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Determination of Sulfite in Beer Samples Using a Fill and Flow Channel Biosensor

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Abstract

A simple method is described to determine sulfite in beer samples using a fill-and-flow channel biosensor. In a rectangular flow cell the sample solution flows through three consecutive zones: over a predictor electrode, an enzyme layer and a detector electrode. Together these three zones enable the differentiation between current due to sulfite and current due to other electroactive species in the sample. The predictor electrode is located upstream, and on the opposite channel wall to the enzyme layer and detector electrode, and is poised at the same potential (+0.65 V versus Ag/AgCl) as the detector electrode. On this electrode the current contribution from all species in the sample solution that are oxidized at that potential is determined. The enzyme layer contains sulfite oxidase, which, in the process of oxidizing sulfite, produces hydrogen peroxide, which itself is reduced by excess sulfite. The current at the downstream detector electrode is therefore different from that at the predictor electrode as a result of the enzyme reaction, and the difference of the currents, corrected for the dimensions of the electrodes, is proportional to the concentration of sulfite. The method enables a straightforward correction of the interfering current at the detector electrode and a determination of the analyte concentration. The effect of interferences from ascorbic acid, ethanol, sorbic acid and tartaric acid in the detection of sulfite is efficiently removed. The concentration of sulfite in a sample of beer measured by the biosensor is equivalent to that measured using a reference method based on the AOAC-recommended Monier-Williams method.

Introduction

Sulfite is adopted as a common preservative in a great variety of foods to prevent enzyme activity causing browning and to inhibit the growth of microorganisms during storage. However, in recent years it has been increasingly realized that sulfite has a potential to cause an adverse reaction in sulfite-sensitive asthmatic individuals [1-3]. Since 1986 the United States Food and Drug Administration (FDA) has required labeling of products containing more than 10 ppm of sulfite (156 μM) in foods or beverages [4]. Since 2002 the Australian Food Standards Code (AFSC) has regulated that any wine containing added sulfites in concentration of 10 ppm or more sold in Australia must be labeled as such. In order to accurately control the quality of manufactured products, a sensitive, easily applied and accurate analytical method for the determination of sulfite is required.

A number of methods have been developed for sulfite determination, which may be classified according to the compounds measured. One approach is to acidify the sample then determine the amount of sulfur dioxide gas formed using gas chromatography [5]. Alternatively sulfur dioxide can be oxidized to sulfuric acid using hydrogen peroxide, followed by an acid-base titration [6]. A typical example of the titration approach is the Monier-Williams method, which is recommended by the Association of Official Analytical Chemists (AOAC) [6]. This method is reliable and economic, but it is complicated, time consuming and lacks good precision. In contrast, a gas chromatography method has been reported that detects sulfur dioxide through extraction from the headspace, with integrated acidification and determination . Gas chromatography is accurate, and has better sensitivity, but it requires expensive instrumentation and skilled operators [5].

In a second class of methods free or complex sulfite ion is determined directly using liquid chromatography [7-10], capillary electrophoresis [11], spectrophotometry [12] or electrochemistry [4, 13-15]. The major difficulty in using liquid chromatography to determine sulfite is in the separation of sulfite from sulfate, because of their very similar retention times. One solution is to use a specific column, which is available commercially but can be very expensive to use. In order to explore more economic methods some chromatographic researchers have developed on-line sample pretreatment, including hyphenation of gas-diffusion separation, which is based on the diffusion separation of sulfur dioxide following on-line oxidation with hydrogen peroxide to sulfate and a final determination of sulfate using ion pair chromatography [16].

Electrochemical sulfite sensors have the potential to be simple, sensitive and reliable to use and hence have attracted attention. The detection systems are usually based on measuring the oxidation current of sulfite directly [14, 15], or that of hydrogen peroxide produced by the reaction with the enzyme sulfite oxidase [4, 13, 17-19]. At the high oxidation potentials required for both sulfite and hydrogen peroxide other electroactive compounds in the sample are also oxidized at the working electrode and produce interfering currents. Various membranes, such as conducting polymers [17-19] and non-conducting polytyramine [4], have been coated on the surfaces of electrodes to prevent interfering species from approaching the working electrode. Although introducing a membrane helps to avoid interferences, this membrane-coated sensor invariably fails to completely remove the interfering compounds and lowers the sensitivity towards sulfite.

