercurous solution with potassium chloride, potassium bromide, and potassium iodide. He used a mercury electrode along with a mercury/mercurous nitrate reference electrode. He found that in a cell composed of mercurous nitrate and mercurous nitrate/mercury, the initial voltage is 0. If potassium chloride is added to mercurous nitrate on one side, mercury (I) chloride is precipitated. This decreased the osmotic pressure of mercury (I) ions on the side and creates a potential difference. This potential difference increases slowly as additional potassium chloride is added, but then increases more rapidly. He found the greatest potential difference is achieved once all of the mercurous nitrate has been precipitated. This was used to discern end points of titrations.

Wilhelm Böttger then developed the tool of potentiometric titration while working at Ostwald's Institute. He used potentiometric titration to observe the differences in titration between strong and weak acids, as well as the behavior of polybasic acids. He introduced the idea of using potentiometric titrations for acids and bases that could not be titrated in conjunction with a colorimetric indicator.

Potentiometric titrations were first used for redox titrations by Crotogino. He titrated halide ions with potassium permanganate using a shiny platinum electrode and a calomel electrode. He said that if an oxidizing agent is added to a reducing solution then the equilibrium between the reducing substance and reaction product will shift towards the reaction product. This changes the potential very slowly until the amount of reducing substance becomes very small. A large change in potential will occur then once a small addition of the titrating solution is added, as the final amounts of reducing agent are removed and the potential corresponds solely to the oxidizing agent. This large increase in potential difference signifies the endpoint of the reaction.



Potentiometric titration is a useful tool for estimating the amounts of isohumulones in hops bitter substances, which are a large component of beer. Isohumulones are seen to combine with metals in stoichiometric proportions. This allows solutions of isohumulones, generally created by extracting isohumulones from hops using isooctane or petroleum and a liquid-liquid extraction, can be titrated with solutions of metal salts using changes in conductivity to determine an end point. The solutions of isohumulones can then be titrated against a metal salt such as nickel acetate or lead acetate. When this titration is done using nickel acetate, two moles of isohumulones combines with one mole of nickel acetate, and each millilitre of titrant contains 1/500 of a mill mole of nickel. This allows the concentration (C) of the bitter substance to be calculated as:

$$C = (mL \text{ of nickel acetate} \times 710 \times 10)/500$$

Potentiometric Measurements

We use a potentiometer to determine the difference between the potential of two electrodes. The potential of one electrode, the working or indicator electrode responds to the analyte's activity, and the other electrode, the counter or reference electrode has a known, fixed potential. In this section we introduce the conventions for describing potentiometric electrochemical cells, and the relationship between the measured potential and the analyte's activity.

We noted that the equilibrium position of a chemical reaction is a function of the activities of the reactants and products, not their concentrations. To be correct, we should write the Nernst equation, in terms of activities. First, concentrations are always easier to calculate than activities. Second, in a redox titration we determine the analyte's concentration from the titration's end point, not from the potential at the end point. The only reasons for calculating a titration curve is to evaluate its feasibility and to help in selecting a useful indicator. In most cases, the error we introduce by assuming that concentration and activity are identical is too small to be a significant concern.

In potentiometry we cannot ignore the difference between activity and concentration. Later in this section we will consider how we can design a potentiometric method so that we can ignore the difference between activity and concentration.

The electrochemical cell consists of two half-cells, each containing an electrode immersed in a solution of ions whose activities determine the electrode's potential. A **salt bridge** containing an inert electrolyte, such as KCl, connects the two half-cells. The ends of the salt bridge are fixed with porous frits, allowing the electrolyte's ions to move freely between the half-cells and the salt bridge. This movement of ions in the salt bridge completes the electrical circuit.

By convention, we identify the electrode on the left as the anode and assign to it the oxidation reaction; thus

$$Zn(s) \leftrightharpoons Zn^{2+}(aq) + 2e^{-}$$

The electrode on the right is the **cathode**, where the reduction reaction occurs.

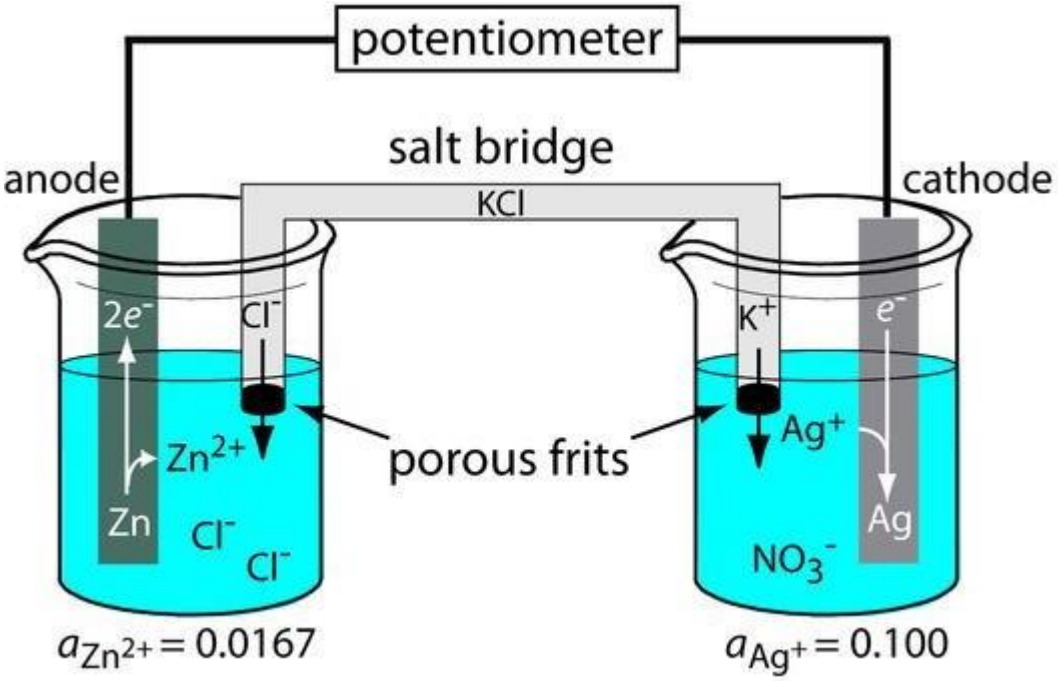
$$Ag^{+}(aq) + e^{-} \leftrightharpoons Ag(s)$$

The reason for separating the electrodes is to prevent the oxidation and reduction reactions from occurring at one of the electrodes. For example, if we place a strip of Zn metal in a solution of AgNO3, the reduction of Ag^{+} to Ag occurs on the surface of the Zn at the same time as a potion of the Zn metal oxidizes to Zn^{2+}. Because the transfer of electrons from Zn to Ag^{+} occurs at the electrode's surface, we cannot pass them through the potentiometer.

The potential of the electrochemical cell is

$$Zn(s) + 2Ag^{+}(aq) \leftrightharpoons 2Ag(s) + Zn^{2+}(aq)$$

We also define potentiometric electrochemical cells such that the cathode is the indicator electrode and the anode is the reference electrode.



Reference Electrodes

In a potentiometric electrochemical cell one half-cell provides a known reference potential and the potential of the other half-cell indicates the analyte's concentration. By convention, the reference electrode is the anode; thus, the short hand notation for a potentiometric electrochemical cell is

reference || indicator

and the cell potential is

$E\text{cell} = E\text{ind} - E\text{ref} + E\text{j}$

The ideal reference electrode provides a stable, known potential so that any change in *E*cell is attributed to analyte's effect on the potential of the indicator electrode. In addition, the ideal reference electrode should be easy to make and to use. Three common reference electrodes are discussed in this section.

Standard Hydrogen Electrode (SHE)

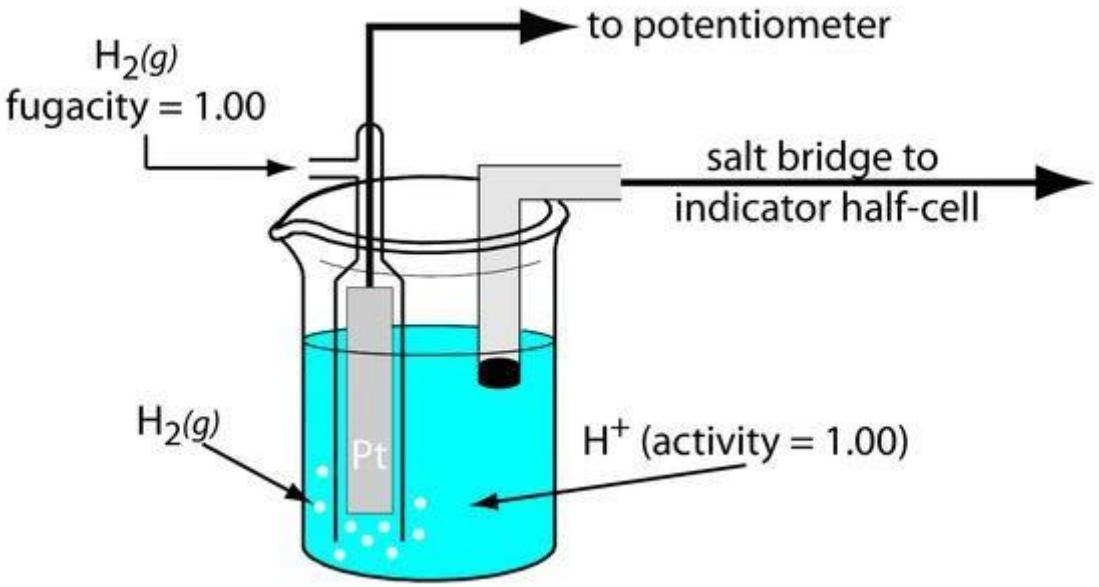
Although we rarely use the **standard hydrogen electrode** (SHE) for routine analytical work, it is the reference electrode used to establish standard-state potentials for other half-reactions. The SHE consists of a Pt electrode immersed in a solution in which the activity of hydrogen ion is 1.00 and in which the fugacity of H2(*g*) is 1.00. A conventional salt bridge connects the SHE to the indicator half-cell. The short hand notation for the standard hydrogen electrode is

$Pt_{(s)}, H_2(g, f_{H2}=1.00) |H^+(aq, a_{H}{}^{+}=1.00) ||$

and the standard-state potential for the reaction

$H^+_{(aq)} + e^- \leftrightharpoons 1/2H2(g)$

is, by definition, 0.00 V at all temperatures. Despite its importance as the fundamental reference electrode against which we measure all other potentials, the SHE is rarely used because it is difficult to prepare and inconvenient to use.



Calomel Electrodes

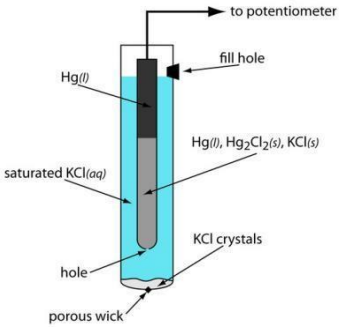
Calomel is the common name for the compound Hg_2Cl_2.

Calomel reference electrodes are based on the following redox couple between Hg2Cl2 and Hg

$Hg_2Cl_2(s)+2e^- \leftrightharpoons 2Hg(l)+2Cl^-(aq)$

The potential of a calomel electrode, therefore, is determined by the activity of Cl^- in equilibrium with Hg and Hg2Cl2

In a **saturated calomel electrode** (SCE) the concentration of Cl^- is determined by the solubility of KCl. The electrode consists of an inner tube packed with a paste of Hg, Hg2Cl2, and KCl, situated within a second tube containing a saturated solution of KCl. A small hole connects the two tubes and a porous wick serves as a salt bridge to the solution in which the SCE is immersed. A stopper in the outer tube provides an opening for adding addition saturated KCl. The short hand notation for this cell is Hg(*l*)|Hg2Cl2(*s*), KCl (*aq*, *sat'd*) ||



Because the concentration of Cl^- is fixed by the solubility of KCl, the potential of an SCE remains constant even if we lose some of the solution to evaporation. A significant disadvantage of the SCE is that the solubility of KCl is sensitive to a change in temperature. At higher temperatures the solubility of KCl increases and the electrode's potential decreases. For example, the potential of the SCE is +0.2444 V at 25°C and +0.2376 V at 35°C. The potential of a calomel electrode containing an unsaturated solution of KCl is less temperature dependent, but its potential changes if the concentration, and thus the activity of Cl^-, increases due to evaporation.

The potential of a calomel electrode is +0.280 V when the concentration of KCl is 1.00 M and +0.336 V when the concentration of KCl is 0.100 M. If the activity of Cl^- is 1.00, the potential is +0.2682 V.

Silver/Silver Chloride Electrodes

Another common reference electrode is the **silver/silver chloride electrode**, which is based on the following redox couple between AgCl and Ag.

$$AgCl(s) + e^- \leftrightharpoons Ag(s) + Cl^-(aq)$$

As is the case for the calomel electrode, the activity of Cl^- determines the potential of the Ag/AgCl electrode; thus

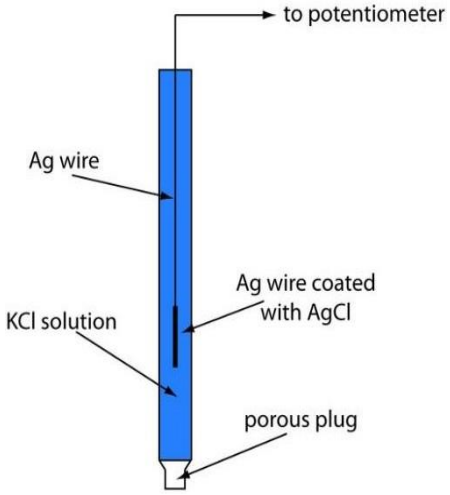
$$E = E°_{AgCl/Ag} - 0.05916 \log a_{Cl^-} = +0.2223\ V - 0.05916 \log a_{Cl}$$

When prepared using a saturated solution of KCl, the potential of a Ag/AgCl electrode is +0.197 V at 25°C. Another common Ag/AgCl electrode uses a solution of 3.5 M KCl and has a potential of +0.205 V at 25°C.

The potential of a Ag/AgCl electrode using a saturated solution of KCl is more sensitive to a change in temperature than an electrode using an unsaturated solution of KCl.

A typical Ag/AgCl electrode consists of a silver wire, the end of which is coated with a thin film of AgCl, immersed in a solution containing the desired concentration of KCl. A porous plug serves as the salt bridge. The electrode's short hand notation is

$Ag(s) \mid AgCl(s), KCl\ (aq, a\mathrm{Cl}^- = x) \parallel$



Potentiometric Titration principle:

A solution of known concentration is titrated with a solution of unknown concentration. The endpoint of the titration is, when the two solutions have the same concentration. The titration is monitored by a potentiometer, which measures the voltage of the solution.

Potentiometric Titration Procedure:

A potentiometric titration procedure is a laboratory technique used to measure the concentration of an analyte in a sample solution by titrating it with a standard solution of known concentration. The analyte is typically an acid or a base. The titration is performed by adding a measured volume of the standard solution to the sample solution, and then measuring the resulting change in the electrical potential (potential) of the solution. The potential is measured with a potentiometer, which is a device that measures the potential difference between two points in a solution. The concentration of the analyte in the sample can be determined from a graph of potential versus volume of standard solution added, which is called a titration curve.

Types of Potentiometric Titration

There are three types of potentiometric titration:

1. Strong acid-strong base titration

2. Weak acid-strong base titration

3. Weak acid-weak base titration

Acid-base titration

An acid-base titration is a common laboratory technique used to determine the concentration of an unknown acid or base solution. It involves the addition of a measured volume of one solution (the titrant) to another solution (the analyte) of unknown concentration until the endpoint of the reaction

is reached. The endpoint is determined by the addition of a indicator that changes color when the reaction is complete. The concentration of the titrant can then be easily determined from its volume and the known concentration of the indicator.

Redox titration

A redox titration is a type of titration in which the concentration of one species is determined by the amount of another species that is titrated into it. The two species are typically oxidized and reduced forms of the same element.

Complexometric titration

A complexometric titration is a type of titration involving the determination of the concentration of an analyte in a sample by titrating it with a titrant that forms a complex with the analyte. The concentration of the titrant is then determined from the amount of titrant used. Complexometric titrations are used to determine the concentration of metals and other ions in solution. The most common type of complexometric titration is the determination of calcium ion concentration in water using EDTA as the titrant.

Precipitation titration

In a precipitation titration, aqueous solutions of two soluble compounds are mixed until one of the compounds precipitates out of solution. The amount of the compound that precipitates is then used to calculate the concentration of the other compound in the original solutions.

The most common type of precipitation titration is the titration of a strong base with a strong acid. In this case, the strong base is added to the acid until the acid is completely neutralized. The amount of base needed to neutralize the acid is then used to calculate the concentration of the acid in the original solution.

Applications of Potentiometric titrations

There are many applications of potentiometric titration. Some common applications include: Determining the concentration of an unknown acid or base in a solution.
Determining the concentration of an unknown salt in a solution. Determining the concentration of an unknown ion in a solution. Determining the end point of a titration reaction.

2. TUBERCULOSIS

Tuberculosis (TB) is a serious illness that mainly affects the lungs. The germs that cause tuberculosis are a type of bacteria. Tuberculosis can spread when a person with the illness coughs, sneezes or sings. This can put tiny droplets with the germs into the air. Another person can then breathe in the droplets, and the germs enter the lungs.

Tuberculosis spreads easily where people gather in crowds or where people live in crowded conditions. People with HIV/AIDS and other people with weakened immune systems have a higher risk of catching tuberculosis than people with typical immune systems. Drugs called antibiotics can treat tuberculosis. But some forms of the bacteria no longer respond well to treatments.

Symptoms

When TB germs survive and multiply in the lungs, it is called a TB infection.

A TB infection may be in one of three stages. Symptoms are different in each stage.

Primary TB infection. The first stage is called the primary infection. Immune system cells find and capture the germs. The immune system may completely destroy the germs. But some captured germs may still survive and multiply.

Most people don't have symptoms during a primary infection. Some people may get flu-like symptoms, such as:

- Low fever.
- Tiredness.
- Cough.

Latent TB infection. Primary infection is usually followed by the stage called latent TB infection. Immune system cells build a wall around lung tissue with TB germs. The germs can't do any more harm if the immune system keeps them under control. But the germs survive.

There are no symptoms during latent TB infection.

Active TB disease. Active TB disease happens when the immune system can't control an infection. Germs cause disease throughout the lungs or other parts of the body. Active TB disease may happen right after primary infection. But it usually happens after months or years of latent TB infection.

Symptoms of active TB disease in the lungs usually begin gradually and worsen over a few weeks. They may include:

- Cough.
- Coughing up blood or mucus.
- Chest pain.
- Pain with breathing or coughing.
- Fever.
- Chills.

- Night sweats.
- Weight loss.
- Not wanting to eat.
- Tiredness.
- Not feeling well in general.

Active TB disease outside the lungs. TB infection can spread from the lungs to other parts of the body. This is called extrapulmonary tuberculosis. Symptoms vary depending on what part of the body is infected. Common symptoms may include:

- Fever.
- Chills.
- Night sweats.
- Weight loss.
- Not wanting to eat.
- Tiredness.
- Not feeling well in general.
- Pain near the site of infection.

Active TB disease in the voice box is outside the lungs, but it has symptoms more like disease in the lungs.
Common sites of active TB disease outside the lungs include:

- Kidneys.
- Liver.
- Fluid surrounding the brain and spinal cord.
- Heart muscles.
- Genitals.
- Lymph nodes.

