SO2SAFE - A FAST AND ACCURATE ELECTROCHEMICAL BIOSENSOR FOR SULPHITE ANALYSIS IN FOOD INDUSTRY

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Abstract- Enzyme-based biosensors are widely used in many applications such as health care, food safety and environmental monitoring. However, the implementation of many of these applications is strongly hindered by their limited stability on time.

This paper reports a disposable electrochemical biosensor based on screen-printing technology for the easy, fast and accurate determination of sulphite in shrimp farming, which shows good sensitivity (62 nA/ppm), reproducibility (RSD = 4%; n = 5), and a high storage stability (8 months).

Keywords:*sulphite, biosensor, screen-printed electrodes, food safety, electrochemical.*

1. INTRODUCTION

Sulphites are food additives that have antioxidant and preservative properties. However, they are recorded as allergens by the main international regulatory bodies on food safety because of their adverse health effects. Hence, sulphites maximum concentration levels in foodstuff are regulated, and must be ensured by the agro-food processing industries [1]. However, current methods used for sulphite level monitoring in the agro-food industry, such as Monier Williams [2], are not solving the problem of the high quality control standards required to comply with new regulation on food safety and consumer health protection.

In the framewok of the SO2SAFE project, financed under the H2020 SME instrument, BIOLAN and CIDETEC reported a disposable electrochemical biosensor based on screen-printing technology for the easy, fast and accurate determination of sulphite in shrimp farming [3].

The sulphite biosensor which is based on a three-electrode configuration (consisting of a graphite counter electrode, a Ag/AgCl pseudoreference electrode and graphite working electrode), is constructed by modifying the working electrode with an electrochemical mediator (potassium ferricyanide) and the Sulphite Oxidase enzyme.

The developed biosensor showed good sensitivity (62 nA/ppm), reproducibility (RSD = 4%; n = 5), and a linear range of 250 – 1000 ppm. In addition, the applicability of the biosensor for the analysis of sodium metabisulphite in shrimp farm samples was demonstrated successfully.

Here, we present the further improvements carried out on the development of the sulphite biosensor. In particular, the improvement of the long-term stability [4].

2. EXPERIMENTAL

2.1 Reagents and solutions

Bovine serum albumin (BSA), mucin from porcin stomach, D-sacarose, D-mannitol,
and sodium sulphite were purchased from Sigma-Aldrich (Madrid, Spain). Sulphite Oxidase (SuOx) enzyme was produced at BIOLAN. All other chemicals employed were of analytical reagent grade. Ultrapure water obtained with a Millipore Elix 3/Milli-Q A10 Gradient purification system from Millipore Ibérica S.A. (Madrid, Spain) was used throughout this work.

The following solutions were prepared and used throughout this work:

- 50 mM succinic buffer pH 5.5, pH 6 and pH 6,5 (SB)
- 100 mM phosphate buffer pH 7.0 (PB)
- 100 mM carbonate buffer pH 10.2, containing 10 mM mannitol (CB)
- Working solutions of sodium sulphite were prepared daily in 100 mM carbonate buffer pH 10.2, containing 10 mM mannitol.

2.2. Materials and instruments

All the electrochemical experiments were performed with a PGSTAT 128N potentiostat-galvanostat from Autolab (KM Utrecht, The Netherlands), using the software package NOVA 1.11 or the portable potentiostat developed by BIOLAN (Fig. 1b).

The disposable screen-printed electrodes (SPEs) consisting of a carbon working electrode, a carbon counter electrode and a silver/silver chloride (Ag/AgCl) pseudoreference electrode shown in Fig. 1a were produced at IK4-CIDETEC, using a Thieme 110E screen-printing machine from Thieme GmbH&Co (Teningen,Germany), an UV tabletop dryer Aktiprint T/A 40-2 from Technigraf (Hessen, Germany) and an oven PN 200 from Carbolite (Derbyshire, UK).

