ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

Determination of urea in commonly consumed Coffee in different parts of India using biosensor prepared by immobilisation of urease on nylon membrane in AISE

Meenakshi [1], Seema Kumari [*]

1,[*] Department of Botany, Baba Mastnath University, Rohtak, Haryana

Email *: kumareeseema2011@gmail.com

Email [1]: kumarim008@gmail.com

ABSTRACT

Coffee, after tea, is the most used non-alcoholic caffeinated beverage. A biosensor was created by immobilising urease enzyme on a nylon membrane and attaching it to an ammonium ion selective electrode (AISE) which was characterised by FTIR and FESEM with particle size between 0.09µm-0.49µm.in diameter .The biosensor showed optimum response within 20s at pH 5.5 in 0.05mM urea conc. in sodium phosphate buffer and 40˚C. It exhibited excellent sensitivity of 38 mV/decade and LOD 0.001 mM, and linear range 0.001 to 0.80 mM. Analytical recovery of added urea which were found to be 99.8%, 101.04%, 108.35%, 103.9%, 98.99%, 104.7%, 99.93%, 102.34%, 101.76%, and 103.02%The average urea conc. in coffee is 0.133 mg/L whereas the average pH is 5.36.

KEYWORDS: Coffee, AISE, Immobilization, Urease, Urea, Biosensor

1. Introduction

Coffee is a renowned beverage that is made from the roasted and ground seeds of tropical evergreen African coffee bushes. One of the three most popular beverages drunk worldwide, along with tea and water, is coffee. It is also one of the most lucrative global commodities. The seeds found in the fruits of the shrubs are believed to be originated somewhere in equatorial Africa, Java, Sumatra, and other Dutch East Indies, West Indies, India, and China. They also grew naturally in the shade of African forests, including those on the islands of Madagascar and Mauritius (formerly known as one among the Mascarene Islands). We roast, grind, and boil green coffee beans to create the well-known beverage that is consumed worldwide. [1]

Coffee is a significant commodity crop that is essential to the socioeconomic well-being of more than 50 nations. It was once thought that coffee, which belongs to the genus Coffea in the family Rubiaceae, originated in tropical Africa. The four sections of the Coffea genus are

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

Agrocoffea, Paracoffea, Mascarocoffea, and Eucoffea. Coffea canephora and Coffea arabica are the two most significant commercial species. Except for C arabica, which has tatraploid 2n=4x=44 chromosomes, all the species are diploid with 2n=2x=22 chromosomes. In comparison to other species, Coffea arabica has been determined to have minimal polymorphismThe most significant chemical found in coffee beans is caffeine, which ranges in concentration from 0.8% to 1.4% in Arabica coffee to 1.7% to 4.0% in canephora. Cellulose, minerals, carbohydrates, lipids, tannin, and polyphenols are some of the other ingredients found in coffee beans. Low temperatures are detrimental to the coffee seed's ability to survive and has an intermediate effect on how coffee is stored. Priming of the seeds is used to improve the uniformity of germination for improved crop establishment since abscisic acid (ABA) induces dormancy and inhibits germination. According to Adepoju (2017)[2], shade has an impact on coffee by increasing bean weight, size, antioxidant activity, total phenolic content, and chlorogenic acid concentration.

The International Coffee Organisation claims that roasted coffee beans are used to make a variety of drinks, and that coffee consumption is rising globally [3]. An amount of ground coffee, referred to as a "dose," is used while making a beverage. It is important to use the right amount of coffee, and this cannot be understated. This grinding-related number affects the extraction process and the final beverage, so it must be reliable and consistent.

To get a uniform powder, the beans must be ground down to a smaller size. In order for soluble and emulsifying ingredients to move into the brew more easily, the main goal is to increase the specified extraction surface, or more particularly, the interface between water and coffee [4]. The amount varies depending on a number of variables, including the brewing technique and formula, including the removal of caffeine and other important bioactive chemicals during extraction [5].

To create coffee powder, specialised grinders are employed. Both domestic and industrial systems use flat and conical burr grinders. Two conical rings are positioned inside of one another in a conical grinder, while two flat rings are parallel to one another in a flat burr grinder. In both types, one ring is rotated by a motor while the other remains fixed. On-demand systems use an accumulation tank for the ground powder instead of a dosing mechanism, which is used in some variants.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

Different-sized grains or particles make up ground coffee powder. A bimodal distribution profile called a particle size curve can be used to represent granules. The "first modal" stands for the high peak, whereas the "second modal" stands for the low peak. A particle can be as little as a few micrometres or as large as a thousand micrometres. Coffee's distinctive aroma is caused by the rapid dissolution of chemical components by hot water and the release of volatiles [6].

A comprehensive study was conducted to assess the urea content in various low-priced coffee brands available in the Indian market, employing a cutting-edge biosensing method. Urea, a naturally occurring compound, is often used unscrupulously to enhance the apparent freshness and aroma of coffee beans. The research aimed to shed light on the potential adulteration and its implications for consumers. Through meticulous biosensing techniques, the study meticulously scrutinized each coffee sample, revealing critical insights into the urea concentrations across different brands. The findings of this investigation serve as a crucial resource in safeguarding the quality and authenticity of affordable coffee options in India, ensuring that consumers can savor their favorite brew with confidence and peace of mind.

Nature contains large amounts of urea, and research into this substance is crucial for agricultural chemistry, food science, environmental monitoring, and therapeutic care [7]. With a molecular weight of 60 g/mol, urea being a little water-soluble molecule contains two nitrogen atoms and is a byproduct of nitrogen and protein metabolism [8]. It is an odourless, colourless solid that is very soluble in water, does not have any acidic or alkaline characteristics, and is almost non-toxic [9,10]. It has been produced as a byproduct during the urea cycle inside the bodies of numerous species.

