EFFICIENCY OF BIOSENSORS PRODUCED WITH LACCASE AND IONIC LIQUIDS IN PESTICIDE QUANTIFICATION

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ABSTRACT

Several studies have been carried out to develop rapid and efficient techniques for quantifying pesticides in water and food. In this context, during the development of this study, the efficiency of biosensors produced using laccase and low-cost ionic liquids to quantify pesticides was evaluated. For this purpose, two different ionic liquids (*n*-butylammonium acetate - IL1 and *n*-butylammonium lactate – IL2)) were tested, with LI2 showing the best results, considering the current densities. Different biosensors produced (LAC1, LAC2, LAC3, LAC4, LAC5, LAC6, and LAC7) were also evaluated concerning the efficiency in quantifying carbendazim through the reduction of the signal related to dopamine, which was also added to the electrolyte solution. The maximum pesticide concentration that could be determined with the biosensor was also determined, which was 7.0 ppm.

Keywords: Laccase. Biosensor. Pesticide. Quantification.

1 INTRODUCTION

There is currently an increase in agricultural productivity and, with this, an increase in the use of agrochemicals for pest control, as described by Caratelli and collaborators (2022), who report that 74.8% of global agricultural production uses pesticides.[1] Therefore, the need for rapid and efficient quantification of the contamination of water, food, and soil by these compounds is highlighted, aiming to control the development of various health problems such as neurological disorders, cancer, and allergies, among others, with high concentrations in organisms can lead to death.[2] To achieve this, several techniques, such as chromatographic techniques (liquid and gaseous), capillary electrophoresis, and spectroscopic techniques (mass, FTIR, nuclear magnetic resonance, and atomic absorption), can be applied. However, their application generates some limitations, such as delays in carrying out the analyses, high costs, the need to apply expensive and complex instrumentation, the need for qualified technicians, and difficulties in handling and preparing samples, which make applying the techniques difficult.[3,4]

In this context, the importance of developing techniques that favor fast, efficient, and low-cost analysis is demonstrated, with biosensors being promising for this purpose in this scenario, leading several researchers to study these devices' application and application reliability. Mainly, enzymes are produced using laccase, acetylcholinesterase, tyrosinase, and peroxidase.[5] They have several advantages compared to other methods, such as low cost, high sensitivity, agility in analysis, and the possibility of use at the cultivation site for quality control.[3] Biosensors are devices that present high specificity, sensitivity, reproducibility, stability, linearity, easy to operate, economical, and possible to be applied in various quantifications, especially enzymatic biosensors, which are devices that can be used to quantify compounds through the evaluation of inhibition.[4] As an example, it is possible to highlight the biosensors produced with acetylcholinesterase, which is inhibited by organophosphate pesticides, the production of biosensors containing laccase, which lead to pesticide degradation mediated by phenolic compounds, and those using organophosphate hydrolase, which leads to hydrolysis of the evaluated pesticide.[4]

Therefore, several studies have been carried out to validate the application of biosensors in the quantification of pesticides such as organophosphates, carbamates, and pyrethroids in food and water, making it possible to highlight enzymatic biosensors and immunosensors. Of this, enzymatic biosensors have stood out for the diversity of quantification techniques that can be applied when using them, such as amperometry, voltammetry, potentiometer, conductometry, colorimetry, and fluorescence; for their production, it is possible to use several enzymes, such as peroxidase, tyrosinase, acetylcholinesterase, laccase, among others.[4,5] Taking into account the importance of carrying out processes that quickly and effectively quantify these compounds, in this work, an initial evaluation of the efficiency of using laccase biosensors in the quantification of the pesticides permethrin and carbendazim was carried out, with different protocols being evaluated. Immobilization of laccase and production of biosensors, as well as determining the minimum and maximum concentration of pesticides, can be determined via potentiometric techniques.

2 MATERIAL & METHODS

Two ionic liquids were initially synthesized, *n*-butylammonium acetate – IL1 and *n*-butylammonium lactate – IL2, to apply them as a binder in the biosensor production stage. They were produced via an acid-base reaction using a stoichiometry of 1:1. The temperature during synthesis was maintained at 10ºC, and the reaction was processed for two h after adding all the acid to the amine. Once the synthesis was complete, the material was left to rest at room temperature for 24 h and characterized via NMR.