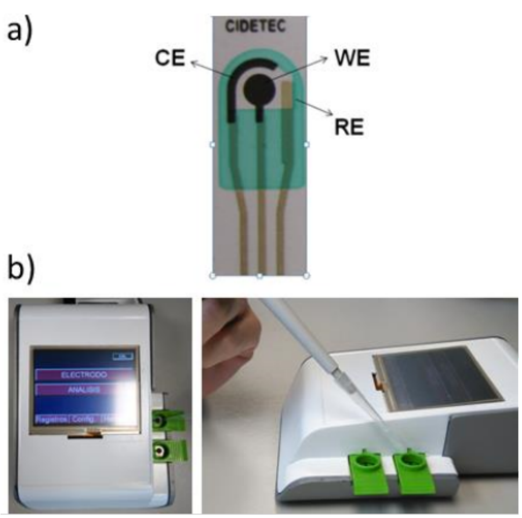

Fig. 1. Screen-printed electrodes produced at IK4-CIDETEC, consisting of a carbon working electrode (WE), a carbon counter electrode (CE), and a Ag/AgCl pseudoreference electrode (RE) (a) and the portable potentiostat developed by BIOLAN (b).

2.3. Preparation of the sulphite biosensor

The sulphite biosensor was constructed by modifying the WE of the SPEs with ferricyanide and SuOx. Firstly, 1.5µl of 60mM ferricyanide prepared in a mixture of ethanol and SB (1:1) were deposited and dried at RT. Then 1.5µl of a mixture of ethanol and water (1:1) were uniformly spread on the WE and 2.5µl of 0.4U/µl of SuOx in PB, containing 1% (w/w) BSA, 1% (w/w) mucin, 5 % (w/w) sacarose and 2.5 % (w/w) mannitol, were added and left drying at 30ºC for 30 minutes.

2.4. Electrochemical assays

The amperometric measurements were performed by covering the three electrode system with 60µl of the corresponding solution and by applying +225mV vs. Ag/AgCl pseudoreference electrode during 60s. The current value recorded at 60s was then used for constructing the calibration curve. For each measurement one biosensor was used and each point was recorded by triplicate.

2.5. Shelf-life determination

In order to evaluate the long-term stability of the developed sulphite biosensor, the calibration curves of the fabricated biosensors were collected 2-4 times per month over a period of 8 months. The fabricated biosensors were vacuum packed and stored at 30ºC until use.

3. RESULTS AND DISCUSSION

3.1. Optimization of biosensor composition

Although the applicability of the developed biosensor for the analysis of sodium metabisulphite in shrimp farm samples was successfully demonstrated in a previous work [3], the biosensor showed short life time and poor stability, a key factor that hinders its successful commercialization.

The poor stability is mainly due to two factors: the first, associated to the increase of the background currents over the time due to the electron transfer reactions that occur between the electrochemical mediator and a portion of enzyme protein, and the second, associated to the enzymatic activity, which decays exponentially with time due to the inactivation of the enzyme.

In order to improve the long term stability of the developed biosensor, different parameters concerning the fabrication of the sulphite biosensors were optimized.

On one hand, with the aim to increase the stability of the background currents, different pH buffers were tested for dissolving the electrochemical mediator, potassium ferricyanide, which is more stable at slightly acidic buffers prepared from carboxylic acids/salts.

Fig. 2 shows the background signals recorded over one week using a batch of SPEs modified with potassium ferricyanide solutions dissolved in different buffers. From the obtained results it can be concluded that the stability of the background currents improves when using succinic buffer pH 5.5 for diluting the potassium ferricyanide.

On the other hand, with the aim to obtain a stable protein formulation and avoid the inactivation of the enzyme, sacarose (as protein stabilizer) in combination with mannitol (as bulking agent) and BSA and mucin (as filling proteins) was used to prevent protein unfolding/aggregation and chemical degradation [4].

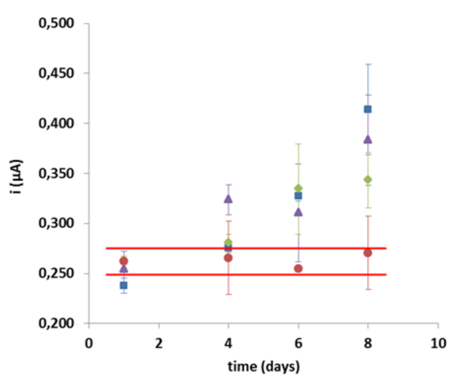

Fig. 2. Blank signals (CB) obtained for the sulphite biosensors prepared using PB pH 7 (blue squares), succinic buffer pH 6.5 (purple triangles), succinic buffer pH 6.0 (green diamonds) and succinic buffer pH 5.5 (red circles) as mediator buffer with respect to the storage time (at 30ºC). Each point represents the current value recorded at 60 seconds (mean value and standard deviation; n = 3). Red lines indicate a 10% deviation from the signal obtained the first day with the biosensors produced using succinic buffer pH 5.5, E = +225mV vs. Ag/AgCl pseudo reference electrode.

