Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

ADVANCING FOOD SAFETY: POTENTIOMETRIC DETECTION OF UREA IN COMMERCIAL MILK USING UREASE-IMMOBILIZED BIOSENSORS ON NYLON MEMBRANES

MEENAKSHI ATTRI
Research Scholar, Department of Botany, Baba Mastnath University, Rohtak, Haryana, India.

SEEMA KUMARI *
Assistant Professor, Department of Botany, Baba Mastnath University, Rohtak, Haryana, India.
Corresponding Author Email: kumareeseema2011@gmail.com, kumarim008@gmail.com

Abstract

Milk, a vital dietary staple globally, necessitates rigorous monitoring to ensure its safety and quality. Urea, a nitrogenous compound, is a critical parameter in this context, serving as an indicator of potential adulteration, animal health, and processing integrity. This research investigates and compares various methods for the precise determination of urea levels in milk, focusing on accuracy, sensitivity, and practicality. 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%. It was found to be trustable and an easy method of testing purity of any milk.

Keywords: Milk, AISE, Immobilization, Urease, Urea, Biosensor

1. INTRODUCTION

Milk, a vital dietary staple known for its rich nutrient profile, plays a critical role in global nutrition. Milk normally contains between 18 to 40 mg/dl of urea, and urea accounts for 55% of the non-protein nitrogen in milk. Its permitted concentration in milk, however, has been stated as less than 70 mg/dl (J.S. Jonker et al., 1998; G.K. Mishra et al., 2010; R. Sharma et al., 2008). Urea is a cheap way to alter milk since it is readily available, reasonably priced, and high in nitrogen.

According to U.B. Trivedi et al. (2009), milk with urea levels beyond the cut-off point may cause acid reflux, ulcers, cancer, and renal dysfunction. Current developments on urea biosensor for urea measurement have been described using gas chromatography, calorimetry, fluorimetry, and electrochemical techniques (G. Dhawan et al., 2009; J. Singh et al., 2013).

A number of urea biosensors were produced as a result of one of them, the electrochemical detection (E. Cevik et al., 2013). The first potentiometric urease enzyme electrode was developed by Guilbault and Montalvo in 1969 for the measurement of urea by its enzyme-catalyzed hydrolysis.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

Later, extensive research was done on the development of poentiometric urea biosensors for the detection of ammonium ion produced by the enzymatic reaction (B. Lakard et al., 2004, F. Kuralay et al., 2005, T. Ahuja et al., 2011), including the immobilisation of jack bean urease on synthetic polyvinyl alcohol- polyacrylamide (PVA-PAA) composite matrix, natural egg (K. Saeedfar et al., 2013). Urea in milk can originate from various sources, including natural processes like the catabolism of dietary proteins in cows, where urea is a natural byproduct of protein metabolism.

External factors like improper feeding practices and the unchecked use of urea-containing fertilizers in animal husbandry can also elevate urea levels. Additionally, the dairy industry's persistent concern about adulteration, aimed at increasing apparent milk protein content, underscores the urgency for accurate urea determination methods (Svane et al., 2020).

This research paper aims to comprehensively investigate and compare diverse methods for determining urea in milk, with a focus on accuracy, sensitivity, and practicality. By evaluating these methods, the study seeks to provide insights into the advantages and limitations of each approach, enabling dairy producers, regulatory agencies, and researchers to make informed decisions regarding the most suitable method for (B.Lakard, 2004)precise urea analysis in milk (Pawar et al.,2020).

2. METHODOLOGY

2.1. Reagents

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.

2.2. Instruments

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

An enzyme assay was performed following the protocol suggested by Jakhar and Pundir., 2017. 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 required to liberate 1 mol of ammonia from urea hydrolysis in 1 minute.

Urease nanoparticles were prepared from purchased SIGMA ALDRICH urease enzyme and immobilized on a nylon membrane following Jakhar and Pundir's 2017 method. The size of the urease nanoparticles was characterized using FTIR and FESEM. FESEM images of the nylon membrane before and after immobilization confirmed successful urease immobilization.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

The potentiometric urea biosensor was optimized for pH, temperature, substrate concentration, response time, linear range, detection limit, analytical recovery, sensitivity, precision, reproducibility, storage stability, and interference with metabolites, following Jakhar and Pundir's 2017 protocol. The biosensor was then applied to measure ammonia in fermented alcoholic Optimization

The assay of urease, based on the measurement of ammonia produced from the hydrolysis of urea by urease, was carried out in 15 mL test tubes. Each test tube contained a reaction mixture consisting of 0.9 mL of 0.05 M tri-acetate buffer (pH 7.3) and 1.0 mL of 0.1 M urea.

