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Amperometric sensor for selective on-site analysis of free sulphite in wines

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Abstract

Accurate and rapid on-site analysis of free SO₂ content is crucial in the process of winemaking from a producer and consumer perspective. Herein, we present an amperometric sensor based on commercially available screen-printed electrodes coupled with an electrochemical oxygen filter. The developed amperometric method gave a linear response in a concentration range up to 200 mg L⁻¹ with a limit of quantification of 7.5 mg L⁻¹. The applicability of the developed sensor was successfully tested on 27 white and red wine samples and compared to the Ripper method (iodometry) which is a standard procedure for free SO₂ determination. The sensor exhibits similar precision and accuracy but shows no interference from oxidizable species such as ascorbic acid, which is a major advantage over iodometric titration. Performance of the sensor was in addition positively evaluated during on-site analysis in a winery.

Keywords

Amperometry, wine, sulphur dioxide reduction, electrochemical oxygen filter, screen printed electrodes

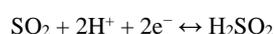
Sulphites are widely used as food preservatives, antioxidants, and anti-browning agents. They are usually added to foods such as dried fruit or vegetables, bottled soft drinks, juices, wine or beer. Sulphites establish an equilibrium between 'free' sulphites (which can be found as a dissolved salt HSO₃⁻; SO₃²⁻, or as molecular SO₂ depending on pH) and 'bound' sulphites that are complexed with carbohydrates, lipids, or proteins in foods. This equilibrium varies in different kinds of foods, and depends on the temperature, pH and macromolecular composition of food. ^{1,2} In winemaking, sulphites also provide microbial stabilization, regulation of off-flavours and preservation of aromas and colour. ³ Many studies have confirmed a sensitivity of some individuals to sulphite additives. Adverse reactions include asthmatic and anaphylactic episodes. ² However, there is minimal risk involved for the general population following the acceptable daily intake of 0 – 0.7 mg / kg of body weight. ^{1,2}

During the winemaking process, sulphite can be added at harvesting, before and after the fermentation process, at the start of the ageing process, and at the bottling stage. A free sulphite concentration between 20 and 40 mg L⁻¹ is advised to guarantee a protection against the oxidation of the wine.³ Total sulphite content in wines must not exceed 150 mg L⁻¹ in red wines and 200 mg L⁻¹ in white / rosé wines (with higher limit for some sweet wines) according to the EU legislative⁴ and OIV (International organisation of vine and wine) recommendations.⁵ Wines containing more than 10 mg L⁻¹ of sulphites must include a statement “contains sulphites” on their label. Sulphites (total content of up to 100 mg L⁻¹) may also be formed by the yeast during the fermentation.⁶ In recent years, following the consumer demand, there is also a tendency to reduce (or completely eliminate) the amount of additives like SO₂ in wines. Therefore, it is important for winemakers to accurately determine the amount of free SO₂ in the different stages of winemaking process to guarantee the quality of the final product for consumers.

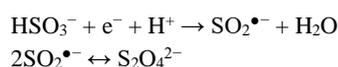
There are currently two methods recommended by OIV for determination of free or total SO₂ content in wines. The Ripper method is an iodometric titration based on the oxidation of SO₂ in an acidified sample with starch as an indicator. To get a total SO₂ content, the wine sample must be pre-treated with a basic solution to release all bound SO₂.⁷ The other method is based on Monier-Williams procedure in which a sample is distilled under inert atmosphere and all SO₂ is then captured in a peroxide solution where it reacts to sulphuric acid. The acid is then determined by acido-basic titration. This method is more accurate with less interfering species, but it is more complicated to set up and the time of analysis is consequently longer.⁸ Determination of sulphites is usually done with automatic titrators⁹ based on iodometry for its simplicity compared to the Monier-Williams procedure. Commercially available and affordable titration kits based on the Ripper method are currently available to winemakers for on-site analysis.¹⁰ However, this method is prone to interferences from any oxidizable compounds such as polyphenols or ascorbates.⁷ Additionally, cheap colorimetric paper strip tests are also available but they obviously offer lower precision and difficult interpretation especially for red wines.¹¹ Recently, a commercial sensor based on electrochemical detection of free SO₂ was also introduced for winemakers.¹²