- Bones and joints.
- Skin.
- Walls of blood vessels.
- Voice box, also called larynx.

Active TB disease in children. Symptoms of active TB disease in children vary. Typically, symptoms by age may include the following:

- **Teenagers.** Symptoms are similar to adult symptoms.
- **1- to 12-year-olds.** Younger children may have a fever that won't go away and weight loss.
- **Infants.** The baby doesn't grow or gain weight as expected. Also, a baby may have symptoms from swelling in the fluid around the brain or spinal cord, including:
  - Being sluggish or not active.
  - Unusually fussy.
  - Vomiting.
  - Poor feeding.
  - Bulging soft spot on the head.
  - Poor reflexes.

The symptoms of tuberculosis are similar to symptoms of many different illnesses. See your health care provider if you have symptoms that don't improve with a few days of rest.

Get emergency care if you have:

- Chest pain.
- Sudden, severe headache.
- Confusion.
- Seizures.
- Difficulty breathing.

Get immediate or urgent care if you:

- Cough up blood.
- Have blood in your urine or stool.

Causes

Tuberculosis is caused by a bacterium called *Mycobacterium tuberculosis.*

People with active TB disease in the lungs or voice box can spread the disease. They release tiny droplets that carry the bacteria through the air. This can happen when they're speaking, singing, laughing, coughing or sneezing. A person can get an infection after inhaling the droplets.

The disease is more likely to spread when people spend a lot of time together in an indoor space. So the disease spreads easily in places where people live or work together for long periods. Also, the disease spreads more easily in crowded gatherings.

A person with a latent TB infection cannot pass the disease to other people. A person taking drugs to treat active TB disease usually can't pass the disease after 2 to 3 weeks of treatment.

Drug-resistant TB

Some forms of the TB bacteria have become drug resistant. This means that drugs that once cured the disease no longer work.

This happens, in part, because of naturally occurring genetic changes in bacteria. A random genetic change in a bacterium might give it some quality that makes it more likely to survive the attack of an antibiotic. If it does survive, then it can multiply.

When antibiotic drugs aren't used correctly or drugs fail to kill all the bacteria for another reason, the conditions are ideal for more-resistant versions of the bacteria to get established and multiply. If these bacteria are passed on to other people, a new drug-resistant strain can grow over time.

Problems that can lead to such drug-resistant strains of bacteria include the following:

- People didn't follow directions for taking the drugs or stopped taking the drugs.
- They weren't prescribed the right treatment plan.
- Drugs were not available.
- The drugs were of poor quality.
- The body didn't absorb the drugs as expected.

Risk factors

Anyone can get tuberculosis, but certain factors increase the risk of getting an infection. Other factors increase the risk of an infection becoming active TB disease.

The Centres for Disease Control and Prevention recommends a TB test for people who have an increased risk of TB infection or active TB disease. Talk to your health care provider if you have one or more of the following risk factors.

Risk of active TB disease

A weakened immune system increases the risk of a TB infection becoming active TB disease. Conditions or treatments that weaken the immune system include:

- HIV/AIDS.

Risk of TB infection

Certain living or working conditions make it easier for the disease to pass from one person to another. These conditions increase the risk of getting a TB infection:

- Living with someone with active TB disease.
- Living or traveling in a country where TB is common, including several countries in Latin America, Africa, Asia and the Pacific Islands.
- Living or working in places where people live close together, such as prisons, nursing homes and shelters for homeless people.
- Living in a community identified as being at high risk of tuberculosis.
- Working in health care and treating people with a high risk of TB.
- Diabetes.
- Severe kidney disease.
- Cancers of the head, neck and blood.
- Malnutrition or low body weight.
- Cancer treatment, such as chemotherapy.
- Drugs to prevent rejection of transplanted organs.
- Long-term use of prescription steroids.
- Use of unlawful injected drugs.
- Misuse of alcohol.
- Smoking and using other tobacco products.

Age and active TB disease

The risk of a TB infection becoming active TB disease changes with age.

- **Under 5 years of age.** Until children reach age 5, they have high risk of a TB infection becoming active TB disease. The risk is greater for children under age 2. Tuberculosis in this age group often leads to serious disease in the fluid surrounding the brain and spinal column, called meningitis.
- **Age 15 to 25.** People in this age group have an increased risk of developing more-severe active TB disease in the lungs.
- **Age 65 and older.** The immune system weakens during older age. Older adults have a greater risk of active TB disease. Also, the disease may be more difficult to treat.

Prevention

If you test positive for latent TB infection, you may need to take drugs to prevent active TB disease.

Preventing the spread of disease

If you have active TB disease, you'll need to take steps to prevent other people from getting an infection. You will take drugs for four, six or nine months. Take all of the drugs as directed during the entire time.

During the first 2 to 3 weeks, you will be able to pass TB bacteria to others. Protect others with these steps:

- **Stay home.** Don't go to work or school.
- **Isolate at home.** Spend as little time as possible among members of your household. Sleep in a separate room.
- **Ventilate the room.** Tuberculosis germs spread more easily in small, closed spaces. If it's not too cold outdoors, open the windows. Use a fan to blow air out. If you have more than one window, use one fan to blow air out and another to blow air in.
- **Wear face masks.** Wear a mask when you have to be around other people. Ask other members of the household to wear masks to protect themselves.
- **Cover your mouth.** Use a tissue to cover your mouth anytime you sneeze or cough. Put the dirty tissue in a bag, seal it and throw it away.

Vaccinations

In countries where tuberculosis is common, infants often are vaccinated with the bacille Calmette-Guerin (BCG) vaccine. This protects infants and toddlers who are more likely to have active TB disease in the fluid surrounding the brain and spinal cord.The vaccine may not protect against

disease in the lungs, which is more likely in the United States. Dozens of new TB vaccines are in various stages of development and testing.

Diagnosis

To diagnosis a tuberculosis (TB) infection, your health care provider will do an exam that includes:

- Listening to you breathe with a stethoscope.
- Checking for swollen lymph nodes.
- Asking you questions about your symptoms.

TB tests

Your health care provider will order tests if:

- Tuberculosis is suspected.
- You were likely exposed to a person with active TB disease.
- You have health risks for active TB disease.

Skin test

A tiny amount of a substance called tuberculin is injected just below the skin on the inside of one forearm. Within 48 to 72 hours, a health care worker will check your arm for swelling at the injection site. The size of the raised skin is used to determine a positive or negative test.

This test is seeing if your immune system reacts, or has made an antibody, to tuberculosis. A positive test indicates you likely have either a latent TB infection or active TB disease. People who had a TB vaccination might get a positive test even if they have no infection.A negative test means that your body didn't react to the test. It doesn't necessarily mean you don't have an infection.

Blood tests

A sample of blood is sent to a lab. One lab test finds out whether certain immune system cells can "recognize" tuberculosis. A positive test shows that you have either a latent TB infection or active TB disease. Other tests of the blood sample can help determine if you have active disease.

A negative result means you likely do not have a TB infection.

X-ray

A chest X-ray can show irregular patches in the lungs that are typical of active TB disease.

Sputum tests

Your health care provider may take a sample of the mucus that comes up when you cough, also called sputum. If you have active TB disease in your lungs or voice box, lab tests can detect the bacteria.

A relatively quick laboratory test can tell if the sputum likely has the TB bacteria. But it may be showing bacteria with similar features.

Another lab test can confirm the presence of TB bacteria. The results often take several weeks. A lab test also can tell if it's a drug-resistant form of the bacteria. This information helps your health care provider choose the best treatment.

Other lab tests

Other lab tests that may be ordered include:

- Breath test.
- Procedure to remove sputum from your lungs with a special tube.
- Urine test.
- Test of the fluid around the spine and brain, called cerebrospinal fluid.

Treatment

If you have a latent TB infection, your health care provider may begin drug treatments. This is especially true for people with HIV/AIDS or other factors that increase the risk of active TB disease. Most latent TB infections are treated for three or four months.

Active TB disease may be treated for four, six or nine months. Specialists in TB treatment will determine which drugs are best for you.

You will have regular appointments to see if you're improving and to watch for side effects.

Take all of the drugs

It is important to take every dose as instructed. And you must complete the full course of treatment. This is important for killing the bacteria in your body and preventing new drug-resistant bacteria.

Your public health department may use a program called directly observed therapy (DOT). With DOT, a health care worker visits you at home to watch you take your dose of drugs.

Some health care departments have programs that let you take your drugs on your own. The Centres for Disease Control and Prevention has printable forms you can use to keep track of your daily doses.

Most common TB drugs

If you have a latent TB infection, you might need to take only one or two types of drugs. Active TB disease requires taking several drugs. Common medications used to treat tuberculosis include:

- Isoniazid
- Rifampicin (Rimactane)
- Rifabutin (Mycobutin)
- Rifapentine (Priftin)
- Pyrazinamide
- Ethambutol (Myambutol)

You may be prescribed other drugs if you have drug-resistant tuberculosis or other complications from your illness.

Medication side effects

Most people can take TB drugs without serious side effects. If you have serious side effects, your care provider may ask you to stop taking a drug. You may have to change the dose of a drug.

Talk to your health care provider if you experience any of the following:

- Upset stomach
- Vomiting
- Loss of appetite
- Severe diarrhea
- Light-colored stool.
- Dark urine.
- Yellowish skin or eye color.
- Changes in vision.
- Dizziness or trouble with balance.
- Tingling in hands or feet.
- Easy bruising or bleeding.
- Unexplained weight loss.
- Unexplained tiredness.
- Sadness or depression.
- Rash.
- Joint pain.

It is important for you to list all drugs, dietary supplements or herbal remedies you take. You may need to stop taking some of these during your treatment.

3. ANTI-TUBERCULAR DRUGS

Tuberculosis is a disease that results from infection with the bacteria *Mycobacterium tuberculosis*. It most commonly affects the lungs but can also affect other areas of the body. The infection can be active or latent, with approximately 10% of latent infections progressing to active status. The disease is spread by droplets from speaking, coughing, and sneezing. In the past, the disease was colloquially known by the name consumption. Diagnosis is via chest X-ray, microbacterial cultures, and tuberculin skin test.

Anti-tubercular medications: rifampicin, isoniazid, pyrazinamide, and ethambutol are FDA-approved for the treatment of *Mycobacterium tuberculosis* infections. The combination and duration on which medications to use for therapy rely on whether the patient has active or latent disease. A feared complication of tuberculosis therapy is multi-drug-resistant tuberculosis (MDR-TB). MDR-TB is distinguished from its resistance to first-line medications isoniazid and rifampicin. Therapy for MDR-TB is steadily advancing, and suggestions are continually changing. Second-line drugs that are in common use for MDR-TB are kanamycin, capreomycin, and amikacin via injections. Fluoroquinolones such as levofloxacin, moxifloxacin, and gatifloxacin are also among the common second-line agents used when drug resistance develops to the first-line agents. Drugs that have recently received FDA approval for multi-drug resistance TB are pretomanid, used in sequence with bedaquiline and linezolid. A more dangerous and uncommon type of MDR-TB is extensively multi-drug resistant tuberculosis (XDR-TB). This infection characteristically shows the resistance to first-line medications rifampicin and isoniazid, one second-line aminoglycoside, and either of the fluoroquinolones.

First Line

- Rifampicin
- Isoniazid
- Pyrazinamide
- Ethambutol

Second Line

- Kanamycin (discontinued use in the USA)
- Streptomycin
- Capreomycin
- Amikacin
- Levofloxacin
- Moxifloxacin
- Gatifloxacin

MDR-TB

- Bedaquiline
- Delamanid
- Linezolid
- Pretoman

MECHANISM OF ACTION

Rifampicin

Rifampicin exerts its effects by reversibly inhibiting DNA-dependent RNA polymerase, which further inhibits bacterial protein synthesis and transcription.

Isoniazid

Isoniazid is a pro-drug that is converted to its active form metabolite by catalase-peroxidase and exerts its action by further inhibiting the biosynthesis of mycolic acid.

Pyrazinamide

Pyrazinamide's mechanism of action remains unknown and not fully understood. Pyrazinamide is converted to its active form pyrazinoic acid and exerts its effect by inhibiting trans-translation and possibly coenzyme A synthesis needed for the bacteria to survive.

Ethambutol

Ethambutol inhibits the enzyme arabinosyl transferases and prevents the biosynthesis of the mycobacterial cell wall.

Aminoglycosides (Streptomycin, Kanamycin, Amikacin)

Aminoglycosides exert their action by binding to the 30S subunit of ribosomes and inhibiting the protein synthesis of the mycobacteria.

Fluoroquinolones (Levofloxacin, Moxifloxacin, Gatifloxacin)

Fluoroquinolones exert their effects by inhibiting DNA gyrase and topoisomerase IV, further inhibiting DNA synthesis within the bacteria.

ADMINISTRATION

Active Tuberculosis

During active disease, there are two phases for treatment: the initiation phase and the continuation phase. The initiation phase consists of two months of rifampicin, isoniazid, pyrazinamide, and ethambutol therapy. This regimen is administered orally daily for eight weeks for a total of 56 doses.

Once completed, isoniazid and rifampicin are continued for an additional four-month for the continuation phase. This regimen is administered orally daily for 18 weeks for a total of 126 doses. For patients that cannot tolerate ethambutol, streptomycin can be substituted.

Rifampicin

10 mg/kg /day

The maximum dose: 600mg

Isoniazid

5 mg/kg/day

The maximum dose: 300mg

Pyrazinamide

25 mg/kg/day

Dosages are adjusted according to weight

Ethambutol

15 to 20 mg/kg/day

Dosages are adjusted according to weight Second-line agents such as kanamycin, capreomycin, amikacin are administered as an injection, and fluoroquinolones such as moxifloxacin, gatifloxacin, and levofloxacin are administered orally. These agents are options when resistance to first-line medication develops.

Latent Tuberculosis

The most typical and used treatment for latent tuberculosis is isoniazid therapy for a duration of nine months. This regimen is administered orally daily for nine months for a total of 270 doses. A three-month combination of isoniazid and rifampicin or a fourth-month duration of rifampicin monotherapy are also possibilities. Isoniazid monotherapy can have an effect of greater than 90% on the latent disease if taken upon completion of the full nine-month duration. Pyridoxine (vitamin B6) use is advised alongside isoniazid therapy, as isoniazid use individually can cause peripheral neuropathy secondary to vitamin B6 deficiency.

Isoniazid

5 mg/kg/day

The maximum dose: 300mg

Vitamin B6

10 to 25 mg/day

Anti-tubercular medications should be taken in the daytime one hour before consuming any meals.

ADVERSE EFFECTS

Rifampicin

- Thrombocytopenia
- Neutropenia
- Orange/Red discoloration of bodily fluids
- CYP450 Inducer

Isoniazid

- Hepatotoxicity
- Vitamin B6 deficiency
- Peripheral Neuropathy

Pyrazinamide

- Hepatotoxicity
- Hyperuricemia
- Arthralgia

Ethambutol

- Optic neuropathy
- Hepatotoxicity

Aminoglycosides (Streptomycin, Kanamycin, Amikacin)

- Ototoxicity
- Nephrotoxicity

Fluoroquinolones (Levofloxacin, Moxifloxacin, Gatifloxacin)

- Tendonitis
- Tendon rupture
- Arthropathy

CONTRAINDICATIONS

Pregnancy: During pregnancy, all anti-tubercular medications are useful for treatment except for aminoglycosides. Aminoglycosides such as streptomycin, amikacin, and kanamycin may exhibit ototoxic effects on the developing foetus and are contraindicated during pregnancy.

In the USA, pyrazinamide use is avoided during pregnancy because it is a possible teratogen.

MONITORING

Liver function tests should be monitored routinely as rifampicin, isoniazid, pyrazinamide, and ethambutol all may exert hepatotoxic effects. A CBC is also required to regularly monitor patients taking rifampicin, as it can lead to thrombocytopenia and neutropenia. Rifampicin also exerts its effects by inducing cytochrome P450 (CYP450), which may cause unwanted drug interactions of medications that are metabolized by the CYP450 system and decrease their clinical efficacy. Isoniazid can cause pyridoxine deficiency that may lead to peripheral neuropathy in patients. The patient can supplement vitamin B6 to prevent this from happening. Pyrazinamide can increase uric acid concentrations and precipitate acute gout flare-ups in predisposed individuals. The recommendation is to monitor uric acid concentrations routinely for patients managed with pyrazinamide.

TOXICITY

All first-line anti-tubercular medications, rifampicin, isoniazid, pyrazinamide, and ethambutol, can exert hepatotoxic effects. A continual rise in liver functions test should prompt discontinuation of treatment. Aminoglycoside-induced nephrotoxicity is reversible when stopping the medication. Renal toxicity depends on the patient if any underlying renal disease is present and on the dose of the medication being administered. Renal insufficiency is avoidable in most patients.

ENHANCING HEALTHCARE OUTCOMES

Rifampicin, isoniazid, pyrazinamide, and ethambutol are first-line antitubercular medications, which are FDA-approved and indicated for the treatment of *Mycobacterium tuberculosis* infections. The care for patients suffering from tuberculosis prompts critical care from an interprofessional team of healthcare professionals as the preventable infectious disease can lead to medication resistance and mortality. These healthcare professionals include a primary care clinician, an infectious disease specialist, a nurse, and a pharmacist. The primary care physicians and specialists should educate the patients about the consequences of non-adherence to pharmacotherapy for the full duration and how resistance to treatment can further develop into MDR-TB and cause mortality.

Primary care clinicians should routinely monitor labs, as all four agents are hepatotoxic drugs. Counselling and careful monitoring should be conducted during pregnancy, as some second-line

medications are teratogenic. Clinicians should be up to date with the newly FDA-approved MDR-TB and their effects in the event drug resistance develops. Interprofessional communication between all team members is key to building patient rapport and developing a therapeutic alliance, so the patients adhere to therapy adequately to eradicate the bacteria and prevent further spread. This interprofessional approach with open communication channels between team members will drive better patient outcomes with fewer adverse events.

4. SENSORS

A sensor is a device that senses chemical, and biological signals and allows them to be analysed and reported. Heat, weight or gravity, noise intensity, pressure, illuminance, vibration, rate of flow of liquids and gases, magnitude of electronic and magnetic fields, and amounts of different compounds in different forms (solid, liquid or gaseous) are all physical characteristics that may be detected. Even though sensors currently are where computers were in 1970, therapeutic uses of sensors are growing off because of improvements in microprocessor technology and chemical science.

Several sensing techniques employed in industry could be used in pharmaceutics, and when novel sensors and micro-electro-mechanical devices which depend on sensors are developed and verified, in future other sectors will utilize these technologies for industrial purposes.

There seems to be a growing convergence between digital technologies and biotechnology in the medical field, and the function of sensing devices, actuators, micromachines, and signal transducers will grow as well.

The development of micro sensor systems for biological applications is gaining popularity. The medical uses of this kind of sensors are immense, notably in point-of-care and intensive care diagnostics. Significant developments are anticipated to deliver the desired enhancements inconvenience, patient safety, affordability, and response time, or even have a significant influence on healthcare in the early twenty-first century. During the last 2 years, the latest findings have emerged, such as sensor convergence with micromachined analysis tools, massive development of geno sensors, the genetic configuration of biorecognition element, extremely fast supervision of dynamic events in microscopic settings, electrodes, and the adoption of innovative sensing materials. Such new domains, coupled with ongoing research into innovative biocatalystsand selective biosensors based on acoustic, electrochemical, optical, or piezoelectric transducers, will undoubtedly increase the therapeutic use of biosensors and electroanalysis.

