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Beyond potentiometry: an amperometric approach to urea detection using antimony electrodes

Justas Miškinis*,

Marius Dagys,

Julija Razumienė,

Marius Butkevičius

*Institute of Biochemistry,
Life Sciences Center,
Vilnius University,
7 Saulėtekio Avenue,
10257 Vilnius, Lithuania*

In this study, we present an amperometric urea biosensor based on a custom-made antimony electrode modified with an enzymatic membrane containing immobilised urease. The biosensor showed a high sensitivity, ranging from 306.6 to 77.5 nA/mM depending on the buffer solution capacity (5–50 mM PBS). Stability tests demonstrated that the sensor retained 65% of its initial activity after 10 days at room temperature. Good repeatability was observed, with relative standard deviations below 10% for 10 replicate measurements at 0.5 mM urea. Validation with aqueous samples showed a strong correlation with a commercial colorimetric assay, with deviations not exceeding 10%. During the tests with biological samples (human saliva and serum), the biosensor reported lower urea concentrations compared with the colorimetric method, indicating that interfering compounds present in complex biological matrices can affect biosensor performance. The developed biosensor represents a simple, cost-effective and adaptable platform for urea determination, showing a strong potential for integration into point-of-care diagnostic systems.

Keywords: antimony, amperometric biosensor, urea

INTRODUCTION

Urea is an important by-product of nitrogen metabolism that helps to safely remove excess nitrogen from the body [1]. Proper regulation of urea levels is critical, as both elevated and decreased concentrations can indicate serious metabolic or renal disease, which is why this compound has a high diagnostic and prognostic value [2]. Most harmful urea concentrations are associated with renal dysfunction, especially acute kidney disease [3]. The number of patients with chronic kidney disease is steadily increasing worldwide [4], emphasising the need for accessible and reliable diagnostic tools. The most common diagnostic methods for urea measurement include colorimetric and chromatographic methods [5, 6]. However, there is an ever-growing research and application of biosensors that provide a cost-effective, rapid, accurate and reliable method for measuring urea concentration in various media [7]. To date, a wide range of urea biosensors have been developed, including electrochemical, optical, piezoelectric and fluorescence-based sensors [8]. For the effective and reliable use of urea biosensors in point-of-care applications, the sensors must have a high stability and sensitivity, require a minimal maintenance and have a straightforward and practical design [9, 10].

The enzymatic breakdown of urea catalysed by urease leads to a change in the pH value of the reaction solution [11]:

$$(NH_2)_2CO + H_2O \rightarrow 2\ NH_3 + CO_2$$

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-.$$

Therefore, the reasonable approach in developing a urea biosensor is to utilise the capability of pH sensitivity of a urease-coated sensor electrode [12], including the use of ion-sensitive field-effect

* Corresponding author. Email: justas.miskinis@bchi.stud.vu.lt

transistor electrodes [13]. However, it is no secret that potentiometric pH electrodes – especially those based on glass – suffer from a relatively poor long-term stability, and a high electrical impedance which increases susceptibility to interference, and often require a careful maintenance to ensure reliable measurements [14]. Therefore, amperometric urea biosensors are currently being developed [15], including the work of our group [16, 17], which offer a better operational stability, a higher signal-to-noise ratio and less interference. In such studies, the surface of the working electrode is prepared to use a pH signal conversion reaction leading to Faradaic current. For example, thermally reduced graphene oxide is used together with urease in the same electrode membrane environment [16].

Here we present an amperometric urea biosensor based on an antimony working electrode modified with an enzymatic membrane containing immobilised urease, which functions without additional pH conversion reactions. The core idea is to exploit the pH sensitivity of the antimony metal surface [18, 19] not in a conventional potentiometric mode, but under applied polarisation, which allows the detection of enzymatically induced pH changes via a resulting Faradaic current, resulting in amperometric rather than a potentiometric system. Such an approach would not be possible with the usual glass electrode systems used in pH measurements, but the metallic nature of antimony allows this. While a differential signal system based on antimony electrodes operating in the circuit of a pH meter has been described [20], to our knowledge, the proposed experiment has not yet been investigated.

This brief study is dedicated to the 70th birthday of Professor Valdemaras Razumas, who had a special interest in the development of non-standardised biosensor approaches such as yeast-based amperometric lactate sensors [21], biosensors based on enzymes entrapped in cubic liquid crystalline phases [22, 23] or self-assembling monolayers [24].