Many recent analytical studies have been dedicated to their determination. SO₂ content in wines was determined by GC-MS,¹³ ICP-MS,¹⁴ microextraction coupled with Surface-Enhanced Raman Scattering,¹⁵ or UV-vis spectrophotometry,¹⁶ conductometry in a flow system based on a gas-diffusion cell,¹⁷ by using solid polymer electrolyte electrodes in a flow system with amperometric detection,¹⁸ and by a device based on a gas-diffusion membrane and pH-ISFET (Ion Selective Field Effect Transistors) system.¹⁹ All these examples are based on methods that are either not suitable for on-site analysis due to the size and the price of the instrumentation, or require some complex sample pre-treatment (extraction, flow systems, gas-diffusion cells, etc.). Both these reasons are a hindrance for a development of a robust, portable and cost-effective sensor. Recent study based on colorimetric assay on a microfluidic paper-based device could be considered as an example of sensor fulfilling these requirements.²⁰

Electrochemical methods of analysis on disposable screen-printed electrodes (SPE) provide a rapid, reliable, and affordable way of in-situ analysis in complex matrices at portable devices.²¹ The analysis of sulphites by electrochemical methods has been extensively studied before. Sulphites are more often determined by oxidation.^{22,23} However, in the case of wines, this approach is sensitive to interference from various molecules: e.g. polyphenols or ascorbates. These interfere with the analysis itself, and also significantly contribute to the electrode passivation, further complicating the analysis.²² On the other hand, reduction does not have interferences in the wine matrix apart from oxygen.²⁴ SO₂ is reduced under acidic conditions (0 < pH < 2) by 2 electrons to sulfoxylic acid H₂SO₂:



At more positive pH (2 < pH < 5) when HSO₃⁻ species is prevalent, reduction occurs by one electron to SO₂^{•-} species, which then dimerizes to S₂O₄²⁻:^{24,25}



Reduction of SO₂ was used for its determination only in limited number of works.^{22,26-31} SO₂ was determined on carbon nanotubes modified electrode in various beverages by square wave voltammetry.²⁶ Gas diffusion extraction was used for sulphite determination by electrochemical methods in several publications by the same group of authors for detection of free SO₂ in matrices of wine and beer. Free SO₂ was selectively extracted from samples and determined by voltammetry in a solution free of any matrix effects. However, experimental procedure requires several steps and determination was only done by standard additions which are not applicable for on-site analysis due to the instability of standard sulphite solutions.²⁷⁻³⁰ A similar approach based on gas-diffusion membrane and electrochemical detection was applied for determination of total SO₂ content in wines.³² Sulphites were also determined by polarography in dried fruit samples. Sample preparation included deoxygenation of buffers (pH 4) and was reported to be difficult considering the volatility of SO₂.³¹ The removal of oxygen proved to be a tricky problem considering the volatility of molecular SO₂ and the necessity to purge the solution before electrochemical analysis with inert gas to remove dissolved oxygen. Our solution of localised oxygen removal using a porous second working electrode (the so-called “oxygen filter”) positioned in the vicinity of a sensing electrode was presented in previous publications.³³⁻³⁶ The oxygen filter has been so far used in an applied electrochemical sensor only for sensing of heavy metals (zinc, lead, cadmium) by stripping chronopotentiometric technique³⁵ and for a fructose biosensor based on NAD⁺/NADH regeneration which is sensitive to the oxygen presence.³³ Here we present a novel use of the oxygen filter fully integrated into an electrochemical sensor designed specifically for on-site amperometric sensing of free SO₂ in complex wine samples. The oxygen filter offers an analysis without sample purging with an inert gas significantly facilitating the use of the method outside of the laboratory. Idea of a

simple on-site analysis is further supported by the use of low-cost commercially available unmodified disposable SPEs, minimal sample pre-treatment, and a robust method with facile automatic evaluation.

Experimental

Chemicals and materials

A stock solution of SO_2 (1 g L^{-1}) was prepared daily from anhydrous Na_2SO_3 (98 %, Fluka) in 10 mL of deionized water purged with nitrogen to obtain a stable solution.¹⁶ Concentration of the solution was checked using iodometry. 0.1 mol L^{-1} HCl-KCl solutions and 0.1 mol L^{-1} Britton-Robinson buffers with given pH were prepared as previously reported for the electrochemical characterisation of the set-up.³⁷ 37 % hydrochloric acid (Fluka) was diluted to 10 mol L^{-1} concentration. pH of buffers and wines was checked using pH meter Meterlab PHM210 (Radiometer Copenhagen). All solutions were prepared using deionized water. Low-sulphite white and red wines were used as model samples for the optimization of the amperometric method and construction of the calibration curves. These wines were identical to wines R1 and W1, but they were opened and then stored in a fridge and used for a month. All experiments with wines were carried out in these matrices unless stated otherwise.