The latest advancements of sensors and biosensors is mostly owing to two factors, one of technological nature and another of sociological nature. For several years, the necessary monitoring to assure people's safety and protection was dependent on separation methods, primarily chromatographic strategies paired with high-sensitivity detection techniques, beginning with mass spectroscopy. This method is highly capital intensive, lengthy, and laborious, thus fast and inexpensive methods are explored. However, civil society is paying more attention and focus to the safety of foods and nutritional supplements, owing to a widespread desire to live a safer life.

BIOSENSORS

A biosensor is a device that detects biological and chemical responses by producing signals corresponding to the concentration of the analyte in the reaction. Biosensors should be incredibly precise and consistent, irrespective of external factors such as pH and temperature.

Biosensors are used in the medicinal field for disease management, drug development, as well as for detecting contaminants, infective microorganisms, and disease markers in biological fluids (blood,

urine, saliva, sweat). The components of a typical biosensor include a bioreceptor, a transducer and a signal amplifier. DNA probes, antibodies, enzymes, and cell receptors that bind with the analyte can all be used as biosensing substances. The transducers, which can be made of acoustic, optical, physicochemical, or piezoelectric material, convert biological input to optical and electrical output.

APPLICATIONS OF BIOSENSORS

Biosensors have been used in a variety of areas, including the food industry, pharmaceutics, and the marine sector. They give more stability and sensitivity than traditional techniques.

- In the case of a biological attack, biosensors have been utilized for strategic operations. The primary goal of these biosensors is to detect and identify organisms that pose a danger on time, known as biowarfare agents (BWAs), like bacteria, toxic chemicals, and viruses. Numerous efforts have been made to design such biosensors utilizing molecular methods capable of recognizing the biomarkers of BWAs.

- Biosensors can be used to evaluate processing conditions passively by analysing the presence of products, biomass, enzymes, antibodies, or by-products of the process. Because of their simple apparatus, impressive selectivity, low costs, and ease of automated processes, biosensors accurately manage the fermentation process and give consistent results.
- Biosensors are widely utilized in the medical community to detect infectious conditions.

- The advent of biosensors as a result of the requirement for simplified, authentic, precise, and low-cost approaches, appears to be advantageous in food processing, control, food validity, safety, and quality. Biosensors have been employed to detect pathogens in food.

- Biosensors have also been used to discover lacking components related to analyte metabolism, modulation, or transportation. A transportation process in phloem loading-sucrose outflow is performed by a fluorescence resonance energy transfer (FRET) sensor for sucrose, which is important for protein detection.
- Fluorescent biosensors have been employed in drug discovery and development to identify drugs using high throughput, high content testing techniques, as well as for the post screening interpretation of the findings and lead optimization. These are thought to be effective methods for the clinical and preclinical analysis of prospective drugs' therapeutic properties, bioavailability, and pharmacokinetics.

5. THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is a chromatography technique that separates components in non-volatile mixtures.

It is performed on a TLC plate made up of a non-reactive solid coated with a thin layer of adsorbent material. This is called the stationary phase. The sample is deposited on the plate, which is eluted with a solvent or solvent mixture known as the mobile phase (or eluent). This solvent then moves up the plate via capillary action. As with all chromatography, some compounds are more attracted to the mobile phase, while others are more attracted to the stationary phase. Therefore, different compounds move up the TLC plate at different speeds and become separated. To visualize colourless compounds, the plate is viewed under UV-light or is stained. Testing different stationary and

mobile phases is often necessary to obtain well-defined and separated spots.

TLC is quick, simple, and gives high sensitivity for a relatively low cost. It can monitor reaction progress, identify compounds in a mixture, determine purity, or purify small amounts of compound.

PROCEDURE

The process for TLC is similar to paper chromatography but provides faster runs, better separations, and the choice between different stationary phases. Plates can be labelled before or after the chromatography process with a pencil or other implement that will not interfere with the process.

There are four main stages to running a thin-layer chromatography plate:

Plate preparation: Using a capillary tube, a small amount of a concentrated solution of the sample is deposited near the bottom edge of a TLC plate. Solvent completely evaporate before the next step. A vacuum chamber may be necessary for non-volatile solvents. To make sure there is sufficient compound to obtain a visible result, the spotting procedure can be repeated. Depending on the application, they may also place different samples in a row, the same distance from the bottom edge; Each sample will move up the plate in its own "lane."

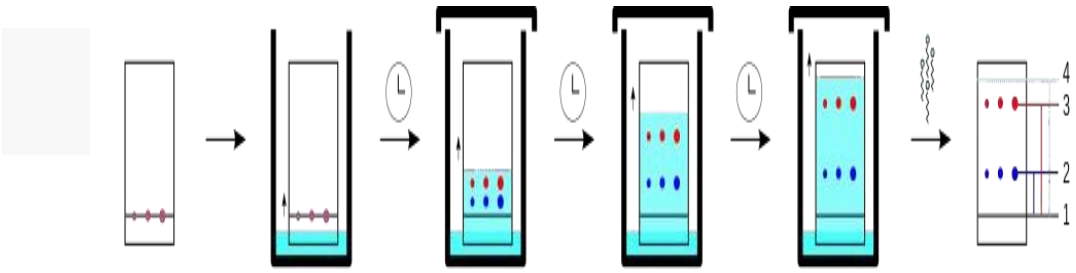
Development chamber preparation: The solvent or solvent mixture is placed into a transparent container (separation/development chamber) to a depth of less than 1 centimetre. They then place a strip of filter paper (aka "wick") along the container wall. This filter paper should touch the solvent and almost reach the top of the container. The container is covered with a lid and the solvent vapours are allowed to saturated the atmosphere of the container. Failure to do so results in poor separation and non-reproducible results.

Development: The TLC plate is placed in the container such that the sample spot(s) are not submerged into the mobile phase. The container is covered to prevent solvent evaporation. The solvent migrates up the plate by capillary action, meets the sample mixture, and carries it up the plate (elutes the sample). The plate is removed from the container before the solvent reaches the top of the plate. Otherwise, the results will be misleading. Without delay, they mark the *solvent front:* the furthest extent of solvent up the plate.

Visualization: The solvent evaporate from the plate. Visualization methods include UV-light, staining, and many more.

SEPARATION PROCESS AND PRINCIPLE

The separation of compounds is due to the differences in their attraction to the stationary phase and because of differences in solubility in the solvent. As a result, the compounds and the mobile phase compete for binding sites on the stationary phase. Different compounds in the sample mixture travel at different rates due to the differences in their partition coefficients. Different solvents, or different solvent mixtures, gives different separation. The retardation factor (*R*f), or *retention factor*, quantifies the results. It is the distance travelled by a given substance divided by the distance travelled by the mobile phase.



In normal phase TLC, the stationary phase is polar. Silica gel is very common in normal-phase TLC. More polar compounds in a sample mixture interact more strongly with the polar stationary phase. As a result, more polar compounds move less (resulting in smaller *R*f) while less polar compounds move higher up the plate (higher *R*f). A more polar mobile phase also binds more strongly to the plate, competing more with the compound for binding sites; A more polar mobile phase also dissolves polar compounds more. As such, all compounds on the TLC plate move higher up the plate in polar solvent mixtures. "Strong" solvents move compounds higher up the plate, whereas "weak" solvents move them less.

If the stationary phase is non-polar, like C18-functionalized silica plates, it is called reverse-phase TLC. In this case, non-polar compounds move less and polar compounds move more. The solvent mixture will also be much more polar than in normal-phase TLC.

SOLVENT CHOICE

An eluotropic series, which orders solvents by how much they move compounds, can help in selecting a mobile phase. Solvents are also divided into solvent selectivity groups. Using solvents with different elution strengths or different selectivity groups can often give very different results. While single-solvent mobile phases can sometimes give good separation, some cases may require solvent mixtures.

In normal-phase TLC, the most common solvent mixtures include ethyl acetate/hexanes (EtOAc/Hex) for less polar compounds and methanol/dichloromethane (MeOH/DCM) for more polar compounds. Different solvent mixtures and solvent ratios can help give better separation. In reverse-phase TLC, solvent mixtures are typically water with a less-polar solvent: Typical choices are water with tetrahydrofuran (THF), acetonitrile (ACN), or methanol.

ANALYSIS

As the chemicals being separated may be colourless, several methods exist to visualize the spots:

- Placing the plate under blacklight (366 nm light) makes fluorescent compounds glow TLC plates containing a small amount of fluorescent compound (usually manganese-activated zinc silicate) in the adsorbent layer allow for visualization of some compounds under UV-C light (254 nm). The adsorbent layer will fluoresce light-green, while spots containing compounds that absorb UV-C light will not.
- Placing the plate in a container filled with Iodine vapours temporarily stain the spots. They typically become a yellow or brown colour.
- The TLC plate can either be dipped in or sprayed with a stain. Many stains exist but some examples include:
  - Potassium permanganate
  - Bromine

- Acidic vanillin
- Phosphomolybdic acid

- In the case of lipids, the chromatogram may be transferred to a polyvinylidene fluoride membrane and then subjected to further analysis, for example, mass spectrometry. This technique is known as far-eastern plot.

PLATE PRODUCTION

TLC plates are usually commercially available, with standard particle size ranges to improve reproducibility. They are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like calcium sulphate (gypsum) and water. This mixture is spread as a thick slurry on an unreactive carrier sheet, usually glass, thick aluminium foil, or plastic. The resultant plate is dried and *activated* by heating in an oven for thirty minutes at 110 °C. The thickness of the absorbent layer is typically around 0.1–0.25 mm for analytical purposes and around 0.5–2.0 mm for preparative TLC. Other adsorbent coatings include aluminium oxide (alumina), or cellulose.

APPLICATIONS

Reaction monitoring and characterization

TLC is a useful tool for reaction monitoring. For this, the plate normally contains a spot of starting material, a spot from the reaction mixture, and a co-spot (or cross-spot) containing both. The analysis will show if the starting material disappeared and if any new products appeared. This provides a quick and easy way to estimate how far a reaction has proceeded. In one study, TLC has been applied in the screening of organic reactions. The researchers react an alcohol and a catalyst directly in the co-spot of a TLC plate before developing it. This provides quick and easy small-scale testing of different reagents.

TLC for reaction monitoring and choosing a purification solvent mixture (left)TLC from the resulting flash column chromatography (right).

Compound characterization with TLC is also possible and is similar to reaction monitoring. However, rather than spotting with starting material and reaction mixture, it is with an unknown and a known compound. They may be the same compound if both spots have the same *R*f and look the same under the chosen visualization method. However, co-elution complicates both reaction monitoring and characterization. This is because different compounds will move to the same spot on the plate. In such cases, different solvent mixtures may provide better separation.

Purity and purification

TLC helps show the purity of a same. A pure sample should only contain one spot by TLC. TLC is also useful for small-scale purification. Because the separated compounds will be on different areas of the plate, a scientist can scrape off the stationary phase particles containing the desired compound and dissolve them into an appropriate solvent. Once all the compound dissolves in the solvent, they filter out the silica particles, then evaporate the solvent to isolate the product. Big preparative TLC plates with thick silica gel coatings can separate more than 100 mg of material.

For larger-scale purification and isolation, TLC is useful to quickly test solvent mixtures before running flash column chromatography on a large batch of impure material. A compound elutes from a column when the amount of solvent collected is equal to 1/*R*f. The eluent from flash column chromatography gets collected across several containers (for example, test tubes) called fractions. TLC helps show which fractions contain impurities and which contain pure compound.

Furthermore, two-dimensional TLC can help check if a compound is stable on a particular stationary phase. This test requires two runs on a square-shaped TLC plate. The plate is rotated by 90º before the second run. If the target compound appears on the diagonal of the square, it is stable on the chosen stationary phase. Otherwise, it is decomposing on the plate. If this is the case, an alternative stationary phase may prevent this decomposition.

TLC is also an analytical method for the direct separation of enantiomers and the control of enantiomeric purity, e.g. active pharmaceutical ingredients (APIs) that are chiral.

6. pH METER

A **pH meter** is a scientific instrument that measures the hydrogen-ion activity in water-based solutions, indicating its acidity or alkalinity expressed as pH. The pH meter measures the difference in electrical potential between a pH electrode and a reference electrode, and so the pH meter is sometimes referred to as a "potentiometric pH meter". The difference in electrical potential relates to the acidity or pH of the solution. Testing of pH via pH meters (**pH-meter**) is used in many applications ranging from laboratory experimentation to quality control.

Principle of operation

Potentiometric pH meters measure the voltage between two electrodes and display the result converted into the corresponding pH value. They comprise a simple electronic amplifier and a pair of electrodes, or alternatively a combination electrode, and some form of display calibrated in pH units. It usually has a glass electrode and a reference electrode, or a combination electrode. The electrodes, or probes, are inserted into the solution to be tested. pH meters may also be based on the antimony electrode (typically used for rough conditions) or the quinhydrone electrode.

In order to accu

rately measure the potential difference between the two sides of the glass membrane reference electrode, typically a silver chloride electrode or calomel electrode are required on each side of the membrane. Their purpose is to measure changes in the potential on their respective side. One is built into the glass electrode. The other, which makes contact with the test solution through a porous plug, may be a separate reference electrode or may be built into a combination electrode. The resulting voltage will be the potential difference between the two sides of the glass membrane possibly offset by some difference between the two reference electrodes, that can be compensated for. The article on the glass electrode has a good description and figure.

The design of the electrodes is the key part: These are rod-like structures usually made of glass, with a bulb containing the sensor at the bottom. The glass electrode for measuring the pH has a glass bulb specifically designed to be selective to hydrogen-ion concentration. On immersion in the solution to be tested, hydrogen ions in the test solution exchange for other positively charged ions on the glass bulb, creating an electrochemical potential across the bulb. The electronic amplifier detects the difference in electrical potential between the two electrodes generated in the measurement and converts the potential difference to pH units. The magnitude of the electrochemical potential across the glass bulb is linearly related to the pH according to the Nernst equation.

The reference electrode is insensitive to the pH of the solution, being composed of a metallic conductor, which connects to the display. This conductor is immersed in an electrolyte solution, typically potassium chloride, which comes into contact with the test solution through a porous ceramic membrane. The display consists of a voltmeter, which displays voltage in units of pH.



On immersion of the glass electrode and the reference electrode in the test solution, an electrical circuit is completed, in which there is a potential difference created and detected by the voltmeter. The circuit can be thought of as going from the conductive element of the reference electrode to the surrounding potassium-chloride solution, through the ceramic membrane to the test solution, the hydrogen-ion-selective glass of the glass electrode, to the solution inside the glass electrode, to the silver of the glass electrode, and finally the voltmeter of the display device. The voltage varies from test solution to test solution depending on the potential difference created by the difference in hydrogen-ion concentrations on each side of the glass membrane between the test solution and the solution inside the glass electrode. All other potential differences in the circuit do not vary with pH and are corrected for by means of the calibration.

For simplicity, many pH meters use a combination probe, constructed with the glass electrode and the reference electrode contained within a single probe. A detailed description of combination electrodes is given in the article on glass electrodes.

The pH meter is calibrated with solutions of known pH, typically before each use, to ensure accuracy of measurement. To measure the pH of a solution, the electrodes are used as probes, which are dipped into the test solutions and held there sufficiently long for the hydrogen ions in the test solution to equilibrate with the ions on the surface of the bulb on the glass electrode. This equilibration provides a stable pH measurement.

pH electrode and reference electrode design

Details of the fabrication and resulting microstructure of the glass membrane of the pH electrode are maintained as trade secrets by the manufacturers. However, certain aspects of design are published. Glass is a solid electrolyte, for which alkali-metal ions can carry current. The pH-sensitive glass membrane is generally spherical to simplify the manufacture of a uniform membrane. These membranes are up to 0.4 millimetres in thickness, thicker than original designs, so as to render the probes durable. The glass has silicate chemical functionality on its surface, which provides binding sites for alkali-metal ions and hydrogen ions from the solutions. This provides an ion-exchange capacity in the range of 10^{-6} to 10^{-8} mol/cm^2. Selectivity for hydrogen

ions (H^+) arises from a balance of ionic charge, volume requirements versus other ions, and the coordination number of other ions. Electrode manufacturers have developed compositions that suitably balance these factors, most notably lithium glass.

The silver chloride electrode is most commonly used as a reference electrode in pH meters, although some designs use the saturated calomel electrode. The silver chloride electrode is simple to manufacture and provides high reproducibility. The reference electrode usually consists of a platinum wire that has contact with a silver/silver chloride mixture, which is immersed in a potassium chloride solution. There is a ceramic plug, which serves as a contact to the test solution, providing low resistance while preventing mixing of the two solutions.

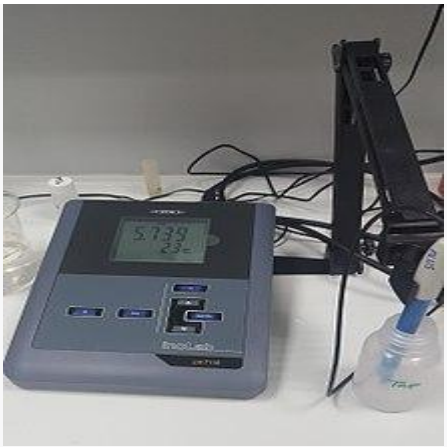
With these electrode designs, the voltmeter is detecting potential differences of

±1400 millivolts. The electrodes are further designed to rapidly equilibrate with test solutions to facilitate ease of use. The equilibration times are typically less than one second, although equilibration times increase as the electrodes age.

Maintenance

Because of the sensitivity of the electrodes to contaminants, cleanliness of the probes is essential for accuracy and precision. Probes are generally kept moist when not in use with a medium appropriate for the particular probe, which is typically an aqueous solution available from probe manufacturers. Probe manufacturers provide instructions for cleaning and maintaining their probe designs. For illustration, one maker of laboratory-grade pH gives cleaning instructions for specific contaminants: general cleaning (15-minute soak in a solution of bleach and detergent), salt (hydrochloric acid solution followed by sodium hydroxide and water), grease (detergent or methanol), clogged reference junction (KCl solution), protein deposits (pepsin and HCl, 1% solution), and air bubbles.

Calibration and operation



The German Institute for Standardization publishes a standard for pH measurement using pH meters, DIN 19263.

Very precise measurements necessitate that the pH meter is calibrated before each measurement. More typically calibration is performed once per day of operation. Calibration is needed because the glass electrode does not give reproducible electrostatic potentials over longer periods of time.

Consistent with principles of good laboratory practice, calibration is performed with at least two standard buffer solutions that span the range of pH values to be measured. For general purposes, buffers at pH 4.00 and pH 10.00 are suitable. The pH meter has one calibration control to set the meter reading equal to the value of the first standard buffer and a second control to adjust the meter

reading to the value of the second buffer. A third control allows the temperature to be set. Standard buffer sachets, available from a variety of suppliers, usually document the temperature dependence of the buffer control. More precise measurements sometimes require calibration at three different pH values. Some pH meters provide built-in temperature-coefficient correction, with temperature thermocouples in the electrode probes.

