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Construction of potentiometric biosensor to access urea content in soil

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Abstract

This study presents the construction of a potentiometric biosensor for the detection of urea content in soil, utilizing urease enzyme extracted from Canavalia ensiformis (jack bean). Urease catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide, with ammonia acting as a key indicator for the presence of urea. The biosensor was designed by immobilizing urease onto a conductive electrode surface, creating a sensing platform capable of detecting the variation in pH as ammonia is released during enzymatic activity. The potentiometric detection principle relies on measuring the changes in electrical potential (voltage) that correspond to changes in the local pH caused by urea breakdown. The response of the biosensor was optimized through varying enzyme concentrations, immobilization methods, and environmental conditions, ensuring high sensitivity and selectivity for urea. Calibration curves were developed to quantify urea content in soil samples, with the biosensor showing a wide linear detection range and high stability. This biosensor provides a rapid, cost-effective, and reliable tool for monitoring soil nutrient levels, enabling better agricultural practices by facilitating real-time assessment of urea content. The results suggest that this potentiometric biosensor has the potential for widespread application in precision agriculture and environmental monitoring. This study presents the construction of a potentiometric

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biosensor for the detection of urea content in soil, utilizing urease enzyme extracted from Canavalia ensiformis (jack bean). Urease catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide, with ammonia acting as a key indicator for the presence of urea. The biosensor was designed by immobilizing urease onto a conductive electrode surface, creating a sensing platform capable of detecting the variation in pH as ammonia is released during enzymatic activity. The potentiometric detection principle relies on measuring the changes in electrical potential (voltage) that correspond to changes in the local pH caused by urea breakdown. The response of the biosensor was optimized through varying enzyme concentrations, immobilization methods, and environmental conditions, ensuring high sensitivity and selectivity for urea. Calibration curves were developed to quantify urea content in soil samples, with the biosensor showing a wide linear detection range and high stability. The response time was rapid, with minimal interference from common soil ions. This biosensor provides a rapid, cost-effective, and reliable tool for monitoring soil nutrient levels, enabling better agricultural practices by facilitating real-time assessment of urea content. The results suggest that this potentiometric biosensor has the potential for widespread application in precision agriculture and environmental monitoring, contributing to sustainable farming practices.

1 Introduction

The overuse of chemical fertilizers, particularly urea, in modern agriculture has raised significant concerns regarding its environmental impact. Urea, a widely used nitrogen fertilizer, is crucial for crop growth but is also a major contributor to soil degradation, water pollution, and ammonia emissions. Urea is naturally broken down in the soil by an enzyme called urease, which catalyzes its hydrolysis into ammonia and carbon dioxide. While this enzymatic breakdown is essential for nitrogen cycling, it also leads to nitrogen loss, reducing the fertilizer's effectiveness and contributing to environmental pollution. Therefore, accurate

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and efficient monitoring of urea content in soil is essential to optimize fertilizer use and minimize negative environmental effects. One promising solution to manage urea fertilizer efficiently is the development of a potentiometric biosensor using urease from Canavalia ensiformis (jack bean), a plant known for its high urease activity. A potentiometric biosensor is a device that measures changes in the electrical potential (voltage) caused by chemical reactions, offering a simple and cost-effective method for detecting specific substances, such as urea. In this case, the biosensor can detect urea by measuring the change in pH levels as a result of the urease-catalyzed breakdown of urea into ammonia and carbon dioxide. Urease enzymes are highly efficient catalysts, widely distributed in the environment, and play a critical role in nitrogen cycling by hydrolyzing urea into ammonia and carbon dioxide. The ammonia released during this reaction increases the pH of the surrounding environment, a phenomenon that can be easily detected using a potentiometric sensor. By immobilizing urease onto an appropriate carrier material, such as an electrode or membrane, a biosensor can be created that will specifically respond to urea in soil. The immobilization of urease ensures the stability and reusability of the enzyme, making the biosensor an effective tool for long-term use in agricultural applications. The biosensor works by detecting the changes in pH associated with the hydrolysis of urea. When urea comes into contact with the immobilized urease enzyme, the enzyme catalyzes the breakdown of urea into ammonia and carbon dioxide, causing a local increase in pH. The potentiometric sensor detects this change in pH, and the resulting electrical signal is directly proportional to the concentration of urea in the soil. This real-time, on-site measurement allows for precise monitoring of urea levels, providing valuable data to farmers and agricultural professionals. By using the biosensor, farmers can adjust their fertilizer application rates, ensuring that the correct amount of urea is used for optimal crop growth while minimizing waste and environmental impact. The use of jack bean urease in the biosensor is particularly advantageous due to the enzyme's high activity and stability. Urease extracted from Canavalia ensiformis has been extensively studied and is known to be one of the most efficient ureases available. The