In previous papers a fill-and-flow channel biosensor for glucose was described [20, 21]. The biosensor possessed upstream electrodes for detecting the current contribution from interfering species, a layer containing glucose oxidase trapped within carbon paste and a downstream detector electrode. This work represented a paradigm shift in dealing with electroactive interferences by quantifying their effect rather than attempting to eliminate it. Quantification of the contribution of interfering species on the detector electrode current was possible because the well-defined velocity profile of the flow through the channel [22] enabled the convective-diffusion equations, describing the movement of all species in solution, to be solved, and hence the concentration of species at any position within the channel could be calculated [23, 24]. In the present paper the fill-and-flow channel biosensor concept is extended to the determination of sulfite, which has the complication of both the product of the enzyme reaction and the analyte itself being electroactive at the same potential. Sulfite oxidase is immobilized in carbon paste with Kel-F oil. One predictor electrode is located upstream of the enzyme layer to determine the total oxidation current of sulfite and interfering species. The detector electrode is located downstream of the enzyme layer to determine the total oxidation current of interfering species and sulfite which survives the passage over the enzyme layer. The difference between the currents on predictor and detector electrode is proportional to the concentration of sulfite, and forms the basis of sulfite determination. A wide range of interfering species, including ethanol, ascorbic acid, sorbic acid and tartaric acid are investigated. Finally sulfite in a beer sample is measured using this channel biosensor. Results from the biosensor are compared with those using a the Monier-Williams method with ICP-AES measurement of sulfur.

Experimental Section

Reagents

Sulfite oxidase (EC 1.8.3.1) from chicken liver, and sorbic acid were from Sigma (Sydney, Australia). Potassium sulfate, sodium sulfite, tris(hydroxymethyl)amino-methane (trizma), potassium chloride, graphite powder (1-2 μm, synthetic), bentonite and polyvinylpolypyrrolidone (PVPP) were from Aldrich Chemicals (Sydney, Australia). Sodium hydroxide, tartaric acid and ascorbic acid were from Ajax Chemicals (Sydney, Australia) whilst hydrochloric acid and hydrogen peroxide were from Asia Pacific Specialty Chemicals (Sydney, Australia). All reagents were of Analytical Grade and were used without further purification. Milli-Q grade water was used for all solutions.

Fill and Flow Channel Biosensor

The flow cell used in this work has been described previously [22]. It comprises a channel unit and cover plate both of which were constructed from Perspex. The channel unit is 20 mm × 2.0 mm with a depth of 0.4 mm. The detector electrode (dimensions: 0.76 mm × 0.81 mm) and auxiliary electrode (dimensions: 5.0 mm × 2.0 mm) were of platinum foil (Aldrich Chemical Company Sydney, Australia), and the Ag/AgCl reference electrode (1.5 mm diameter) was purchased from Clark Electromedical Instruments (Sydney, Australia). The reference electrode is exposed to the solution, the chloride in which determines the reference potential. The upstream predictor electrode was also of platinum foil (dimensions: 5.0 mm × 2.0 mm). The flow rate was controlled using a hydrophilic porous plug prepared from polymer sheeting generously supplied by Austral Cathay Trading Pty. Ltd.(Sydney, Australia). Electrochemical experiments using a fill-and-

flow channel biosensor require poising a potential on two working electrodes and hence were performed with a bi-potentiostat (AFCBP1, Pine Instrument Company, Grove City, Pennsylvania, USA).

The enzyme was immobilized in a well of dimensions 3.0 mm × 1.6 mm, and depth 0.2 mm, located 0.33 mm upstream of the detector electrode. 50 U of sulfite oxidase was mixed for 40 minutes with 480 mg carbon paste containing Kel-F oil. The resulting paste was packed tightly into the well in the cover plate and smoothed flush with the cover plate using weighing paper. The confinement of the enzyme reaction to the surface of the enzyme layer prepared in this manner was verified previously by comparing the response characteristics to a true monolayer of enzyme fabricated via the covalent attachment of the enzyme to a self-assembled monolayer [21].