Electrochemical apparatus and methods

Screen-printed electrodes (DRP-110) containing carbon ink working and counter electrodes, and silver ink quasi-reference electrode were obtained from Dropsens / Metrohm France. Electrodes were connected to a bipotentiostat Dropsens $\mu\text{Stat ECL}$ controlled by Dropview 8400 software. A platinum mesh (aperture: 0.12 mm, wire diameter: 0.04 mm) for the construction of the oxygen filter was obtained from Goodfellow France. 3D printer Prusa I3 MK3S+ (Prusa Research, Czech Republic) was used for 3D printing. The software Blender (Blender studio) was used for the creation of the 3D model of the electrochemical cell. Polyethylene terephthalate glycol (PETG) and polylactic acid (PLA) polymers (both Prusa Research) were used for the prints. Nitrile rubber coated nylon diaphragm sheeting of 0.25 mm thickness (Coruba, UK) was cut out to the appropriate size to act as an O-ring.

Cyclic voltammetry and linear sweep voltammetry were usually carried out at 50 mV s^{-1} scan rate. Amperometric detection was carried out at the SPE at the potential -0.65 V with 1 s sampling rate. The oxygen filter was connected as a second working electrode. Potential of -0.3 V was applied at the oxygen filter for 60 s before the start of the scan at the SPE and was kept on for the whole experiment. Current value was evaluated after 240 s as an average value of the current from the last 10 points. SPEs could be used throughout the day for several experiments (maximum was 15 wine samples in a day) with no observable degradation of SO_2 signal. All potential values were reported versus silver quasi reference electrode.

Analytical procedures

At first, the cell was assembled by placing first SPE (used as a working electrode only) in the designed place in a cell. The cell was then enclosed using a sealing and all 3D printed parts as shown in Fig. 1A. After the introduction of a sample, the cell was closed using a cap with a second SPE (used as a reference and a counter electrode). The fully assembled cell could be used for several experiments with various wine samples.

For the construction of a standard calibration and first experiments with low sulphite wines the procedure was the following: (i) 10 mL of the sample solution (wine sample) was added to the cell and then purged with nitrogen (to limit the reaction of SO₂ with oxygen); (ii) 100 µL of 10 mol L⁻¹ HCl was added to modify the pH to the optimal conditions. HCl was used as it also provided a constant high concentration of Cl⁻ ions necessary to stabilize the potential of silver quasi-reference electrode; (iii) Given amounts of SO₂ standard were then pipetted to the sample in the cell.

For the determination in wine samples: (i) 10 mL of wine was pipetted into the cell (List of wines is given in Table S1); (ii) 100 µL of 10 mol L⁻¹ HCl was added followed by a gentle stir of a solution; (iii) Amperometric scan was then immediately started; (iv) Concentration of free SO₂ was then calculated by dividing the current with the obtained standard slope separately for red and white wines.

Iodometric titration

Method for iodometric titration of free SO₂ was taken from OIV method ⁵. Metrohm DMS Titrimo716 automatic titrator controlled by Tiamo 1.3 software was utilized. End point detection was carried out potentiometrically at the inflex point of the titration curve. 25 mL of wine sample was diluted with 35 mL of deionized water and after the addition of 5 mL of 25% H₂SO₄, the titration was immediately started. Titration solution was 0.01 M solution of I₂ prepared from 0.1 N Iodine solution (SDS France) that was standardized against As₂O₃. ³⁸ All measurements were repeated in duplicate for every wine sample. In a case of high interferent (ascorbic acid) concentration, 5 mL of 7 g L⁻¹ acetaldehyde solution in ethanol was added to the wine sample and let rest for at least 30 mins. Free SO₂ concentration was then obtained by calculating the difference between the first run (SO₂ + interferents) and the second run (interferents only). This was done for wine samples that showed at least twice higher free SO₂ concentration measured by iodometry than by amperometry.