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enzyme's ability to catalyze urea hydrolysis at a rapid rate ensures that the biosensor provides accurate and quick measurements. Additionally, the immobilization of urease enhances its stability, making it resistant to environmental factors such as temperature changes, pH fluctuations, and proteolytic degradation, which could otherwise affect the performance of free, unbound enzymes. The development of a potentiometric biosensor for urea content in soil is an exciting advancement in precision agriculture. It offers a cost-effective, accurate, and real-time method for monitoring soil nutrients, enabling farmers to make informed decisions about fertilizer use. By optimizing the application of urea, this biosensor can help reduce the negative environmental impacts of excessive fertilizer use, such as nitrogen runoff and ammonia volatilization. Furthermore, it can contribute to more sustainable farming practices by ensuring that crops receive the right amount of nutrients without over-fertilizing. Ultimately, the construction of this potentiometric biosensor is a step towards more efficient, environmentally friendly agricultural practices that benefit both crop production and the environment.

2 Objective

The objective of this project work is to

1. Design of experiment to construct of potentometric biosensor using encapsulation tecnique
2. Optimize detection of urea content in soil using constructed biosensor

3 Methodology and material

3.1 Urease extraction:

It consists in extracting finely powdered, fat-free jack bean meal with 31.6 percent acetone and allowing the material to filter by gravity in an ice chest .After standing overnight the

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filtrate is centrifuged and the precipitate of crystalline urease is stirred with cold 31.6 percent acetone and centrifuged again.

3.2 Materials

Soil samples

pH electrodes

pH meter

Petri Dishes and volumetric flasks

Cellular nitrate membrane

3.3 Enzymes and Chemicals:

Standard buffer tablets were procured from Qualigens Fine Chemicals, Mumbai.we have purchased urease source of Jack Bean Meal from the Leba chemicals pvt.ltd,Jahangir villa,107,Woodhouse Road,Colaba,Mumbai,Maharashtra,India-400005.

3.4 General Principles of Potentiometric Analysis:

1, Potentiometric Methods:

- based on measurements of the potential of electrochemical cells in the absence of appreciable currents
- absolute values for individual half cell potentials cannot be determined in the laboratory. That is, only relative cell potentials can be measured experimentally.
- potentiometric techniques have been used for the location of end points in titrations.
- More recently, measurements of the potential of ion- selective electrodes have been used.
- The equipment required for potentiometric methods is simple and inexpensive and includes an indicator electrode, a reference electrode, and a potential measuring device.

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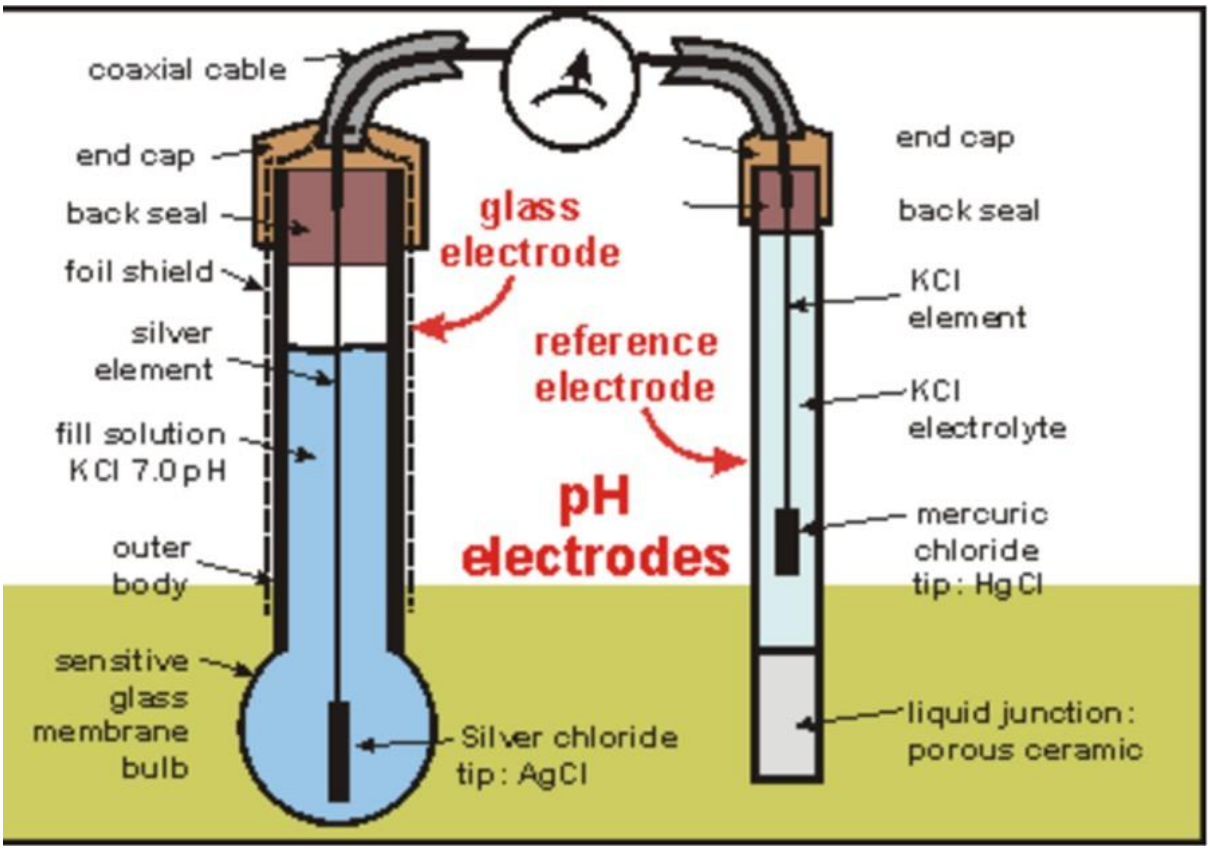