EXPERIMENTAL

Reagents

Urease from *Canavalia ensiformis* (Jack bean) (82 kU/g) was purchased from Sigma-Aldrich. Human saliva samples (from donors with no known health conditions) were purchased from Lee Biosolutions. Human serum was purchased from Sigma-Aldrich. All other reagents were of analytical grade and were purchased from Sigma-Aldrich.

Electrode preparation

First, an antimony rod (d = 2 mm) was produced. In short, antimony granules were placed in a glass tube with a diameter of 2 mm and melted with a gas burner. After the glass cooled down and the antimony solidified completely, the glass was carefully broken, and the antimony rod was cut into 3–5 mm pieces. A copper wire was soldered to a piece of antimony and attached to an acrylic housing using a two-component epoxy adhesive. Finally, the antimony surface was polished to a mirror finish with sandpaper and polished with 0.3 μm aluminum oxide. The electrode was then cleaned in an ultrasonic bath for 10 min to remove any remaining impurities.

Preparation of the urea-sensing membrane

To facilitate the replacement of the biosensor's recognition element, the urease was immobilised on a perforated polyethylene membrane, to which an O-ring was attached to ensure an easy and stable fixation of the membrane. 10 μL of the urease immobilisation mixture – consisting of 30 mg/mL bovine serum albumin, 20 mg/mL urease and 1.5% glutaraldehyde – was applied to the membrane and allowed to dry at room temperature. The prepared membranes were stored at 4°C until use.

Chronoamperometric measurements

All measurements were performed with a three-electrode system consisting of a titanium plate as counter electrode, an Ag/AgCl electrode as reference electrode and an antimony electrode equipped with a urease membrane as working electrode. A Gamry 300 potentiostat system was used for all experiments. Measurements were performed in the 20 mM potassium phosphate buffer with 100 mM KCl (pH 7.2) at 25°C (unless otherwise stated). The working electrode was polarised at –425 mV.

The measurements were performed in a 1 mL electrochemical cell under continuous stirring with a magnetic stirrer. For the determination of urea in an aqueous solution, 50 μL of a urea solution of the desired concentration was added to the cell (all concentrations are given as the final

concentration in the measuring cell). For the sample analysis, 50 µL of the sample was used, whereby all concentrations indicated were adjusted for dilution.

Colorimetric urea determination (Sigma-Aldrich MAK006)

Urea concentration was quantified using the Sigma-Aldrich Urea Assay Kit (MAK006), in which urea is determined by a coupled enzymatic reaction that produces a colorimetric product measurable at 570 nm. All reagents were prepared and handled according to the manufacturer's instructions. For calibration, a 0.5 mM urea working solution was prepared by diluting the 100 mM urea standard with the urea assay buffer. Aliquots of 0, 2, 4, 6, 8 and 10 µL of the 0.5 mM standard were added to the wells of a 96-well plate, and the volume in each well was adjusted to 50 µL with the urea assay buffer. Subsequently, 50 µL of the reaction mixture (for the composition see the Table) was added to each well. The contents were mixed thoroughly with an automatic pipette and incubated for 60 min at 37°C in the dark. The absorbance was then measured at 570 nm. Samples were analysed without an additional pretreatment, except for dilution, to ensure that absorbance values fell within the range of the calibration curve. Urea concentrations in the samples were calculated by subtracting the blank value from the sample value and determining the final value from the calibration curve.

Table. **Reaction mix composition**

Reagent	Sample blank, µL	Samples and standards, µL
Urea assay buffer solution	44	42
Peroxidase substrate	2	2
Enzyme mix	2	2
Developer	2	2
Converting enzyme	0	2

RESULTS AND DISCUSSION

To determine the initial potential of the working electrode, measurements of the open circuit potential (OCP) were performed, which showed that the OCP between the Ag/AgCl and the antimony electrode with the urea detection membrane was approximately –430 mV. A potential of –425 mV was selected for the further experiments. Applying a more positive potential resulted in an increased background current and a reduced biosensor sensitivity. Conversely, applying a more negative potential caused the background current to shift into the negative range and significantly decreased the sensitivity as well. Calibration solutions in the range of 0.1–2 mM urea were used for the measurements, reflecting the expected concentrations in biological samples (saliva and blood serum) after applying a tenfold dilution. According to the literature, the average urea concentration in human saliva is approximately 2 mM, increasing to around 7 mM in patients with chronic kidney disease. In blood serum, the corresponding values are about 5 mM in healthy individuals and up to 18 mM in chronic kidney disease patients [25]. The biosensor is also capable of measuring urea concentrations higher than 2 mM; however, after such measurements, the sensor requires a longer rinsing period and additional time to reach a stable background current. Since the performance of the sensor can be influenced by the buffer capacity, its effect was also evaluated. For this purpose, potassium phosphate buffer solutions with concentrations of 5, 10, 20 and 50 mM (each containing 100 mM KCl) were used. The results obtained are shown in Fig. 1. Based on the data obtained, the sensitivity of the urea biosensor was evaluated. It was found that the sensitivity at –425 mV vs Ag/AgCl depends on the buffer capacity and varies between 306.6 and 77.5 nA/mM when 5 and 50 mM PBS are used, respectively.