Additionally, the bulk of food items include urea, which raises the blood urea level. The goal of the current study is to use a biosensor to measure the amount of urea in coffee.

Urea is broken down by a dinickel enzyme called urease (EC 3.5.1.5) ammonia (NH_3) and carbonic acid (H_2CO_3) via carbamic acid (H_2NCOOH) (Fig. 1).1. In aqueous solutions, bicarbonate (HCO_3) and ammonium (NH_4^+) ions, respectively, balance carbonic acid and NH_3 levels. Invertebrates, fungi, bacteria, and plants all generate urease, and most species have a high degree of structural and functional similarity. Two Ni^{2+} ions are joined by a carbamylated lysine and hydroxyl group in the urease active site [11].

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

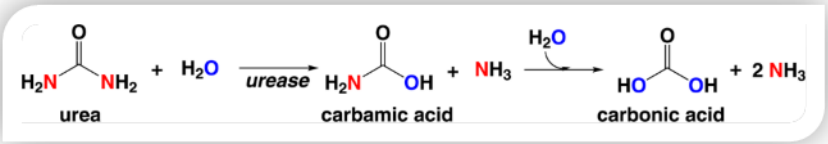


Fig.1 schematic breakdown of urea by urease in its byproducts

Over time, researchers have utilized a range of methods to assess urea concentration in beverages. Historical approaches include colorimetric techniques like the Berthelot Reaction and Diacetyl Monoxime Method, as well as enzymatic assays employing urease. These methods evolved to include amperometric analysis, ion-selective electrodes, and advanced techniques such as LC-MS, Capillary Electrophoresis, and NMR. This historical perspective highlights the enduring relevance of these methods in beverage analysis.

For determining the physiological status of important electrolytes, potentiometry using polymeric membrane ion-selective electrodes (ISEs) is a well-established analytical technique. Potentiometric determination of urea boasts several distinct advantages when compared to alternative analytical methods, rendering it a preferred choice in specific contexts. One of its key strengths lies in its high sensitivity, enabling the accurate quantification of even minute concentrations of urea within a sample. This attribute proves particularly valuable in applications where trace levels of urea need to be precisely measured. Additionally, potentiometric sensors can be tailored for high selectivity towards urea, effectively reducing interference from other substances present in the sample, thus ensuring the reliability and precision of results. Furthermore, the non-destructive nature of this method allows the sample to remain intact, permitting subsequent analyses if necessary. Its simplicity and speed make it suitable for routine analyses and high-throughput applications, and it can be applied to a wide range of sample types, including liquids and solids. Cost-effectiveness, minimal chemical waste generation, and real-time monitoring capabilities further bolster the appeal of potentiometric determination, making it a versatile and eco-friendly choice in the realm of analytical chemistry [12].

Academics from a wide range of fields are interested in monitoring ammonium ion concentration since it is a crucial indicator for environmental and medical applications. For instance, ammonium is thought to be both a potential biomarker of an enzyme byproduct in crucial physiological reactions as well as a natural indicator of water quality. Due to

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

advantages like cost-effectiveness, user-friendliness, and miniaturisation ability, which enables easy portable measurements, potentiometric ion-selective electrodes (ISEs) have piqued the scientific community's interest among traditional analytical methods used to detect ammonium ions [13].

The uremic toxin with the greatest plasma concentration has a variety of effects that are both direct and indirect on numerous organs and tissues. The substance urea is detrimental to the kidneys (indirectly fostering renal fibrosis), the fat cells (causing resistance to insulin), other components of the blood (inflicting erythropoietin carbamylation), the circulatory system (CVS), and the digestive tract (causing epithelial barrier disintegrate and microbiome alteration).[14]

When evaluating coffee's quality and authenticity, the amount of urea in it must be known. The analysis of urea in food and beverages, including coffee, has used a variety of analytical techniques. Enzymatic assay is one often employed technique that depends on the enzymatic degradation of urea by urease and the subsequent measurement of the ammonia or other reaction products [15]. Urea concentrations in coffee samples have also been determined using chromatographic methods such gas chromatography (GC) and high-performance liquid chromatography (HPLC) [16]. With their great sensitivity and selectivity, these techniques enable precise determination of urea levels. As quick and non-destructive approaches for urea analysis in coffee, spectroscopic techniques including Fourier-transform infrared spectroscopy (FTIR) and near-infrared spectroscopy (NIRS) have showed promise. Additionally, mass spectrometry (MS) techniques, such as liquid chromatography-mass spectrometry (LC-MS), have been employed for the identification and quantification of urea in coffee [17]. These various analytical techniques offer academics and business experts a variety of choices for precisely determining the urea content in coffee samples.

2. Methodology

2.1 Materials used: The reagents required for the procedures include urease, sodium phosphate buffer, tris-acetate buffer, ethanol, glutaraldehyde, cysteamine dihydrochloride, chitosan, methanol, deionized water, 0.1 M NaCl (reference filling solution), ISAB (Internal Standard Addition Buffer), 25% glutaraldehyde solution (for the preparation of 2.5% glutaraldehyde), Nessler's reagent, tri-chloroacetic acid (TCA), and absolute ethanol.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

2.2 Instruments used: Digital ion meter, Water bath,, Sonicator, UV spectrophotometer, Weighing balance,Magnetic stirrer, Centrifuge, Ammonium ion selective electrode:, FTIR, FESEM.