Figure 1:

2, Potentiometric working:

A potentiometric biosensor is an analytical device that measures changes in electrical potential (voltage) caused by biochemical reactions. When an enzyme is immobilized on the surface of an electrode, it catalyzes a specific reaction, leading to a change in ion concentration (e.g., H^+, NH_4^+) that can be detected potentiometrically. Below is a detailedexplanation of how a potentiometric biosensor works with an immobilized enzyme, using urease as an example.

3.4.1

Principle of Operation

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4 Enzyme Immobilization:

• The enzyme (e.g., urease) is immobilized on the surface of an ion-selective electrode (ISE) or pH-sensitive electrode.

• Immobilization methods include entrapment (e.g., in a polymer matrix likecalcium alginate), adsorption, or covalent binding.

5 Biochemical Reaction:

• The immobilized enzyme catalyzes a specific reaction. For urease:

Urea+H2O→Urease2NH3+CO2Urea+H2O Urease 2NH3+CO2

• The ammonia (NH_3) produced reacts with water to form ammonium ions (NH_4^+) and hydroxide ions (OH^-), leading to a change in pH:

NH3+H2O→NH4++OH−NH3+H2O→NH4++OH−

6 Potentiometric Detection:

• The change in pH or ammonium ion concentration is detected by the ion-selective electrode (e.g., pH electrode or ammonium ion-selective electrode).

• The electrode generates a potential difference (mV) proportional to the concentration of the target ion (H^+ or NH_4^+).

7 Signal Measurement:

• The potential difference is measured using a potentiometer or pH meter.

• The signal is correlated with the concentration of the analyte (e.g., urea) using a calibration curve.

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7.1

7.1.1

Working Mechanism

8 Sample Introduction:

• The sample solution containing urea is introduced to the biosensor.

9 Enzyme Reaction:

• Urease catalyzes the hydrolysis of urea, producing ammonia and carbon dioxide.

10 PH Change:

• Ammonia reacts with water, increasing the pH of the solution near the electrode surface.

11 Potential Change:

• The pH-sensitive electrode detects the change in H^+ ion concentration, generating a potential difference.

12 Signal Output:

• The potentiometer records the potential change, which is proportional to the urea concentration.