3.2. Performance of the developed biosensor

The developed biosensor shows good reproducibility (RSD 4%, n=5), sensitivity (62 nA/ppm) and a linear range between 250 and 1000 ppm with a good detection limit (2 ppm) and quantification limit (5 ppm). Fig. 3 shows the calibration curve obtained with the new optimized prototype using standard solutions of sulphite prepared in CB.

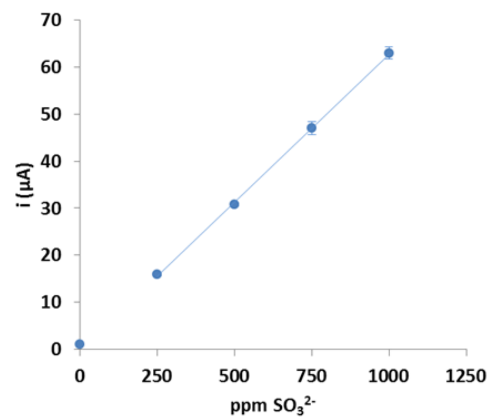

Fig. 3. Calibration curve for sulphite obtained with the developed biosensors. Each point represents the current value recorded at 60 seconds (mean value and standard deviation; n = 5). E = +225mV vs. Ag/AgCl pseudo reference electrode.

3.3. Biosensor stability

In order to check the long term stability of the optimized biosensor, a batch of 250 sulphite biosensors was prepared as described in previous sections, sealed in vacuum bags in order to prevent from humidity related degradation and stored at 30ºC until use. The long-term stability testing has been performed at 30ºC, in an accelerated ageing time (AAT) study using a conservative ageing factor (Q_{10}=2), in order to establish a tentative shelf life time until the results of real time aging studies are completed [5].

At the beginning of the study, the calibration curves as well as the blank measurements were recorded once per week. Later on, the frequency was changed to twice per month. Fig. 4 shows the mean value of the slopes obtained for the different calibration curves recorded during this period of

time. Upper and lower control limits indicate a 10% deviation of the slope value obtained the first day.
As it can be seen, the slope remains almost constant (± 10%) after 8 months at 30ºC.
Moreover, applying conservative ageing factors (Q_{10}=2 and 23ºC as RT), the shelf-life of sulphite biosensors can be extrapolated to 12 months until the real stability at RT has been confirmed.

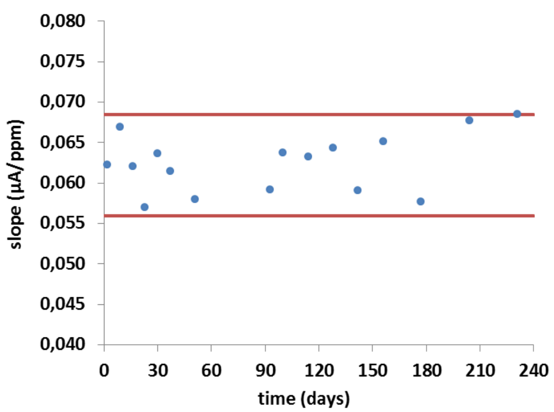

Fig. 4. Long-term stability chart obtained for the sulphite biosensors stored at 30ºC. Each point represents the mean value of the slopes (blue dots). Upper and lower limits (red lines) indicate a 10 % deviation of the slope value obtained the first day.

4. CONCLUSIONS

A disposable electrochemical biosensor based on screen-printing technology for the easy, fast and accurate determination of sulphite in shrimp farming, which shows good sensitivity (62 nA/ppm), reproducibility (RSD = 4%; n = 5), and a high storage stability (8 months at 30ºC) has been reported.
It has been shown that the operational stability of the biosensor greatly improves by immobilizing the enzyme in a suitable reagent matrix.
Moreover, the use of thermal accelerated ageing studies can be applied to the shelf-life determination of electrochemical biosensors and provide preliminary indication of how design improvements can affect the stability in a timely manner. Thus, the application of concept and knowledge commonly used on the pharmaceutical industry can serve to improve
and test the stability of electrochemical biosensors.
Further work will be focused in evaluating the biosensor performance in shrimp aquaculture facilities in Ecuador and Honduras in order to demonstrate SO2SAFE biosensor operation in real environment.

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