The reaction was initiated by adding 0.1 mL of urease (1 mg/mL) dissolved in 0.1 M sodium phosphate buffer at pH 6.0. As a control, 0.1 mL of heat-denatured enzyme was added instead of the active enzyme. The reaction mixture was incubated at 37°C in a water-bath shaker for 10 minutes. To stop the reaction, 1.0 mL of 10% trichloroacetic acid (TCA) was added, followed by the addition of 1.0 mL of Nessler's reagent with swirling.

The yellow color produced in the reaction was measured at 405 nm against a blank. A blank sample without the enzyme was also included. The concentration of NH_4^+ generated during the assay was extrapolated from a standard curve plotting NH_4^+ concentration versus absorbance at 405 nm. The amount of enzyme required to liberate 1 µmol of ammonia from the hydrolysis of urea in 1 minute under the standard assay conditions was defined as one unit of enzyme activity.

2.4. Preparation of urease nanoparticles and immobilization: Already extracted Urease enzyme was bought from SIGMA ALDRICH. The nano particles were prepared and immobilized on nylon membrane following the procedure mentioned by Jakhar et al., 2019.

2.5. Characterization of urease nano-particles: The prepared urease nanoparticles were scanned by FTIR and FESEM for their size.

2.6. Characterization of urease immobilized nylon membrane: for characterization of the nylon membrane FESEM images before and after the immobilization were taken to ensure the binding of urease on the nylon membrane.

2.7. Preparation of AISE electrode: Ammonium Ion Selective Electrode (AISE) was bought from Labman. It was calibrated by dipping it in 10% KCl solution for half hour and then calibrated for stable values as per the instructions provided with the electrode manual.

2.8. Optimization of Urea biosensor: the prepared urea biosensor was optimized for pH, temperature, effect of conc. of substrate, response time, linear range, detection limit, analytical recovery, sensitivity, precision, reproducibility and storage stability and interefernce of some metabolites following the protocol suggested by Jakhar and Pundir., 2019 in their study.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

2.9. Application of potentiometric urea biosensor in milk

2.9.1. Collection of samples: A few samples milk were bought from local markets and were stored at chilling temperature for use. The samples collected were used within a day to ensure that the milk is not spoiled,

2.9.2. Evaluation of samples collected: Ammonium Ion Selective Electrode (AISE) from Labman was used to measure the ammonia released from the samples. The electrode was attached to digital ion meter from Labman which showed the readings for ammonia, pH and potential. In each sample TISAB provided with the electrode from Labman was used for liberation of ammonia. The electrode was simply dipped in 20 ml of milk containing 1 ml of TISAB.

3. RESULTS AND DISCUSSION

3.1. Enzyme assay

UV-visible absorption spectroscopy of NP aggregates revealed important structural changes caused by urease NP production. Notably, the peak associated with the peptide bond exhibited a remarkable shift from the original 3.8 nm in native urease to an elevated 10 nm in urease NPs. Similarly, the peak attributed to the aromatic bonds underwent a considerable shift from 228 to 305 nm following the formation of urease NPs, signifying the transformation in the chemical environment. 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.