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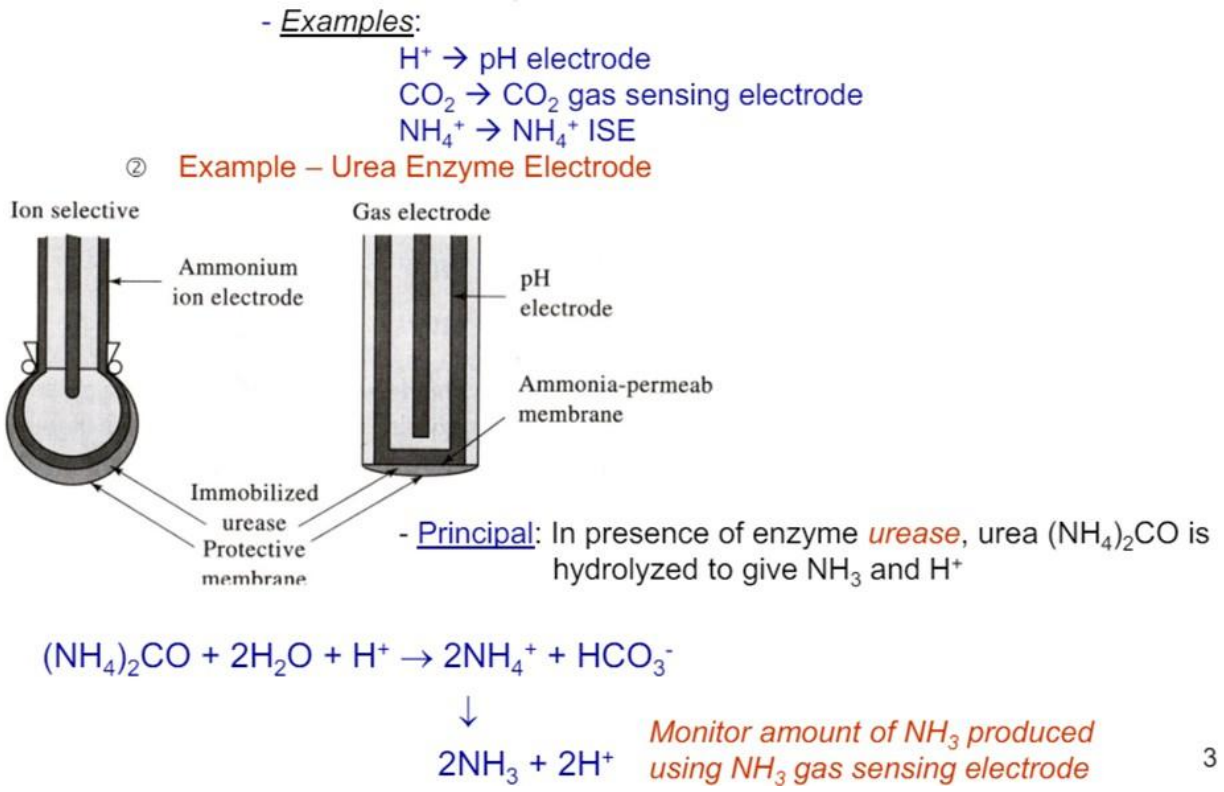

Figure 2:

12.1 Entrapment of Urease on pH Probe and Urease Assay:

To immobilize urease enzyme on a pH probe using sodium alginate and calcium chloride for the development of a potentiometric biosensor and to determine the activity of both free and immobilized urease.

12.1.1

Materials Required:

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13 Enzyme: Urease (source: jack bean or microbial

14 Sodium Alginate: For gel preparation

15 Calcium Chloride ($CaCl_2$: For cross-linking sodium alginate to form gel beads

16 Cellulose Nitrate Membrane: To support the immo- bilized enzyme

17 Glass pH Electrode: Standardized for potentiometric measurements

18 PH Meter: For measuring changes in pH

19 Urea Solution: Saturating concentration for enzyme activity assay

20 Nessler's Reagent: For ammonia detection

21 Magnetic Stirrer: To maintain uniform conditions during the assay

22 Buffer Solutions: Phosphate buffer (pH 5 3 for optimal

enzyme activity

23 Other Chemicals: Deionized water, stabilizers, and

Step 1: Preparation of Alginate Gel for Urease Immobilization

25 Sodium Alginate Solution:

Dissolve sodium alginate (2-3% w/v) in deionized water to form a homogeneous solution.

Add urease enzyme to the sodium alginate solution and mix gently to ensure uniform distribution.

26 Calcium Chloride Solution:

Prepare a 2% (w/v) calcium chloride solution in deionized water.

27 Formation of Alginate Gel Beads:

Using a syringe or dropper, drop the sodium alginate-urease mixture into the calcium chloride solution.

Allow the droplets to remain in the $CaCl_2$ solution for 10-15 minutes to form calcium alginate gel beads with entrapped urease.

Wash the beads with deionized water to remove excess $CaCl_2$.

Step 2: Immobilization of Urease on pH Probe

28 Preparation of Cellulose Nitrate Membrane:

Cut a standard cellulose nitrate membrane to fit the bulb of the glass pH electrode.

Soak the membrane in deionized water to ensure flexibility.

29 Coating the pH Electrode:

Attach the calcium alginate gel beads (with entrapped urease) to the cellulose nitrate membrane.

Carefully wrap the membrane around the bulb of the glass pH electrode, ensuring the gel layer is uniform.

Secure the membrane in place using a non-reactive adhesive or tape.

30 Formation of Enzyme Layer:

Allow the gel to dry slightly, forming a stable layer of calcium alginate with immobilized urease around the pH electrode.

Step 3: Assembly of the Biosensor

31 Connection to pH Meter:

Connect the glass pH electrode (with immobilized urease) to a pH meter for potentiometric measurements.

Calibrate the pH meter using standard buffer solutions (e.g., pH 4.0, 7.0, and 10.0).

32 Preparation of Urea Solution:

Prepare a saturating concentration of urea solution (e.g., 100 mM) in phosphate buffer (pH 5.3).

