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Simultaneous amperometric determination of malic and gluconic acids in wine using screen-printed carbon electrodes

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ABSTRACT

The selective and simultaneous amperometric determination of malic and gluconic acids was carried out using a screen-printed design with two working carbon electrodes. One of the two working electrodes was modified with gold nanoparticles, tetrathiafulvalene (TTF) and malate quinone oxido-reductase enzyme for the sensitive detection of malic acid. Analogously, the other working electrode was modified with TTF and gluconate dehydrogenase enzyme for the sensitive detection of gluconic acid. The array of biosensors responds to gluconic and malic acids giving each anodic currents (+100 mV and pH 6.5), with detection capabilities of 1.89 μM ($\alpha = \beta = 0.05$) and 0.79 μM to malic and gluconic acids, respectively, at room temperature. The method has been applied to the determination of both analytes in wine, obtaining successful results.

1. Introduction

Wineries impose new trends in winemaking, based on biological technologies, to optimize the process and improve the final product. An important group of compounds in wine are low molecular weight organic acids, since they can influence organoleptic properties (flavour, colour, and aroma) or even the stability and microbiologic control of these beverages [1–3]. Among them, malic [4–11] and gluconic [12–17] acids are two essential components, which come directly from the grape and/or from the processes which are subjected, such as alcoholic or malolactic fermentation [2,18]. The analysis of these acids allows to check the process of maturation of grapes and to control the evolution of the acidity of wines during the several stages of their elaboration process [2,19,20].

Several spectroscopic, chromatographic and enzymatic methods have been developed for the determination of malic and gluconic acids separately. Biosensors, in particular amperometric biosensors, offer fast, cheap and smart easy to handle devices able to selectively detect and quantify malic [4,7–11,19–26] and gluconic acids [13,17,27,28] in wine samples. They can be envisaged as serious competitors for conventional techniques, especially when using disposable transducers such as screen-printed electrodes (SPEs), representing an attractive alternative for small industries [25]. Screen-printing apart from miniaturization allows efficient large-scale production of sensors of relatively low cost and high reproducibility, which are of special interest for simple and fast electrochemical determination of a range of analytes [29].

The demand for multianalyte sensing device is increasing, especially in biomedical, biotechnological, industrial and environmental applications [30]. Undoubtedly, the use of a multiparameter approach that permits to determine malic and gluconic acids in a single analysis would simplify the detection procedure and reduce costs. Up to now, these analytes have been simultaneously determined in wine by near infrared reflectance spectroscopy [31], as well as in honey [32] and vinegar [33] by HPLC.

Thus, the aim of this work has been approaching the advantages of electrochemical biosensors to the detection and quantification of malic and gluconic acids in a single analysis. The application of screen-printed technology allows the design of setups containing electrodes of different functions. In this case, a disposable array containing two working electrodes modified with malate quinone oxido-reductase enzyme (MQO) [26] and gluconate dehydrogenase enzyme (GADH) [28] has been made up for the simultaneous analysis of these compounds in wine samples. Each enzyme has been strictly deposited on the surface of the corresponding screen-printed carbon working electrode by cross-linking with glutaraldehyde (GA) and bovine serum albumin (BSA) [34–36],

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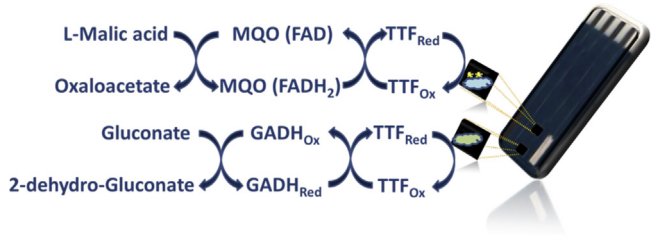

Fig. 1. Schematic view of the dual amperometric biosensor for malic and gluconic acids determination.

avoiding mixing that can compromise the biosensor specificity and "cross-talk" effects [30]. Both biosensors are based on the regeneration of a suitable mediator, tetrathiafulvalene (TTF), at the electrode surface at a low operational potential, in such a way that the anodic currents registered can be related to the malic and gluconic acids concentration into the electrochemical cell, avoiding interferences (Fig. 1) [26,28].

2. Materials and methods

2.1. Chemicals and reagents

Carbon (C200802P2, Gwent Electronic Materials, Torfaen, UK) Ag/AgCl (Electrodag 6037 SS, Acheson Colloiden, Scheemda, The Netherlands), and dielectric (D2071120D1 Gwent Electronic Materials, Torfaen, UK) inks were used in the fabrication of the disposable transducers.

All reagents used were of analytical-reagent grade. Milli-Q water (Millipore, Bedford, MA, USA) was used for preparing aqueous solutions.

MQO (EC 1.1.99.16, 1.0 U μL^{-1}) and GADH (EC 1.1.99.3, 2.8 U μL^{-1}) were purchased from Biolan Microbiosensores (Parque Tecnológico de Vizcaya, Zamudio, Spain). TTF was obtained from Acros Organics (Geel, Belgium). L-Malic acid, BSA, GA and gluconic acid were provided by Sigma–Aldrich (Steinheim, Germany).