Procedure

When using the channel biosensor the potentials of the detector electrode and predictor electrodes were set to the same value, +0.65 V vs. Ag/AgCl. The chosen potential was the lowest potential which at which a saturation current was achieved without electrode fouling [25]. The potential of the reference electrode was established by the concentration of the chloride in the flow solution (0.05 M). All measurements of sulfite were performed in solutions buffered to pH 8.5 using a solution of 0.05M tris buffer containing 0.05 M potassium chloride. This pH was chosen as it was close to the enzyme optimum [13, 17, 26-31] and stable electrochemistry was observed at this pH. At pH values below 7.0 electrode fouling was observed. When operating the channel biosensor, initially buffer solution was injected into the inlet reservoir, and allowed to drain through the channel. After the background current reached steady state and the reservoir had drained, 500 μL of buffered analyte solution was added to the reservoir. The current was recorded after 30 s. After each measurement the reservoir was washed using the corresponding buffer solution until a stable background current was reached once again. Samples were typically measured in triplicate.

For the measurement of sulfite in beer using the channel biosensor the response was calibrated between 0.01 and 1.0 mM with standard sulfite solution. The beer sample was prepared by a three-step treatment. First, every 100 mL beer sample was mixed with 1 g bentonite, stirred for 2 minutes, and filtered followed by a similar procedure with 1 g PVPP. Then, a 9.0 mL aliquot of the pretreated sample was mixed with 0.5 mL 1 M tris solution containing 1 M potassium chloride, and 0.2 mL 1 M sodium hydroxide were added into a 10 mL volumetric flask, diluted to the mark using Milli-Q water and filtered. The pH of the prepared sample was caused to be 8.5, because in more acidic conditions sulfite is not stable, and in a open beer will be converted to sulfur dioxide and lost.

The measurement results from use of the biosensor were compared with those from the standard Monier-Williams method [6, 32], but with sulfur levels determined by ICP-AES rather than GC. To 300 mL of the same beer used for the channel biosensor was added 90 mL of 4 M hydrochloric acid and 10 mL of Milli-Q water were then added to a round bottomed flask. The sample was refluxed for 2 hours during which time all the sulfite was converted to sulfur dioxide. The volatile SO_2 that reached the top of the condenser was collected in two receptor flasks containing 40 mL of de-aerated 3% hydrogen peroxide solution. The solutions in the two flasks were transferred to 100 mL flasks and diluted to the mark. The sulfate contents of these solutions were measured using a GBC Integra ICP-AES spectrometer with twenty-two channel polychromator and monochromator

(GBC Scientific Equipment Pty Ltd, Victoria, Australia). The standard solution for ICP-AES measurements was 96.40 ppm potassium sulfate in 3% aqua-regia solution. The blank solution was 3% hydrogen peroxide in Milli-Q water.

Results and Discussion

Measurement principle

In the typical configuration of the channel biosensor as depicted in Figure 1, an enzyme layer is located upstream, but on the same side of the channel as the detector electrode. In the case of measurement of glucose with glucose oxidase, the product of the enzyme reaction, hydrogen peroxide, is oxidized at the downstream electrode to give a current which is indicative of the concentration of glucose in the sample being analyzed. The relationship of the current to analyte concentration is typically complicated by other redox active species in the sample which also oxidize at the detector electrode and add an interfering signal to the detector electrode current. We have shown previously, for a glucose biosensor employing glucose oxidase, that when a predictor electrode is located upstream, and on the opposite wall, to the enzyme layer then the contribution of interfering species to the detector electrode current can be quantified [24, 33]. In particular the current at each electrode is proportional to the width (w) times the length$^{2/3}$ ($x^{2/3}$). In any comparison between the currents at electrodes, a factor $\frac{w_D x_D^{2/3}}{w_P x_P^{2/3}}$ must be applied. For the dimensions of the biosensor here, this factor is 0.119 ± 0.008. The determination of sulfite using sulfite oxidase in a channel biosensor is, however, more complicated. The complications arise from two aspects of the chemistry of sulfite. First, sulfite is also electroactive at the potentials used, and therefore both the predictor and detector electrode currents will have a contribution from sulfite oxidation. Secondly, because sulfite oxidase oxidizes sulfite to sulfate with hydrogen peroxide as a product of the enzyme reaction, sulfite is further removed by reaction with enzyme and of remaining sulfite with product hydrogen peroxide. Therefore, unlike the glucose biosensor, hydrogen peroxide does not reach the detector electrode because it is reduced by sulfite which survived the passage over the enzyme layer. This dual oxidation of sulfite by both the enzyme and hydrogen peroxide produced in the enzyme reaction, leads to a significant difference in currents between predictor and detector electrodes and thus allows operation of the sensor. (If electrochemically-active hydrogen peroxide simply replaced electrochemically-active sulfite, there would be only a small difference in currents). An expression for the difference in currents can now be formulated. At +0.65 V versus Ag/AgCl, the current observed at the predictor electrode (I_P) comes from interfering species, I_P(int) and sulfite, I_P(sulfite).