Step 4: Urease Activity Assay

33 Free Urease Activity:

Add a known amount of free urease enzyme to the urea solution.Incubate the mixture at 37°C with constant stirring using a magnetic stirrer.

After a fixed time interval (e.g., 5 minutes), take an aliquot of the solution and mix it with Nessler's reagent.

Measure the absorbance of the solution at 425 nm using a spectrophotometer to determine the amount of ammonia liberated.

34 Immobilized Urease Activity:

Immerse the pH electrode (with immobilized urease) in the urea solution.

Monitor the change in pH using the pH meter as the urease catalyzes the hydrolysis of urea to ammonia and carbon dioxide.

Record the pH change over time and calculate the amount of ammonia produced based on the calibration curve.

35 Nessler's Reagent Calibration:

Prepare a series of ammonium chloride solutions of known concentrations.Mix each solution with Nessler's reagent and measure the absorbance at 425 nm.Plot a calibration curve of absorbance vs. ammonia concentration.

Step 5: Calculation of Urease Activity

36 Definition of Urease Unit:

One unit of urease activity is defined as the amount of enzyme required to liberate 1 μmol of ammonia per minute at pH 5.3 and 37°C.

37 Activity Calculation:

For free urease: Calculate the amount of ammonia liberated using the Nessler's reagent calibration curve and express the activity in units.

For immobilized urease: Calculate the amount of ammonia produced based on the pH change and express the activity in units.

Expected Outcomes:

38 Successful immobilization of urease on the pH probe using calcium alginate gel

38.1 Linear relationship between urea concentration and pH change for the immobilized urease biosensor

3. Determination of urease activity for both free and immobilized enzyme, with immobilized urease showing slightly reduced activity due to diffusion limitations.

39 Data Analysis:

40 Calibration Curve for Ammonia:

- Plot absorbance (at 425 nm) vs. ammonia concentration to determine the relationship.

41 Urease Activity:

- Calculate the activity of free and immobilized urease using the formula:

Urease Activity (Units)=Amount of Ammonia Liberated (μmol)Time (min)Urease Activity (Units)=Time (min)Amount of Ammonia Liberated (μmol)

42 Comparison of Free and Immobilized Urease:

- Compare the activity of free and immobilized urease to assess the efficiency of the immobilization process.

