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Abstract

Miniaturized Flow-System Integrating Enzymatic Electrochemical Biosensors for Monitoring the Malolactic Fermentation of Red Wines †

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During the malolactic fermentation (MLF) of red wines, L-malic acid is mainly converted to L-lactic acid. The concentration of both acids along the process have a significant influence on the quality of the final wine, therefore real-time monitoring of the MLF would be interesting for the winemaking industry. The traditional methods used at present require laboratory equipment; therefore, the results are not known in real-time. In order to solve that issue, a miniaturized and portable flow-system device that integrates amperometric enzymatic biosensors is proposed in this work. The system allows the simultaneous determination of both acids (L-lactic and L-malic acids) involved in the MLF of red wines using very low samples and reagents volumes. Biosensors for lactic and malic acids are based on thin film platinum microelectrodes fabricated with standard photolithographic techniques and polypyrrol enzymatic membranes. The electrode cell is based on a silicon chip with four electrodes in parallel: a 2.5 mm^2 pseudo-reference electrode (p-RE), two 2.5 mm^2 working electrodes (WE) and a 5 mm^2 counter electrode (CE). This cell architecture allows the integration of the two biosensors for the determination of both acids in the same chip. The surface of the platinum WEs is electro-modified with a three-dimensional polypyrrole membrane by applying the potentiostatic conditions in PB solutions containing the monomer and the entrapped chemical reagents. The fabrication conditions for both electrosynthesized biosensors were optimized previously by our group [1,2]. The sensor chip is implemented in a low-cost, robust and portable polymethyl methacrylate (PMMA) and pressure-sensitive adhesive (PSA) module with the required fluidic channels for the management of samples and reagents and a small chamber for the electrochemical cell (10 μL).

The analytical performance of both biosensors was studied by chronoamperometry. The L-lactate biosensor had a sensitivity of $-173 \pm 8 \times 10^2$ μA M^{-1} cm^{-2} (r = 0.997, n = 7) in a linear range from 5×10^{-6} M to 1×10^{-4} M and a LOD (3σ IUPAC criterion) of $3.2 \pm 0.3 \times 10^{-6}$ M. Regarding the L-malate biosensor, a sensitivity of $(5.53 \pm 0.6) \times 10^2$ mA M^{-1} cm^{-2} (r = 0.997, n = 5) in a linear range from 1×10^{-7} M to 1×10^{-6} M and a LOD of $6.7 \pm 0.2 \times 10^{-8}$ M have been obtained. The RSD was calculated for both biosensors using three biosensors fabricated under the same conditions on the same day, obtaining a value lower than 8% and 6% for the L-lactate and the L-malate biosensor, respectively. Both biosensors showed long-term stability, retaining more than the 90% of their initial sensitivity after more than 30 days, meeting the requirements of the proposed application.

Finally, the fluidic system was applied to the monitoring of the malolactic fermentation of samples recollected during this process for several red wines, with the results obtained by the proposed system showing excellent agreement with those obtained with the standard method.

Conflicts of Interest: The authors declare no conflict of interest.

References

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