CHEMICAL ENGINEERING TRANSACTIONS
VOL. 32, 2013
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at: www.aidic.it/cet
Chief Editors: Sauro Pierucci, Jiří J. Klemeš

ISBN 978-88-95608-23-5; **ISSN** 1974-9791
DOI: 10.3303/CET1332062

Analysis of Potential Applicability of the Potentiometric Urea Biosensor to Real Samples

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Since the production and use of chemical products are performing a marked growth, the charge, for fast and efficient mechanisms of environmental pollution control and monitoring, has increased. This charge has been made even more present in the rural scenario, especially with regard to fertilizers and nitrogen compounds, which have a high pollution potential. Several methods are used to urea determination, but usually they require a pretreatment or they are unsuitable for monitoring in situ. For this reason, urea biosensor can be a fast and cost-effective analytical technique to be used in extensive monitoring programs.
Urea enzyme sensors based on potentiometric detection of ammonium ion, ammonia gas and carbon dioxide have been reported. In this study, the potential applicability of the potentiometric urea biosensor to real samples has been studied. The biocomponent used was jack beans, *Canavalia ensiformis*, a vegetal tissue rich in urease. The vegetal tissue was immobilized on nylon net by glutaraldehyde in order to improve the enzyme stability and the lifetime of the urea biosensor. Moreover, an ammonium ion-selective electrode was been chosen as the transducer.
The instrument was applied to commercial urea fertilizer solutions and an environmental sample, the sugar-cane vinasse. The linear concentration range calibration curve was 1–20 ppm urea with a response time of 15 min. Urea biosensor could be reused for 70 days and the response was more than 95 % reproducible. Moreover, the urea biosensor was tested in real samples with qualitative promising responses.

1. Introduction

Nitrogen compounds are pollutants commonly found in industrial effluents, being its determination of extreme environmental importance. Furthermore, the presence of urea in agricultural land as a pollutant due to excessive fertilizers use is also widely known. Urea can stress the environment because it decomposes to ammonia, which is very toxic, and so it can pollute the streams and rivers when released (Chagas, 2007).
Presenting the advantages of providing fast responses, low cost and low waste generation, in relation to the classical methodology, biosensors (Figure 1) appear as a viable alternative for these analyzes (Silva et al., 2011b). These are integrated instruments capable of providing analytical specific information, quantitative or semi-quantitative, by the use of a biological component and a transducer element (Thévenot et al., 1999).
In this paper, the behavior of a potentiometric urea biosensor, previously developed by Silva (2011), was analyzed, when it is applied in a commercial fertilizer sample and a real sample (the sugar-cane vinasse).

Please cite this article as: Flores R.O., Silva L.M.C., Melo A.F., Salgado A.M., 2013, Analysis of potential applicability of the potentiometric urea biosensor to real samples, Chemical Engineering Transactions, 32, 367-372 DOI: 10.3303/CET1332062

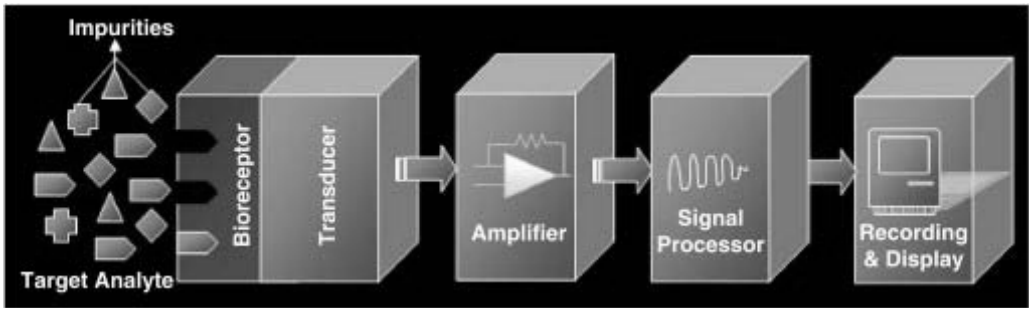

Figure 1: Biosensor general schema (Silva et al., 2011a).