The calibration process correlates the voltage produced by the probe (approximately 0.06 volts per pH unit) with the pH scale. Good laboratory practice dictates that, after each measurement, the probes are rinsed with distilled water or deionized water to remove any traces of the solution being measured, blotted with a scientific wipe to absorb any remaining water, which could dilute the sample and thus alter the reading, and then immersed in a storage solution suitable for the particular probe type.

TYPES OF pH METERS



A simple pH meter



Soil pH meter

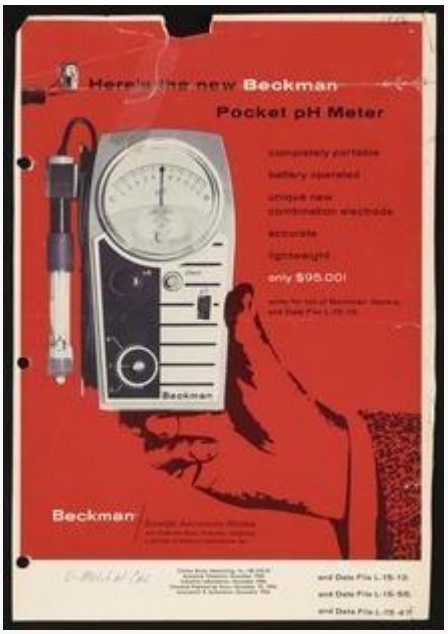
In general there are three major categories of pH meters. Benchtop pH meters are often used in laboratories and are used to measure samples which are brought to the pH meter for analysis. Portable, or field pH meters, are handheld pH meters that are used to take the pH of a sample in a field or production site. In-line or in situ pH meters, also called pH analysers, are used to measure pH continuously in a process, and can stand-alone, or be connected to a higher level information system for process control.

pH meters range from simple and inexpensive pen-like devices to complex and expensive laboratory instruments with computer interfaces and several inputs for indicator and temperature measurements to be entered to adjust for the variation in pH caused by temperature. The output can be digital or analogue, and the devices can be battery-powered or rely on line power. Some versions use telemetry to connect the electrodes to the voltmeter display device.

Specialty meters and probes are available for use in special applications, such as harsh environments and biological microenvironments. There are also holographic pH sensors, which allow pH

measurement calorimetrically, making use of the variety of pH indicators that are available. Additionally, there are commercially available pH meters based on solid state electrodes, rather than conventional glass electrodes.

HISTORY



"Here's the new Beckman Pocket pH Meter", 1956

The concept of pH was defined in 1909 by S. P. L. Sørensen, and electrodes were used for pH measurement in the 1920s.

In October 1934, Arnold Orville Beckman registered the first patent for a complete chemical instrument for the measurement of pH, U.S. Patent No. 2,058,761, for his "acidimeter", later renamed the pH meter. Beckman developed the prototype as an assistant professor of chemistry at the California Institute of Technology, when asked to devise a quick and accurate method for measuring the acidity of lemon juice for the California Fruit Growers Exchange (Sunkist).

On April 8, 1935, Beckman's renamed National Technical Laboratories focused on the manufacture of scientific instruments, with the Arthur H. Thomas Company as a distributor for its pH meter. In its first full year of sales, 1936, the company sold 444 pH meters for $60,000 in sales. In years to come, the company sold millions of the units. In 2004 the Beckman pH meter was designated an ACS National Historic Chemical Landmark in recognition of its significance as the first commercially successful electronic pH meter.

The Radiometer Corporation of Denmark was founded in 1935, and began marketing a pH meter for medical use around 1936, but "the development of automatic pH-meters for industrial purposes was neglected. Instead American instrument makers successfully developed industrial pH-meters with a wide variety of applications, such as in breweries, paper works, alum works, and water treatment systems."

In the 1940s the electrodes for pH meters were often difficult to make, or unreliable due to brittle glass. Dr. Werner Ingold began to industrialize the production of single-rod measuring cells, a combination of measurement and reference electrode in one construction unit, which led to broader acceptance in a wide range of industries including pharmaceutical production.

Beckman marketed a portable "Pocket pH Meter" as early as 1956, but it did not have a digital read-out. In the 1970s Jenco Electronics of Taiwan designed and manufactured the first portable digital pH meter. This meter was sold under the label of the Cole-Parmer Corporation.

APPLICATIONS

The rate and outcome of chemical reactions taking place in water often depends on the acidity of the water, and it is therefore useful to know the acidity of the water, typically measured by means of a pH meter. Knowledge of pH is useful or critical in many situations, including chemical laboratory analyses. pH meters are used for soil measurements in agriculture, water quality for municipal water supplies, swimming pools, environmental remediation; brewing of wine or beer; manufacturing, healthcare and clinical applications such as blood chemistry; and many other applications.

Advances in the instrumentation and in detection have expanded the number of applications in which pH measurements can be conducted. The devices have been miniaturized, enabling direct measurement of pH inside of living cells. In addition to measuring the pH of liquids, specially designed electrodes are available to measure the pH of semi-solid substances, such as foods. These have tips suitable for piercing semi-solids, have electrode materials compatible with ingredients in food, and are resistant to clogging.

7. MASS SPECTROMETRY

Mass spectrometry (**MS**) is an analytical technique that is used to measure the mass-to-charge ratio of ions. The results are presented as a *mass spectrum*, a plot of intensity as a function of the mass-to-charge ratio. Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures.

A mass spectrum is a type of plot of the ion signal as a function of the mass-to-charge ratio. These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical identity or structure of molecules and other chemical compounds.

In a typical MS procedure, a sample, which may be solid, liquid, or gaseous, is ionized, for example by bombarding it with a beam of electrons. This may cause some of the sample's molecules to break up into positively charged fragments or simply become positively charged without fragmenting. These ions (fragments) are then separated according to their mass-to-charge ratio, for example by accelerating them and subjecting them to an electric or magnetic field: ions of the same mass-to-charge ratio will undergo the same amount of deflection.[1] The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the signal intensity of detected ions as a function of the mass-to-charge ratio. The atoms or molecules in the sample can be identified by correlating known masses (e.g.: an entire molecule) to the identified masses or through a characteristic fragmentation pattern.

HISTORY OF MASS SPECTROMETRY

In 1886, Eugen Goldstein observed rays in gas discharges under low pressure that travelled away from the anode and through channels in a perforated cathode, opposite to the direction of negatively charged cathode rays (which travel from cathode to anode). Goldstein called these positively charged anode rays "Kanalstrahlen"; the standard translation of this term into English is "canal rays". Wilhelm Wien found that strong electric or magnetic fields deflected the canal rays and, in 1899, constructed a device with perpendicular electric and magnetic fields that separated the positive rays according to

their charge-to-mass ratio (*Q/m*). Wien found that the charge-to-mass ratio depended on the nature of the gas in the discharge tube. English scientist J. J. Thomson later improved on the work of Wien by reducing the pressure to create the mass spectrograph.

The word *spectrograph* had become part of the international scientific vocabulary by 1884. Early *spectrometry* devices that measured the mass-to-charge ratio of ions were called *mass spectrographs* which consisted of instruments that recorded a spectrum of mass values on a photographic plate. A *mass spectroscope* is similar to a *mass spectrograph* except that the beam of ions is directed onto a phosphor screen. A mass spectroscope configuration was used in early instruments when it was desired that the effects of adjustments be quickly observed. Once the instrument was properly adjusted, a photographic plate was inserted and exposed. The term mass spectroscope continued to be used even though the direct illumination of a phosphor screen was replaced by indirect measurements with an oscilloscope. The use of the term *mass spectroscopy* is now discouraged due to the possibility of confusion with light spectroscopy. Mass spectrometry is often abbreviated as *mass-spec* or simply as *MS*.

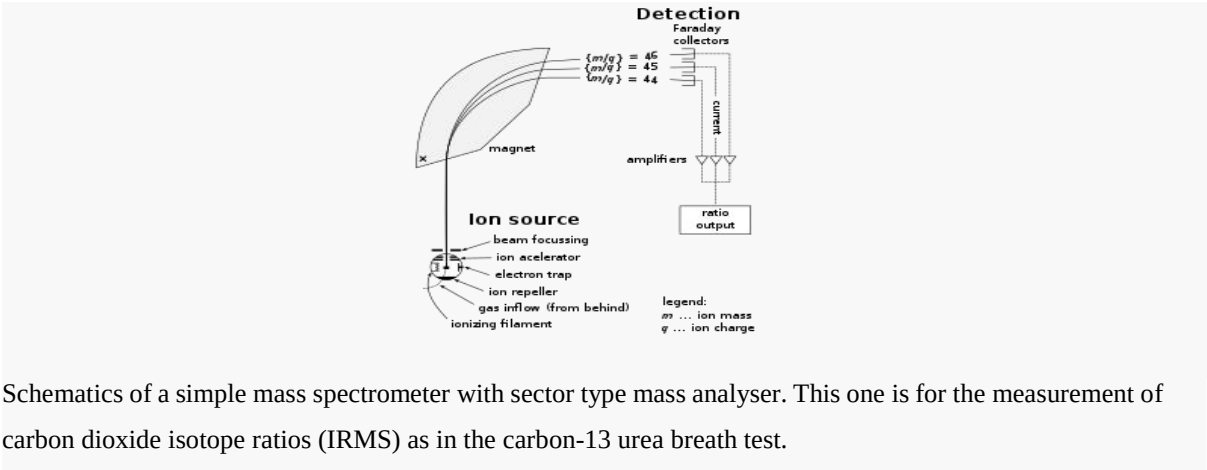
Modern techniques of mass spectrometry were devised by Arthur Jeffrey Dempster and F.W. Aston in 1918 and 1919 respectively.

Sector mass spectrometers known as calutrons were developed by Ernest O. Lawrence and used for separating the isotopes of uranium during the Manhattan Project.[9] Calutron mass spectrometers were used for uranium enrichment at the Oak Ridge, Tennessee Y-12 plant established during World War II.

In 1989, half of the Nobel Prize in Physics was awarded to Hans Dehmelt and Wolfgang Paul for the development of the ion trap technique in the 1950s and 1960s.

In 2002, the Nobel Prize in Chemistry was awarded to John Bennett Fenn for the development of electrospray ionization (ESI) and Koichi Tanaka for the development of soft laser desorption (SLD) and their application to the ionization of biological macromolecules, especially proteins.

PARTS OF MASS SPECTROMETRY



Schematics of a simple mass spectrometer with sector type mass analyser. This one is for the measurement of carbon dioxide isotope ratios (IRMS) as in the carbon-13 urea breath test.

A mass spectrometer consists of three components: an ion source, a mass analyser, and a detector. The *ionizer* converts a portion of the sample into ions. There is a wide variety of ionization techniques, depending on the phase (solid, liquid, gas) of the sample and the efficiency of various ionization mechanisms for the unknown species. An extraction system removes ions from the sample, which are then targeted through the mass analyser and into the *detector*. The differences in masses of

the fragments allows the mass analyser to sort the ions by their mass-to-charge ratio. The detector measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present. Some detectors also give spatial information, e.g., a multichannel plate.

Theoretical example

The following describes the operation of a spectrometer mass analyser, which is of the sector type. (Other analyser types are treated below.) Consider a sample of sodium chloride (table salt). In the ion source, the sample is vaporized (turned into gas) and ionized (transformer into electrically charged particles) into sodium (Na^+) and chloride (Cl^-) ions. Sodium atoms and ions are monoisotopic, with a mass of about 23 u. Chloride atoms and ions come in two stable isotopes with masses of approximately 35 u (at a natural abundance of about 75 percent) and approximately 37 u (at a natural abundance of about 25 percent). The analyser part of the spectrometer contains electric and magnetic fields, which exert forces on ions traveling through these fields. The speed of a charged particle may be increased or decreased while passing through the electric field, and its direction may be altered by the magnetic field. The magnitude of the deflection of the moving ion's trajectory depends on its mass-to-charge ratio. Lighter ions get deflected by the magnetic force more than heavier ions (based on Newton's second law of motion, $F = ma$). The streams of sorted ions pass from the analyser to the detector, which records the relative abundance of each ion type. This information is used to determine the chemical element composition of the original sample (i.e. that both sodium and chlorine are present in the sample) and the isotopic composition of its constituents (the ratio of ^{35}Cl to ^{37}Cl).

CREATING IONS

Surface ionization source at the Argonne National Laboratory linear accelerator.

The ion source is the part of the mass spectrometer that ionizes the material under analysis (the analyte). The ions are then transported by magnetic or electric fields to the mass analyser.

Techniques for ionization have been key to determining what types of samples can be analysed by mass spectrometry. Electron ionization and chemical ionization are used for gases and vapours. In chemical ionization sources, the analyte is ionized by chemical ion-molecule reactions during collisions in the source. Two techniques often used with liquid and solid biological samples include electrospray ionization (invented by John Fenn) and matrix-assisted laser desorption/ionization (MALDI, initially developed as a similar technique "Soft Laser Desorption (SLD)" by K. Tanaka for which a Nobel Prize was awarded and as MALDI by M. Karas and F. Hillenkamp).

Hard ionization and Soft ionization



Quadrupole mass spectrometer and electrospray ion source used for Fenn's early work

In mass spectrometry, ionization refers to the production of gas phase ions suitable for resolution in the mass analyser or mass filter. Ionization occurs in the ion source. There are several ion sources available; each has advantages and disadvantages for particular applications. For example, electron ionization (EI) gives a high degree of fragmentation, yielding highly detailed mass spectra which when skilfully analysed can provide important information for structural elucidation/characterisation and facilitate identification of unknown compounds by comparison to mass spectral libraries obtained under identical operating conditions. However, EI is not suitable for coupling to HPLC, i.e. LC-MS, since at atmospheric pressure, the filaments used to generate electrons burn out rapidly. Thus EI is coupled predominantly with GC, i.e. GC-MS, where the entire system is under high vacuum.

Hard ionization techniques are processes which impart high quantities of residual energy in the subject molecule invoking large degrees of fragmentation (i.e. the systematic rupturing of bonds acts to remove the excess energy, restoring stability to the resulting ion). Resultant ions tend to have *m/z* lower than the molecular ion (other than in the case of proton transfer and not including isotope peaks). The most common example of hard ionization is electron ionization (EI).

Soft ionization refers to the processes which impart little residual energy onto the subject molecule and as such result in little fragmentation. Examples include fast atom bombardment (FAB), chemical ionization (CI), atmospheric-pressure chemical ionization (APCI), atmospheric-pressure photoionization (APPI), electrospray ionization (ESI), desorption electrospray ionization (DESI), and matrix-assisted laser desorption/ionization (MALDI).

Inductively coupled plasma

Inductively coupled plasma (ICP) sources are used primarily for cation analysis of a wide array of sample types. In this source, a plasma that is electrically neutral overall, but that has had a substantial fraction of its atoms ionized by high temperature, is used to atomize introduced sample molecules and to further strip the outer electrons from those atoms. The plasma is usually generated from argon gas, since the first ionization energy of argon atoms is higher than the first of any other elements except He, F and Ne, but lower than the second ionization energy of all except the most electropositive metals. The heating is achieved by a radio-frequency current passed through a coil surrounding the plasma.

Photoionization mass spectrometry

Photoionization can be used in experiments which seek to use mass spectrometry as a means of resolving chemical kinetics mechanisms and isomeric product branching.[14] In such instances a high energy photon, either X-ray or UV, is used to dissociate stable gaseous molecules in a carrier gas of He or Ar. In instances where a synchrotron light source is utilized, a tuneable photon energy can be utilized to acquire a photoionization efficiency curve which can be used in conjunction with the charge ratio m/z to fingerprint molecular and ionic species. More recently atmospheric pressure photoionization (APPI) has been developed to ionize molecules mostly as effluents of LC-MS systems.

Ambient ionization

Some applications for ambient ionization include environmental applications as well as clinical applications. In these techniques, ions form in an ion source outside the mass spectrometer. Sampling becomes easy as the samples don't need previous separation nor preparation. Some examples of ambient ionization techniques are DESI, SESI, LAESI, desorption atmospheric-pressure chemical ionization (DAPCI), and desorption atmospheric pressure photoionization DAPPI among others.

Other ionization techniques

Others include glow discharge, field desorption (FD), fast atom bombardment (FAB), thermo spray, desorption/ionization on silicon (DIOS), Direct Analysis in Real Time (DART), atmospheric pressure chemical ionization (APCI), secondary ion mass spectrometry (SIMS), spark ionization and thermal ionization (TIMS).

Mass Selection

Mass analysers separate the ions according to their mass-to-charge ratio. The following two laws govern the dynamics of charged particles in electric and magnetic fields in vacuum:

$\mathbf{F} = Q\,(\mathbf{E} + \mathbf{v} \times \mathbf{B})$ (Lorentz force law);

$\mathbf{F} = m\mathbf{a}$ (Newton's second law of motion in the non-relativistic case, i.e. valid only at ion velocity much lower than the speed of light).

Here **F** is the force applied to the ion, m is the mass of the ion, **a** is the acceleration, Q is the ion charge, **E** is the electric field, and $\mathbf{v} \times \mathbf{B}$ is the vector cross product of the ion velocity and the magnetic field

Equating the above expressions for the force applied to the ion yields:
$(m/Q)\,\mathbf{a} = \mathbf{E} + \mathbf{v} \times \mathbf{B}$

This differential equation is the classic equation of motion for charged particles. Together with the particle's initial conditions, it completely determines the particle's motion in space and time in terms of m/Q. Thus, mass spectrometers could be thought of as "mass-to-charge spectrometers". When presenting data, it is common to use the (officially) dimensionless m/z, where z is the number of elementary charges (e) on the ion (z=Q/e). This quantity, although it is informally called the mass-to-charge ratio, more accurately speaking represents the ratio of the mass number and the charge number, z.

There are many types of mass analysers, using either static or dynamic fields, and magnetic or electric

fields, but all operate according to the above differential equation. Each analyser type has its strengths and weaknesses. Many mass spectrometers use two or more mass analysers for tandem mass spectrometry (MS/MS). In addition to the more common mass analysers listed below, there are others designed for special situations.

There are several important analyser characteristics. The mass resolving power is the measure of the ability to distinguish two peaks of slightly different *m/z*. The mass accuracy is the ratio of the *m/z* measurement error to the true m/z. Mass accuracy is usually measured in ppm or milli mass units. The mass range is the range of *m/z* amenable to analysis by a given analyser. The linear dynamic range is the range over which ion signal is linear with analyte concentration. Speed refers to the time frame of the experiment and ultimately is used to determine the number of spectra per unit time that can be generated.

Sector instruments

A sector field mass analyser uses a static electric and/or magnetic field to affect the path and/or velocity of the charged particles in some way. Sector instruments bend the trajectories of the ions as they pass through the mass analyser, according to their mass-to-charge ratios, deflecting the more charged and faster-moving, lighter ions more. The analyser can be used to select a narrow range of *m/z* or to scan through a range of *m/z* to catalog the ions present.

Time-of-flight

The time-of-flight (TOF) analyser uses an electric field to accelerate the ions through the same potential, and then measures the time they take to reach the detector. If the particles all have the same charge, their kinetic energies will be identical, and their velocities will depend only on their masses. Ions with a lower mass will reach the detector first. However, in reality, even particles with the same m/z can arrive at different times at the detector, because they have different initial velocities. The initial velocity is often not dependent on the mass of the ion, and will turn into a difference in the final velocity. Because of this, ions with the same m/z ratio will reach the detector at a variety of times, which broadens the peaks shown on the count vs m/z plot, but will generally not change the central location of the peaks, since the starting velocity of ions is generally centred at zero. To fix this problem, time-lag focusing/delayed extraction has been coupled with TOF-MS.