After synthesis, they were used to produce biosensors based on chitosan, graphite, laccase, and ionic liquid or mineral oil with binder. The addition of bovine serum albumin to biosensor production was also evaluated. The biosensors produced (LAC1:

chitosan-immobilized laccase + graphite + *n*-butylammonium acetate; LAC2: chitosan-immobilized laccase + graphite + *n*-butylammonium lactate; LAC3: chitosan-immobilized laccase + graphite + mineral oil; LAC4: laccase + graphite + serum albumin + glutaraldehyde + *n*-butylammonium lactate; LAC5: laccase + graphite + chitosan + 4 drops of *n*-butylammonium lactate; LAC6: laccase + graphite + chitosan + 5 drops of *n*-butylammonium lactate; LAC7: laccase + graphite + chitosan + 6 drops of *n*-butylammonium lactate) were compared with biosensors produced using mineral oil as a binder. The efficiency of using *n*-butylammonium lactate as a binder was verified, the ideal concentration of laccase to be added for the production of the biosensor was verified, as well as the maximum concentration of pesticide carbedazim possible to be quantified using the biocomponent produced was evaluated. Quantifications were carried out via cyclic voltammetry (potential range: -1 and 1 mV/s, two scans per analysis, a rate of 0.3 mV/s, and a time interval of 24.4 ms) or square pulse voltammetry (potential range: -1 and 1 mV/s, rate of 5 mV, amplitude of 30 mV, frequency of 10 Hz, and current range of 1 mA).

3 RESULTS & DISCUSSION

Two ionic liquids were synthesized: *n*-butylammonium acetate (LI1) and *n*-butylammonium lactate (LI2) (Figure 1), characterized via H1-NMR and C13-NMR. After obtaining and characterizing the ionic liquids (ILs), an initial assessment of the efficiency of applying ILs in the production of biosensors as a binder was carried out (Figure 2A). It is possible to verify that using LIs led to a broadening of the curve and better sensitivity when using LI2 (Table 1). Therefore, it was selected during the optimization stage of biosensor production using different matrices. This way, different biosensors (LAC4, LAC5, LAC6, and LAC7) were produced, with greater efficiency observed when applying the LAC6 biosensor (Figure 2B, Table 1).

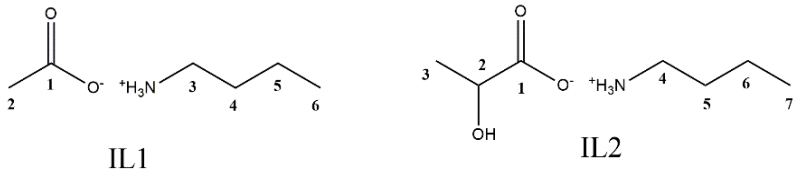

Figure 1 Chemical structure of synthesized ionic liquids

IL-1: H^1NMR (600 MHz, CDCl3): δ 1.89 (s, 3H), 2.80 (t, 2H), 1.61 (m, 2H), 1.38 (m, 2H), 0.92 (t, 3H), 7.22 (s, 3H). C^{13}NMR (150 MHz, CDCl3): δ 178.69, 30.16, 39.20, 24.12, 19.30, 13.39.

IL-2: H^1NMR (600 MHz, CDCl3): δ 4.03 (m, 1H), 2.86 (d. 3H), 3.26 (t, 2H), 1.42 (m, 2H), 1.32 (m. 2H), 0.95 (t. 3H), 6.62 (s. 3H). C^{13}NMR (150 MHz, CDCl3): δ 181.78, 67.77, 21.22, 39.81, 29.67, 19.25, 13.81.