2.3 Assay of free Urease enzyme

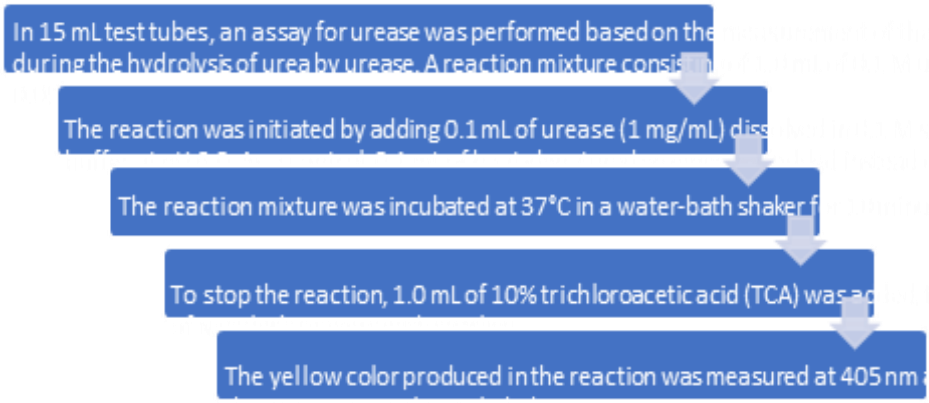
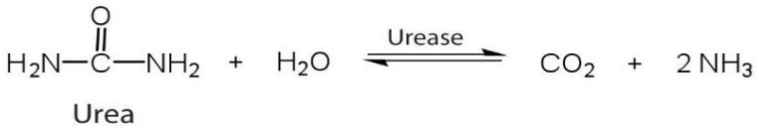


Fig. 2: stepwise representation of enzyme assay.

A standard curve mapping NH4+ concentration vs absorbance at 405 nm was used to extrapolate the concentration of NH4+ created throughout the experiment. Under standard test circumstances, one unit of enzyme activity was defined as the quantity of enzyme

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

required to liberate 1 mol of ammonia from urea hydrolysis in 1



Urea

minute.

2.4 Preparation of urease nanoparticles and immobilization: Already extracted Urease enzyme was bought from SIGMA ALDRICH. The nano particles were produced and immobilised on nylon membrane using the method described by Jakhar and Pundir., 2017 [18].

2.5 Characterization of free urease nano-particles: The size of the produced urease nanoparticles was determined using FTIR and FESEM.

2.6 Characterization of urease immobilized nylon membrane: FESEM pictures of the nylon membrane before and after immobilisation were acquired to ensure urease immobilisation on the nylon membrane.

2.7 Preparation of AISE electrode: Labman supplied the Ammonium Ion Selective Electrode (AISE). It was calibrated by dipping it in a 10% KCl solution for half an hour and then calibrating for stable values according to the electrode manual.

2.8 Optimization of Potentiometric Urea biosensor: Following the protocol suggested by Jakhar and Pundir., 2017 in their study, the prepared urea biosensor was optimised for pH, temperature, effect of substrate concentration, response time, linear range, detection limit, analytical recovery, sensitivity, precision, reproducibility, storage stability, and intereference of some metabolites.

2.9 Application of potentiometric urea biosensor in fermented alcoholic beverages

2.9.1 Collection of samples: as many samples of coffee powder were collected from local market nad online stores.

2.9.2 Evaluation of samples collected: The ammonia emitted from the samples was measured using a Labman Ammonium Ion Selective Electrode (AISE). The electrode was electrically attached to a digital ion metre from Labman, which displayed the values of ammonia, pH, and potential. TISAB equipped with a Labman electrode was utilised in each sample to liberate ammonia. Simply dip the electrode in 20 ml of alcohol containing 1 cc of TISAB.

3. Results

3.1 Enzyme assay

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

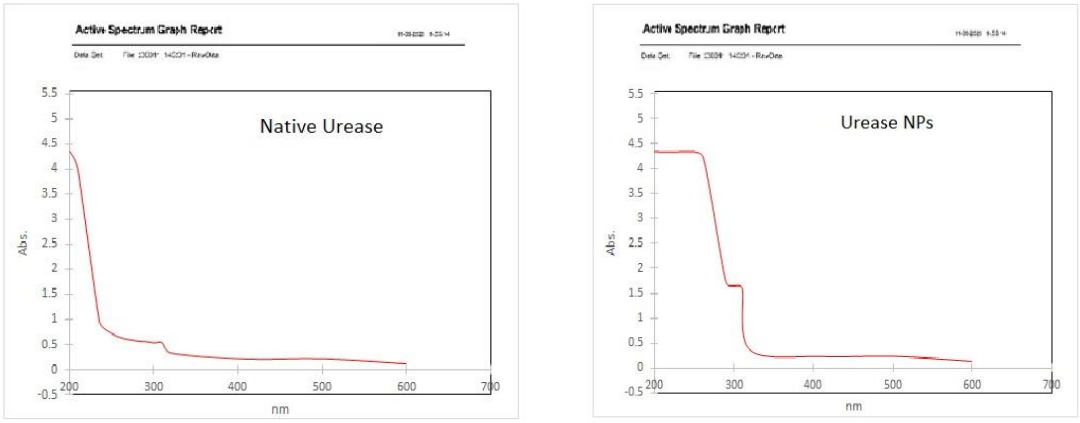


Fig.3 Spectrophotometric graph indicating enzyme activity

UV-visible absorption spectroscopy of NP aggregates revealed important structural changes caused by urease NP production. The dramatic shifts in absorption peaks, together with the increased absorbance within the aggregates, all contributed to the successful formation of urease NP aggregates while preserving the enzyme's unique molecular structure..