Quadrupole mass filter

Quadrupole mass analysers use oscillating electrical fields to selectively stabilize or destabilize the paths of ions passing through a radio frequency (RF) quadrupole field created between four parallel rods. Only the ions in a certain range of mass/charge ratio are passed through the system at any time, but changes to the potentials on the rods allow a wide range of m/z values to be swept rapidly, either continuously or in a succession of discrete hops. A quadrupole mass analyser acts as a mass-selective filter and is closely related to the quadrupole ion trap, particularly the linear quadrupole ion trap except that it is designed to pass the untrapped ions rather than collect the trapped ones, and is for that reason referred to as a transmission quadrupole. A magnetically enhanced quadrupole mass analyser includes the addition of a magnetic field, either applied axially or transversely. This novel type of instrument leads to an additional performance enhancement in terms of resolution and/or sensitivity depending upon the magnitude and orientation of the applied magnetic field. A common variation of the transmission quadrupole is the triple quadrupole mass spectrometer. The "triple quad" has three consecutive quadrupole stages, the first acting as a mass filter to transmit a particular incoming ion to the second quadrupole, a collision chamber, wherein that ion can be broken into fragments. The third quadrupole also acts as a mass filter, to transmit a particular

fragment ion to the detector. If a quadrupole is made to rapidly and repetitively cycle through a range of mass filter settings, full spectra can be reported. Likewise, a triple quad can be made to perform various scan types characteristic of tandem mass spectrometry.

ION TRAPS

Three-dimensional quadrupole ion trap

The quadrupole ion trap works on the same physical principles as the quadrupole mass analyser, but the ions are trapped and sequentially ejected. Ions are trapped in a mainly quadrupole RF field, in a space defined by a ring electrode (usually connected to the main RF potential) between two endcap electrodes (typically connected to DC or auxiliary AC potentials). The sample is ionized either internally (e.g.: with an electron or laser beam), or externally, in which case the ions are often introduced through an aperture in an endcap electrode.

There are many mass/charge separation and isolation methods but the most commonly used is the mass instability mode in which the RF potential is ramped so that the orbit of ions with a mass $a > b$ are stable while ions with mass b become unstable and are ejected on the z-axis onto a detector. There are also non-destructive analysis methods.

Ions may also be ejected by the resonance excitation method, whereby a supplemental oscillatory excitation voltage is applied to the endcap electrodes, and the trapping voltage amplitude and/or excitation voltage frequency is varied to bring ions into a resonance condition in order of their mass/charge ratio.

Cylindrical ion trap

The cylindrical ion trap mass spectrometer (CIT) is a derivative of the quadrupole ion trap where the electrodes are formed from flat rings rather than hyperbolic shaped electrodes. The architecture lends itself well to miniaturization because as the size of a trap is reduced, the shape of the electric field near the centre of the trap, the region where the ions are trapped, forms a shape similar to that of a hyperbolic trap.

Linear quadrupole ion trap

A linear quadrupole ion trap is similar to a quadrupole ion trap, but it traps ions in a two dimensional quadrupole field, instead of a three-dimensional quadrupole field as in a 3D quadrupole ion trap. Thermo Fisher's LTQ ("linear trap quadrupole") is an example of the linear ion trap.

A toroidal ion trap can be visualized as a linear quadrupole curved around and connected at the ends or as a cross-section of a 3D ion trap rotated on edge to form the toroid, donut-shaped trap. The trap can store large volumes of ions by distributing them throughout the ring-like trap structure. This toroidal shaped trap is a configuration that allows the increased miniaturization of an ion trap mass analyser. Additionally, all ions are stored in the same trapping field and ejected together simplifying detection that can be complicated with array configurations due to variations in detector alignment and machining of the arrays.

As with the toroidal trap, linear traps and 3D quadrupole ion traps are the most commonly miniaturized mass analysers due to their high sensitivity, tolerance for mTorr pressure, and capabilities for single analyser tandem mass spectrometry (e.g.: product ion scans).

Orbitrap



Orbitrap mass analyser

Orbitrap instruments are similar to Fourier-transform ion cyclotron resonance mass spectrometers (see text below). Ions are electrostatically trapped in an orbit around a central, spindle shaped electrode. The electrode confines the ions so that they both orbit around the central electrode and oscillate back and forth along the central electrode's long axis. This oscillation generates an image current in the detector plates which is recorded by the instrument. The frequencies of these image currents depend on the mass-to-charge ratios of the ions. Mass spectra are obtained by Fourier transformation of the recorded image currents.

Orbitraps have a high mass accuracy, high sensitivity and a good dynamic range.

Fourier-transform ion cyclotron resonance



Fourier-transform mass spectrometry (FTMS), or more precisely Fourier-transform ion cyclotron Fourier-transform ion cyclotron resonance mass spectrometer

Resonance MS, measures mass by detecting the image current produced by ions cyclotroning in the presence of a magnetic field. Instead of measuring the deflection of ions with a detector such as an electron multiplier, the ions are injected into a Penning trap (a static electric/magnetic ion trap) where they effectively form part of a circuit. Detectors at fixed positions in space measure the electrical signal of ions which pass near them over time, producing a periodic signal. Since the frequency of an ion's cycling is determined by its mass-to-charge ratio, this can be deconvoluted by performing a Fourier transform on the signal. FTMS has the advantage of high sensitivity (since each ion is "counted" more than once) and much higher resolution and thus precision.

Ion cyclotron resonance (ICR) is an older mass analysis technique similar to FTMS except that ions are detected with a traditional detector. Ions trapped in a Penning trap are excited by an RF electric field until they impact the wall of the trap, where the detector is located. Ions of different mass are resolved according to impact time.

Detectors

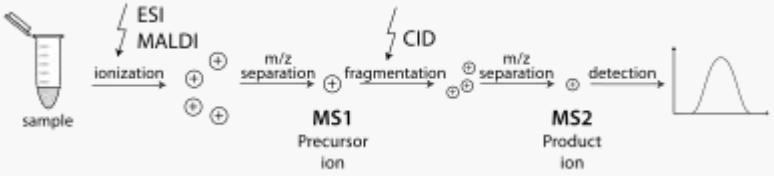


A continuous dynode particle multiplier detector

The final element of the mass spectrometer is the detector. The detector records either the charge induced or the current produced when an ion passes by or hits a surface. In a scanning instrument, the signal produced in the detector during the course of the scan versus where the instrument is in the scan (at what *m/Q*) will produce a mass spectrum, a record of ions as a function of *m/Q*.

Typically, some type of electron multiplier is used, though other detectors including Faraday cups and ion-to-photon detectors are also used. Because the number of ions leaving the mass analyser at a particular instant is typically quite small, considerable amplification is often necessary to get a signal. Microchannel plate detectors are commonly used in modern commercial instruments. In FTMS and Orbitraps, the detector consists of a pair of metal surfaces within the mass analyser/ion trap region which the ions only pass near as they oscillate. No direct current is produced, only a weak AC image current is produced in a circuit between the electrodes. Other inductive detectors have also been used.

TANDEM MASS SPECTROMETRY



Tandem mass spectrometry for biological molecules using ESI or MALDI

A tandem mass spectrometer is one capable of multiple rounds of mass spectrometry, usually separated by some form of molecule fragmentation. For example, one mass analyser can isolate one peptide from many entering a mass spectrometer. A second mass analyser then stabilizes the peptide ions while they collide with a gas, causing them to fragment by collision-induced dissociation (CID). A third mass analyser then sorts the fragments produced from the peptides. Tandem MS can also be done in a single mass analyser over time, as in a quadrupole ion trap. There are various methods for fragmenting molecules for tandem MS, including collision-induced dissociation (CID), electron capture dissociation (ECD), electron transfer dissociation (ETD), infrared multiphoton dissociation (IRMPD), blackbody infrared radiative dissociation

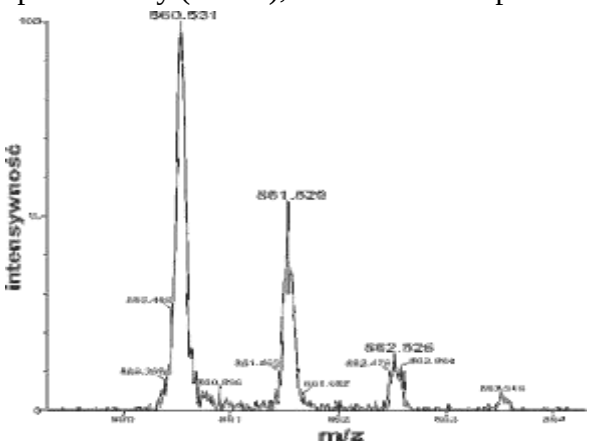
(BIRD), electron-detachment dissociation (EDD) and surface-induced dissociation (SID). An important application using tandem mass spectrometry is in protein identification.

Tandem mass spectrometry enables a variety of experimental sequences. Many commercial mass spectrometers are designed to expedite the execution of such routine sequences as selected reaction monitoring (SRM) and precursor ion scanning. In SRM, the first analyser allows only a single mass through and the second analyser monitors for multiple user-defined fragment ions. SRM is most often used with scanning instruments where the second mass analysis event is duty cycle limited. These experiments are used to increase specificity of detection of known molecules, notably in pharmacokinetic studies. Precursor ion scanning refers to monitoring for a specific loss from the precursor ion. The first and second mass analysers scan across the spectrum as partitioned by a user-defined *m/z* value. This experiment is used to detect specific motifs within unknown molecules. Another type of tandem mass spectrometry used for radiocarbon dating is accelerator mass spectrometry (AMS), which uses very high voltages, usually in the mega-volt range, to accelerate negative ions into a type of tandem mass spectrometer.

Common mass spectrometer configuration and techniques

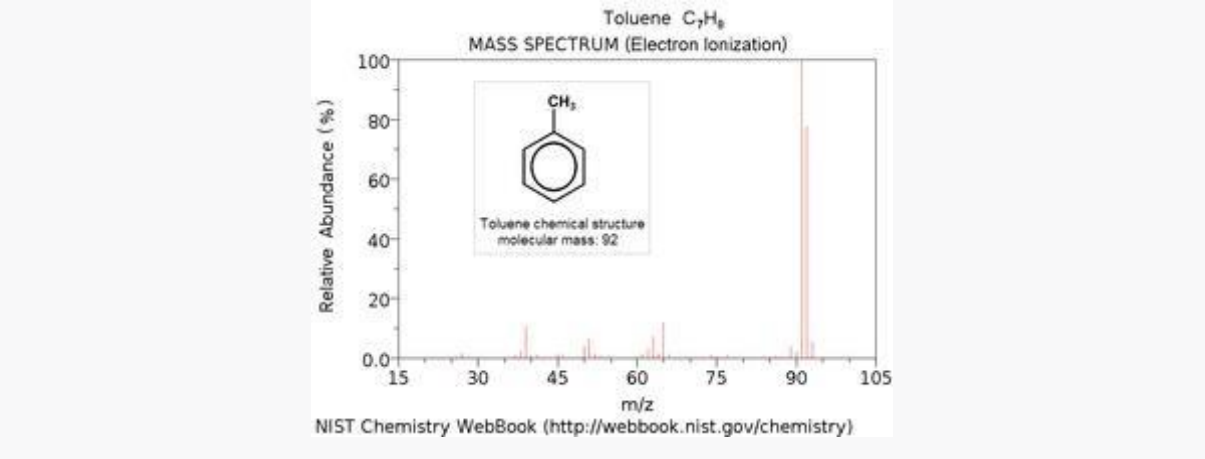
When a specific combination of source, analyser, and detector becomes conventional in practice, a compound acronym may arise to designate it succinctly. One example is MALDI-TOF, which refers to a combination of a matrix-assisted laser desorption/ionization source with a time-of-flight mass analyser. Other examples include inductively coupled plasma-mass spectrometry (ICP-MS), accelerator mass spectrometry (AMS), thermal ionization-mass spectrometry (TIMS) and spark source mass spectrometry (SSMS).

Certain applications of mass spectrometry have developed monikers that although strictly speaking would seem to refer to a broad application, in practice have come instead to connote a specific or a limited number of instrument configurations. An example of this is isotope-ratio mass spectrometry (IRMS), which refers in practice to the use of a limited number of sector.



Mass spectrum of a peptide showing the isotopic distribution

Interpretation of mass spectra



Since the precise structure or peptide sequence of a molecule is deciphered through the set of fragment masses, the interpretation of mass spectra requires combined use of various techniques. Usually, the first strategy for identifying an unknown compound is to compare its experimental mass spectrum against a library of mass spectra. If no matches result from the search, then manual interpretation or software assisted interpretation of mass spectra must be performed. Computer simulation of ionization and fragmentation processes occurring in mass spectrometer is the primary tool for assigning structure or peptide sequence to a molecule. An *a priori* structural information is fragmented *in silico* and the resulting pattern is compared with observed spectrum. Such simulation is often supported by a fragmentation library that contains published patterns of known decomposition reactions. Software taking advantage of this idea has been developed for both small molecules and proteins.

Analysis of mass spectra can also be spectra with accurate mass. A mass-to-charge ratio value (*m/z*) with only integer precision can represent an immense number of theoretically possible ion structures; however, more precise mass figures significantly reduce the number of candidate molecular formulas. A computer algorithm called formula generator calculates all molecular formulas that theoretically fit a given mass with specified tolerance.

A recent technique for structure elucidation in mass spectrometry, called precursor ion fingerprinting, identifies individual pieces of structural information by conducting a search of the tandem spectra of the molecule under investigation against a library of the product-ion spectra of structurally characterized precursor ions.

Applications

Mass spectrometry has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now commonly used in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds. Quantitation can be relative (analysed relative to a reference sample) or absolute (analysed using a standard curve method).

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

A few of the disadvantages of the method is that it often fails to distinguish between optical and geometrical isomers and the positions of substituent in o-, m- and p- positions in an aromatic ring. Also, its scope is limited in identifying hydrocarbons that produce similar fragmented ions.

8. SOLUBILITY STUDIES

In chemistry, **solubility** is the ability of a substance, the solute, to form a solution with another substance, the solvent. **Insolubility** is the opposite property, the inability of the solute to form such a solution.

The extent of the solubility of a substance in a specific solvent is generally measured as the concentration of the solute in a saturated solution, one in which no more solute can be dissolved. At this point, the two substances are said to be at the solubility equilibrium. For some solutes and solvents, there may be no such limit, in which case the two substances are said to be "miscible in all proportions" (or just "miscible").

The solute can be a solid, a liquid, or a gas, while the solvent is usually solid or liquid. Both may be pure substances, or may themselves be solutions. Gases are always miscible in all proportions, except in very extreme situations, and a solid or liquid can be "dissolved" in a gas only by passing into the gaseous state first.

The solubility mainly depends on the composition of solute and solvent (including their pH and the presence of other dissolved substances) as well as on temperature and pressure. The dependency can often be explained in terms of interactions between the particles (atoms, molecules, or ions) of the two substances, and of thermodynamic concepts such as enthalpy and entropy.

Under certain conditions, the concentration of the solute can exceed its usual solubility limit. The result is a supersaturated solution, which is metastable and will rapidly exclude the excess solute if a suitable nucleation site appears.

The concept of solubility does not apply when there is an irreversible chemical reaction between the two substances, such as the reaction of calcium hydroxide with hydrochloric acid; even though one might say, informally, that one "dissolved" the other. The solubility is also not the same as the rate of solution, which is how fast a solid solute dissolves in a liquid solvent. This property depends on many other variables, such as the physical form of the two substances and the manner and intensity of mixing.

The concept and measure of solubility are extremely important in many sciences besides chemistry, such as geology, biology, physics, and oceanography, as well as in engineering, medicine, agriculture, and even in non-technical activities like painting, cleaning, cooking, and brewing. Most chemical reactions of scientific, industrial, or practical interest only happen after the reagents have been dissolved in a suitable solvent. Water is by far the most common such solvent.

The term "soluble" is sometimes used for materials that can form colloidal suspensions of very fine solid particles in a liquid. The quantitative solubility of such substances is generally not well-defined, however.

Quantification of solubility

The solubility of a specific solute in a specific solvent is generally expressed as the concentration of a saturated solution of the two. Any of the several ways of expressing concentration of solutions can be

used, such as the mass, volume, or amount in moles of the solute for a specific mass, volume, or mole amount of the solvent or of the solution.

Per quantity of solvent

In particular, chemical handbooks often express the solubility as grams of solute per 100 millilitres of solvent (g/(100 mL)), or as grams of solute per decilitre of solvent (g/dL); or, less commonly, as grams of solute per litre of solvent (g/L). The quantity of solvent can instead be expressed in mass, as grams of solute per 100 grams of solvent (g/(100 g)), or as grams of solute per kilogram of solvent (g/kg). The number may be expressed as a percentage in this case, and the abbreviation "w/w" may be used to indicate "weight per weight". (The values in g/L and g/kg are similar for water, but that may not be the case for other solvents.)

Alternatively, the solubility of a solute can be expressed in moles instead of mass. For example, if the quantity of solvent is given in kilograms, the value is the molality of the solution (mol/kg).

Per quantity of solution

The solubility of a substance in a liquid may also be expressed as the quantity of solute per quantity of *solution*, rather than of solvent. For example, following the common practice in titration, it may be expressed as moles of solute per litre of solution (mol/L), the molarity of the latter.

In more specialized contexts the solubility may be given by the mole fraction (moles of solute per total moles of solute plus solvent) or by the mass fraction at equilibrium (mass of solute per mass of solute plus solvent). Both are dimensionless numbers between 0 and 1 which may be expressed as percentages (%).

Liquid and gaseous solutes

For solutions of liquids or gases in liquids, the quantities of both substances may be given volume rather than mass or mole amount; such as litre of solute per litre of solvent, or litre of solute per litre of solution. The value may be given as a percentage, and the abbreviation "v/v" for "volume per volume" may be used to indicate this choice.

Conversion of solubility values

Conversion between these various ways of measuring solubility may not be trivial, since it may require knowing the density of the solution — which is often not measured, and cannot be predicted. While the total mass is conserved by dissolution, the final volume may be different from both the volume of the solvent and the sum of the two volumes.

Moreover, many solids (such as acids and salts) will dissociate in non-trivial ways when dissolved; conversely, the solvent may form coordination complexes with the molecules or ions of the solute. In those cases, the sum of the moles of molecules of solute and solvent is not really the total moles of independent particles solution. To sidestep that problem, the solubility per mole of solution is usually computed and quoted as if the solute does not dissociate or form complexes -- that is, by pretending that the mole amount of solution is the sum of the mole amounts of the two substances.

Qualifiers used to describe extent of solubility

The extent of solubility ranges widely, from infinitely soluble (without limit, i.e. miscible) such as ethanol in water, to essentially insoluble, such as titanium dioxide in water. A number of other descriptive terms are also used to qualify the extent of solubility for a given application. For example, U.S. Pharmacopoeia gives the following terms, according to the mass m_{SV} of solvent

required to dissolve one unit of mass m_{su} of solute: (The solubilities of the examples are approximate, for water at 20-25 °C.)