$$I_P = I_P(\text{sulfite}) + I_P(\text{int}) \quad (1)$$

The current observed at the detector electrode (I_D) similarly comes from interfering species, I_D(int), and remaining sulfite, I_D(sulfite).

$$I_D = I_D(\text{sulfite}) + I_D(\text{int}) \quad (2)$$

The separation across the channel, and a sufficient flow rate, ensures that each electrode independently samples the electroactive species present at its surface, as described in a previous

work [24]. Therefore the current from interfering species at the predictor electrode will be related to the current at the detector electrode by .

$$I_D(\text{int}) = \beta\, I_P(\text{int}) \quad (3)$$

where β is the factor that accounts for the different dimensions of the electrodes (here 0.119). However, $I_P(\text{sulfite}) > \beta\ I_D(\text{sulfite})$ for the reasons given above. The sulfite removed by the processes at the enzyme is twice the amount oxidised by the enzyme (as 1 mol sulfite reacts with 1 mol hydrogen peroxide), and in the linear region of the enzyme reaction this is proportional to the bulk concentration of sulfite. As the sulfite current is proportional to the concentration of sulfite at the detector electrode

$$I_D(\text{sulfite}) = 2\,F\,k\,(\,[SO_3^{2-}] - 2\alpha[SO_3^{2-}]\,) = 2\,F\,k(1 - 2\alpha)\,[SO_3^{2-}] \quad (4)$$

where $[SO_3^{2-}]$ is the bulk sulfite concentration, k is the electrochemical rate constant for sulfite oxidation, F the Faraday constant, and α is a constant which is the fraction of sulfite concentration consumed at the enzyme layer. At the predictor electrode the sulfite current is simply

$$I_P(\text{sulfite}) = 2\,F\,k\,[SO_3^{2-}] \quad (5)$$

Therefore the difference in the currents at predictor and detector electrodes corrected for the geometries of the electrodes is

$$\Delta I = \beta\, I_P - I_D = \beta\,(I_P(\text{sulfite}) + I_P(\text{int})) - (I_D(\text{sulfite}) + I_D(\text{int})) = \beta\ \ I_P(\text{sulfite}) - I_D(\text{sulfite})$$

$$= 2Fk\,\{\,(1 - \beta) + 2\alpha)\,\}\,[SO_3^{2-}] = \text{const}\,[SO_3^{2-}] \quad (6)$$

Thus ΔI is directly proportional to bulk sulfite concentration. The contributions to the currents at each electrode and the passage of species through the channel are summarized in Figure 1.

