Sensors and Actuators B 192 (2014) 56–59

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

GADH screen-printed biosensor for gluconic acid determination in wine samples

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ARTICLE INFO

Article history:
Received 22 July 2013
Received in revised form
25 September 2013
Accepted 22 October 2013
Available online 30 October 2013

Keywords:
Screen-printed carbon electrodes
Chronoamperometric biosensor
Gluconate dehydrogenase
Tetrathiafulvalene
Gluconic acid

ABSTRACT

Gluconate dehydrogenase (GADH) biosensors have been developed for the satisfactory determination of gluconic acid in wine samples, without any pretreatment. The biosensors have been fabricated by cross-linking immobilization of GADH onto screen-printed carbon electrodes, containing the mediator tetrathiafulvalene (TTF). Chronoamperograms have been registered at +100 mV vs. screen-printed Ag/AgCl electrode by successive additions of a gluconic acid solution in the concentration range from 9.0 to 131.4 μM. This method shows a reproducibility of 8.1% ($n = 3$) related to the slopes of these calibration curves and a repeatability of 3.2% ($n = 5$). The procedure has shown an average capability of detection of 9.0 μM for a probability of false positive and negative of 0.05. Wine samples have been analyzed with these biosensors, obtaining satisfactory results.

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1. Introduction

Wine industry has become a huge business worldwide with a high turnover, which requires a continuous development in technology. A small step can make a significant improvement in technology that leads to saving a lot of money for wineries. In this way, this work shows a simple, selective and cheap method for gluconic acid determination. It is well known that gluconic acid is an organic compound with special interest in wine science, since is the main indicator of infection of grapes by *Botrytis Cinerea* [1]. It also plays an important role in the physical and chemical stability, as well as in the sensory properties of wine [2].

Gluconic acid has been determined in different samples by several methods, such as chromatography [3–15], electrophoresis [5,16–21], infrared spectroscopy [22,23], UV/Vis spectrophotometry [24], chemiluminescence [25] and fluorescence [26]. Taking into account figures of merit as accuracy, improved sensitivity and cost-effectiveness, electroanalytical methods have attracted much attention. Consequently, enzyme based electrochemical biosensors have been developed as an attractive alternative for gluconic acid determination [27–30]. In this way, gluconate kinase (GK) and 6-phospho-D-gluconate dehydrogenase (6PGDH) based biosensors have been described for the amperometric detection of gluconic acid, at an operational potential of +800 mV [27,28]. This large overpotential can be minimized by the use of the enzyme gluconate dehydrogenase (GADH) and electron-transfer mediators, such as *p*-benzoquinone [29] and tetrathiafulvalene (TTF) [30] (Table 1). This last concept can be easily improved by transferring it to disposable electrodes, such as screen-printed carbon electrodes (SPCEs) [31–33], which allow carrying out decentralized assays. Thus, this work aims to develop an amperometric biosensor based on the immobilization of GADH enzyme by cross-linking onto TTF modified SPCEs ($SPC_{TTF}Es$). The incorporation of the redox mediator into the carbon ink, which is screen-printing to define the working electrode, simplifies the measurement procedure. Moreover, the great performance of GADH allows the fabrication of a disposable biosensor for the sensitive and selective detection of gluconic acid.

2. Experimental

2.1. Reagents

Electrodag 418 Ag ink and Electrodag 6037 SS Ag/AgCl ink (Acheson Colloiden, Scheemda, The Netherlands), as well as C10903P14 carbon ink and D2071120D1 dielectric ink (Gwent Electronic Materials, Torfaen, UK) were used in the fabrication of SPCEs.

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http://dx.doi.org/10.1016/j.snb.2013.10.098

Table 1
Analytical characteristics of GADH based biosensors for gluconic acid determination.

Electrode	Enzyme	Working potential (mV)	K_M^{app} (mM)	Linear range (μM)	Reproducibility (% RSD)	Capability of detection (μM)	Sample	Refs.
Composite paste	GK + 6PGDH	+800 vs. SCE	0.52 ± 0.05	7–250	1.7	Not shown	White wine	[27]
SPCE	GK + 6PGDH	+800 vs. $SP_{Ag/AgCl}E$	0.22 ± 0.02	7.5–71.4	2.9	7.5	Red wine	[28]
Carbon paste	GADH	+400 vs. SCE	2.1	100–4000	4.8	Not shown	Not shown	[29]
Gold	GADH	+150 vs. SCE	0.21 ± 0.01	0.6–20	7.4	0.2	Wine and must	[30]
SPCE	GADH	+100 vs. $SP_{Ag/AgCl}E$	0.11 ± 0.03	9.0–131.4	8.1	9.0	Red Wine	This work

All reagents used were of analytical-reagent grade. All solutions were prepared with water purified with a Milli-Q apparatus from Millipore (Bedford, MA, USA).

TTF was obtained from Acros (Acros Organics, Geel, Belgium).

GADH (E.C. 1.1.99.3; 2.2 U/μL) was purchased from BIOLAN Microbiosensores S.L. (Parque Tecnológico de Vizcaya, Zamudio, Spain).

Glutaraldehyde (GA), bovine serum albumin (BSA) and gluconic acid were obtained from Sigma (Sigma-Aldrich, Steinheim, Germany).