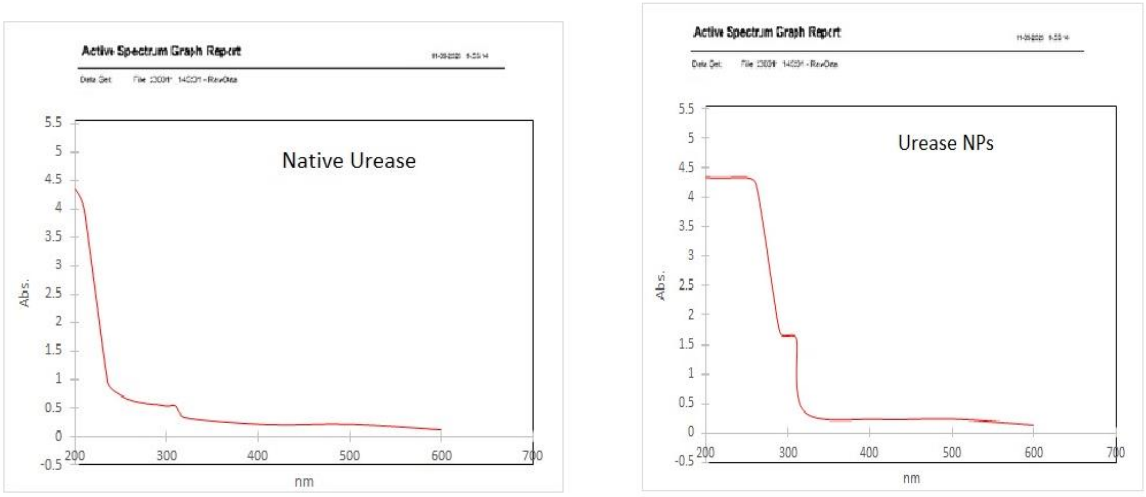


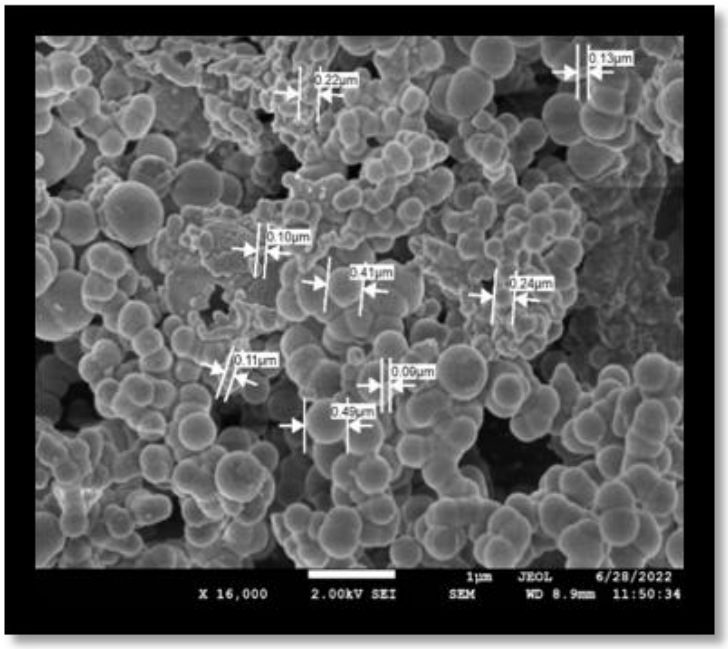
Fig 1: Spectroscopic Images of Urease Enzyme

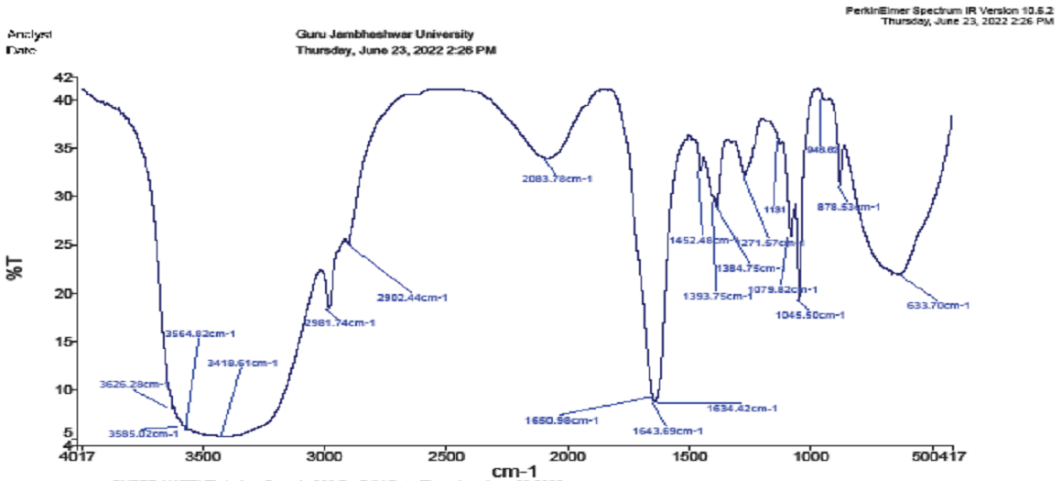
Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

3.2. Preparation of urease nano-particles:: At 4°C, urease nanoparticles (NPs) were produced 58 by desolvation with ethanol. We cross-linked the aggregates using glutaraldehyde, interacting with -NH2 groups introduced via cysteamine dihydrochloride, to ensure their long-term stability and enzymatic activity.

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..

This finding suggests that each spherical urease NP was generated by the aggregation of 14 to 18 native urease molecules.





Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

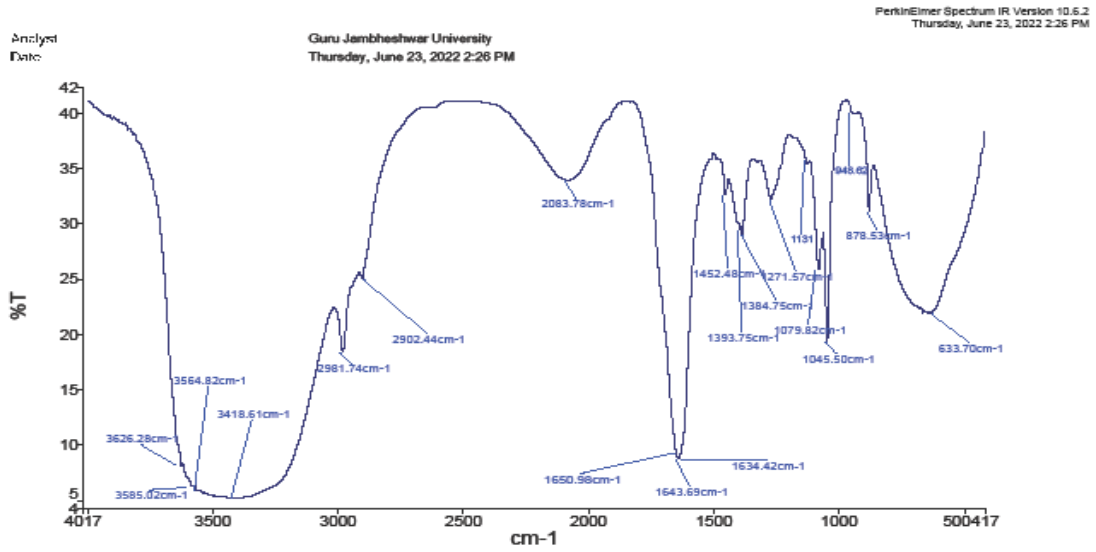


Fig 3: FTIR graphs of urease nanoparticles showing curve 1 and curve 2

The study's figure 1 illustrate structural and chemical transformations. Curve 1 displays the FTIR spectrum of untreated urease (4000-400 cm^{-1}), and Curve 2 shows urease nanoparticles. Key findings include transmittance 70 peaks at 3418.61 cm^{-1} and 3410.0 cm^{-1} for -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). 74 These wavelength observations align with those reported by Jakhar and Pundir in their 2017 paper.

3.4. Characterization of nylon membrane by FESEM: Untreated Nylon membrane showed a distinct hollow beaded structure under FESEM. In contrast, Nylon membrane coated with urease nanoparticles displayed scattered clusters of these particles, forming bead-like patterns on the surface. The enzyme, immobilized this way, retained 79 86.71% of its initial activity, comparable to the native enzyme. The conjugation process achieved a density of 80 1.64 mg/cm^2, indicating increased enzyme activity due to covalent urease nanoparticle immobilization on the Nylon membrane.

3.5. Building of potentiometric urea biosensor

An ammonium ion selective electrode (AISE) was utilized in conjunction with urease nanoparticle (NPs) aggregates-bound Nylon membrane to create a potentiometric urea biosensor. This biosensor arrangement involved attaching the Nylon membrane containing aggregates of urease NPs lower, more sensitive region of the AISE. This combined setup was then connected to a digital ion meter.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

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 which have been shown in figure 4.

