



Miniaturized Platinized-Pt Electrodes for Voltammetric Determination of Ammonia: Optimization of Urease Mass from *Canavalia Ensiformis* for Urea Conversion

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RESUMO

A amônia e a ureia são compostos nitrogenados comumente encontrados em ambientes aquáticos e associados à poluição de origem industrial. A urease é uma enzima capaz de hidrolisar a ureia, formando NH_3 and CO_2 , tornando possível a sua quantificação. Neste trabalho, avaliou-se a eficiência de diferentes massas de urease na conversão de ureia em NH_3 , seguido da quantificação por voltametria de pulso diferencial utilizando um eletrodo miniaturizado de Pt em meio alcalino. Os resultados mostraram que a conversão espontânea da ureia foi limitada, atingindo 55,86%, enquanto o uso de 10 mg de urease promoveu uma conversão de 96,61%. 20 mg de urease não apresentaram um aumento significativo, portanto, os resultados indicaram que 10 mg é a massa ideal de enzima, garantindo eficiência e uso racional. O método desenvolvido mostrou-se promissor para a determinação de NH_3 em amostras ambientais, combinando seletividade enzimática com sensibilidade eletroquímica.

Palavras-chave: Voltametria de Pulso Diferencial, Amônia, Urease, Ureia.

Introduction

The presence of nitrogen-containing compounds such as ammonia (NH_3) and urea in natural waters is directly related to pollution processes of industrial origin and can cause significant impacts on aquatic life and water quality^{1,2}. In Brazil, the maximum allowable concentration of NH_3 in natural waters is regulated by CONAMA and depending on the pH of the medium; for environments with pH above 8.5, the maximum permitted is 13 mg L^{-1} ³. In this context, the development of analytical methods for the quantification of NH_3 is very important. Electroanalytical methods are promising because they offer low sample and reagent consumption, high sensitivity, simple instrumentation, and low LOD and LOQ values. However, since urea is not an electroactive compound under certain conditions, it is indirect determination can be carried out through the enzymatic action of urease, which converts urea into NH_3 and CO_2 . Therefore, the aim of this work was to evaluate the mass of urease required to convert urea into NH_3 using a miniaturized Pt electrode in alkaline medium.

Experimental

Urease from Canavalia Ensiformis

The urease employed in this study exhibited high purity and was stored under appropriate refrigerated conditions to preserve its enzymatic activity.

Pt electrodeposition on miniaturized electrode

Pt particles were electrodeposited under controlled potential conditions (-0.2 V vs. $\text{Ag}/\text{AgCl}/\text{KCl}_{\text{sat}}$). Electrodeposition was assumed to proceed with 100% current efficiency, and the

electrical charge passed was used to estimate the Pt electrodeposited mass.

Electrochemical Measurements

All electrochemical experiments were carried out with a 0.5 mm diameter Pt working electrode using $\text{KOH } 0.1 \text{ mol L}^{-1}$ as the supporting electrolyte and a stainless-steel cylinder as the counter electrode. The electrochemical cell was composed of an eppendorf tube containing 3 mL of supporting electrolyte solution. The enzymatic conversion of urea to ammonia was evaluated by varying the mass of urease added to the system, aiming to determine the optimal enzyme mass for complete hydrolysis and subsequent electrochemical detection of NH_3 .

Results and Discussion

A urea solution of known concentration was prepared, and different masses of urease were added to it. Aliquots were collected every 5 minutes and transferred to the electrochemical cell containing the supporting electrolyte and differential pulse voltammograms were then recorded. Figure 1A shows the NH_3 concentration as a function of the total evaluation time (50 minutes). In the absence of urease, the conversion of urea to NH_3 was minimal, indicating a slow process with low conversion efficiency. However, when different masses of urease were used, the conversion of urea began within the first few minutes, resulting in higher NH_3 concentrations over time. The best performance was observed with 10 mg of urease. Therefore, to assess the conversion efficiency, a triplicate experiment was conducted, as shown in Figure 1B. A conversion of $90.45 \pm 9.77\%$ was achieved, indicating that nearly all the urea

in solution was converted to NH₃.

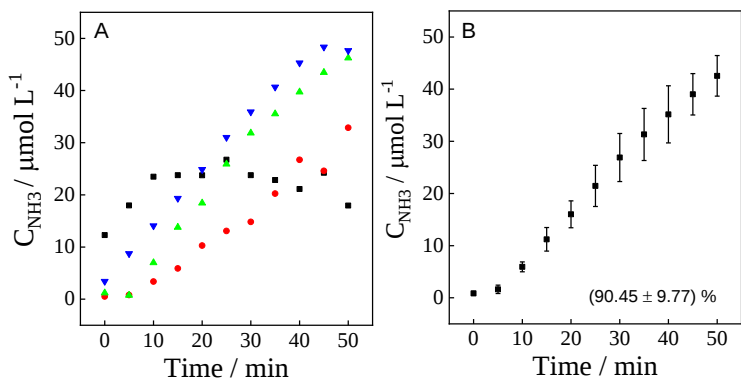


Figure 1. A) NH₃ concentration as a function of time for different urease masses. (■) without enzyme, (●) 5 mg, (▲) 10 mg, (▲) 20. B) Replicated measurements (n = 3) of NH₃ concentration over time using 10 mg of urease.

Table 1 presents the percentage of urea-to-ammonia conversion obtained with different urease masses. The theoretical concentration of ammonia expected from complete hydrolysis of urea was 47.62 $\mu\text{mol L}^{-1}$ for all conditions tested. In the absence of urease, the conversion efficiency was limited to 55.86%, indicating slow and inefficient spontaneous hydrolysis. With increasing enzyme mass, a progressive improvement in conversion was observed. At 5 mg of urease, the conversion reached 68.66%, while 10 mg resulted in 96.61%, demonstrating that a moderate amount of enzyme is sufficient for nearly complete conversion within the evaluated time frame. Interestingly, with 20 mg of urease, the measured concentration slightly exceeded the theoretical value, reaching 101%. This small deviation can be attributed to experimental variations and suggests that 10 mg is an optimal mass, balancing enzyme use and conversion efficiency.

Table 1. Percentage of urea-to-ammonia conversion using different urease masses.

Enzyme mass / mg	Theoretical concentration / $\mu\text{mol L}^{-1}$	Maximum concentration / $\mu\text{mol L}^{-1}$	Conversion / %
0	47.62	26.74	55.86
5	47.62	32.87	68.66
10	47.62	46.25	96.61
20	47.62	48.35	101

Conclusions

The results demonstrated that urease was efficient in converting urea into NH₃, showing a significant improvement in the conversion percentage compared to the absence of the enzyme. Spontaneous urea conversion was limited, reaching approximately 55%, while the addition of urease promoted a marked increase in efficiency, reaching 96.61% with 10 mg of enzyme. Increasing the enzyme mass to 20 mg did not result in a significant improvement in conversion, indicating that 10 mg of urease is sufficient to achieve good performance with rational enzyme use. These results reinforce the potential of urease for the conversion of urea into NH₃, aiming at its determination by voltammetric techniques in samples of environmental interest.

Acknowledgments

FAPEMIG and CAPES

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