Statistical evaluation

Statistics were calculated with the help of Origin software and Microsoft Excel. All errors are given at 95% confidence limit. Errors in standard slope are calculated at 95% confidence limit as 1.96-times standard deviation of the fitted curve and converted to relative error which is then

applied to every result. ³⁹ Limit of quantification was calculated as a concentration corresponding to a 10-fold standard deviation of a slope of a fitted concentration curve. ³⁹ T-test was used to evaluate the accuracy of the results obtained by two analytical methods. Critical value t_s for two-tailed test ($P = 0.05$, 4 degrees of freedom) was 2.78. ³⁹

Elimination of false positive results

In the cases of wines with low free SO_2 concentration (below 15 mg L^{-1}), a procedure was devised to eliminate current readings that did not correspond to SO_2 signal and only represented the charging current. This was done by evaluating the average current value of 10 data points and comparing them to the average current value of previous 10 data points. This constraint can be expressed by an equation:

$$\left(\frac{\bar{i}_{(n-10;n-20)}}{\bar{i}_{(n;n-10)}} - 1 \right) \times 100\% < 2\%$$

where \bar{i} is an average current of n^{th} to $(n-m)^{\text{th}}$ data points. This evaluation was done for every data point in the exported data file of the amperometric curve. Two amperometric recordings (false positive and correct reading) and the calculation of their decrease is shown in Table S4. Only data points at the end of an amperometric recording were considered (from 200 to 240 s). If the equation was valid for data points in this range (decrease less than 2%), this result is correct and the obtained concentration reading corresponds to free SO_2 concentration. If the current decrease is more than 2% in the evaluated range, this sample is a false positive and the concentration of free SO_2 was labelled to be below LOQ of the method. This principle is also graphically illustrated in Fig. S6B with total of 10 amperometric recordings from 8 wine samples. The critical value of 2% clearly divides false positive and correct readings.

Results

Sensor cell design with built-in oxygen filter

This work started with a careful design and optimization of the electrochemical cell that would incorporate an oxygen filter and could be used with SPEs. The previous cell design presented in a previous publication for analysis of metals was a starting point.³⁵ In that work, oxygen filter was a single part of a cell consisting of several pieces and quite complicated assembly. The aim was to create and design a more robust and more user-friendly electrochemical cell. 3D printing with PETG thermoplastic polymer was used for this purpose. The printed cell was used for the period of several months with no obvious leaking or degradation of plastic while working with wine samples.

3D model of the cell can be seen in Fig 1A. It consists of two 3D-printed plastic parts put together with the help of magnets inserted into the models during 3D-printing process. The lower part (in red on Fig 1A) is designed to host SPE. The upper part (in blue in Fig 1A) has an oxygen filter (shown as (1) on Fig 1A) incorporated in it. The oxygen filter was constructed

with 1, 2, or 4 layers of platinum mesh and was inserted into the plastic during 3D printing. The distance between the oxygen filter and SPE was controlled with an O-ring made from nitrile rubber of 250 μm thickness. The 15 mL plastic tube with a cut-out bottom was then attached with epoxy glue into the top part. A sample was placed as a column of liquid in an enclosed space. This prevented a rapid escape of SO_2 and created a barrier for oxygen coming into the sample from the air. A scheme illustrating this function of the oxygen filter is in Fig 1B. The cell was finally assembled and ready for measurements with some other 3D printed parts (printed in grey plastic in Fig 1A) in order to facilitate the handling of the cell.

At the start of the experimental work, a set-up using only one single SPE ((3) in Fig 1A) was at first used. However, this configuration was found to be unsuitable for a robust sensor design. It was found that the proximity of reference and counter electrodes in a thin layer with the working electrode and the oxygen filter causes a rapid increase of observed current on both working electrodes. This can be explained by unfavourable feedback loop caused by the products of a reaction at a counter electrode that are then reduced at working electrodes. Therefore, a second SPE (acting only as a reference and a counter electrode) had to be placed further from the working electrode to prevent this issue. The second SPE was inserted into the cap of the cell ((6) in Fig 1A). There was no significant change in parameters of determination of SO_2 when using one or two SPEs.

The cell was at first optimized in order to maximize the efficiency of oxygen removal. It was proven that with the increasing number of layers (1, 2 or 4), the effectiveness of the oxygen filter was improved (the time after no O_2 response was observed by CV was decreased). We used several commercially available sealing rubber-based materials of various thickness to observe the effect of a distance between the oxygen filter and the sensing electrode. As expected, the speed of oxygen removal increased with shorter distance between the filter and SPE. However, a seal with a thickness, d , lower than 250 μm did not keep the cell properly tight and the solution leaked. Therefore, a seal with 250 μm thickness and the oxygen filter consisting of 4 layers of platinum mesh were used for this work (Fig S1). In the later parts of the work, after obtaining the optimal conditions for SO_2 determination, the parameters such as the applied potential and time of operation of the filter before the detection at the sensing electrode were further optimized.