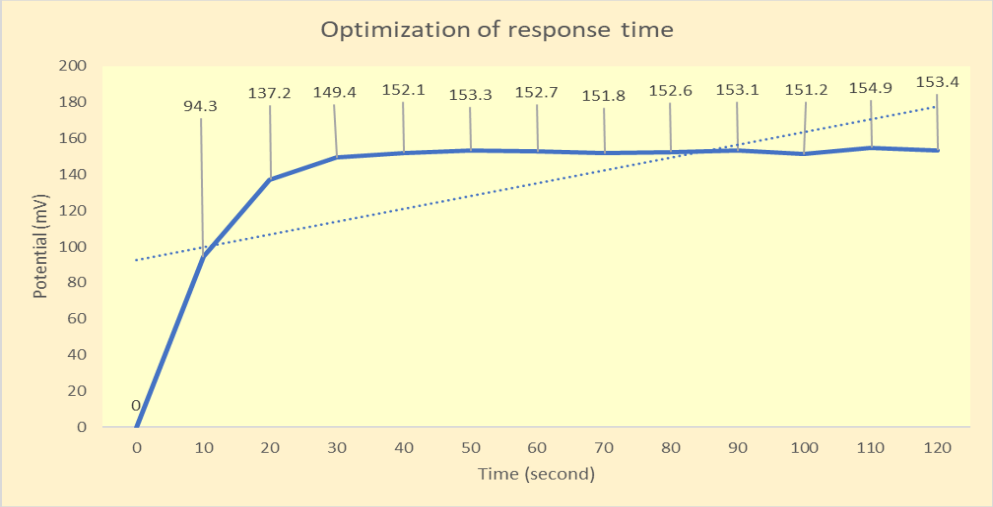


Fig 4: Voltage vs Time graph for optimization of response time

The biosensor based on immobilised urease NP aggregates revealed in fig 5. Shows 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.

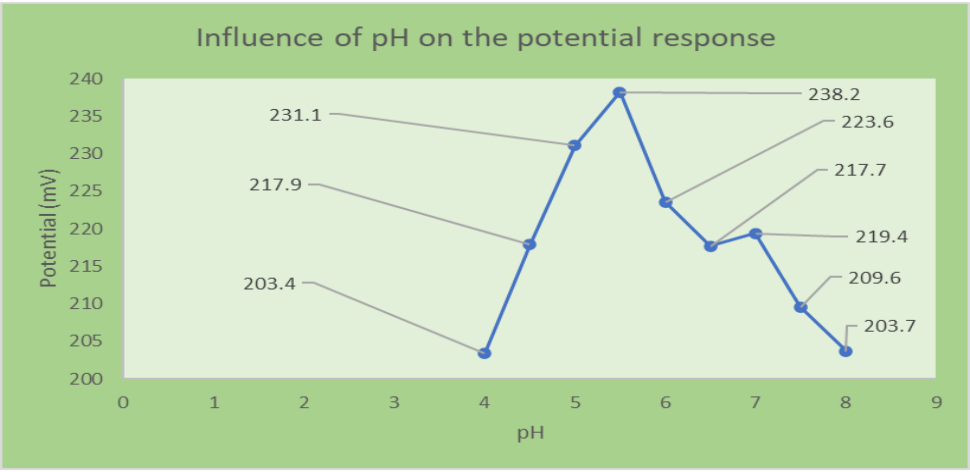


Figure 5: Influence of pH on the potential response of urea biosensor based on urease NPs/Nylon membrane

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 which is indicated by the peak in fig. 6.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

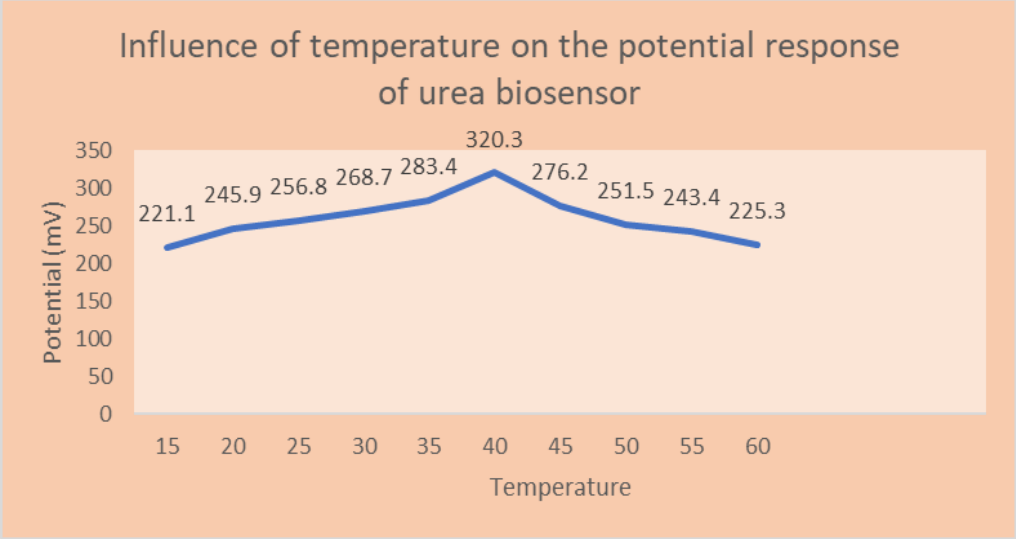


Fig 6: Voltage vs Temperature graph for optimization temperature

3.6.2. Effect of conc. of substrate (urea)

Notably, the present urea biosensor's working range was expanded from 0.001 to 0.08 mM as indicated in the graph in fig. 7.