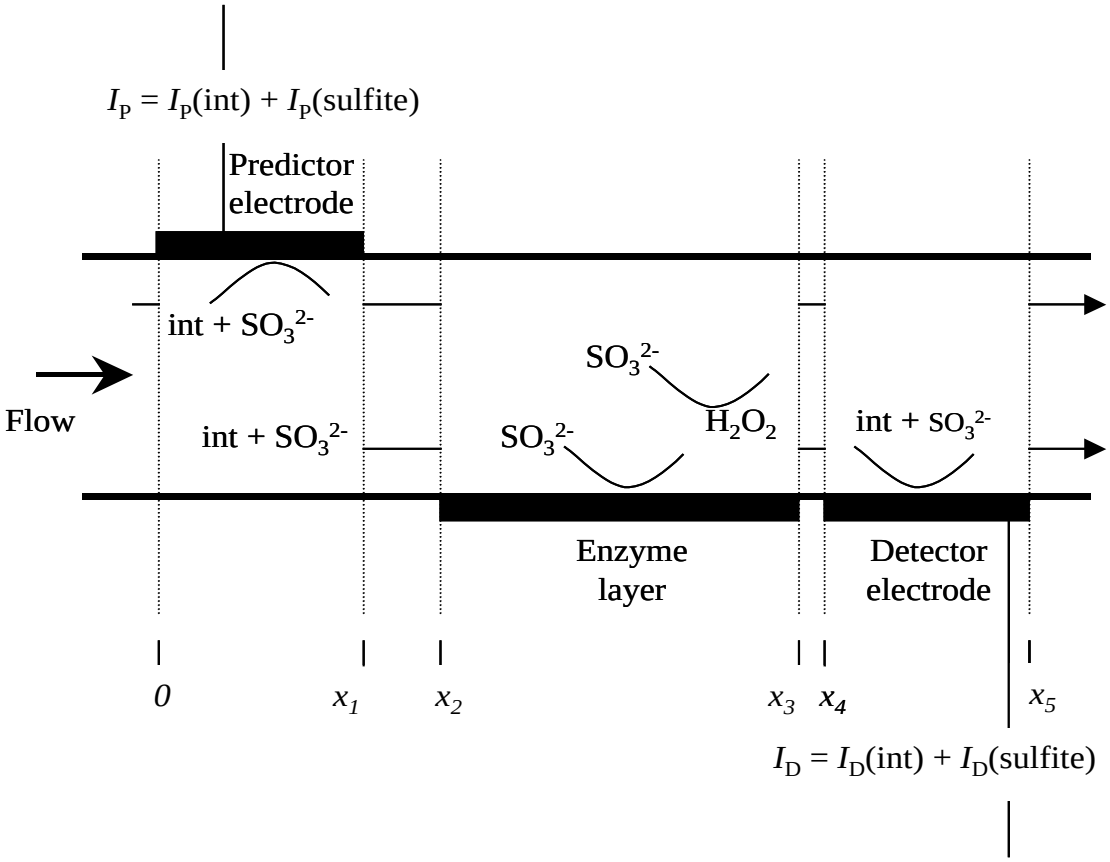
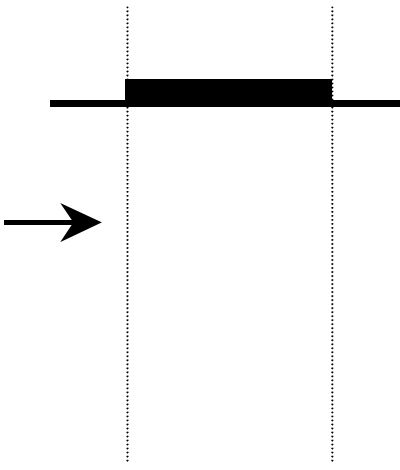


Figure 1: Schematic section of the channel biosensor showing three regions and the species oxidized either by the enzyme or electrochemically. int = interfering species,. Subscripts P and D indicate oxidation of interfering species and sulfite on predictor and detector electrode.

Determination of Sulfite

The current at the detector and current at the predictor corrected for the dimensions of the electrode (βI_P , with $\beta = 0.119$) are shown in Figure 2.