Figure 3: **Formation of Gel Layer**

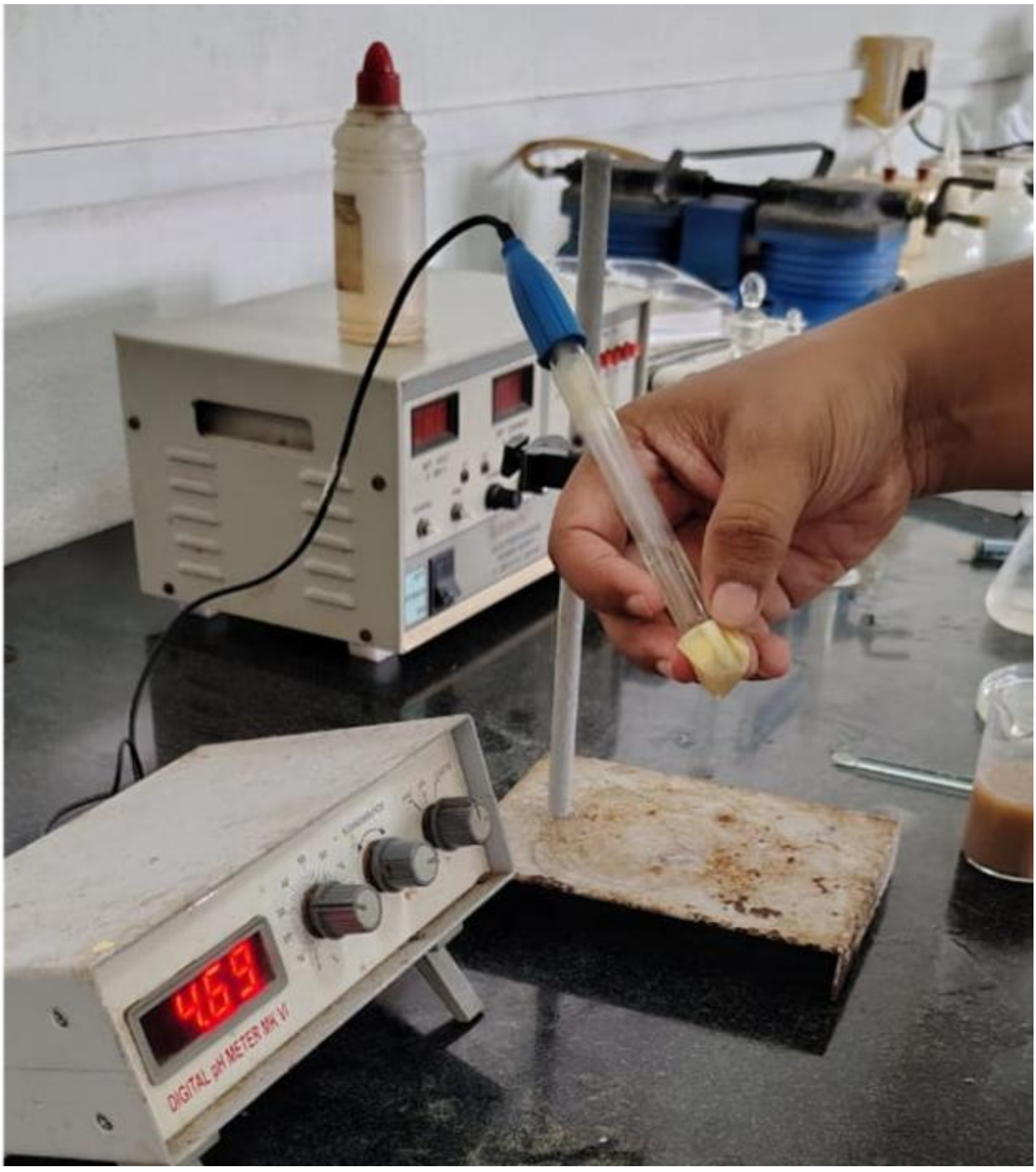

Figure 4: **Fig : Entrapment of Gel Layer**

Figure 5: **Fig : Entrapment of Gel Layer**

Figure 6: **Cellulose Nitrate Membrane**

42.1 Evaluation of the Biosensor:

Potentiometric measurement of urease activity was made using the biosensor by immersing and incubating the bulb in a beaker consisting of soil solution of pH 4.3 at 37°C with constant stirring at a moderate speed. When the electrode potential across the two leads of the biosensor reached a stable value, aliquots of urea at concentrations of 20, 40, 60, 80, and 100 mg/dL were used for calibration and plotting a graph.

Figure 7: **Soil Sample**

43 RESULTS AND DISCUSSION

43.1 Standardization of urea by optical density and fertility of soil based on pH levels

The standardization of urea by optical density(OD) was performed using a spectrophotometer at 540 nm.The results are presented above.The stansard curve shows a linear relationship between OD and urea concerntration,allowing for accurate determination of