50 mM phosphate buffer pH 6.5 (Panreac, Barcelona, Spain), containing 100 mM of KCl (Merck, Darmstadt, Germany), was used as supporting electrolyte solution. pH values were adjusted using a 2.0 M solution of NaOH (JT Baker, Deventer, Netherlands).

2.2. Apparatus

Screen-printed carbon electrodes (SPCEs) were produced on a DEK 248 printing machine (DEK, Weymouth, UK). pH of solutions was measured with a HI 221 pH meter (Hanna instruments, USA). A PalmSens® portable electrochemical bipotentiostat with the PS Trace program (PalmSens® Instruments BV, Houten, The Netherlands) was used for electrochemical measurements.

2.3. Electrodic systems

A new device for the simultaneous analysis of malic and gluconic acids has been developed. The home-made screen-printed transducers have been designed including two different working electrodes (area, 4 mm^2) connected in array mode. The four-electrode array was built by sequential printing of different layers, corresponding to conductive paths, counter electrode, working electrodes and dielectric protective layer, using different formulation of pastes that were cured according to the manufacturer's specifications [34–36].

2.3.1. Deposition of gold nanoparticles onto the SPCEs

Gold nanoparticles (AuNPs) based SPCEs (AuNPs-SPCEs) were fabricated by electrochemical deposition of AuNPs onto one of the carbon working electrodes at room temperature, according to the procedure described anywhere else [37]. Briefly, a volume of 200 μL of 1.0 mM $HAuCl_4$, prepared in 500 mM H_2SO_4, was dropped onto the SPCEs and a potential of +180 mV vs Ag/AgCl SPE was applied at the corresponding working electrode during 10 s.

2.3.2. Immobilization of MQO onto the screen-printed working electrode

1.0 μL of a BSA solution (3%, w/v), 1.0 μL of a GA solution (5%, w/v), 1.0 μL of a TTF solution (1%, w/v) and 4.0 μL of MQO solution were dropped onto the working electrode surface AuNPs-SPCEs (MQO-TTF-AuNPs-SPCEs) [26]. The mixture was left to react 90 min at 4 °C.

2.3.3. Immobilization of GADH onto the screen-printed working electrode

1.0 μL of a BSA solution (3%, w/v), 1.0 μL of a GA solution (5%, w/v), 1.0 μL of a TTF solution (1%, w/v) and 2.0 μL of GADH solution were dropped onto the working electrode surface of SPCEs (GADH-TTF-SPCEs) [28]. The mixture was left to react 90 min at 4 °C.

2.4. Measuring amperometric procedures

All measurements were made at room temperature in a cell containing 5.0 mL of supporting electrolyte, with constant stirring. Working electrodes operated in array mode at +100 mV vs. Ag/AgCl SPE. The corresponding sample, containing both malic and gluconic acids, was added into the electrochemical cell after reaching a stable baseline.

3. Results and discussion

The four-electrode array, containing two working electrodes, was used for the development of a dual-biosensor for the simultaneous detection of malic and gluconic acids. One of the two working electrodes was modified with AuNPs, which enhanced the oxidation current registered, TTF as redox mediator and MQO for the sensitive detection of malic acid [26]. Analogously, the other working electrode was modified with TTF and GADH for the sensitive detection of gluconic acid [28]. Then, the catalytic activity of MQO and GADH towards malic and gluconic acids was simultaneously evaluated by amperometry. As it was observed for the individual assays, anodic currents were also obtained when MQO-TTF-AuNPs-SPCEs and GADH-TTF-SPCEs in array mode were at least polarized at +100 mV vs. Ag/AgCl SPE in supporting electrolyte adjusted to pH 6.5 [26,28]. Enzyme-free TTF-AuNPs-SPCEs and TTF-SPCEs modified with GA and BSA were also evaluated towards malic and gluconic acids, correspondingly. No electrochemical signals were obtained with these control electrodes, which highlight the relation between the concentration of malic and gluconic acids and anodic current registered using the dual-biosensor. In this way, linear responses in malic acid concentration range from 1.89 till 150.66 μM and 0.79 to 437.06 μM for gluconic acid were obtained.

Under the above experimental conditions, the cross-talk was also checked by injecting gluconic acid or malic acid standard solutions. Fig. 2 shows the electrochemical responses recorded. Only the MQO-TTF-AuNPs-SPCE registered any current in presence of malic acid and, analogously, the GADH-TTF-SPCE responds exclusively towards gluconic acid.

The performance of this new procedure for malic and gluconic acids detection and quantification were measured by its precision, in terms of reproducibility, and its capability of detection. With this aim, several calibration curves were performed, at +100 mV vs. Ag/AgCl SPE in supporting electrolyte adjusted to pH 6.5, using different MQO-TTF-AuNPs-SPCEs and GADH-TTF-SPCEs, by successive additions of a solution of malic and gluconic acids (Table 1).