3.2 Preparation of urease nano-particles:

At 4°C, urease nanoparticles (NPs) were produced by desolvation with ethanol. This procedure decreased the hydration layer surrounding urease molecules, boosting interactions such as Vander Waals, hydrophobic, and electrostatic forces, resulting in stable urease-NP aggregates. We cross-linked the aggregates using glutaraldehyde, interacting with -NH2 groups introduced via cysteamine dihydrochloride, to ensure their long-term stability and enzymatic activity. These urease-NP aggregates had considerably higher enzymatic activity, which was most likely due to increased active site exposure and possible structural changes during the aggregating process.

3.3 Characterization of urease nano-particles

FESEM was used to examine the the shape and dimensions of aggregates of urease NPs, as shown in the figure 4. The sizes of the urease NPs ranged from 90 to 100 nm, with an average diameter of 96 nm. In contrast, native monomeric urease had a diameter of 13 nm as measured by TEM in a 1992 study by Turbett et al. This finding suggests that each spherical urease NP was generated by the aggregation of 14 to 18 native urease molecules.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

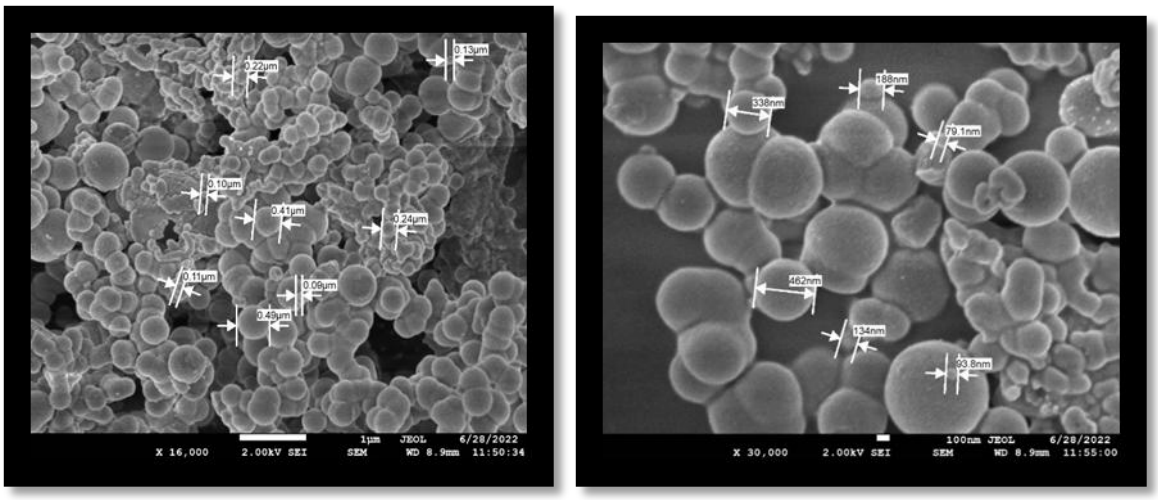
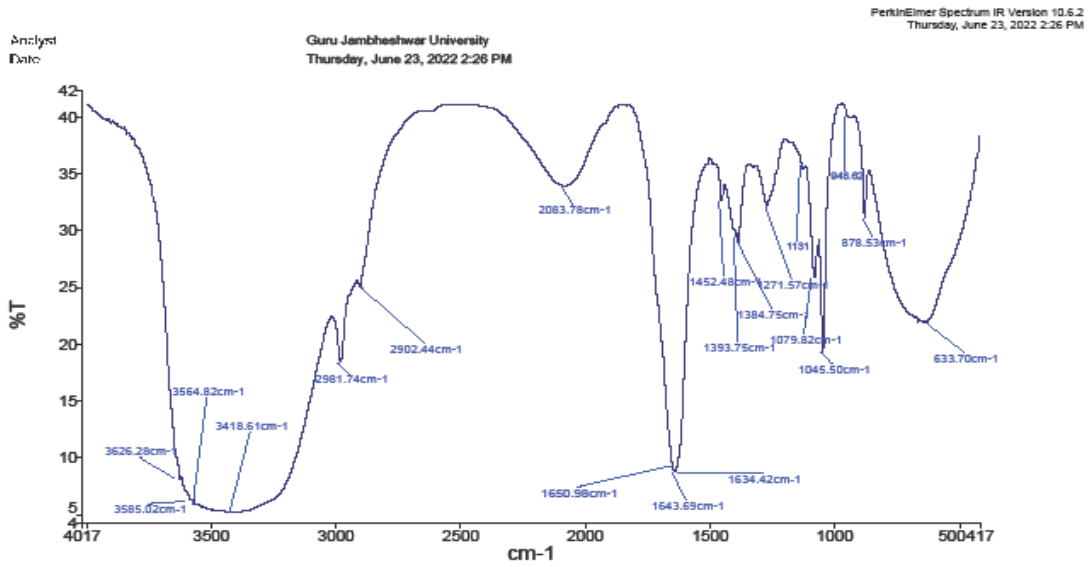


FIG. 4. FESEM image of urease nanoparticles

At 4°C, urease nanoparticles (NPs) were produced using the desolvation procedure with ethanol. Lowering the moisture layer around the urease molecules caused the aggregation, allowing interactions like Vander Waals forces, hydrophobic forces, and electrostatic forces to occur. The aggregates were practically permanently cross-linked with glutaraldehyde, assisted by -NH2 groups from cysteamine dihydrochloride, while retaining their structures and enzymatic activity. The resulting urease-NP aggregates had dramatically increased enzymatic activity, which was most likely due to increased active site exposure and probable structural changes during aggregation.



ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

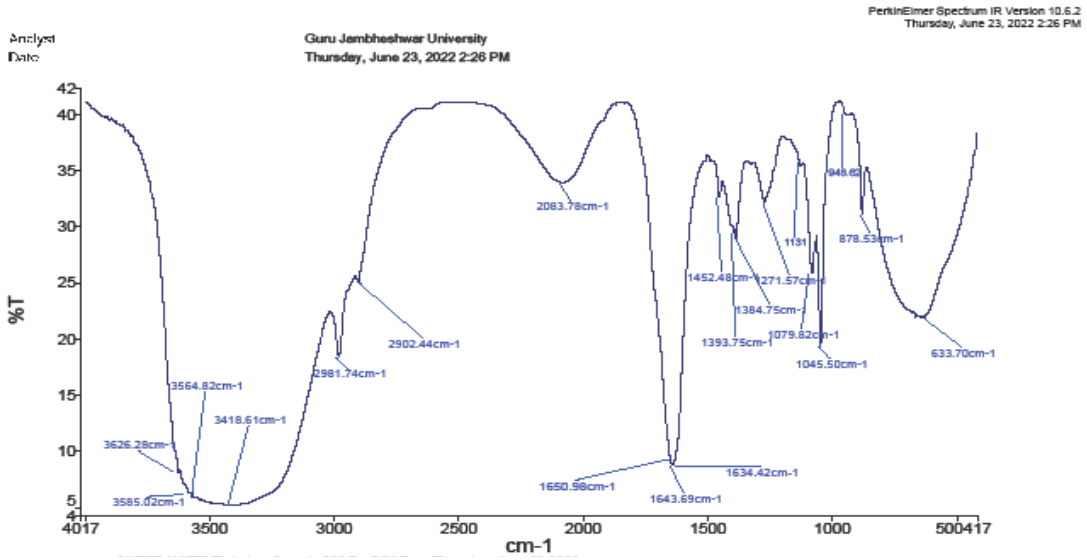


Fig. 5 FTIR graphs of urease nanoparticles

The study's figures highlight structural and chemical transformations. Curve 1 depicts the FTIR spectrum of untreated urease (4000-400 cm^{-1}), while Curve 2 shows aggregated urease nanoparticles. Key findings include transmittance peaks at 3418.61 cm^{-1} and 3410.0 cm^{-1} indicating –NH and –OH groups from cysteamine dihydrochloride. Peaks at 2083.78 cm^{-1} and 2079.31 cm^{-1} represent N-H and C=N stretching vibrations. Vibrations at 1638.19 cm^{-1} and 1635.42 cm^{-1} suggest C=C stretching vibrations from glutaraldehyde. Other significant peaks include 1271.57 cm^{-1} and 1269.47 cm^{-1} (C-N stretching), 1079.82 cm^{-1} and 1079.34 cm^{-1} (C-O stretching), 878.53 cm^{-1} and 878.34 cm^{-1} (=C-H bending), and 633.70 cm^{-1} and 627.64 cm^{-1} (C-H and C=C bending). The curves observed at different wavelengths indicated different bond stretching which are in similar trend as reported by Jakhar and Pundir in their paper in 2017.

3.4 Characterization of nylon membrane by FESEM

The untreated Nylon membrane's scanning electron microscopy (FESEM) pictures revealed a characteristic hollow beaded structure. In contrast, the Nylon membrane coated with aggregated urease nanoparticles had clusters of these nanoparticles scattered across the membrane's surface in bead-like patterns. This finding establishes the attachment and immobilisation of aggregates of urease-NPs on the Nylon membrane.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

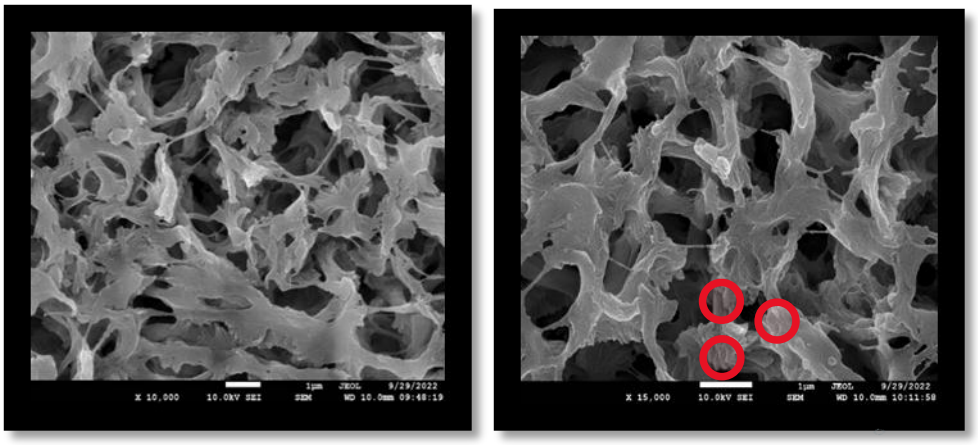


Fig.6 FESEM images of nylon membrane before and after immobilization of urease nano particles

The enzyme immobilised in this manner retained 86.71% of its initial activity, which was comparable to that of the native enzyme. Furthermore, the conjugation process produced a density of 1.64 mg/cm2. This result implies an increase in enzyme activity as a result of the covalent immobilisation of urease nanoparticles onto the Nylon membrane. The application of glutaraldehyde coupling, which coupled the amino groups of cysteamine-dihydrochloride made urease enzyme nps functional to the CHIT-decorated Nylon membrane, assisted immobilisation.