100 mM pH 6 phosphate buffer ($NaH_2PO_4 \cdot 2H_2O$, Merck, Darmstadt, Germany) containing 100 mM of KCl (Merck, Darmstadt, Germany), was used as supporting electrolyte. NaOH (J.T. Baker, Deventer, The Netherlands) was used to adjust the pH value.

2.2. Apparatus

$SPC_{TTF}Es$ were produced on a DEK 248 printing machine (DEK, Weymouth, UK) using screen polyester mesh and polyurethane squeegees.

Electrochemical measurements were made with a PalmSens handheld potentiostat (Palm Instruments BV, Houten, The Netherlands).pH of solutions was measured with a Hanna instruments HI 221 pH meter (USA).

2.3. Biosensors manufacturing

$SPC_{TTF}Es$ (Working area, 15.90 mm^2) were home-produced according to the procedure described anywhere else [28]. In this case, the working electrode ink was prepared by thoroughly mixing the carbon ink with TTF (3% w/w) and immediately screen-printed.

3 μL of a mixture of GADH, GA (5%) and BSA (6%) solution, in the volume ratio 8:6:7, were dropped onto the working electrode surface. The mixture was left to react for 90 min at 4 °C. The biosensors were kept at 4 °C prior to use.

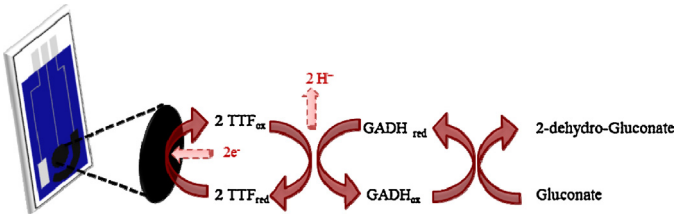
2.4. Measuring chronoamperometric procedures

All measurements were carried out in a batch system with constant stirring, at room temperature, in a cell containing 5 mL of the supporting electrolyte solution.

The chronoamperometric detection has been performed by measuring the anodic current due to the oxidation of TTF at a potential of +100 mV vs. screen-printed Ag/AgCl reference electrode, except for the optimization process. The corresponding gluconic sample was added after reaching a stable baseline.

3. Results and discussion

Gluconic acid concentration in a solution can be related to the electrocatalytic oxidation current of TTF recorded using a GADH modified $SPC_{TTF}E$ (Scheme 1). In this work, GADH was immobilized at the surface of the working electrodes by a simple cross-linking procedure. BSA was used as non-active protein in order to prevent the loss of enzymatic activity that can cause the cross-linker GA [33,34]. Thus, GA/BSA volume ratios used to build the biosensors,



Scheme 1. Enzymatic reaction mechanism at the working electrode surface.

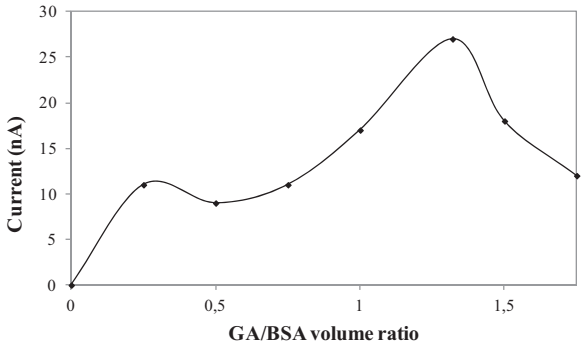


Fig. 1. Current registered with different GA/BSA ratios for biosensors optimization at +100 mV vs. screen-printed Ag/AgCl electrode and 100 mM phosphate buffer solution pH 6.

in the range from 0 to 1.8, were analyzed in order to maximize the chronoamperometric signal registered related to a 12.5 μM gluconic acid solution. As it can be seen in Fig. 1, the optimum GA/BSA volume ratio obtained was 1.3.

The main variables that can influence the chronoamperometric response, namely working potential and pH, were also optimized. Fig. 2 shows the cyclic voltammogram recorded in 100 mM of KCl using a $SPC_{TTF}E$, with an oxidation peak close to +150 mV vs.

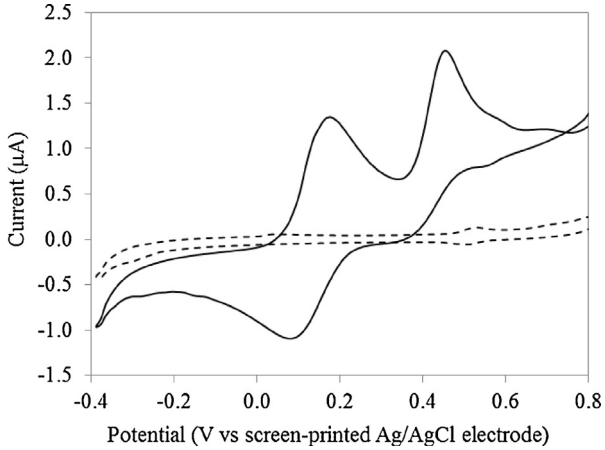


Fig. 2. Cyclic voltammograms recorded in a 100 mM KCl solution using a SPCE (dot line) and a $SPC_{TTF}E$ (solid line).

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