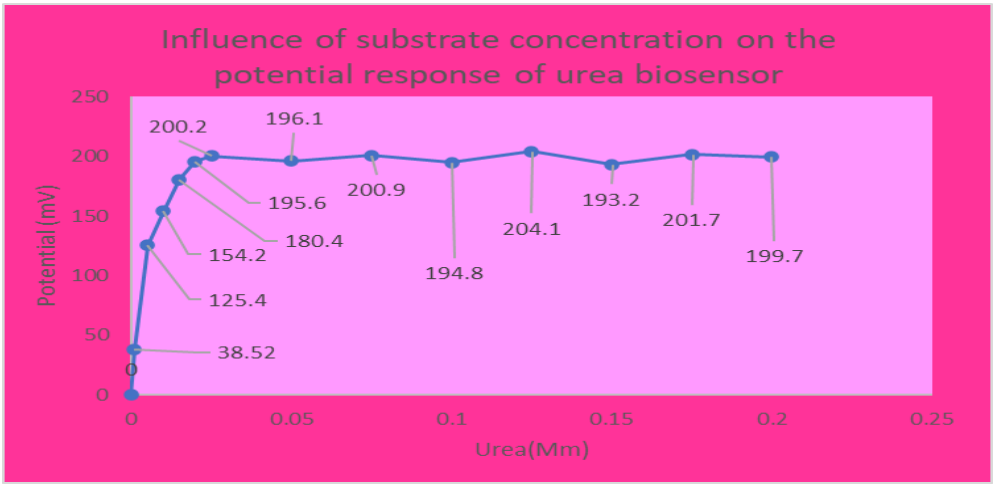


Fig 7: Voltage vs conc. Of urea graph for optimization of effect of conc. Of substrate

3.6.3. Lower detection limit

The current biosensor's detection limit was determined to be 0.1 mol/L, demonstrating its great sensitivity in monitoring urea contents. This can be calculated from the graph provided in fig 8.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

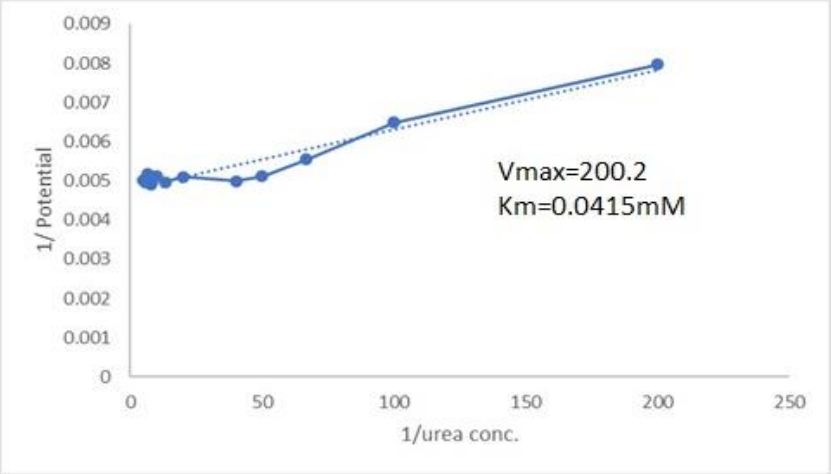


Fig 8: Graph indicating Vmax and Km of the experiment.

3.6.4. Sensitivity

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

3.7. Application

The above made biosensor was used for detection of urea in commercially available milk packets in the northern region of India. Among the brands, Vita recorded a urea concentration of 0.128 mg/L, Nandani had 0.121 mg/L, Namaste India registered 0.133 mg/L, and Heritage had 0.125 mg/L. Amul's urea concentration was 0.118 mg/L, Prabhat stood at 0.173 mg/L, Verka at 0.104 mg/L, and Saras at 0.135 mg/L. Dolda had a urea concentration of 0.124 mg/L, Mother Dairy recorded 0.117 mg/L, Patanjali had 0.128 mg/L, Lakshay showed 0.121 mg/L, Parag at 0.112 mg/L, Paras at 0.105 mg/L, and Baba at 0.109 mg/L.