The stability of the sensor was also evaluated. The measurements were performed while the biosensor was stored at room temperature. It was found that the biosensor remained functional for at least 10 days and retained 65% of its original activity compared to day 1.

Before performing biosensor tests with biological samples (human serum and saliva), the repeatability of the biosensor measurements was evaluated. 10 independent measurements were performed with a 0.5 mM urea solution. The average response was 0.45 ± 0.08 mM and 0.48 ± 0.04 mM using 5 mM and 20 mM PBS, respectively.

Prior to the measurements with biological samples, the biosensor was tested with aqueous solutions, and the results were compared with those of the colorimetric method. Test solutions with

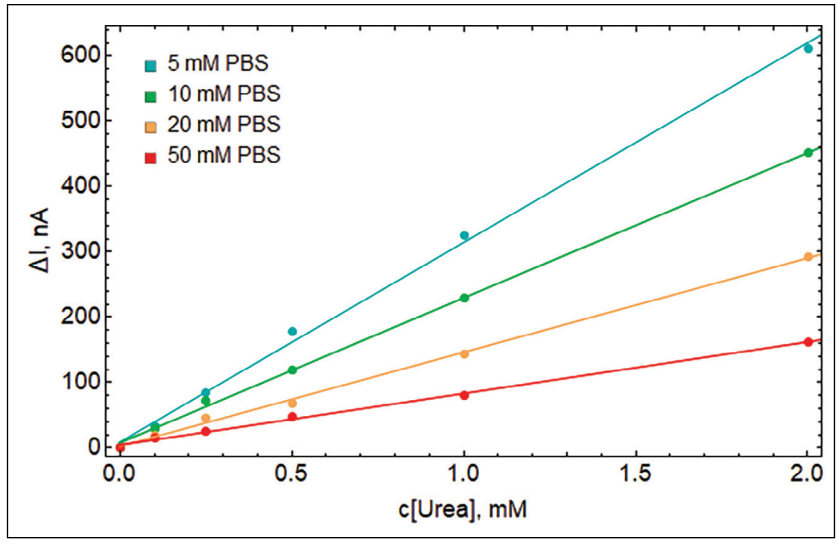

Fig. 1. Calibration curve of a urea biosensor in different PBS solutions. Points are the measured values. Straight line is a linear model, and the average correlation coefficient is 0.99

unknown urea concentrations were prepared by mixing PBS and 2 mM urea in PBS in random ratios. The results showed a good correlation between the two methods, with the maximum difference not exceeding 10% (Fig. 2. Urea samples 1–3).

Measurements were then carried out with blood serum and saliva using 5 and 20 mM PBS buffer solutions. It was found that the urea concentrations determined with the biosensor in both serum and saliva were lower than those determined with the colorimetric method. In saliva, on the other hand, the values determined with the biosensor were closer to the values determined with the colorimetric assay. The higher apparent concentrations observed with the colorimetric method are probably due to the presence of ammonium ions in saliva, which was confirmed by control experiments. When the urea-converting enzyme was omitted, a clear colour change was still observed in the saliva samples. According to the manufacturer's protocol, such colour changes can be caused by ammonium ions, $NAD^+/NADP^+$, and pyruvate in the sample.

Since the urea concentrations determined by the colorimetric method were relatively similar for