2. Material and methods

2.1 Biocomponent: vegetal tissue

The biocomponent used was jack beans, *Canavalia ensiformis* (Figure 2), a vegetal tissue rich in urease enzyme (EC 3.5.1.5) (Luca and Reis, 2001). The hydrolysis of urea to yield ammonia (NH_3) and carbamate was made by enzyme (urease) catalysis, the latter compound decomposes spontaneously to generate a second molecule of ammonia and carbon dioxide (CO_2) (Takishima et al., 1988).

Figure 2: Canavalia ensiformis beans photo.

2.2 Jack beans preparation and immobilization

For the use of biocomponent in the biosensor, the beans were manually peeled off and chopped into small pieces with an electric grinder. The biocomponent were being used as a powder, with a particle size less than or equal to 3.0 mm. The material already in the powder form was stored in a refrigerator at 4 ºC in hermetically sealed container, to further use in the immobilization process.

The immobilization of the *Canavalia ensiformis* (powder) was performed according Júnior (1995). The final configuration of the procedure, in brief, urease was covalently immobilized on nylon net according to the following procedure: 0.2 g of biocomponent (powder) was placed under a nylon screen and 200.0 mL of 12.5 % glutaraldehyde solution were added. Then, another net was placed on top. The system rested at room temperature (24 °C ± 1 °C) for 20 min. Subsequently, it was immersed in distilled water for 15 min and then in sodium phosphate buffer pH 6.0 for the same time. The immobilized biocomponent was used after storage for 24 h in the refrigerator, at 4 ºC. This immobilization procedure is shown in Figure 3.

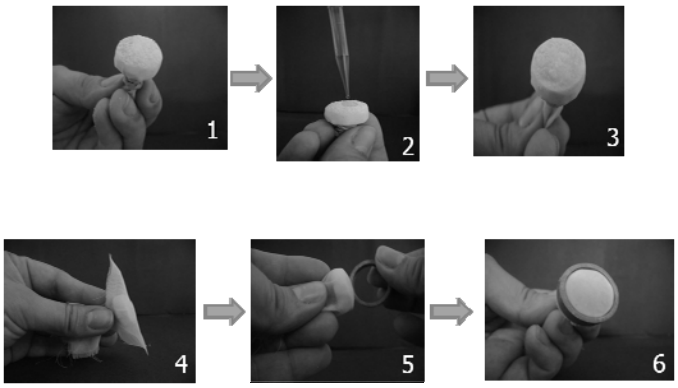

Figure 3: Procedure step sequence of powdered jack bean immobilization (Silva, 2011)

2.3 Biosensor curve calibration

Calibration curves of developed urea biosensor (Figure 4) were carried out by injection of urea standard solutions (1.0 to 20.0 ppm, diluted with sodium phosphate buffer pH 6.0) at a flow rate of 40.0 mL/min (Silva, 2011). After the solution has completed in the reaction chamber, the pump was turned off and the electrode was immersed and kept in contact with the solution for 15 min (time reaction) (Silva, 2011). After each sample analysis, the system was thoroughly rinsed with distilled water for 2 min. The potentiometric measurements were made at room temperature (24 °C ± 1 °C).

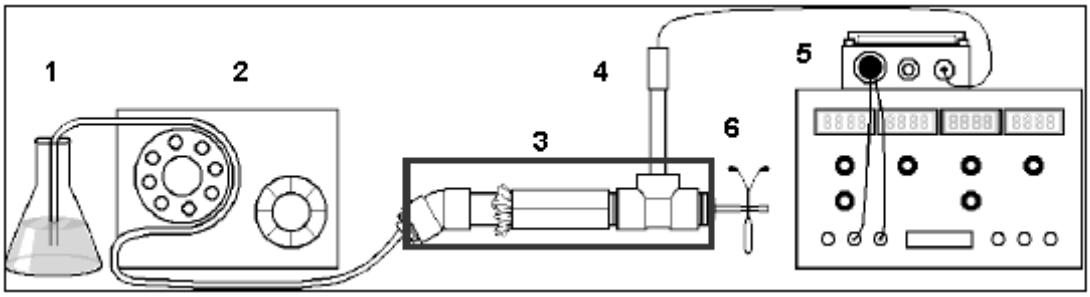

Figure 4: Scheme of the urea biosensor. The set up consists of: (1) urea standard solution or real sample; (2) peristaltic pump; (3) reaction chamber; (4) ammonium ion-selective electrode, (5) potentiostat e (6) waste sample