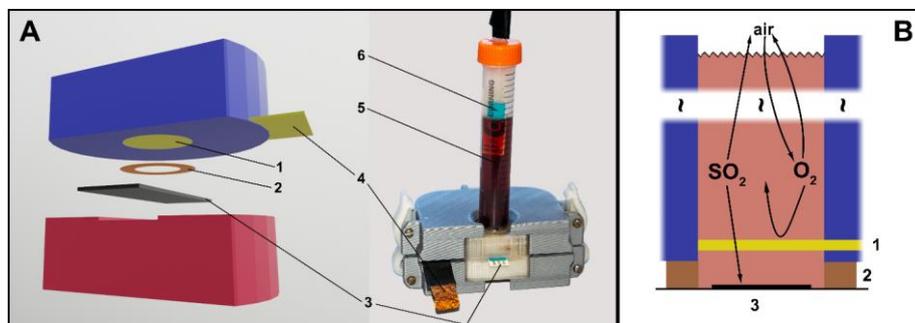


Fig 1: (A) 3D model of the cell (on the left) and photograph (on the right) of the assembled cell with a red wine sample. Oxygen filter made of a platinum mesh (1) is inserted into the 3D printed upper part. Upper and lower parts are put together and sealed with the rubber O-ring (2) ($d = 250 \mu\text{m}$) placed on top of the SPE acting as the working electrode only (3). The connection of the oxygen filter to the bipotentiostat as a second working electrode is made with a copper tape (4). Wine sample (5) was pipetted into the cell from the top. SPE acting as a reference and a counter electrode was integrated in the cap (6). (B) Scheme illustrating the removal of the oxygen in the vicinity of the sensing electrode for the determination of SO_2 in wines.

Voltammetric detection of SO_2

The investigation into SO_2 sensor was started by confirming the reported pH dependency of SO_2 reduction on a carbon SPE.^{24,25} This reaction was studied in solutions of pH 0-6 (Fig S2). The highest peak currents were observed for pH 0 and 1 and a continuous decrease of the peak current was then observed from pH 2 until 6 in accordance with previously reported results.^{24,25} The potential applied at the oxygen filter was varied with pH to avoid hydrogen evolution and is summarized in Table S2.

To be selective, the oxygen filter must not react with SO_2 . This was confirmed by cyclic voltammetry showing that no current increase was observed on a platinum mesh used as the oxygen filter when 200 mg L^{-1} of SO_2 was added in the acidified low-sulphite white wine (Fig 2A). The only variation observed after an addition of this high concentration of sulphite in a solution was a decrease of cathodic current at the platinum mesh, possibly because of the removal of trace oxygen in the solution or at the electrode surface by the reaction with sulphite. When the oxygen filter was active, the height of the peak current measured on SPE by CV increased linearly with SO_2 concentration in the range of $10\text{-}100 \text{ mg L}^{-1}$ in the optimal conditions in HCl-KCl buffer pH 1 (Fig S3).

During the next part of the investigation, we were focused on the application directly in wine samples. Therefore, only the addition of the HCl acid was carried out to adjust the pH of the wine close to 1. The attempt at the determination of SO_2 in the wine matrix gave about 3-times lower peak height (compared to the buffer solution) and more pronounced distortion of the SO_2

peak, giving two maxima (Fig 2B). This complicated the evaluation as only the first maximum could give a linear response with increasing concentration. These phenomena caused by the wine matrix rendered a voltammetric sensor based on carbon SPEs hardly applicable. The design of the voltammetric sensor for the eventual commercial use is in general more complex due to the complexity of the proper peak evaluation. On the other hand, amperometric sensors are much easier to design since the obtained current reading can be directly converted into the concentration value, bringing much less complexity into the design of a commercial sensor. One of obvious examples are commercial glucometers, which mostly work on the principle of amperometry.⁴⁰ Amperometric response is less prone to signal distortion than voltammetric response and it allows easier signal evaluation. Therefore, a possibility of amperometric sensor was closely investigated next.