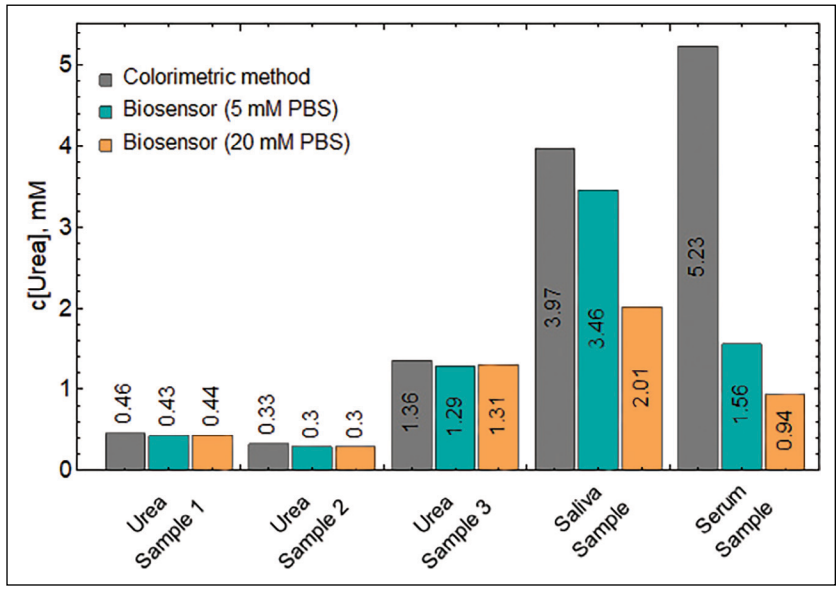

Fig. 2. Comparison of urea concentration values determined by the biosensor and the colorimetric method

both sample types, while the electrochemical biosensor showed much larger differences, this can be explained by the influence of ammonium ions on the electrochemical measurements. According to the literature, the concentration of NH_4^+ in saliva is around 11 mM [26], while in blood serum it is only between 11 and 50 μM [27].

A key aspect of this research was the unconventional use of an antimony electrode in the amperometric mode. Instead of detecting pH changes potentiometrically, the sensor quantified enzymatically induced pH shifts through Faradaic currents. The proposed electrode design represents a promising alternative to conventional methods for urea determination. Nevertheless, the biosensor has certain limitations. The detection mechanism of the antimony electrode is essentially based on the equilibrium between its metallic and oxidised forms, which means that large variations in the composition of the sample can lead to a signal drift and reduced reproducibility. However, extensive studies still need to be carried out to thoroughly explore the biosensor's operating mechanisms and its expected response in different media and under different environmental conditions.

CONCLUSIONS

In this study, we presented a custom-made antimony electrode modified with an enzymatic membrane containing immobilised urease. The experimental results showed good results and proved that the electrode can determine urea concentrations in aqueous solutions. During the measurements, the sensor performed well with a sensitivity of 306.6 to 77.5 nA/mM using 5 and 50 mM PBS, respectively. In addition, the electrode proved suitable for the detection of urea in complex biological matrices, including saliva and serum, and showed reasonable response times. These results provide a solid basis for future investigations into the applicability of the biosensor in biochemical analysis systems.

The electrode introduced by our research group is characterised by a relatively simple structure. The sensor consists of an antimony electrode to which an enzymatic membrane is attached. The maintenance of such a device is simple and only requires a regular replacement of the enzymatic membrane and recalibration of the electrode. This design allows the sensor to be adapted for point-of-care applications, especially when integrated into semi-automated analytical platforms that can provide fast and accurate measurements at a lower cost per sample than diagnostic laboratories.

This work has shown that an antimony-based biosensor with an enzymatic membrane can successfully determine the urea concentration in solutions and biological samples under the conditions tested. However, a detailed analysis of the reactions taking place on the electrode surface was beyond the scope of this study. Overall, the results underline the potential of antimony-based enzymatic biosensors as a practical and cost-effective tool for clinical urea determination.

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References

1. A. Varcoe, D. Halliday, E. Carson, P. Richards, A. Tavill, *Am. J. Clin. Nutr.*, **31(9)**, 1601 (1978).
2. O. Adeyomoye, C. Akintayo, K. Omotuyi, A. Adewumi, *Indian J. Nephrol.*, **32(6)**, 539 (2022).
3. J. Himmelfarb, T. A. Ikizler, *Kidney Int.*, **71(10)**, 971 (2007).
4. J. Guo, W. Jiao, S. Xia, et al., *BMC Nephrol.*, **26(1)**, 136 (2025).
5. B. V. M. Silva, I. T. Cavalcanti, M. M. S. Silva, R. F. Dutra, *Talanta*, **117**, 431 (2013).
6. D. Yang, Y. Zhou, Y. Zhou, J. Han, Y. Ai, *Biosens. Bioelectron.*, **133**, 16 (2019).
7. M. Regiart, A. Ledo, E. Fernandes, et al., *Biosens. Bioelectron.*, **199**, 113874 (2022).
8. P. Mehrotra, *J. Oral. Biol. Craniofac. Res.*, **6(2)**, 153 (2016).
9. C. Dincer, R. Bruch, E. Costa-Rama, et al., *Adv. Mater.*, **31**, 30 (2019).
10. J. Wang, *Biosens. Bioelectron.*, **21(10)**, 1887 (2006).
11. H. L. Mobley, R. P. Hausinger, *Microbiol. Rev.*, **53(1)**, 85 (1989).
12. D. Chirizzi, C. Malitesta, *Sens. Actuators B Chem.*, **157(1)**, 211 (2011).
13. O. A. Boubriak, A. P. Soldatkin, N. F. Starodub, A. K. Sandrovsky, A. K. El'skaya, *Sens. Actuators B Chem.*, **27(1–3)**, 429 (1995).
14. A. J. Bard, L. R. Faulkner, H. S. White, *Electrochemical Methods: Fundamentals and Applications*, John Wiley & Sons (2022).
15. A. Pizzariello, *Talanta*, **54(4)**, 763 (2001).
16. J. Razumiene, V. Gureviciene, I. Sakinyte, L. Rimsevicius, V. Laurinavicius, *Sensors*, **20(16)**, 4496 (2020).