Term	**Range**	**Example**	**g/dL**	m_{sv}/m_s **u**
Very soluble	<1	calcium nitrate	158.7	0.63
Freely soluble	1 to 10	calcium chloride	65	1.54
Soluble	10 to 30	sodium oxalate	3.9	26
Sparingly soluble	30 to 100	-	-	-
Slightly soluble	100 to 1000	calcium sulphate	0.21	490
Very slightly soluble	1000 to 10,000	dicalcium phosphate	0.02	5000
Practically insoluble or insoluble	≥ 10,000	barium sulphate	0.000245	409000

The thresholds to describe something as insoluble, or similar terms, may depend on the application. For example, one source states that substances are described as "insoluble" when their solubility is less than 0.1 g per 100 mL of solvent.

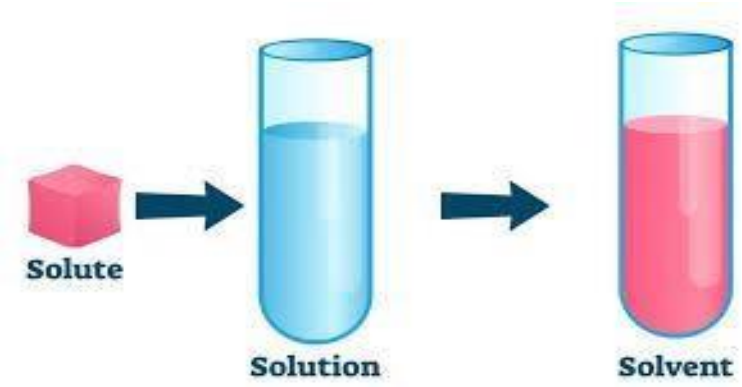
Molecular view

Solubility occurs under dynamic equilibrium, which means that solubility results from the simultaneous and opposing processes of dissolution and phase joining

(e.g. precipitation of solids). The solubility equilibrium occurs when the two processes proceed at equal and opposite rates.

The term *solubility* is also used in some fields where the solute is altered by solvolysis. For example, many metals and their oxides are said to be "soluble in hydrochloric acid", although in fact the aqueous acid irreversibly degrades the solid to give soluble products. It is also true that most ionic solids are dissolved by polar solvents, but such processes are reversible. In those cases where the solute is not recovered upon evaporation of the solvent, the process is referred to as solvolysis. The thermodynamic concept of solubility does not apply straightforwardly to solvolysis.

When a solute dissolves, it may form several species in the solution. For example, an aqueous suspension of ferrous hydroxide, $Fe(OH)_2$, will contain the series $[Fe(H_2O)_x(OH)_x]^{(2x)+}$ as well as other species. Furthermore, the solubility of ferrous hydroxide and the composition of its soluble components depend on pH. In general, solubility in the solvent phase can be given only for a specific solute that is thermodynamically stable, and the value of the solubility will include all the species in the solution (in the example above, all the iron-containing complexes).



Factors affecting solubility

Solubility is defined for specific phases. For example, the solubility of aragonite and calcite in water are expected to differ, even though they are both polymorphs of calcium carbonate and have the same chemical formula.

The solubility of one substance in another is determined by the balance of intermolecular forces between the solvent and solute, and the entropy change that accompanies the solvation. Factors such as temperature and pressure will alter this balance, thus changing the solubility.

Solubility may also strongly depend on the presence of other species dissolved in the solvent, for example, complex-forming anions (ligands) in liquids. Solubility will also depend on the excess or deficiency of a common ion in the solution, a phenomenon known as the common-ion effect. To a lesser extent, solubility will depend on the ionic strength of solutions. The last two effects can be quantified using the equation for solubility equilibrium.

For a solid that dissolves in a redox reaction, solubility is expected to depend on the potential (within the range of potentials under which the solid remains the thermodynamically stable phase). For example, solubility of gold in high-temperature water is observed to be almost an order of magnitude higher (i.e. about ten times higher) when the redox potential is controlled using a highly oxidizing Fe_3O_4-Fe_2O_3 redox buffer than with a moderately oxidizing Ni-NiO buffer.

Solubility (metastable, at concentrations approaching saturation) also depends on the physical size of the crystal or droplet of solute (or, strictly speaking, on the specific surface area or molar surface area of the solute). For quantification, see the equation in the article on solubility equilibrium. For highly defective crystals, solubility may increase with the increasing degree of disorder. Both of these effects occur because of the dependence of solubility constant on the Gibbs energy of the crystal. The last two effects, although often difficult to measure, are of practical importance. For example, they provide the driving force for precipitate aging (the crystal size spontaneously increasing with time).

AIM AND OBJECTIVES

Aim:

Toperform a stability indicating potentiometric method for determination of anti-tubercular drugs using sensors.

Objectives:

To perform the stability studies using potentiometric method

- Validation of anti-tubercular drugs for determining the stability of isoniazid, rifampicin and ethambutol in tablets using ion selective electrodes by potentiometric method.
- To evaluate the performance of the sensors (sensor free and sensor doped) for its selectivity, sensitivity and stability indicating capability.

Plan of work:

The work is planned to carry out as given below:

1. Collection of relevant literature
2. Methodology
3. Solubility studies
4. Results and discussion
5. Conclusion

REVIEW OF LITERATURE

1. Dauda K Tolulope, *et al* (2022): The copper, nickel and zinc complexes of isoniazid (ISO, isonicotinic acid hydrazide), an anti-tubercular drug, have been investigated to determine whether these metal-ions improve the permeability and bioavailability of the drug. Since such properties of isonazid depend on its solution speciation, the latter was investigated by determining the equilibrium constants for the reaction of H^+, Cu(II), Ni(II) and Zn(II) with isonazid in aqueous solution at 25.00 ± 0.01 °C and 0.15 M (NaCl), using glass electrode potentiometry. The structure of the copper complex of isoniazid was investigated using ultraviolet-visible spectroscopy. The results support the structures postulated from the potentiometric data. This study also considered membrane permeability using a Franz cell and octanol/water distribution coefficients. Distribution coefficient studies showed that ISO and its metal complexes are hydrophilic. The incorporation of a metal-ion improves the hydrophilicity of the ligand. The presence of a metal ion greatly enhanced the permeation of ISO through an artificial membrane in the order Cu(II) > Ni(II) > Zn(II) > ISO at pH 2 and Zn(II) > Ni(II) > ISO > Cu(II) at pH 4.

2. Laura Bulagrariu catalina siriteanu *et al* (2022): In this study, a simple spectrophotometric method was developed for the quantitative determination of rifampicin in aqueous solution. The method is based on the property of rifampicin to modify, in aqueous media at pH of 7.0, the degree of ionization of the functional groups. After ionization, a coloured compound is formed, which can be

analysed spectrophotometrically. The visible spectra of the yellow compound were recorded at pH of 7.0, in different buffer solutions (phosphate, hexamethylenetetramine (HMT) and borax) and the qualitative characteristics were determined. The highest absorbance was obtained in phosphate buffer, at □ = 470 nm, against distilled water, and these conditions were selected for quantitative determinations. This procedure allows the quantitative analysis of rifampicin in a wide concentration range (0.01 – 0.16 mmol·L-1), with a detection limit of 0.0002 mmol·L-1 . The very good correspondence between the calculated and experimental values of rifampicin concentration shows that this method is valid and can be used for quantitative analysis, at least for laboratory studies.

3. Mahmoud A. Tantawy, *et al* (2022): It presents a novel potentiometric approach for the determination of palonosetron HCl using two sensors; ionophore-free and ionophore-doped ones. The two sensors successfully determined the cited drug in the range of 1×10^{-5}–1×10^{-2} M with respective Nernstian slopes of 54.9 ± 0.25 and 59.3 ± 0.16 mV/decade. Incorporating calix[8]arene as an ionophore resulted in a lower detection limit (LOD = 3.1×10^{-6} M) and enhanced selectivity when compared to the ionophore-free sensor (LOD = 7.9×10^{-6} M). This modification was also associated with faster response for the ionophore-doped sensor (response time = 20 s) compared to the ionophore-free one (response time = 30 s). The two sensors showed a stable response over a pH range of 3.0–8.0. They successfully determined palonosetron HCl in presence of its oxidative degradation products. They were also used for direct determination of the drug in commercially available parenteral solution without any interference from other dosage forms' additives.

4. Mahmoud A. Tantawy IsraaA. Wahba *et al* (2022): Solid contact ion selective electrodes are extensively utilized owing to their marvelous performance over traditional liquid contact ones. The main drawback of those solid contact electrodes is aqueous layer formation which affects their constancy. Herein and to overcome this common drawback, a carbon paste electrode containing poly(3,4-ethylenedioxythiophene) was constructed and used for determination of probenecid at variant pH values. This modification decreased the potential drift down to 0.8 mV/h and improved its stability over 30 days. A Nernstian slope of − 57.8 mV/decade associated with a linear range of 1.0×10^{-6}–
1.0×10^{-2} mol/L was obtained. The modified carbon paste electrode successfully detected up to 8.0 $\times 10^{-7}$ mol/L probenecid. Results of this modified carbon paste electrode were also compared to unmodified one.

5. **Sara Motamen *et al* (2020) :** *Mycobacterium tuberculosis* (*Mtb*) remains a deadly pathogen two decades after the announcement of tuberculosis (TB) as a global health emergency by the World Health Organization. Medicinal chemistry efforts to synthesize potential drugs to shorten TB treatments have not always been successful. Here, we analyze physiochemical properties of 39 TB drugs and 1271 synthetic compounds reported in 40 publications from 2006 to early 2020. We also propose a new TB space of physiochemical properties that may provide more appropriate guidelines for design of anti-TB drugs. In this analysis, they evaluated 1271 synthetic anti-tubercular compounds using their physiochemical properties. In this analysis, they revealed that log *P* is a critical property which showed the most difference between TB drugs and the synthetic compounds. A huge shift to larger values for clog *P* was observed among the synthetic compounds compared to TB drugs. Selection of the second most apparent variation led to an analysis of clog *P* against PSA. Combining the 3 parameters led us to propose clog *P*, MW, and PSA as the three important properties arising from this analysis. Clog *P*–MW and clog *P*–PSA provide the largest discrimination. Also, a new TB space with more appropriate values of MW ≤ 500, −4 ≤ clog *P* ≤ 3

and 30 ≤ PSA ≤ 140 Å is proposed.

6. Fatehy Abdel-Haleem *et al* (2018): We report on highly-sensitive ion-selective electrodes (ISEs) for potentiometric determining of galantamine hydrobromide (GB) in physiological fluids. Galantamine hydrobromide (GB) was selected for this study due to its previous medical importance for treating Alzheimer's disease. Three different types of ISEs were investigated: PVC membrane electrode (PVCE), carbon-paste electrode (CPE), and coated-wire electrode (CWE). In the construction of these electrodes, galantaminium–reineckate (GR) ion-pair was used as a sensing species for GB in solutions. The modified carbon-paste electrode (MCPE) was prepared using graphene oxide (MCPE-GO) and sodium tetrakis (trifluoromethyl) phenyl borate (MCPESTFPB) as ion-exchanger. The potentiometric modified CPEs (MCPE-GO and MCPE-STFPB) show an improved performance in term of Nernstian slope, selectivity, response time, and response stability compared to the unmodified CPE. The prepared electrodes PVCE, CWE, CPE, MCPE-GO and MCPE-STFPB show Nernstian slopes of 59.9, 59.5, 58.1,
58.3 and 57.0 mV/conc. decade, and detection limits of 5.0×10-6, 6.3×10-6, 8.0×10-6, 6.0×10-6 and 8.0×10-6 mole L -1 , respectively. The prepared ISEs also show high selectivity against cations (i.e. Na^{+},K^{+},$2NH^{4}$,Ca^{2},Al^{3+},Fe^{3+}), amino acids (i.e. glycine, L-alanine alanine), and sugars (i.e. fructose, glucose, maltose, lactose). The prepared ISEs are applicable for determining GB in spiked serums, urines, and pharmaceutical preparations, using a standard addition and a direct potentiometric method. The fast response time(<10 seconds), long lifetime (1-5 weeks), reversibility and stability of the measured signals facilitate the application of these sensors for routine analysis of the real samples.

7. Hanan A. Merey *et al* (2017): A new fabricated ion selective electrode was described for the determination ropinirole HCl by direct potentiometry in pure drug substance and in pharmaceutical formulation. The proposed sensor was fabricated using ammonium reineckate (AR) as a cationic exchanger and 2-nitrophenyl octylether as a plasticizer in a polymeric matrix of polyvinyl chloride (PVC). Nernstian response of 55.1 mV/decade was obtained by the proposed sensor over a wide RP concentration range (10–5 to 1×10–2 mol/L) in the pH range of 3–7. The proposed sensor was stable for two weeks without any change in sensitivity. The proposed electrode shows good selectivity for the analysis of RP in the presence of up to 30% of its induced alkaline degradation product and in the presence of common inorganic and organic species commonly present in pharmaceutical dosage form. Statistical comparison between the results obtained by the proposed sensor for the analysis of ropinirole HCl in dosage form and the reported one shows no significant difference with respect to accuracy and precision.

8. Sumit Chakraborty *et al* (2015): Modern tuberculosis (TB) chemotherapy is widely viewed as a crowning triumph of antiinfectives research. However, only one new TB drug has entered clinical practice in the past 40 years while drug resistance threatens to further destabilize the pandemic. Here, we review a brief history of TB drug development, focusing on the evolution of mechanism(s)-of-action studies and key conceptual barriers to rational, mechanism-based drugs

9. **Camila B. Perantoni *et al* (2013):** Ethambutol (ETB) is a first-line antitubercular drug effective against actively growing Mycobacterium tuberculosis. Resistance of the mycobacterium to ethambutol among tuberculosis (TB) patients results from inadequate or inappropriate dosing of treatment or using low quality medication. It is therefore necessary to develop reliable methods for determining ethambutol metabolic profiles of patients at point of care for proper dosing. Herein an efficient ETB sensor device is illustrated. It consists of a graphite-polyurethane composite electrode. In order to characterise the electrochemical behaviour of ethambutol at pH = 8.0 voltammetric studies were performed. The detector was assembled in a flow injection apparatus and operated at +1.2 V (vs.

Ag/AgCl(NaCl sat.)). The influence of sample volume and flow rate was studied. The linear response for the method was extended up to a 1.1 mmol L-1 ethambutol solution with a detection limit of 0.0634 mmol L-1. The reproducibility of current responses for injections of 0.7 mmol L-1 ethambutol solution was evaluated to be 5.1% (n = 30) and the analytical frequency was 161 determinations h-1. Two different samples were successfully analysed and the results were in good agreement with those obtained using capillary zone electrophoresis (CZE).

10. Dmitriy. A. Golovko *et al* (2011) : A new potentiometric titration method using a Pt wire as an indicator electrode was developed for quantitative determination of ferrate(VI) in the submillimolar to millimolar concentration range in a strong alkaline solution. A Ag/AgCl electrode was the reference electrode and the titrant used was chromium(III) hydroxide solution. The developed method is relatively simple and faster than the colorimetric titration technique. The method eliminates human error associated with the endpoint detection and increases accuracy of the results with respect to the colorimetric titration. The method was applied to study the decay of synthesized ferrate(VI) in alkaline solution.

11. R. Govindan *et al* (2010): A potentiometric method was developed for the determination of lithium in ethanol medium using ammonium fluoride as titrant and fluoride ion selective electrode for end point detection. The precision of the measurements for samples containing 1 to 3 mg of Li was found to be better than 0.5%. The developed methodology was then employed for the determination of concentration of lithium (Li) in a complex chemical mixture containing hexamethylenetetramine (HMTA), formaldehyde and ammonium nitrate, which are the wash streams generated in the sol–gel process employed for preparation of Li2TiO3 microspheres. The concentration data profile agreed with the isotopic profile obtained by thermal ionization mass spectrometry.

12. **Mingming Liu *et al* (2010):** In this work, two allyl-functionalised ionic liquids (ILs), 1-allyl-3-methylimidazolium hexafluorophosphate and 1-allyl-3-methylimidazolium bis(trifluoromethanesulphonyl)imide, were used as selective coating materials to prepare chemically bonded ILs-based organic–inorganic hybrid solid phase microextraction fibres. These fibres were prepared with the aid of γ-methacryloxypropyltrimethoxysilane as bridge using sol–gel method and free radical cross-linking technology. The underlying mechanisms of the sol–gel reaction were proposed, and the successful binding of these functional ILs to the sol–gel substrate was confirmed by Fourier transform infrared spectroscopy. These IL-based sol–gel coatings had porous surface structure, high thermal stability, a wide range of pH stability, strong solvent resistance and good coating preparation reproducibility. They also had high selectivity and sensitivity towards strong polar phenolic environmental estrogens (PEEs) and aromatic amines due to the strong electrostatic interactions, hydrogen bonding and π–π interactions provided by the special molecular structure of these imidazolium ILs. Moreover, their characteristics were somewhat different depending on the type of anions in the IL structure. The practical applicability of these IL-based sol–gel coatings was evaluated through the analysis of PEEs in two real water samples. The detection limits were quite low, varying from 0.0030 to 0.1248 μg L^{-1}. The linearity was very good in the range of 0.1 to 1000 μg L^{-1} for most analytes, and the relative standard deviation values were below 6%. The relative recoveries were between 83.1 and 104.1% for lake water and between 89.1 and 97.1% for sewage drainage outlet water.

13. Zhehao Zhang *et al* (2009) : We built an integrated solid-contact ion-selective electrode (SCISE) system with the functionality of self-calibration. A multiplexed SCISE sensor (K+ and NO3 − vs. Ag/AgCl) was fabricated on printed-circuit board (PCB) substrates and was subsequently embedded into a microfluidic flow cell for self-calibration and flow-through analysis. A PCB circuit that includes modules for both sensor readout and fluid control was developed. The sensors showed a fast

and near-Nernstian response (56.6 for the K+ electrode and −57.4 mV/dec for the NO_3^- electrode) and maintained their performance for at least three weeks. The sensors also showed a highly reproducible response in an automated two-point calibration, demonstrating the potential for in situ monitoring. Lastly, the sensor system was successfully applied to measure mineral nutrients in plant sap samples.

14. **Gamal Abdel-Hafiz Mostafa Mohamed Mahmoud Hefnawy *et al* (2007):** The construction and general performance characteristics of two novel potentiometric membrane sensors responsive to the acebutolol are described. The sensors are based on the use of ion-association complexes of acebutolol (AC) with tetraphenylborate(TPB) (I) and phosphomolybdate(PM) (II) as exchange sites in a PVC matrix. The sensors show a fast, stable and near- Nernstian for the mono charge cation of AC over the concentration range 1×10^{-3} - $\sim10^{-6}$ M at 25 °C over the pH range 2.0 - 6.0 with cationic slope of 51.5 ± 0.5 and 53.0 ± 0.5 per concentration decade for AC-I and AC-II sensors respectively. The lower detection limit is 6×10^{-6} M and 4×0^{-6} M with the response time 20-30 s in the same order of both sensors. Selectivity coefficients of AC related to a number of interfering cation and some organic compounds were investigated. There are negligible interferences are caused by most of the investigated species. The direct determination of 3 - 370 µg/ml of AC shows an average recovery of 99.4 and 99.5% and a mean relative standard deviation of 1.5% at 100.0 µg/ml for sensor I and II respectively. The results obtained by determination of AC in tablets using the proposed sensors which comparable favorably with those obtained by the British pharmacopoeia method. In the present investigation the electrodes have been utilized as end point indicator for some precipitation titration reactions.