2.4 Repeatability

The repeatability of the urea biosensor response was also studied by measuring this response (n = 5) when it was used into urea solution (10 ppm) under optimum working conditions. The assays were performed according to Section 2.3.

2.5 Determination of urea content in sugar-cane vinasse

Determination of urea concentration in real sample was performed as described in Section 2.3. Since this is a complex matrix, the sample was diluted with sodium phosphate buffer pH 6.0. The urea concentration (10 ppm) was artificially added to the sample as compared to the sample without addition.

2.6 Determination of urea content in commercial urea fertilizer

Determination of urea concentration in fertilizer sample was performed as described in Section 2.3. Commercial urea fertilizer solutions were passed through the instrument at concentrations 0.1, 1.0 and 10.0 ppm diluted with sodium phosphate buffer pH 6.0.

3. Results and Discussion

3.1 Typical calibration curve of the urea biosensor

A corresponding change of potential versus the urea concentration could be observed. Different urea concentrations would cause potential changes, due to ammonia generation. The typical calibration curve of the urea biosensor could be obtained correlating the natural logarithm (ln) concentration of urea and the

transducer output value (mV) as seen in Figure 5. The developed equipment was reused for more than 70 days, with 95 % of reproducibility (software STATISTICA Trial Version (StatSoft, Inc. 1984-2011) (Silva, 2011). The results are in agreement with the literature and were found values ranging from several weeks to months (Singh et al., 2008; Dhawan et al., 2009).

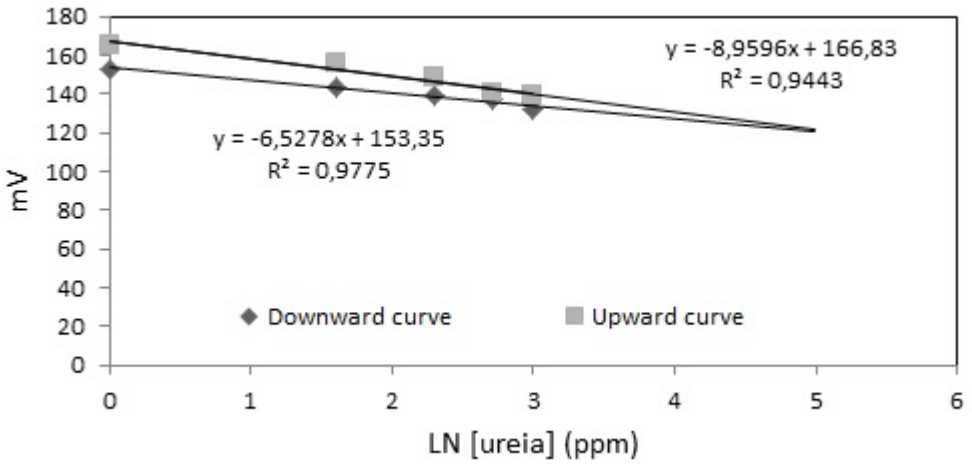

Figure 5: Typical calibration curves for urea detection under optimum working conditions.

3.2 Repeatability

Repeatability of the biosensor was also studied for phenol concentration of 10 ppm (n = 5) under the optimum working conditions. According to the results obtained from the experiments, the standard deviation (SD) and coefficient of variation (CV %) were found to be ± 0.09 mg/L and 3.2 %, respectively.