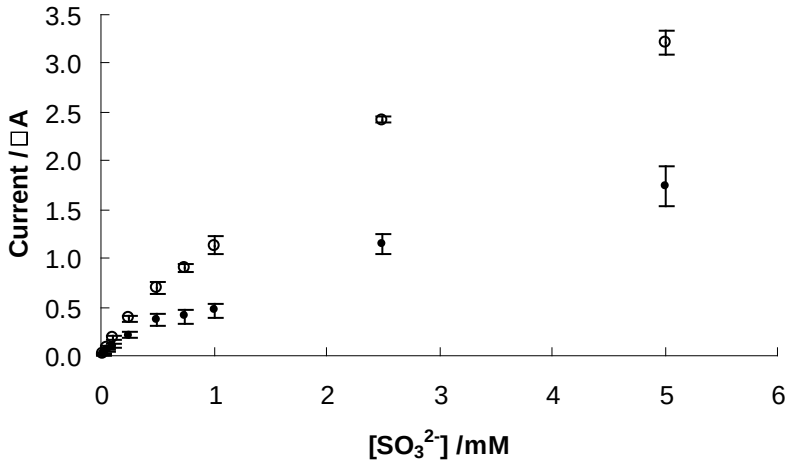


Figure 2: Open circles: The current at the predictor electrode corrected for its dimensions (see text). Closed circles: current at the detector electrode. Error bars are 95% confidence intervals calculated from six replicate measurements. The currents were measured at +0.65 V vs. Ag/AgCl and 30 s after the injection of samples.

Error bars are 95% confidence intervals calculated from six replicate measurements at each concentration. As predicted by theory, the detector current is less than that calculated from the current at the predictor electrode because of reactions of sulfite discussed above. The difference between the currents is proportional to sulfite concentration, and this is shown in Figure 3.

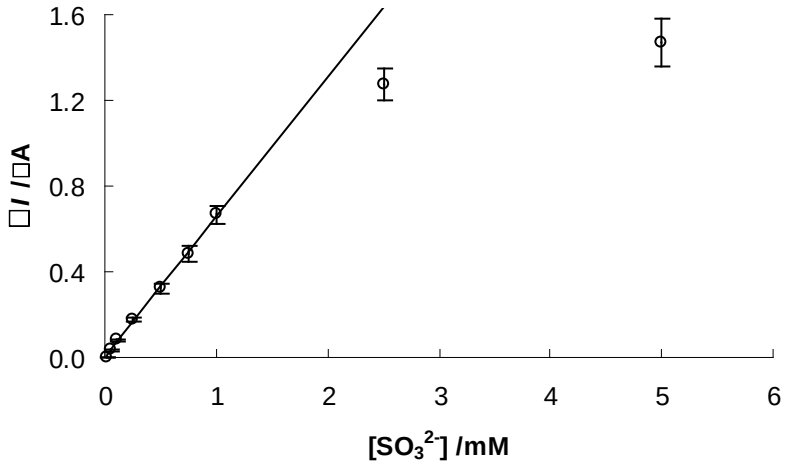


Figure 3: Difference in corrected predictor and detector currents as a function of sulfite concentration. The line is the calibration function ΔI /μA = (0.654 ± 0.029) c /mM + (0.005 ± 0.015).

The linear region, from 0.01 mM to 1.00 mM, followed the equation

$$\Delta I\ /\mu A = (0.654 \pm 0.029)\ c\ /mM + (0.005 \pm 0.015) \quad (7)$$

were c is the concentration of sulfite, with standard error of the regression = 0.011 μA, and 95% confidence interval on a measurement of 0.500 mM sulfite of ± 0.045 mM.

Compared with the sulfite biosensor fabricated by Situmorang *et al* [4] using electrodeposited polytyramine, this fill and flow biosensor has a wider linear range and better response time, but at the cost of poorer sensitivity and detection limit. The usability of the fill-and-flow sensor is also better, making it potentially more useful for unskilled workers testing beverages.

Effects of interfering species

The impact of interferences when monitoring sulfite is compounded by the low concentration of sulfite in beer, of the order of several ppm, which precludes dilution of the beer as a viable option. The effects of ethanol, ascorbic acid, sorbic acid and tartaric acid were investigated. Sulfite standards (0.50 mM) were prepared with ethanol contents in the range of 2.5% to 15% (v/v%). Each sample was measured in triplicate. The effect of ethanol on the sulfite concentration measured by the channel biosensor is shown in Table 1.

Table 1: The measured sulfite concentration of 0.5 mM sulfite in solutions with different ethanol concentrations.