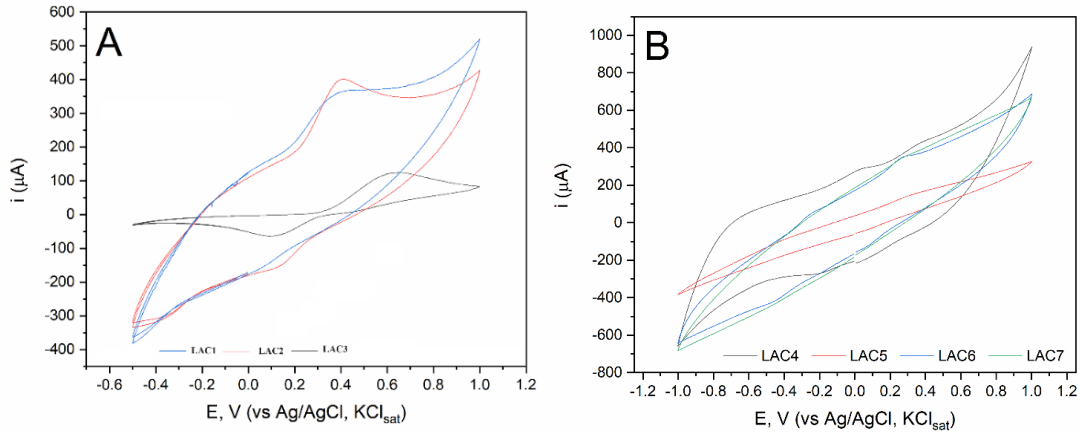

Figure 2 Voltammograms obtained during the characterization of biosensors produced using dopamine solution as electrolyte

Table 1 Surface tension data was obtained for each cultivation condition.

Sample	i_p^a (mA.cm^{-2})	i_p^c (mA.cm^{-2})	Epc (V vs. Ag/Ag)	Epa (V vs. Ag/Ag)	Epc-Epa (V vs. Ag/Ag)
LAC1	0.359	-0.168	0.029	0.383	-0.354
LAC2	0.401	-0.155	0.127	0.408	-0.281
LAC3	0.121	-0.063	0.095	0.605	-0.510
LAC4	0.287	-0.272	-0.211	0.035	-0.246
LAC5	0.148	-0.270	-0.201	0.317	-0.518
LAC6	0.351	-0.427	-0.433	0.242	-0.674
LAC7	0.348	-0.419	-0.428	0.237	-0.665

After analyzing the results, it is possible to verify that using *n*-butylammonium lactate significantly increases the anodic current density. It is possible to confirm that the cathodic current density remains practically constant. Regarding the increase in the concentration of *n*-butylammonium lactate, it is possible to verify that more than five drops reduced the anodic and cathodic current densities. The process can be considered quasi-reversible regarding the mechanisms of the electroactive species. After selecting the best electrode, the behavior of the LAC6 electrode in the quantification of the pesticide carbedazim was evaluated, and it was verified that the minimum concentration determined via square pulse voltammetry was 7.0 ppm (Figure 3).

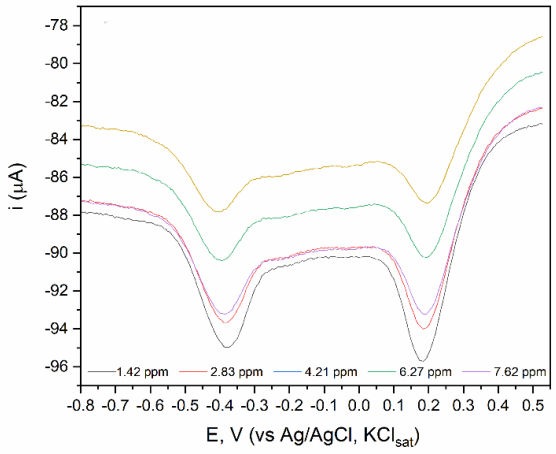

Figure 3 Potentiometric data obtained during quantification of carbedazim

4 CONCLUSION

The presented study demonstrates the effectiveness of using biosensors produced with laccase and ionic liquids, particularly n-butylammonium lactate, to quantify pesticides in solutions. The synthesis and characterization of two ionic liquids, n-butylammonium acetate, and n-butylammonium lactate, provided a new approach to improve biosensor performance. Among the biosensors tested, the biosensor produced with chitosan, graphite, and n-butylammonium lactate (LAC6) exhibited superior sensitivity and efficiency in pesticide detection. Thus, this work highlights the potential of laccase-based biosensors as an economical, rapid, and reliable method for pesticide quantification. Future research should explore further optimization and validation of these biosensors in diverse environmental and agricultural contexts, aiming to increase their robustness and applicability in real-world scenarios.

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