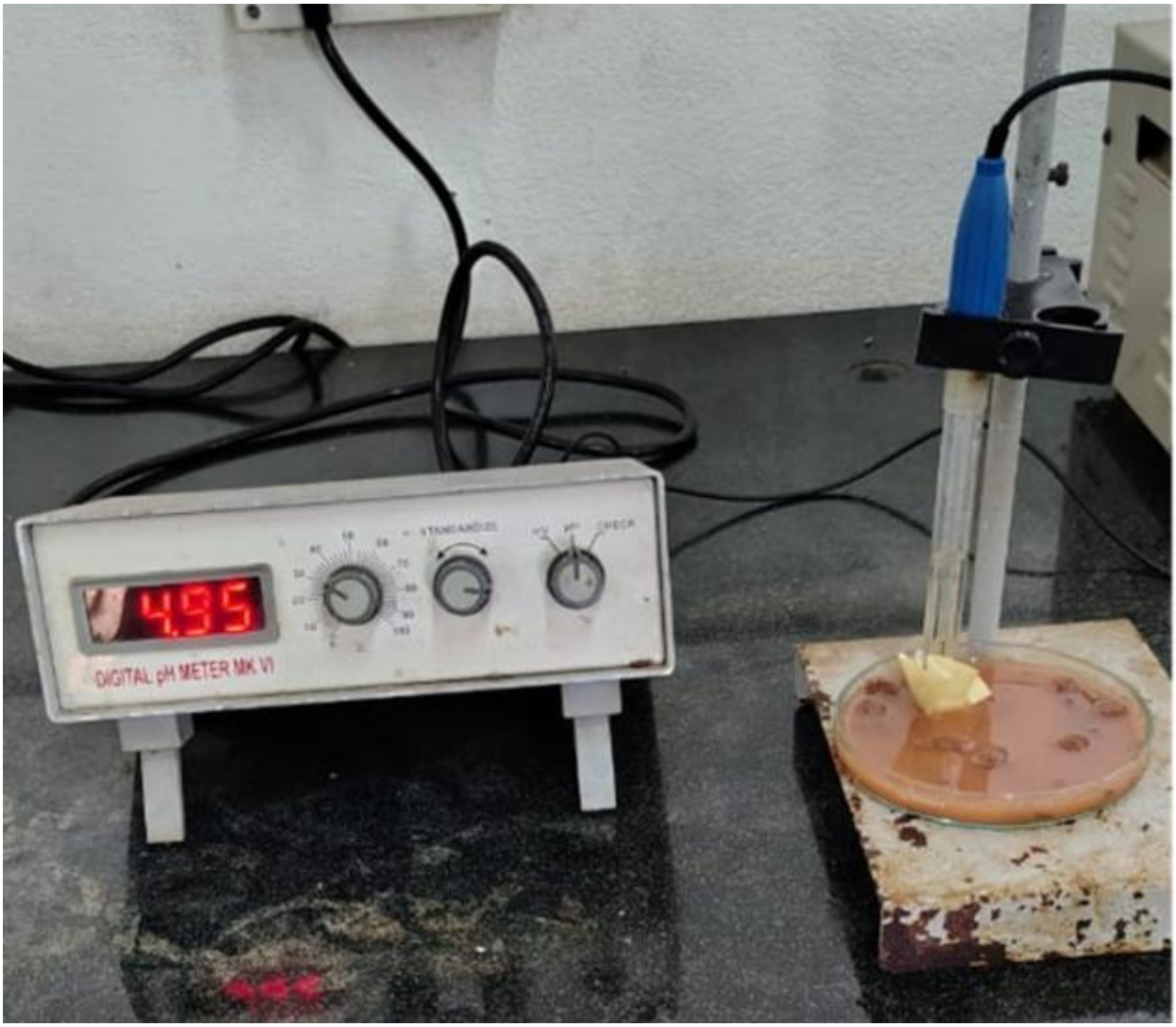

Figure 8: **Calculating the pH Reading**

urea concerntration using OD measurements.

The fertility of soil samples with varying the pH levels was evaluated with based on parameters such as ammonium content.The optimal pH range between the soil fertility is 4 and 7.5,where nutrient availability and organic matter are highest.soils with pH levels outside this range may require amendments to adjust pH and improve fertility.