17. V. Laurinavicius, J. Razumiene, V. Gureviciene, *IEEE Sens. J.*, **13(6)**, 2208 (2013).
18. B. H. Kee, W.-S. Sim, W. Chew, *Anal. Chim. Acta*, **571(1)**, 113 (2006).
19. J. Bobacka, A. Ivaska, A. Lewenstam, *Chem. Rev.*, **108(2)**, 329 (2008).
20. J. Kulys, V. Gurevičienė, V. Laurinavičius, A. Jonuška, *Biosensors (Basel)*, **2(1)**, 35 (1986).
21. J. Kulys, L. Wang, V. Razumas, *Electroanalysis*, **4(5)**, 527 (1992).
22. V. Razumas, J. Kanapienienė, T. Nylander, S. Engström, K. Larsson, *Anal. Chim. Acta*, **289(2)**, 155 (1994).
23. T. Nylander, C. Mattisson, V. Razumas, Y. Miezis, B. Håkansson, *Colloids Surf. A Physicochem. Eng. Asp.*, **114**, 311 (1996).
24. B. Kazakevičienė, G. Valincius, G. Niaura, et al, *Langmuir*, **23(9)**, 4965 (2007).
25. D. Pandya, A. K. Nagrajappa, K. S. Ravi, *J. Clin. Diagn. Res.*, **10(10)**, 58 (2016).
26. M. Bhogadia, M. Edgar, K. Hunwin, G. Page, M. Grootveld, *Metabolites*, **13(7)**, 792 (2023).
27. A. Calvo-Lopez, B. Rebollo-Calderon, A. Ormazábal, et al., *Anal. Chim. Acta*, **1205**, 339782 (2022).

Justas Miškinis, Marius Dagys, Julija Razumienė, Marius Butkevičius

UŽ POTENCIOMETRIJOS RIBŲ: AMPEROMETRINIS KARBAMIDO NUSTATYMO METODAS, NAUDOJANT ANTIMONIO ELEKTRODUS

S a n t r a u k a

Šiame tyrime pristatomas amperometrinis šlapalo biojutiklis, sukurtas naudojant specialiai pagamintą stibio elektrodą, padengtą fermentine membrana su imobilizuota ureaze. Biojutiklis pasižymėjo dideliu jautriu – nuo 77,5 iki 306,6 nA/mM, priklausomai nuo buferinio tirpalo talpos (5–50 mM PBS). Stabilumo tyrimai parodė, kad po 10 dienų laikymo kambario temperatūroje jutiklis išsaugojo 65 % pradinio aktyvumo. Buvo nustatytas geras matavimų atsikartojamumas: atliekant 10 pakartotinių matavimų su 0,5 mM šlapalo tirpalu, santykinis standartinis nuokrypis nesiekė 10 %. Testuojant biojutiklį su vandeniniais mėginiais, gauta gera koreliacija su komerciniu kolorimetriniu šlapalo nustatymo rinkiniu – skirtumai neviršijo 10 %. Tyrimuose su biologiniais mėginiais (seilėmis ir serumu) biojutiklis parodė mažesnes šlapalo koncentracijas nei kolorimetrinis metodas, o tai leidžia teigti, kad kiti biologinių mėginių komponentai gali daryti įtaką biojutiklio veikimui. Sukurtas biojutiklis yra sąlyginai paprastas, ekonomiškas ir tinkamas šlapalo nustatymui, taip pat turi potencialą būti integruotas į greitosios diagnostikos sistemas.