3.5 Construction of potentiometric urea biosensor

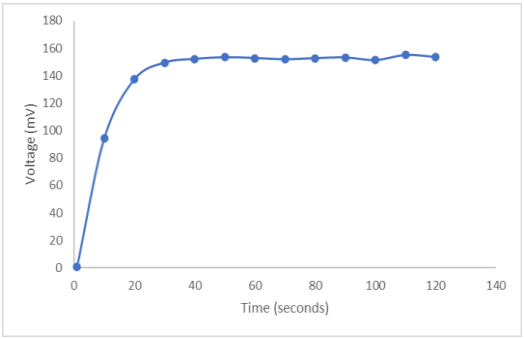
In order to construct a desired potentiometric biosensor for detection of urea, an ammonium ion selective electrode (AISE) was used in conjunction with a urease nanoparticle (NPs) aggregates-bound Nylon membrane. The Nylon membrane containing aggregation of urease NPs was attached to the lower, more sensitive area of the AISE in this biosensor setup. This integrated arrangement was then linked to a digital ion metre.

The potentiometric response was based on the AISE's properties. This method has several advantages, including a simple procedure, a reasonably quick response time, nondestructive examination, a large linear range with reasonable selectivity, and extensive use in quantifying different ions. A potential difference is formed over time in this example due to the changing concentration of NH_4^+ in the reaction buffer. This change in potential is caused by the urease's enzymatic hydrolysis of urea on the NC membrane, which results in the production of NH_4^+ and HCO_3^-. An ammonium ion selective electrode (NH_4^+ selective electrode) was used to measure this potential shift.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

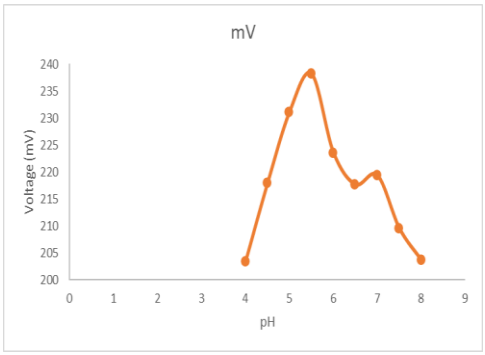
3.6 Optimization of urea biosensor:-

3.6.1 Optimization for Response time, temperature and pH



The biosensor's response time was evaluated at 10-second intervals from 10 seconds to 120 seconds. The reaction time recorded in this investigation was found to be comparable to that of numerous previously described potentiometric urea biosensors..

Fig. 7. Voltage vs Time graph for optimization of response time



The biosensor based on immobilised urease NP aggregates revealed its maximal response at a pH of 5.5, which is much less than free urease, which performs best at a pH of 7.0. This shift towards lower pH for maximal activity could be due to a possible decrease in the availability of -NH2 functional groups within the enzyme structure..

Figure 8: Influence of ph on the potential response of urea biosensor based on urease NPs/Nylon membrane

The optimal temperature for incubating the urease enzyme was discovered to be between 35 and 45°C, with the maximum activity recorded at 40°C. This temperature is higher than that of the native urease enzyme, which operates best at 25°C.

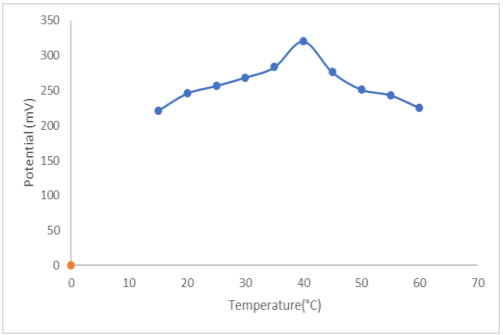


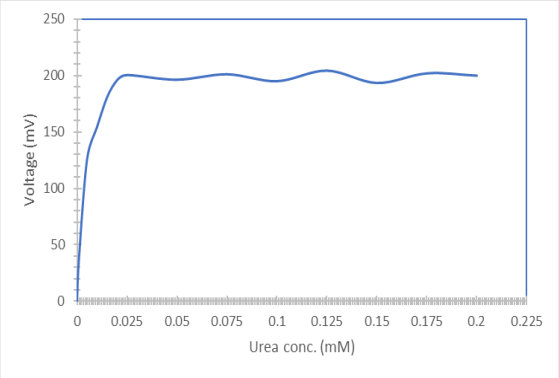
Fig. 9. Voltage vs Temperature graph for optimization temperature

The higher thermo-stability of the engineered nanoparticle (ENP) aggregates can be related to the increase in the optimal temperature of the urease enzyme.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

This improved stability is because of enzyme molecule aggregation and crosslinking with ENPs, which provides a more robust environment for the enzyme to function efficiently at elevated temperatures..

3.6.2. effect of conc. of substrate (urea)



The biosensor's reaction and urea concentration were shown to have a hyperbolic correlation ranging from 1 to 350 M. The reaction was continuously steady above 0.025 mM. Notably, the present urea biosensor's working range was expanded from 0.001 to 0.08 mM. This range outperforms the capabilities of previous potentiometric urea biosensors.

Fig. 10. Voltage vs conc. Of urea graph for optimization of effect of conc. Of substrate

3.6.3. Lower detection limit

The current biosensor's detection limit was determined to be 1 mol/L, demonstrating its great sensitivity in monitoring urea contents. This detection limit is significantly lower than that of numerous previously published potentiometric urea biosensors based on different matrices. It is also worth noting that the detection limit of the current biosensor beats reference methods, including the enzymic colorimetric method, which has a detection limit of 0.0005 M. These findings point to the present biosensor's excellent potential for precisely monitoring urea amounts with high sensitivity and efficiency.