3.3 Application in sugar-cane vinasse

The results obtained when the urea biosensor was applied to sugar-cane vinasse samples are shown in Table 1. Over the assay days, there was an increase in the measured value of the urea concentration added. This fact indicated that possibly some component(s) present(s) in the real sample, that might be inhibiting the urease enzyme present in the immobilized biocomponent, was suffering degradation during the days of storage. Also, as can be seen in Table 1, the urea biosensor was able to respond quantitatively the increase of urea in the samples, although the instrument had not been able to give the precise urea concentration.

Table 1: Results of potentiometric urea biosensor applying in sugar-cane vinasse samples over 23 days.

Storage time of sample under refrigeration (days)	Line equation (biosensor calibration curve)	Real sample concentration (without addition of urea) (A)	Real sample concentration (with addition of urea) (B)	Variation of concentrations (B-A)
7	y= -6.411x + 105.33	8.00 ppm	10.93 ppm	2.93 ppm
20	y= -4.6277x + 97.1	8.87 ppm	13.66 ppm	4.79 ppm
23	y= -8.3291x + 152.82	15.48 ppm	25.03 ppm	9.55 ppm

3.4 Application in commercial urea fertilizer

The results obtained when the urea biosensor was applied to commercial urea fertilizer samples are shown in Figure 6. As can be seen in Figure 6, the higher substrate concentration in the sample, the lower transducer response (mV) had been detected. So, the urea biosensor was able to respond quantitatively the increase of urea in the samples, although the instrument had not been able to give the precise urea concentration.

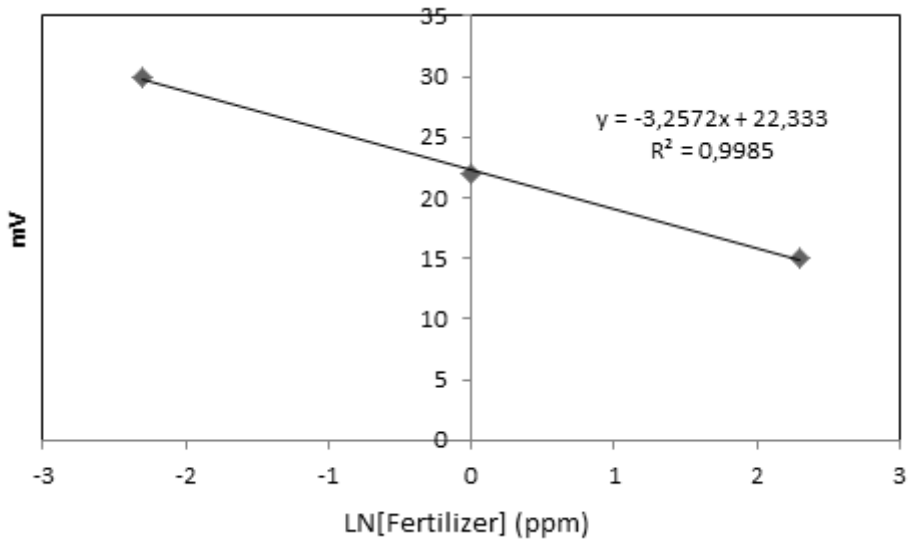

Figure 6: Curve relating the transducer response (mV) for each fertilizer concentration applied to the urea biosensor.

The application of biosensors in real samples, especially in the environmental area, is still a challenge. Most urea biosensors were used to measure urea levels in standard solutions. The environmental samples are highly complex and its components can promote the interference in the analysis, such as inhibition of the biological component of the biosensor. When used in real samples, the urea biosensors were used in the clinical samples (urine and blood) (Sigh et al., 2008).

4. Conclusions

The results obtained showed a promising behavior of the developed urea biosensor for environmental applications, being able to generate significant responses, although having a high complexity in the both analyzed samples, this complexity had caused interference in the analysis. More analysis with urea biosensor will be made submitting it to different environmental samples. Moreover, tests have been made in order to study the pesticide detection (atrazine) by urease inhibition process, using this urea biosensor.

Acknowledgment

The authors thank the financial support of the National Council for Scientific and Technological Development (CNPq) and National Institute of Metrology, Quality and Technology (INMETRO).

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