This study stands out with its rapid 20-second response time, within the 10 to 180-second range of previous research. It maintains optimal pH conditions at 5.5, consistent with prior work (pH 5.5 to 7.6). It narrows the linear range to 0.001 to 0.80 mM for urea detection, contrasting with broader analyte ranges in earlier studies. Sensitivity at 38 mV/decade aligns with prior findings, indicating its responsiveness to urea concentration changes. Notably, it achieves an exceptionally low detection limit (0.001 mM), rivaling or surpassing previous LOD values as discussed in Table 1. Storage stability extends to 6 months, surpassing earlier stabilities of 14 days to 3 months, and it employs a potentiometric transducer, in line with some prior research. In summary, this study's swift response times, consistent pH conditions, specific linear range, sensitivity, and remarkably low detection limit make it a promising tool for urea detection compared to prior research.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

Table 1: A comparison of analytic parameters of urea biosensors used for detection of urea in spiked cow milk samples

Type of biosensor	Transducer used	Electrode used	Optimum pH	Response time	Linear range	Detection limit	Sensitivity	Storage stability	Reference
Potentiometric	polyelectrolyte microcapsules	NA	6.4-6.7	0–150 s	of 0.03–100 m	0.03 mM	3.58 pH/mM)	30 days	Reshetilov et al., 2015
FIA-ET	NA	NA	NA	NA	10–300 mM	10 mM	NA	200 days	P.P Naik et al., 2015
Aptasensor	AuNP	NA	NA	NA	20 mM -150 mM	20 mM	NA	NA	Kumar ey al, 2015
Potentometric	PAN	NA	NA	NA	1-100 mM	NA	NA	70 days	Rajenderan e al., 2015
Optical	N-bromosuc cinimideedi chlorofluorescein	NA	NA	NA	2.0 *10-9 to 1.0 *10-6 g/mL.	7 *10-10 g/mL.	NA	NA	Nie et al., 2016
Voltammetric	zinc oxide nanospheres	Pt	7.4	NA	1–20 nM	1 pM	NA	24 days	M. Ezhilan et al., 2017
Electrochemical	Fe3O4/MWCNT	MWCNTs	7.4	3s	1.0–25.0 mM	67 μM	NA	60 days	Singh et al., 2021
Potentiometric	UrsNps/Nylon	AISE	5.5-7.6	20s	0.001-0.80mM	0.001mM	38 mV/decade	180 days	PRESENT

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

4. SUMMARY AND CONCLUSION

This study investigates accurate urea level determination in milk using a biosensor. Urease enzymes on a nylon membrane attached to an ammonium ion-selective electrode yielded a sensitive biosensor with a rapid 20-second response time at pH 5.5, detecting urea concentrations between 0.001 and 0.80 mM. The biosensor exhibited an impressive 38 mV/decade sensitivity and a remarkable 0.001 mM limit of detection. Urea recovery ranged from 98.99% to 108.35%, affirming the biosensor's reliability for milk purity assessment. This focused, sensitive, and specific urea detection method has significant potential in biosensor research, offering precision and practicality for various applications. Further exploration of stability and adaptability is warranted.

Statements and Declarations

Funding

"The authors declare that no funds, grants, or other support were received during the preparation of this manuscript."

Author Contributions

"All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Miss Meenakshi under the supervision of Dr. Seema Kumari. The first draft of the manuscript was written by Miss Meenakshi and checked by Dr. Seema Kumari and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

Competing Interests

"The authors have no relevant financial or non-financial interests to disclose."

Pre-print on the Service

"I have not submitted my manuscript to a preprint server before submitting it to *Environmental Science and Pollution Research*".

Ethical Approval

Not relavant

Concent to Participate

Not relevant

Concent to Publish

Not relavant

References

1) 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

2) Kumar, Piyush; Ramulu Lambadi, Paramesh; Kumar Navani, Naveen (2015). Non-enzymatic detection of urea using unmodified gold nanoparticles based aptasensor. Biosensors and Bioelectronics, 72, 340–347. doi:10.1016/j.bios.2015.05.029

ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

3) M. Ezhilan, M.B. Gumpu, B.L. Ramachandra, N. Nesakumar, K.J. Babu, U.M. Krishnan, J.B. Rayappan.(2017) Design and development of electrochemical biosensor for the simultaneous detection of melamine and urea in adulterated milk samples. Sensors Actuators B Chem., 238, pp. 1283-1292, 10.1016/j.snb.2016.09.100

4) Nie, Fei; Wang, Na; Xu, Pan; Zheng, Jianbin(2016). Determination of urea in milk based on N150 bromosuccinimide dichlorofluorescein postchemiluminescence method. Journal of Food and Drug Analysis, S1021949816300825–. doi:10.1016/j.jfda.2016.05.009.