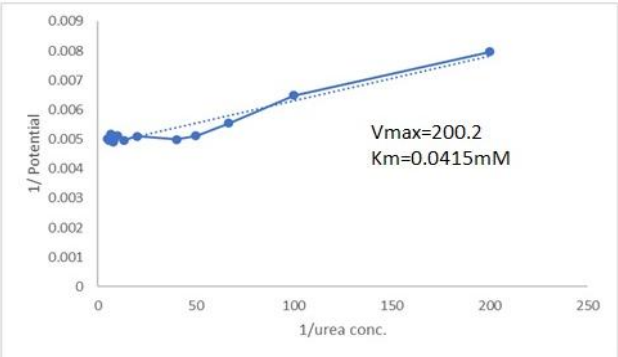


Fig. 11. Graph indicating Vmax and Km of the experiment.

3.6.4. Sensitivity

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

The current improved urea biosensor has a sensitivity of 38 mV/decade, demonstrating its higher performance when compared to previously reported potentiometric urea biosensors based on diverse materials and techniques.

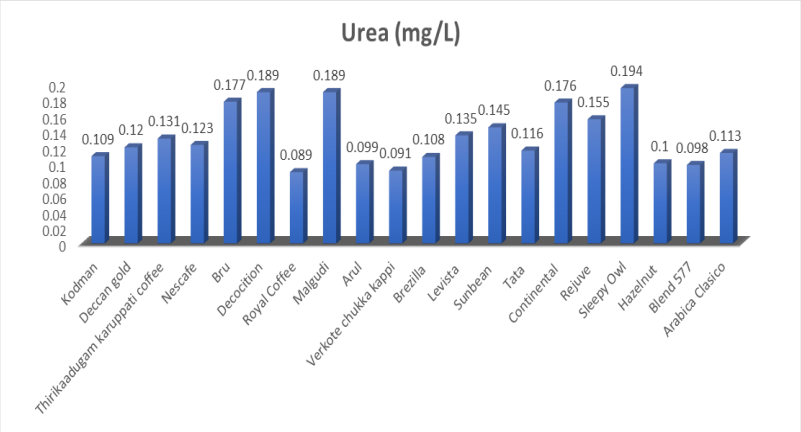


Fig12. Graph indicating concentration of urea of different coffee.

The average urea conc. in coffee is 0.133 mg/L whereas the average pH is 5.36 and potential difference is found to be 106.8 mV.

Discussion

In a study conducted by Menon and Girish in 2017 [19], a specific transducer/support system was employed for the detection of uric acid. The transducer utilized was RB-capped TGA/GNP, with a gold (Au) electrode. Immobilization of the urease enzyme was achieved through an entrapment method. The linear range for urea detection using this system ranged from 9.90 nmol/L to 65.4 nmol/L, with a detection limit of 9.71 nmol/L. The storage stability of this system was reported to be 60 days.

This research offers insights into a specific configuration for urea detection, showcasing the importance of transducer design and immobilization techniques in analytical chemistry. In light of the properties of the commercially available coffee that were examined, this work is innovative. So, it can be considered a novel work in this approach. Additionally, no such information is offered on the websites of the aforementioned brands. Despite the fact that information on their making processes and ingredients is available online. However, various studies have been done on several chemical ingredients found in coffee. Along with this its

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

medical significance has also been studied by many researchers. Urea has the highest plasma concentration of any uremic toxin and has both direct and indirect effects on various organs and tissues. So excess intake of it may increase blood toxicity. Hence, this study could be useful for analysing the addition of this substrate with every cup.

Summary and conclusion

Coffee, the second most widely consumed non-alcoholic caffeinated beverage after tea, is a ubiquitous part of modern society. In this study, a biosensor was developed by immobilizing urease enzyme on a nylon membrane and attaching it to an ammonium ion selective electrode (AISE). Characterization of the enzyme electrode revealed particle sizes ranging from 0.09µm to 0.49µm in diameter, confirmed through Fourier transform infrared spectroscopy (FTIR) and Field emission scanning electron microscopy (FESEM). The biosensor exhibited optimal response conditions at a pH of 5.5, 0.05mM urea concentration in sodium phosphate buffer, and a temperature of 40°C, with a remarkable sensitivity of 38 mV/decade, an ultra-low detection limit of 0.001 mM, and a broad linear range from 0.001 to 0.80 mM. Analytical recovery of added urea ranged from 98.99% to 108.35%.

Considering the average urea concentration in coffee is approximately 0.133 mg/L, with an average pH of 5.36 and a measured potential difference of 106.8 mV, this study sheds light on the chemical composition of coffee. Although coffee consumption has been associated with various health benefits when consumed in moderation, the specific role of urea in coffee remains relatively unexplored, presenting an intriguing avenue for further research.

In conclusion, the development and characterization of the urease-based biosensor presented in this study provide valuable insights into the composition of coffee, a widely consumed beverage. The biosensor's impressive sensitivity, low detection limit, and broad linear range make it a promising tool for urea detection, potentially applicable in various analytical contexts. Furthermore, the analytical recovery rates for added urea underscore the reliability of the biosensor.