METHODOLOGY

1. Isoniazid+Rifampicin (Ia=Ionophore-free, Ia1=Ionophore-doped):

Isoniazid+Rifampicin tablet was kept for reflux with 6% H_2O_2 for 6hrs, then the cold water was added to it, filtered and kept for drying. The degradation product was confirmed by Thin Layer Chromatography (TLC) using the solvent system, ethyl acetate and ethanol in 7:3 ratio. Further, the degradation product was confirmed by Mass Spectra. After this ammonia and methylene blue dye were added to the degradation product (isoniazid+rifampicin). Ammonia and methylene blue dye were used as ionophore-doped sensor instead of calixarene. Ionophore-doped is compared with ionophore-free one in terms of anti-tubercular drugs determination.

2. Ethambutol (IIa=Ionophore-free, IIa2=Ionophore-doped):

Ethambutol tablet was kept for reflux with 6% H_2O_2 for 6hrs, then the cold water was added to it, filtered and kept for drying. The degradation product was confirmed by Thin Layer Chromatography (TLC) using the solvent system, ethyl acetate and ethanol in 7:3 ratio. Further, the degradation product was confirmed by Mass Spectra. After this ammonia and methylene blue dye were added to the degradation product (ethambutol). Ammonia and methylene blue dye were uesd as ionophore-doped sensor instead of calixarene. Ionophore-doped is compared with ionophore-free one in terms of anti-tubercular drugs determination.

3. Combination of Isoniazid+Rifampicin and ethambutol (IIIa=Ionophore-free, IIIa3=Ionophore-doped):

Isoniazid+Rifampicin and ethambutol were kept for reflux with 6% H_2O_2 for 6hrs, then the cold water was added to it, filtered and kept for drying. The degradation product was confirmed by Thin Layer Chromatography (TLC) using the solvent system, ethyl acetate and ethanol in 7:3 ratio. Further, the degradation product was confirmed by Mass Spectra. After this ammonia and methylene blue dye were added to the degradation product (isoniazid+rifampicin). Ammonia and methylene blue dye were used as ionophore-doped sensor instead of calixarene. Ionophore-doped is compared with ionophore-free one in terms of anti-tubercular drugs determination.

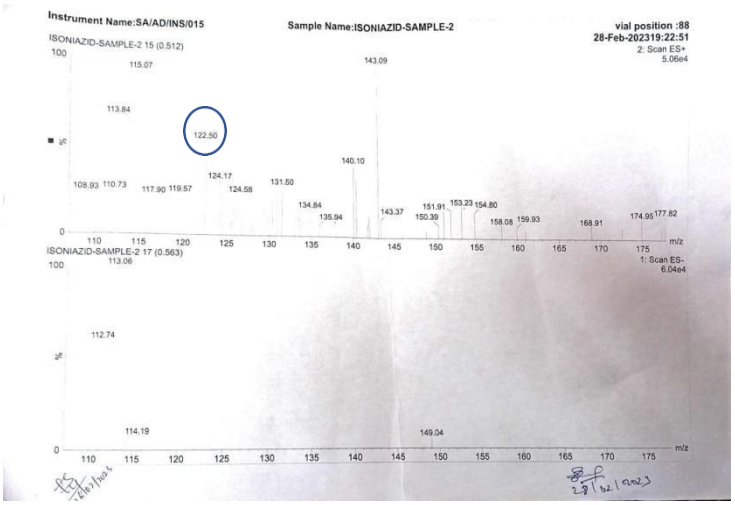
SOLUBILITY STUDIES

S.NO	CHEMICALS	ISONIAZID+ RIFAMPICIN(Ia)	ETHAMBUTOL (IIa)	COMBINATION (IIIa)
1.	Hexane	Insoluble	Soluble	Insoluble
2.	Chloroform	Insoluble	Insoluble	Insoluble
3.	Ethyl acetate	Soluble	Soluble	Soluble
4.	Ethanol	Sparingly Soluble	Sparingly Soluble	Soluble
5.	Water	Insoluble	Insoluble	Insoluble

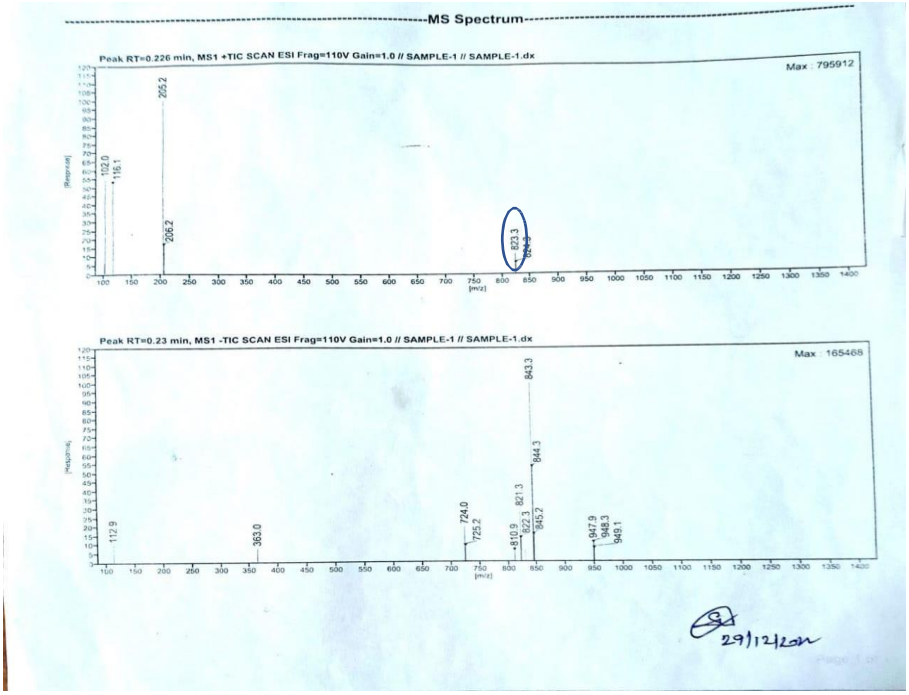
MASS SPECTROMETRY

In the context of stability-indicating potentiometric methods for the determination of antitubercular drugs, mass spectrometry can be used to verify the stability of the drug in the presence of other components in the sample matrix. Mass spectrometry can be used to confirm the identity of the drug and to verify that the drug is not degrading or undergoing chemical changes during the analysis. The mass spectrum obtained from a sample can provide information about the molecular weight and chemical structure of the drug, as well as any impurities that may be present. By comparing the mass spectrum of the drug in the sample to that of reference standard, it is possible to confirm the identity and purity of the drug. Overall, mass spectrometry can play an important role in the development and validation of stability-indicating potentiometric methods for the determination of anti-tubercular drugs providing valuable information about the chemical integrity of the drug in complex matrices.

1. Isoniazid+rifampicin(Ia)

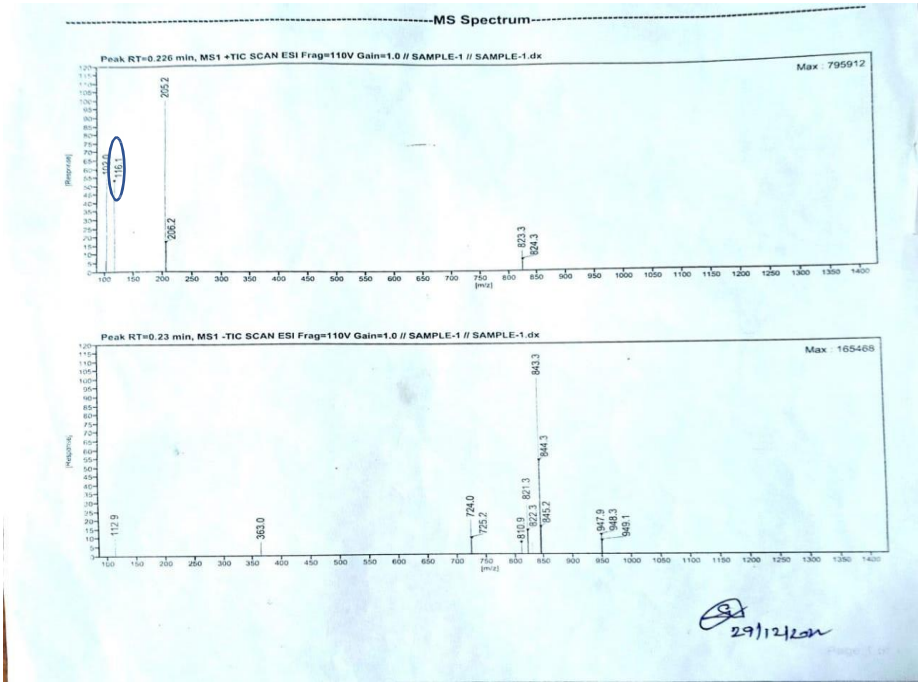


Mol.wt of Isoniazid: 137.139g/mol Ion product of Isoniazid: 122.50



Mol.wt of Rifampicin: 822.94g/mol Ion product of Rifampicin: 823.3

2. Ethambutol:

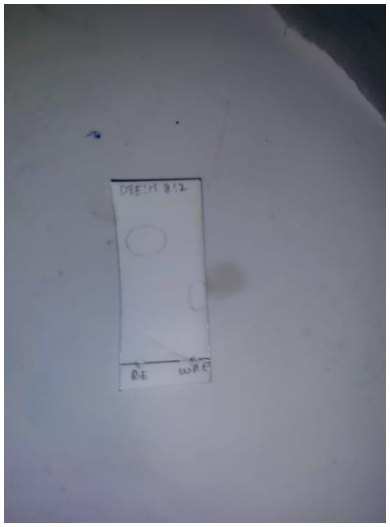


Mol. wt of Ethambutol: 204.31g/mol Ion product of Ethambutol: 116

THIN LAYER CHROMATOGRAPHY



Isoniazid+rifampicin



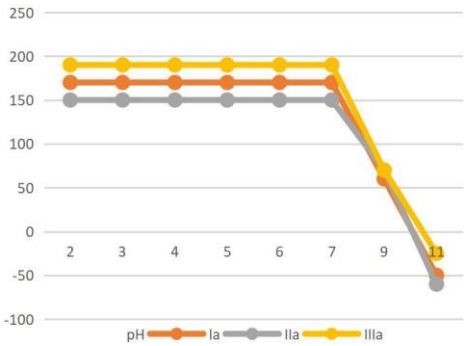
Ethambutol

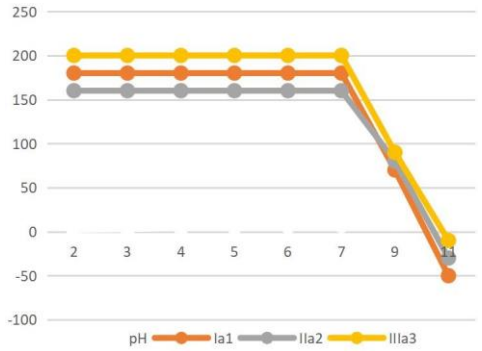


Combination
(Isoniazid+rifampicin & ethambutol)

EFFECT OF pH

The effect of pH of anti-tubercular drugs by using 1×10^{-2} M and 1×10^{-3} M concentration at different pH values, ranging from 2.0-11.0. The obtained potentials were recorded at each different pH values (2 to 11).





E(mv) **E(mv)**

pH pH

Effect of pH on the performance of Ionophore-free sensor performance of Ionophore-doped sensor (Ia, IIa, IIIa)

Ia=Isoniazid+rifampicin

IIa=Ethambutol

IIIa=Combination (Isoniazid+rifampicin & ethambutol)

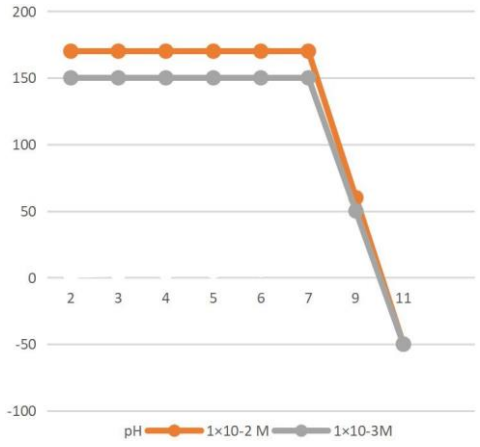
Effect of pH on the (Ia1, IIa2, IIIa3)

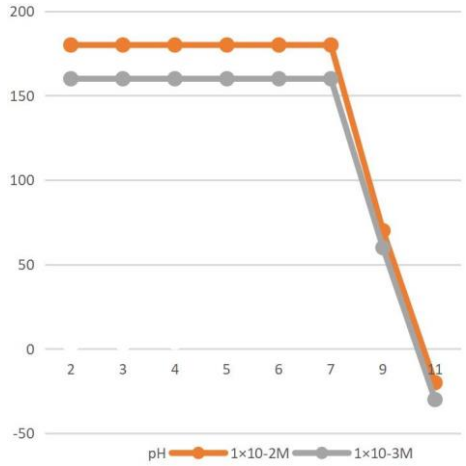
Ia1=Isoniazid+rifampicin

IIa2=Ethambutol

IIIa3=Combination (Isoniazid+rifampicin & ethambutol)

1. **Isoniazid+rifampicin:**



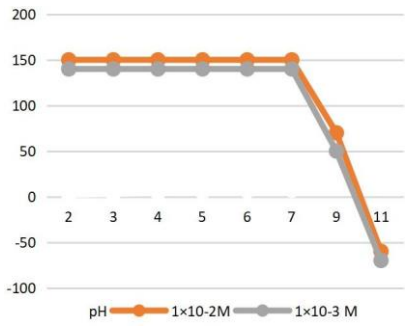


E(mv)

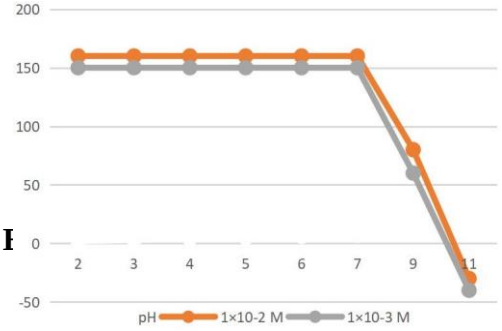
pH pH

Ia = Ionophore-free sensor **Ia1 = Ionophore-doped sensor**

2. **Ethambutol:**



E(mv)



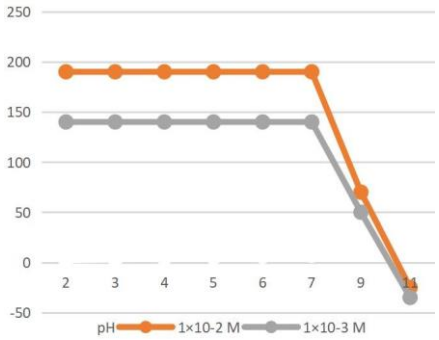
I

pH pH

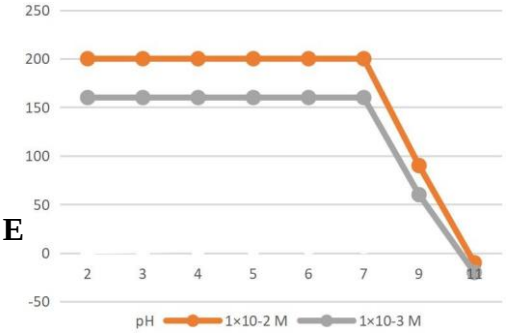
IIa=Ionophore-free sensor **IIa2=Ionophore-doped sensor**

3. Combination (Isoniazid+rifampicin and ethambutol):

E(mv)



E

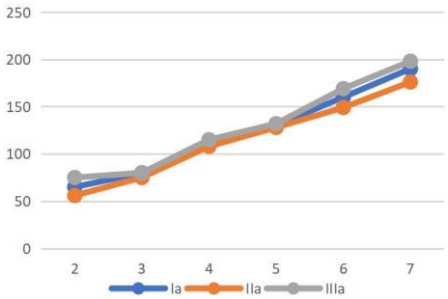


pH pH

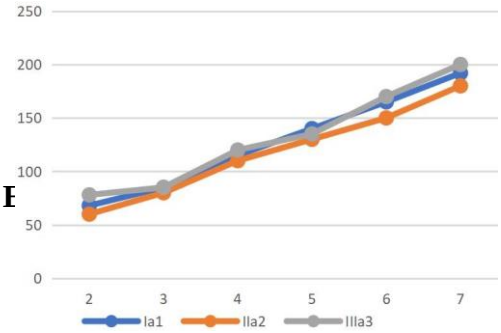
IIIa=Ionophore-free sensor **IIIa3=Ionophore-doped sensor**

EFFECT OF CONCENTRATION

E(mv



I



Conc(µg/ml) **Conc(µg/ml)**

Effect of concentration of Ionophore-free sensor (Ia, IIa, IIIa)

Ia=Isoniazid+rifampicin

IIa=Ethambutol

IIIa=Combination (Isoniazid+rifampicin & ethambutol)

Effect of Concentration of Ionophore-doped sensor (Ia1, IIa2, IIIa3)

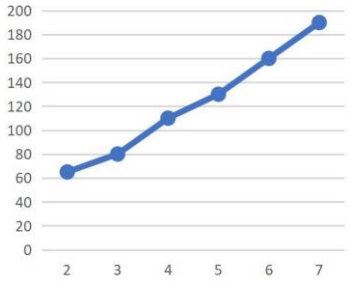
Ia1=Isoniazid+rifampicin

IIa2=Ethambutol

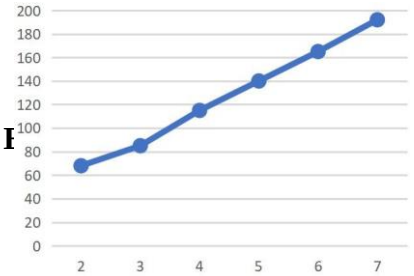
IIIa3=Combination (Isoniazid+rifampicin & ethambutol)

1. Isoniazid+rifampicin:

E(mv)



I



Conc(µg/ml) **Conc(µg/ml)**

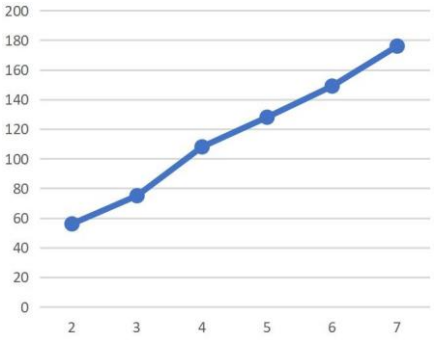
Ia=Ionophore-free sensor **Ia1=Ionophore-doped sensor**

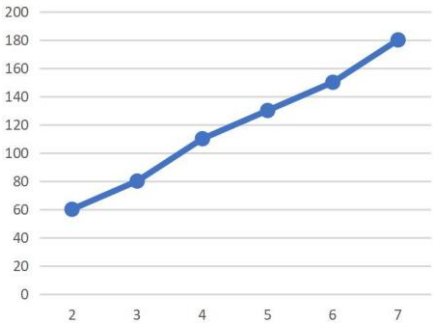
S.No	Conc(ug/ml)	E(mv)
1.	2	65
2.	3	80
3.	4	110
4.	5	130
5.	6	160
6.	7	190

S.No	Conc(ug/ml)	E(mv)
1.	2	68
2.	3	85
3.	4	115
4.	5	140
5.	6	165
6.	7	192

Ia=Ionophore-free sensor **Ia1=Ionophore-doped sensor**

2. Ethambutol:





E(mv)