Ethanol concentration (v/v %)	Measured sulfite/ mM (s, n = 3)
2.5%	0.50 (0.01)
5.0%	0.46 (0.04)
10.0%	0.35 (0.01)*
15.0%	0.20 (0.01)**

Difference between spike and measurement : * significant at 95% probability, ** significant at 99% probability

The results show that when the content of ethanol is lower than 5.0 %, the measured concentration of sulfite is in good agreement with spiked value. But when the content of ethanol is over 10.0 %, the measured values are significantly smaller than the spiked values. Fortunately for

many beers the ethanol content is about 5 % and therefore this method can give adequate results. In the case of ethanol the interference does not contribute to the detector electrode current since no significant oxidation or reduction current is measured at +0.65 V vs. Ag/AgCl. Thus it appears the ethanol is influencing the enzyme activity [34]. When the enzyme activity is inhibited less sulfite will be oxidized and less hydrogen peroxide will be produced, and there will be a smaller current difference. Note that this type of interferent, one that interferes with the enzyme reaction, cannot be compensated for using the predictor electrode method [23, 24].

The influence on the measured current of three electroactive compounds, commonly found in beverages, ascorbic acid, sorbic acid and tartaric acid, was assessed by spiking 0.50 mM sulfite solutions with a solution of each species. Table 2 shows a comparison of the concentration of sulfite calculated from the detector electrode current with and without correction from the current at the predictor electrode.

Table 2: Measured sulfite concentrations of samples containing 0.5 mM sulfite in the presence of different concentrations of interfering species.

Interfering species	Concentration of species /mM	Measured sulfite /mM (*s*)	
		With predictor electrode	Without predictor electrode
ascorbic acid	0.10	0.55 (0.01, *n*=6)*	0.93 (0.13, *n*=6)**
ascorbic acid	0.20	0.53 (0.01, *n*=6)	1.75 (0.10, *n*=6)**
ascorbic acid	0.50	0.47 (0.01, *n*=6)	4.12 (0.06, *n*=6)**
sorbic acid	0.10	0.51 (0.01, *n*=3)	0.62 (0.02, *n*=3)*
tartaric acid	0.10	0.54 (0.01, *n*=3)	0.64 (0.02, *n*=3)**

Difference between spike and measurement : * significant at 95% probability, ** significant at 99% probability.

Table 2 shows that the use of the predictor electrode allows a more accurate measure of the sulfite concentration in the sample. Without compensation for these electroactive interferences the channel biosensor over-predicts the concentration of sulfite in the sample, but when the corrected current from the predictor electrode is subtracted from the detector current, adequate recovery of spiked samples is obtained.

Analysis of sulfite in a beer

The beer was pretreated first with bentonite to remove proteins [35] and second with PVPP to remove polyphenolic compounds [36]. The removal of these components was necessary to prevent electrode fouling. Analysis of six sub-samples of the beer using the biosensor gave a result of 0.12

± 0.02 mM; and the analysis of three samples by the reference method 0.11 ± 0.03 mM. A Student-t test of the means, with the assumption of unequal variances, gave a probability that the two mean results came from populations with the same mean of 0.15.

Conclusions

A fill and flow channel biosensor with a predictor electrode was developed to determine sulfite. The basis of determination is the current difference between predictor and detector electrodes. This strategy can efficiently correct for the interfering current at the detector electrode. Acceptable repeatability (RSD% < 10 %) was obtained. The interferences of ethanol, ascorbic acid, sorbic acid and tartaric acid were investigated. Experimental results indicated that the measured sulfite concentrations were in good agreement with spiked values. Finally, this method was used to determine sulfite in a beer sample. The detected concentrations were compared with values obtained from a reference method based on the Monier-Williams method.