While coffee has been linked to several health benefits when consumed moderately, the specific influence of urea in coffee warrants further investigation. This study opens the door to exploring the role of urea in coffee and its potential implications for human health. Future research in this area may contribute to a more comprehensive understanding of coffee's

chemical components and their effects on the human body, ultimately offering insights that can benefit both coffee enthusiasts and researchers in the field of nutrition and health.

Future perspectives:

The future of potentiometric urea determination in coffee, involving urease enzymes and ion-selective electrodes (ISEs), holds significant promise. It will advance quality control in the coffee industry, reinforcing consumer trust and ensuring adherence to food safety standards. As technology evolves, we can expect refinements and cost-effective enhancements. Automation may streamline the process, and its eco-friendly attributes align with sustainability efforts. Moreover, its potential for assessing various quality parameters underscores its versatility and potential impact on the coffee industry's future.

References:

1. Farah, Adriana. *Coffee in Health and Disease Prevention || The Coffee Plant and Beans* , (2015*), 5–10.* doi:10.1016/B978-0-12-409517-5.00001-2.
2. Adepoju, A.F. "Coffee: Botanty, Distribution, Diversity, Chemical Composition and Its Management." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 10.7 (2017): 57-62 DOI: 10.9790/2380-1007035762
3. ICO. International Coffee Organization., (2018) https://www.ico.org/monthly_coffee_trade_stats.asp. Accessed 2 Sep 2020.
4. Andueza S, Paz De Peña M, Cid C.Chemical and sensorial characteristics of espresso coffee as affected by grinding and torrefacto roast. J Agric Food Chem (2003) 51:7034–7039. https://doi.org/10.1021/jf034628f
5. Angeloni G, Guerrini L, Masella P, Bellumori M, Daluiso S, Parenti A, Innocenti M What kind of coffee do you drink? An investigation on effects of eight different extraction methods. (2019) Food Res Int. https://doi.org/10.1016/j.foodres.2018.10.022
6. Cordoba N, Fernandez-Alduenda M, Moreno FL, Ruiz Y Coffee extraction: a review of parameters and their influence on the physicochemical characteristics and flavour of coffee brews. Trends Food Sci Technol (2020) 96:45–60.
7. Pundir, C.S.; Jakhar, Seema; Narwal, Vinay (2018). *Determination of urea with special emphasis on biosensors: A Review. Biosensors and Bioelectronics, (), S0956566318307668*–. doi:10.1016/j.bios.(2018).09.067 .

8. Raymond Vanholder and others, Urea and chronic kidney disease: the comeback of the century? (in uraemia research), *Nephrology Dialysis Transplantation*, Volume 33, Issue 1, January (2018), Pages 4–12, https://doi.org/10.1093/ndt/gfx039
9. Dhawan, G., Sumana, G., & Malhotra, B. D. Recent developments in urea biosensors. Biochemical Engineering Journal, 44(1), 42–52.(2009) doi:10.1016/j.bej.2008.07.004
10. Wei, L.-F., & Shih, J.-S. Fullerene-cryptand coated piezoelectric crystal urea sensor based on urease. Analytica Chimica Acta, 437(1), (2001)77–85. doi:10.1016/s0003-2670(01)00941-2
11. Svane, S., Sigurdarson, J.J., Finkenwirth, F. *et al.* Inhibition of urease activity by different compounds provides insight into the modulation and association of bacterial nickel import and ureolysis. *Sci Rep* **10**, 8503 (2020). https://doi.org/10.1038/s41598-020-65107-9
12. Jiawang Ding, Wei Qin, Recent advances in potentiometric biosensors, TrAC Trends in Analytical Chemistry, Volume 124, (2020),115803, ISSN 0165-9936, https://doi.org/10.1016/j.trac.2019.115803.
13. Cuartero, MarÃa; Colozza, Noemi; FernÃ¡ndez-PÃ©rez, Bibiana M.; Crespo, GastÃ³n A. *Why ammonium detection is particularly challenging but insightful with ionophore-based potentiometric sensors- an overview of the progress in the last 20 years. The Analyst, 145(9),(2020) 3188 3210.* doi:10.1039/d0an00327a.
14. Colombo, Graziano, Alessandra Altomare, Emanuela Astori, Lucia Landoni, Maria Lisa Garavaglia, Ranieri Rossi, Daniela Giustarini, Maria Chiara Lionetti, Nicoletta Gagliano, Aldo Milzani, and Isabella Dalle-Donne. "Effects of Physiological and Pathological Urea Concentrations on Human Microvascular Endothelial Cells" *International Journal of Molecular Sciences* 24,(2023) no. 1: 691. https://doi.org/10.3390/ijms24010691.
15. Köseoglu M, Özdemir D, Fenderya N, et al. Determination of urea in urine and saliva by a novel conductometric biosensor. J Biotechnol. (2016);232:42-48.
16. Malinowska M, Szymandera-Buszka K. Determination of free amino acids in tea and coffee samples by different chromatographic techniques. J Chromatogr A. (2008);1205(1-2):105-114.
17. Melini V, Acquistucci R. Determination of caffeine, theobromine, and theophylline in cocoa samples by fast high-performance liquid chromatography. J Agric Food Chem. (2008);56(14):5736-5740.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

18. Jakhar, Seema; Pundir, C.S . *Preparation, characterization and application of urease nanoparticles for construction of an improved potentiometric urea biosensors. Biosensors and Bioelectronics, (2017), S0956566317306115–* . doi:10.1016/j.bios.2017.09.005

19. Menon, S., & Girish Kumar, K. A fluorescent biosensor for the determination of xanthine in tea and coffee via enzymatically generated uric acid. LWT, 86, (2017)8-13. https://doi.org/10.1016/j.lwt.2017.07.031.