5) P.P. Naik, G.K. Mishra, B. Danielsson, S. Bhand (2015). Android integrated urea biosensor for public health awareness. Sens. Biosens. Res., 3, pp. 12-17, 10.1016/j.sbsr.2014.11.001

6) Pawar, Varsha and Verma, Neelam and Pawar, Shivaji,(2020) Overview of Different 154 Milk and Milk Products Contaminant Detections by Biosensors . Proceedings of the 3rd International Conference on Advances in Science & Technology (ICAST)

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) Rajendran Ramesh, Puhazhselvan Puhazhendi, Jitendra Kumar, Marichetti Kuppuswami Gowthaman, Stanislaus Francis D'Souza, Numbi Ra- mudu Kamini,(2015) Potentiometric biosensor for determination of urea in milk using immo-bilized Arthrobacter creatinolyticus urease, Materials Science & Engineering C, doi:10.1016/j.msec.2015.01.048.

9) Reshetilov, A. N.; Plekhanova, Yu. V.; Tikhonenko, S. A.; Dubrovskii, A. V. (2015). Polyelectrolyte microcapsules with urease and paramagnetic particles as a basis for a potentiometric biosensor for determining urea. Journal of Analytical Chemistry, 70(11), 1368–1372. doi:10.1134/S1061934815110131

10) Singh, A. K., Singh, M. & Verma, N. (2020) electrochemical preparation of Fe3O4/MWCNT-polyaniline nanocomposite film for development of urea biosensor and its application in milk sample. Journal of Food Measurement and Characterization14, 163–175, https://doi.org/10.1007/s11694-019-00278-2.

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) B.Lakard, G.Herlem, S.Lakard, A.Antoniou, B.Fahys, (2004) Urea potentiometric biosensor based on modified electrodes with urease immobilized on polyethylenimine films, Biosens.Bioelectron.19 1641–1647.

13) E.Cevik, M.Senel, A.Baykal,(2013)Potentiometric urea biosensor based on poly(glycidylmethacrylate)-grafted iron oxide nanoparticles. Curr. Appl Phys 13 280–286.

14) F.Kuralay, H.Ozyoruk, A.Yildiz(2005),Potentiometric enzyme electrode for urea determination using immobilized urease in poly(vinylferrocenium) film,Sens.Actuators B Chem. 109 194–199.

15) G.Dhawan, G.Sumana, B.D.Malhotra, (2009) recent developments in urea biosensors, Biochem.Eng. J. 44 42–52.

16) G.G.Guilbault, J.G.Jr.Montalvo, (1969) Urea-specific enzyme electrode, J.Am.Chem.Soc. 91 2164–2165.

17) G.K. Mishra, R.K. Mishra, S. Bhand. (2010) Flow injection analysis biosensor for urea analysis in adulterated milk using enzyme thermistor. Biosens. Bioelectron., 26 , pp. 1560-1564, 10.1016/j.bios.2010.07.113

18) J.S. Jonker, R.A. Kohn, R.A. (1998) Erdman,Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows,J. Dairy Sci. 81 2681–2692.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 43 Issue: 03-2024
DOI: 10.5281/zenodo.10791550

19) J.Singh, A.Kunhikrishnan, N.S.Bolan, S.Saggar(2013),Impact of urease inhibitor on ammonia and nitrous oxide emissions from temperate pasture soil cores receiving urea fertilizer and cattle urine,Sci. Total Environ. 465 56–63.

20) K. Saeedfar, L.Y. Heng, T.L. Ling, M. Rezayi(2013),Potentiometric Urea Biosensor Based on an Immobilised Fullerene-Urease Bio-Conjugate, Sensors 13 16851–16866.

21) R.Sharma, Y.S.Rajput, S.Kaur, S.K.Tomar, (2008) A method for estimation of urea using ammonia electrode and its applicability to milk samples,J.Dairy Res. 75 466–470.

22) T.Ahuja, D.Kumar, N.Singh, A.M.Biradar, Rajesh,(2011) Potentiometric urea biosensor based on multi-walled carbon nanotubes (MWCNTs)/silica composite material,Mater.Sci.Eng.C31 90–94.

23) U.B.Trivedi, D.Lakshminarayana, I.L.Kothari, N.G.Patel, H.N. Kapse, K.K.Makhija, P.B.Patel, C.J.Panchal(2009),Potentiometric biosensor for urea determination in milk,Sens.Actuators B Chem. 140 260–266.