Conc(µg/ml) **Conc(µg/ml)**

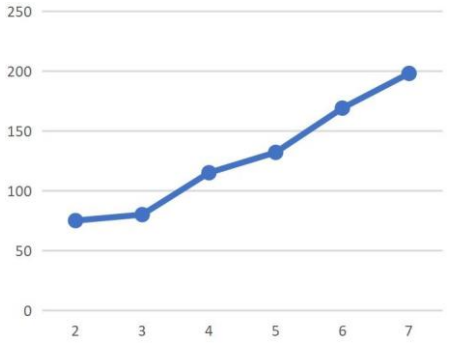
IIa=Ionophore-free sensor **IIa2=Ionophore-doped sensor**

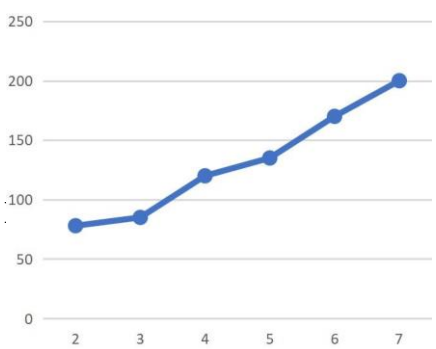
S.No	Conc(ug/ml)	E(mv)
1.	2	56
2.	3	75
3.	4	108
4.	5	128
5.	6	149
6.	7	176

S.No	Conc(ug/ml)	E(mv)
1.	2	60
2.	3	80
3.	4	110
4.	5	130
5.	6	150
6.	7	180

IIa=Ionophore-free sensor **IIa2=Ionophore-doped sensor**

3. Combination (Isoniazid+rifampicin & ethambutol):





E(mv)

Conc(µg/ml) **Conc(µg/ml)**

IIIa=Ionophore-free sensor **IIIa3=Ionophore-doped sensor**

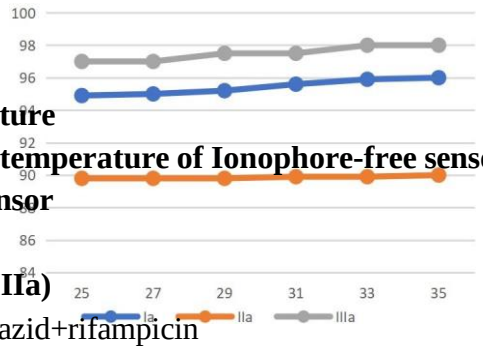
S.No	Conc(ug/ml)	E(mv)
1.	2	75
2.	3	80
3.	4	115
4.	5	132
5.	6	169
6.	7	198

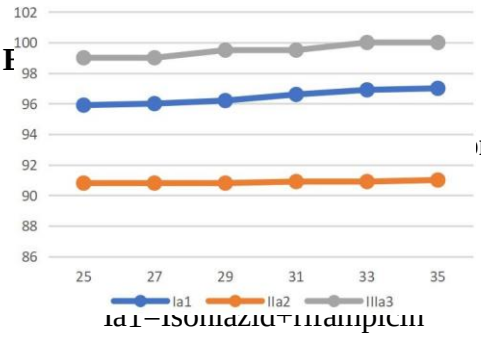
S.No	Conc(ug/ml)	E(mv)
1.	2	78
2.	3	85
3.	4	120
4.	5	135
5.	6	170
6.	7	200

IIIa=Ionophore-free sensor **IIIa3=Ionophore-doped sensor**

EFFECT OF TEMPERATURE:

The impact of temperature on the suggested ISE's response was investigated. When temperature is increased between 25 and 35 °C, a reasonably stable response is seen. 1×10^{-2} M and 1×10^{-3}M standard solutions were utilised. These findings show that the suggested electrode is thermally stable up to 35 °C.





E(mv) **E**

Temperature

Effect of temperature of Ionophore-free sensor **nophore-doped sensor**

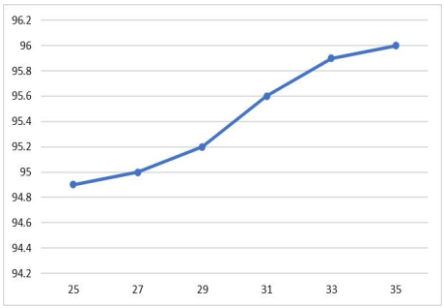
(Ia, IIa, IIIa)

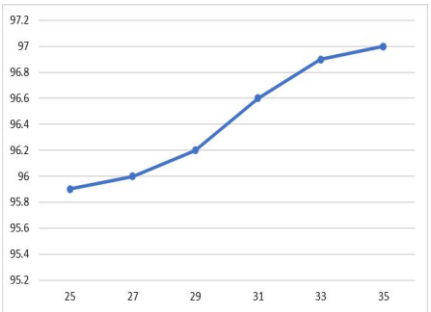
Ia = Isoniazid+rifampicin Ia1=Isoniazid+rifampicin

IIa = Ethambutol IIa2=Ethambutol

IIIa = Combination (Isoniazid+rifampicin & ethambutol) IIIa3=Combination (Isoniazid+rifampicin & ethambutol)

1. Isoniazid+rifampicin:





E(mv)

Temperature **Temperature**

Ia=Ionophore-free sensor **Ia1=Ionophore-doped sensor**

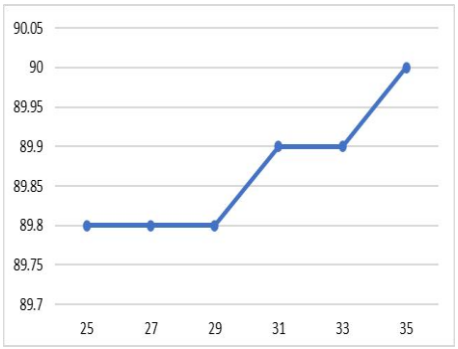
S.No	Temperature	E(mv)
1.	25	94.9
2.	27	95
3.	29	95.2
4.	31	95.6
5.	33	95.9
6.	35	96

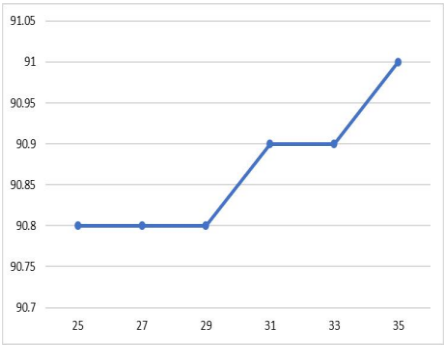
S.No	Temperature	E(mv)
1.	25	95.9
2.	27	96
3.	29	96.2
4.	31	96.6
5.	33	96.9
6.	35	97

Ia=Ionophore-free sensor **Ia1=Ionophore-doped sensor**

2. Ethambutol:

E(mv)





Temperature **Temperature**

IIa=Ionophore-free sensor **IIa2=Ionophore-doped sensor**

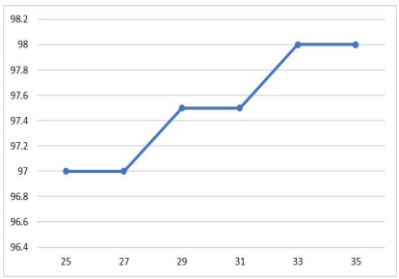
S.No	Temperature	E(mv)
1.	25	89.8
2.	27	89.8
3.	29	89.8
4.	31	89.9
5.	33	89.9
6.	35	90

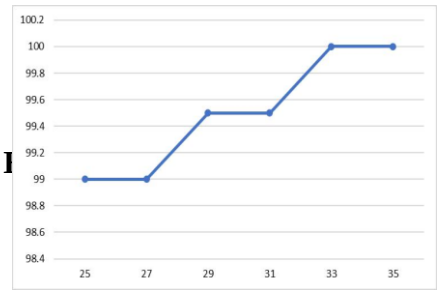
S.No	Temperature	E(mv)
1.	25	90.8
2.	27	90.8
3.	29	90.8
4.	31	90.9
5.	33	90.9
6.	35	91

IIa=Ionophore-free sensor **IIa2=Ionophore-doped sensor**

3. Combination (Isoniazid+rifampicin & ethambutol):

E(mv)





Temperature
IIIa=Ionophore-free sensor

S.No	Temperature	E(mv)
1.	25	97
2.	27	97
3.	29	97.5
4.	31	97.5
5.	33	98
6.	35	98

Temperature
IIIa3=Ionophore-doped sensor

S.No	Temperature	E(mv)
1.	25	99
2.	27	99
3.	29	99.5
4.	31	99.5
5.	33	100
6.	35	100

IIIa=Ionophore-free sensor **IIIa3=Ionophore-doped sensor**

Physical stability of the compounds:

Parameters	Storage conditions	Months			
		0	3rd	6th	9th
Colour	5°C±3°C	White	White	White	White
	30°C±2°C	White	White	White	White
Odour	5°C±3°C	Characteristic	Characteristic	Characteristic	Characteristic
	30°C±2°C	Characteristic	Characteristic	Characteristic	Characteristic
Atmospheric oxidation	5°C±3°C	No change	No change	No change	No change
	30°C±2°C	No change	No change	No change	No change
pH (2-11)	5°C±3°C	No change	No change	No change	No change
	30°C±2°C				

RESULTS AND DISCUSSION

1. Effect of pH:

Two separate solutions of concentrations 1.0×10^{-2} and 1.0×10^{-3} mol L^{-1} of Ia, IIa, IIIa, Ia1, IIa2, and IIIa3 were used to illustrate the effect of pH on the sensors. The potential readings for 1.0×10^{-2} mol L^{-1} and 1.0×10^{-3} mol L^{-1} were fairly over the pH range of 2.0-11.0 were prepared by mixing equal volumes of 0.04 M acetic acid, 0.04 M boric acid and 0.04 M phosphoric acid. The required pH values were adjusted using 0.2 M NaOH standard solution. In Ionophore-free sensor, the IIIa showed the highest potential energy with 190 E(mv) at pH 2 when compared to Ia with P.E. 170 E(mv) at pH 2 and IIa with P.E. 150 E(mv) at pH 2. In Ionophore-doped sensor, the combination IIIa3 showed the P.E 200 E(mv) at pH 2 when compared to Ia1 with P.E. 180 E(mv) at pH 2 and IIa2 with P.E of 160 E(mv) at pH 2.

2. Effect of Concentration:

The results have shown that potential energy increases as the concentration increases. In IIIa Ionophore-free concentration 2µg/ml, the P.E. is 75 E(mv) and at 7µg/ml, the P.E. is 198 E(mv). In

IIa at 2µg/ml, the P.E. is 56 E(mv) and at 7µg/ml, the P.E. is 176 E(mv). In Ia at 2µg/ml, the P.E. is 65 E(mv) and at 7µg/ml, the P.E. is 190 E(mv). In IIIa3 at 2µg/ml, the P.E. is 78 E(mv) and at 7µg/ml, the P.E. is 200 E(mv). In IIa2 at 2µg/ml, the P.E. is 60 E(mv) and at 7µg/ml, the P.E. is 180 E(mv). In Ia1 at 2µg/ml, the P.E. is 68 E(mv) and at 7µg/ml, the P.E. is 192 E(mv).

3. Effect of Temperature:

The impact of temperature on the suggested ISEs response was investigated. When the temperature is increased between 25 and 35ºC, a reasonably stable response is seen at 1×10^{-2} M and 1×10^{-3} M standard solutions. For IIIa 1×10^{-2} M at temperature 35ºC, the P.E. is 98 E(mv) and at 25ºC the P.E. is 97 E(mv). For IIIa3 at 35ºC, the P.E. is 100 E(mv) and at 25ºC, the P.E. is 99 E(mv).

In IIa 1×10^{-2} M at 35ºC, the P.E. is 90 E(mv) and at 25ºC, the P.E. is 89.8 E(mv). For IIa2 at 35ºC, the P.E. is 91 E(mv) and at 25ºC, the P.E. is 90.8 E(mv). For Ia at 35ºC, the P.E. is 96 E(mv) at 25ºC, the P.E. is 94.9 E(mv). For Ia1 at 35ºC, the P.E. is 97 E(mv) and at 25ºC, the P.E. is 95.9 E(mv).

Physical stability:

On each study, there was no change in colour, odour and atmospheric oxidation for 9 months at two different storage temperatures (5°C±3°C and 30°C±2°C). The pH readings as also shown no change (pH 2-11).

CONCLUSION

The compounds are acidic, showing maximum result at pH 2. The compounds have shown more P.E. in both ionophore-free and ionophore-doped sensors.

The impact of temperature on the ion selective electrodes, there is a stable response between 25ºC and 35ºC. Among all the compounds the ionophore-doped sensors have shown more and stable response.The incorporation of ammonia and methylene blue improved the limit of detection as well as the selectivity of the proposed sensor towards the degradation products. It also gives the direct determination of the degradation product.

This stability study was found to be sensitive, rapid, easy to use, selective, simple and more economic.

REFERENCES

1. Elham Pahlavani, Hadi Kargar, and Nahid Sepehri Rad. “A study on antitubercular and antimicrobial activity of isoniazid Derivative.” *Zahedan Journal of Research in Medical Sciences* 17.7 (2015). https://brieflands.com/articles/zjrms-1010.html

2. Zhang, Zhehao, Elena Boselli, and Ian Papautsky. “Potentiometric Sensor System with Self-Calibration for Long-Term, In Situ Measurements.” *Chemosensors* 11.1 (2023): 48. https://www.mdpi.com/2227-9040/11/1/48

3. Attri, S., S. v. Rana, K. Vaiphei, C. P. Sodhi, R. Katyal, R. C. Goel, C. K. Nain, and K. Singh. “Isoniazid and rifampicin–induced oxidative hepatic injury–protection by N-acetylcysteine. Human & experimental toxicology 19, no. 9 (2000): 517-522.

https://journals.sagepub.com/doi/pdf/10.1191/096032700674230830

4. Drobniewski, F. A., and S. M. Wilson. "The rapid diagnosis of isoniazid and rifampicin resistance in Mycobacterium tuberculosis—a molecular story." *Journal of medical microbiology* 47, no. 3 (1998): 189-196.

https://www.microbiologyresearch.org/content/journal/jmm/10.1099/00222615-47-3-189

5. Wypych G. "PVC degradation and stabilization." Elsevier; 2020 Mar 11. https://books.google.com/books?hl=en&lr=&id=k-XEDwAAQBAJ&oi=fnd&pg=PP1&dq=+PVC+degradation+and+stabilization+&ots=oLYF6MWPpP&sig=0N036Ja9bdcw8fshboq4AQPkISQ

6. Motamen, Sara, and Ronald J. Quinn. "Analysis of approaches to anti-tuberculosis compounds." *ACS omega* 5.44 (2020): 28529-28540.

https://dx.doi.org/10.1021/acsomega.0c03177

7. Merey, Hanan A., and Shimaa A. Atty. "Eco-friendly Stability-Indicating Potentiometric Method for the Determination of Ropinirole Hydrochloride." (2017): 1044-1056.

https://www.researchgate.net/publication/322402216

8. Tolulope, Dauda K., Graham E. Jackson, Mino R. Caira, and Ahmed N. Hammouda. "Potentiometric and spectroscopic study of isoniazid–an anti-tubercular drug." *South African Journal of Chemistry* 76 (2022): 65-71. https://www.ajol.info/index.php/sajc/article/view/241105

9. Tantawy, Mahmoud A., Dalia A. Elshabasy, Nadia F. Youssef, and Sawsan M. Amer. "Stability indicating potentiometric method for the determination of palonosetron HCl using two different sensors." *Scientific Reports* 12, no. 1 (2022): 12966. https://www.nature.com/articles/s41598-022-17349-y

10. Tanasă, IUSTINA-DENISA, et al. "Spectrophotometric determination of rifampicin in aqueous solution." *Bul. Inst. Politeh. Iaşi* 68 (2022): 25-34.

http://www.bipcic.icpm.tuiasi.ro/pdf/2022/3/bipi_cic_2022_3_02.pdf

11. Mostafa, Gamal Abdel-Hafiz, Mohamed Mahmoud Hefnawy, and Abdulrahman Al-Majed. "PVC membrane sensors for potentiometric determination of acebutolol." *Sensors* 7.12 (2007): 3272-3286. https://www.mdpi.com/1424-8220/7/12/3272

12. Abdel-Haleem, F. M., Saad, M., Barhoum, A., Bechelany, M., & Rizk, M. S. (2018). "PVC membrane, coated-wire, and carbon-paste ion-selective electrodes for potentiometric determination of galantamine hydrobromide in physiological fluids." *Materials Science and Engineering: C*, *89*, 140-148. https://hal.umontpellier.fr/hal-01822485/document

13. Chakraborty, Sumit, and Kyu Y. Rhee. "Tuberculosis drug development: history and evolution of the mechanism-based paradigm." *Cold Spring Harbor perspectives in medicine*5.8(2015):a021147. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4526730/

14. Tantawy, Mahmoud A., Israa A. Wahba, Samah S. Saad, and Nesrin K. Ramadan. "Two fabricated carbon paste electrodes for novel potentiometric determination of probenecid in dosage form and human plasma." *Scientific Reports* 12, no. 1 (2022): 20418. https://www.nature.com/articles/s41598-022-24920-0

15. Zhang, Zhehao, Elena Boselli, and Ian Papautsky. "Potentiometric Sensor System with Self-Calibration for Long-Term, In Situ Measurements." *Chemosensors* 11.1 (2023): 48. https://www.mdpi.com/2227-9040/11/1/48

16. Silvester, Debbie S., Ewa Grygolowicz-Pawlak, and Eric Bakker. "Potentiometric determination of coextraction constants of potassium salts in ion-selective electrodes utilizing a nitrobenzene liquid membrane phase.'' *Analytica chimica acta* 683.1 (2010):92-95.

https://www.sciencedirect.com/science/article/abs/pii/S0003267010012729

17. Liu, Mingming, Xin Zhou, Yingyi Chen, Hanlan Liu, Xionghan Feng, Guohong Qiu, Fan Liu, and Zhaorui Zeng. "Innovative chemically bonded ionic liquids-based sol–gel coatings as highly porous, stable and selective stationary phases for solid phase microextraction." *Analytica chimica acta* 683, no. 1 (2010): 96-106. https://www.sciencedirect.com/science/article/pii/S0003267010012407

18. Wöllner, K., X. Chen, E. Kremmer, and P. M. Krämer. "Comparative surface plasmon resonance and enzyme-linked immunosorbent assay characterisation of a monoclonal antibody with N-acyl homoserine lactones." *Analytica chimica acta* 683, no.1(2010):113-118.

https://www.sciencedirect.com/science/article/pii/S0003267010012997

19. Golovko, Dmitriy A., Virender K. Sharma, V. I. Suprunovich, O. V. Pavlova, I. D. Golovko, Karel Bouzek, and Radek Zboril. "A simple potentiometric titration method to determine concentration of ferrate (VI) in strong alkaline solutions." *Analytical letters* 44, no. 7 (2011): 1333-1340.

https://www.tandfonline.com/doi/abs/10.1080/00032719.2010.511748

20. Govindan, R., D. Alamelu, Raju V. Shah, TV Vittal Rao, Y. R. Bamankar, A. R. Parab,

K. Sasi Bhushan, S. K. Mukerjee, and S. K. Aggarwal. "Determination of lithium by potentiometry using fluoride ion selective electrode." *Analytical Methods* 2, no. 11 (2010): 1752-1755. https://pubs.rsc.org/en/content/articlehtml/2010/ay/c0ay00275e

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