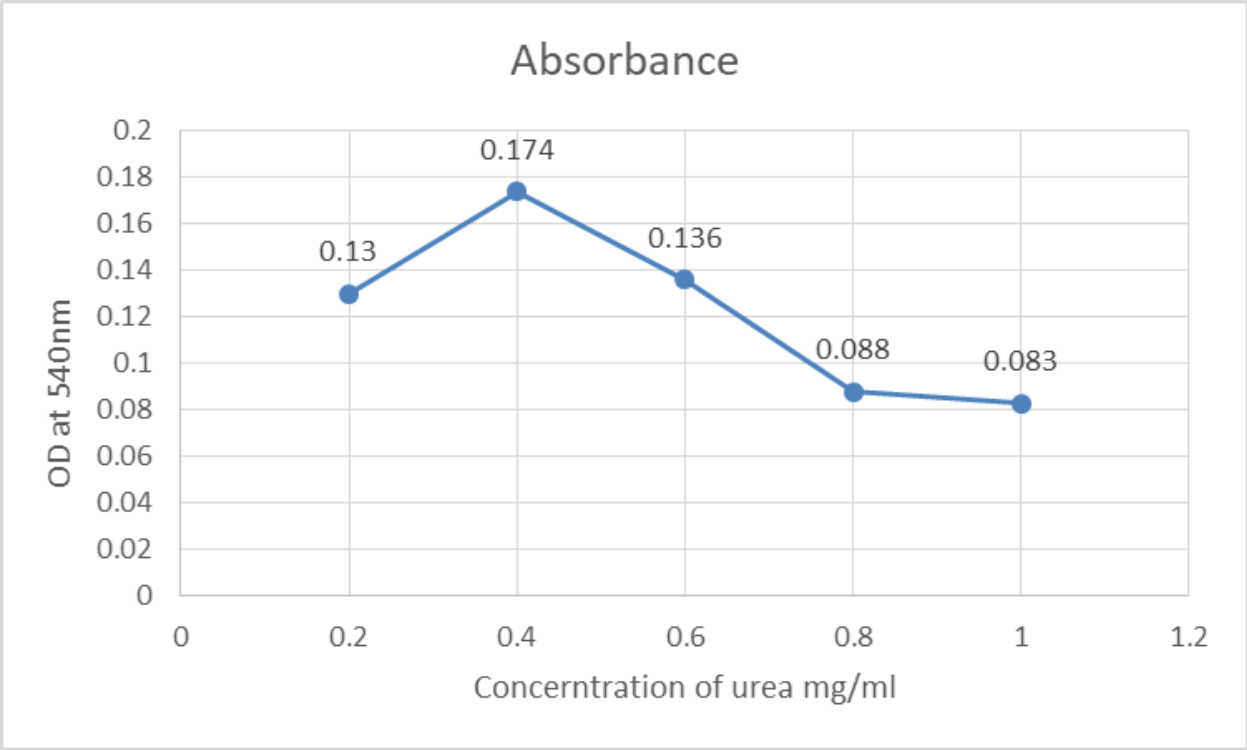

Figure 9: **standardization of urea by optical density .**

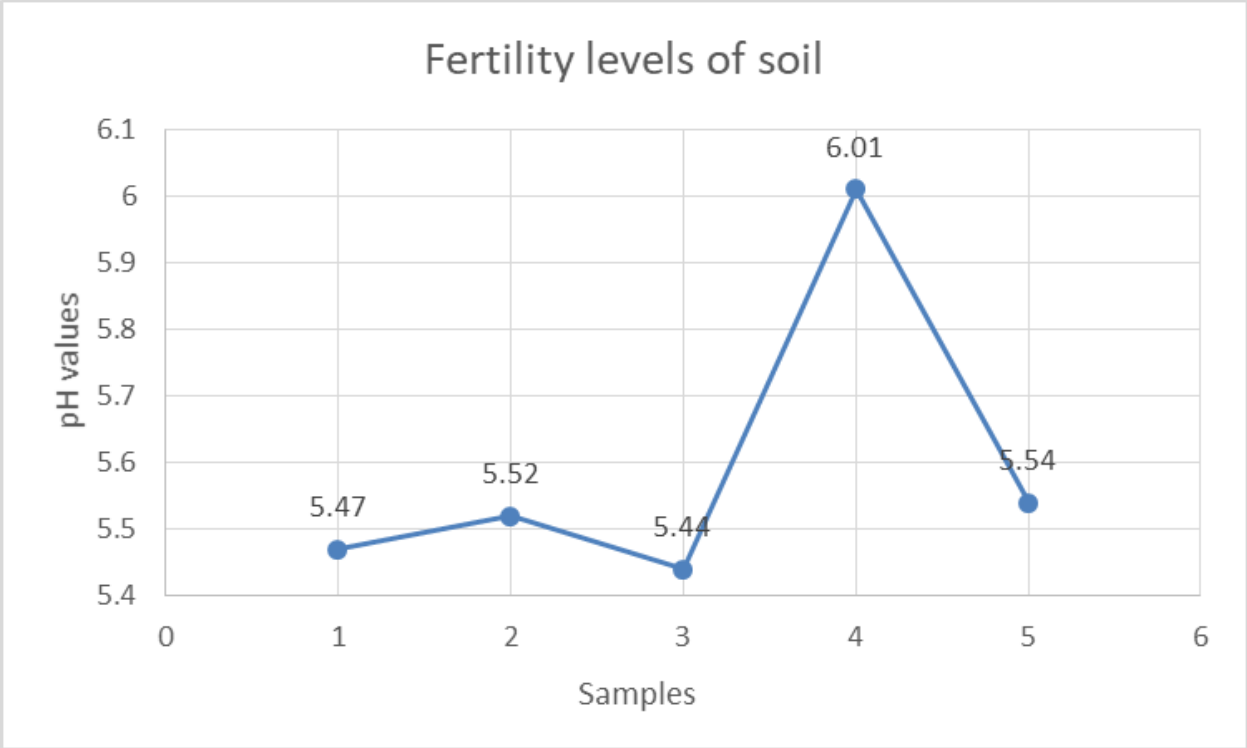

Figure 10: **fertility of soil based on pH levels.**

44 Conclusion

In conclusion, the application of a potentiometric biosensor for evaluating soil fertility through the measurement of ammonia concentration represents a significant advancement in agricultural science and soil management. This innovative approach offers a precise, real-time method for ensuring soil health, which is critical for optimizing crop yield and promoting sustainable agricultural practices and prosperity. The biosensor's ability to provide accurate and timely data on ammonia levels allows farmers to make informed decisions regarding fertilizer application and desired crops and controlled environment for both risk assessment and application , ultimately leading to improved soil fertility and enhanced productivity. Also, the addition of such technology into routine soil testing can contribute to more efficient use of resources and reduction in environmental impact. As agricultural practices continue to evolve, the potentiometric biosensor stands out as a valuable tool for ensuring soil quality and supporting the long-term sustainability of farming . Future research and development will likely expand its capabilities, further enhancing its effectiveness and applicability in diverse agricultural settings.The gadget size can be reduced and used minimal terms for convenience and if possible it can be developed to a commercial product similar to the blood glucose testing device where it is compact and mobile. for convenience,cost reduction and maintenance etcTherefore the biosensor with standardized knowledge can be utilized for the determination of ammonia content with help of ph that changes in a controlled environment from urease enzyme interaction with urea present in the soil that is filtered through cellulose nitrate me