References

[1] H. Vally, A. Carr, J. El-Saleh, P. Thompson, *Journal of Allergy and Clinical Immunology* **1999**, *103*, 41.
[2] H. Vally, P.J. Thompson, *Thorax* **2001**, *56*, 763.
[3] D.H. Allen, *Food Technology in Australia* **1985**, *37*, 506.
[4] M. Situmorang, D.B. Hibbert, J.J. Gooding, D. Barnett, *Analyst* **1999**, *124*, 1775.
[5] M. Munar, M.J. Maurice, A. Kluessendorf, *Journal of the American Society of Brewing Chemists* **1994**, *52*, 168.
[6] Official Methods of Analysis of the Association of Official Analytical Chemists, AOAC, Arlington, VA, 1984.
[7] G. Jankovskiene, V. Paliulionyte, A. Padarauskas, *Chemia Analityczna* **2002**, *47*, 847.
[8] Y. Miura, M. Hatakeyama, T. Hosino, P.R. Haddad, *J. Chromatogr. A* **2002**, *956*, 77.
[9] J. Rethmeier, A. Rabenstein, M. Langer, U. Fischer, *J. Chromatogr. A* **1997**, *760*, 295.
[10] Y. Michigami, M. Morooka, K. Ueda, *J. Chromatogr. A* **1996**, *732*, 403.
[11] G. Jankovskiene, Z. Daunoravicius, A. Padarauskas, *J. Chromatogr. A* **2001**, *934*, 67.
[12] X.F. Yang, X.Q. Guo, Y.B. Zhao, *Analytica Chimica Acta* **2002**, *456*, 121.
[13] V.J. Smith, *Anal. Chem.* **1987**, *59*, 2256.
[14] I.G. Casella, R. Marchese, *Analytica Chimica Acta* **1995**, *311*, 199.
[15] R. Carballo, V.C. Dall'Orto, A. Lo Balbo, I. Rezzano, *Sensors and Actuators B* **2003**, *88*, 155.
[16] S. Faldt, B. Karlberg, W. Frenzel, *Fresenius Journal of Analytical Chemistry* **2001**, *371*, 425.
[17] T. Yao, M. Satomura, T. Nakahara, *Talanta* **1994**, *41*, 2113.
[18] S.B. Adeloju, S.J. Shaw, G.G. Wallace, *Electroanalysis* **1994**, *6*, 865.
[19] S.B. Adeloju, J.N. Barisi, G.G. Wallace, *Anal. Chim. Acta* **1996**, *332*, 145.
[20] M. Zhao, D.B. Hibbert, J.J. Gooding, *Biosensors Bioelectronics* **2003**, *18*, 827.
[21] D. Losic, M. Zhao, J.G. Shapter, J.J. Gooding, *Electroanalysis* **2003**, *15*, 183.
[22] P.R. Unwin, R.G. Compton, *Comprehensive Chemical Kinetics* **1989**, *29*, 173.
[23] M. Zhao, D.B. Hibbert, J.J. Gooding, *Analytical Chemistry* **2003**, *75*, 593.
[24] M. Zhao, I.S. Harding, D.B. Hibbert, J.J. Gooding, *Electroanalysis* **2004**, *16*, 1221.
[25] M. Zhao, Ph.D., The University of New South Wales, Sydney, 2004.
[26] L.T. Ng, Y.J. Yuan, H.J. Zhao, *Electroanalysis* **1998**, *10*, 1119.
[27] J. Svitel, M. Stredansky, A. Pizzariello, S. Miertus, *Electroanalysis* **1998**, *10*, 591.
[28] M.O. Rezende, H.A. Mottola, *Analyst* **1994**, *119*, 2093.
[29] U. Korell, R.B. Lennox, *J. Electroanal. Chem.* **1993**, *351*, 137.
[30] M.A. Nabirahni, R.R. Vaid, *Analytical Letters* **1991**, *24*, 551.
[31] A. Mulchandani, C.A. Groom, J.H.T. Luong, *Journal of Biotechnology* **1991**, *18*, 93.
[32] D. Barnett, *Food Technology in Australia* **1985**, *37*, 503.
[33] R.G. Compton, J.J. Gooding, A.C. Fisher, B.A. Cole, T.I. Cox, *J. Phys. Chem.* **1994**, *98*, 2446.
[34] J. Cano, L. Murillo, J. Delgado, O. Carreras, *Nutrition* **2003**, *19*, 778.
[35] K.J. Siebert, P.Y. Lynn, *Journal of the American Society of Brewing Chemists* **1997**, *55*, 73.
[36] K.J. Siebert, P.Y. Lynn, *Journal of Agricultural and Food Chemistry* **1997**, *45*, 4275.