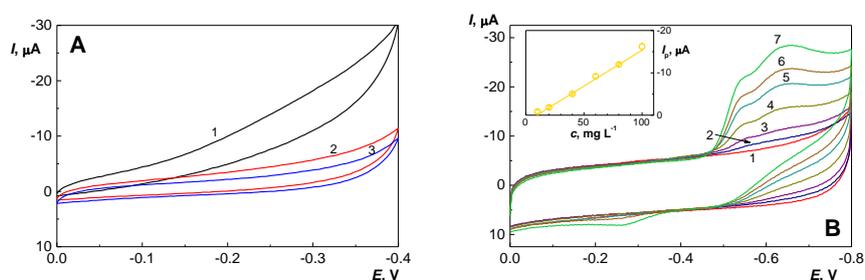


Fig 2: (A) Cyclic voltammograms ($v = 5 \text{ mV s}^{-1}$) obtained at the oxygen filter in the acidified low-sulphite white wine sample (pH 1.4). Curve 1 corresponds to the reduction of dissolved oxygen in wine at the oxygen filter. Curves 2 and 3 were recorded after the removal of oxygen by nitrogen purging in the absence (2) and in the presence (3) of $200 \text{ mg L}^{-1} \text{ SO}_2$ (curve 3). (B) Cyclic voltammograms ($v = 50 \text{ mV s}^{-1}$) of SO_2 ($c = 0$ (1); 10 (2); 20 (3); 40 (4); 60 (5); 80 (6); 100 (7) mg L^{-1}) obtained on SPE in deoxygenated acidified low-sulphite white wine sample (pH 1.4). Oxygen filter was set at a potential -0.3 V . Concentration dependencies are shown in the inset.

Amperometry of SO_2

For amperometric sensing of SO_2 , the use of the oxygen filter is crucial. Amperometric responses of oxygen and SO_2 cannot be separated, therefore, if there was some oxygen present in the wine, the sensor would overestimate the amount of SO_2 . The use of the oxygen filter allows the separation of oxygen and SO_2 signals. If we consider an experiment with a red wine sample that has been opened for some time, we can assume that it is saturated with dissolved oxygen. If no oxygen filter was used, the recorded current after an addition of $500 \mu\text{g}$ of SO_2 to 10 mL of sample was lower than in the blank wine sample (see curves 1, 2 in Fig 3). This was caused by the reaction of added SO_2 and oxygen which decreased oxygen concentration in a solution. If the oxygen filter was used for an analysis of the same sample (see curves 3, 4 in Fig 3), the current value in blank was significantly lower as no oxygen was present near the SPE

surface and the same addition of 500 μg of SO_2 correctly increased the current value. This experiment proves that the accurate determination of SO_2 is impossible without the electrochemical removal of oxygen.

The parameters of the method related to the oxygen filter were optimized next. The optimal potential for oxygen filtering was -0.3 V (Fig S4A). Potentials closer to 0 V did not provide effective oxygen removal and evolution of hydrogen bubbles was observed at potentials more negative than -0.3 V , which could block the contact of electrodes with the solution during analysis. Moreover, it was determined that by starting the oxygen filter 60 s before the amperometric detection scan, it was possible to rapidly achieve the steady state on SPE (Fig S4B) with no significant change using a longer time of oxygen filtration.

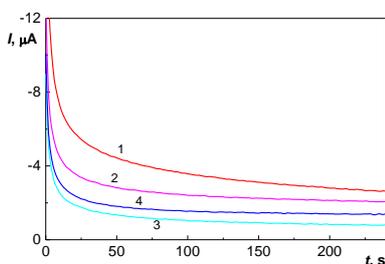


Fig 3: Amperometric recordings obtained on SPE in 10 mL of the acidified low-sulphite red wine sample (pH 1.6) with the oxygen filter not connected (curves 1, 2) and the filter set at the potential -0.3 V (curves 3, 4). Curves 1 and 3 were measured with dissolved oxygen in the wine sample, curves 2 and 4 were measured after the addition of 500 μg of SO_2 .

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The amount of HCl added (pH) was additionally optimized in a narrower range of pH from 0.7 to 2 for amperometric detection (Fig S5A). It was found out that at pH 0.7 the signal of SO_2 started to significantly decrease. This decrease can be probably attributed to too rapid SO_2 evaporation and its escape from the solution. Therefore, the addition of 100 μL of 10 M HCl was chosen as optimal for several reasons. The final pH of wine samples (see Tab S3) was always noted after acidification to check that it was in the range of 1.3 to 1.6. This is due to the wine acting as a buffer, and we can assume that depending on a type of wine, the buffering capacity would differ. The current variation for SO_2 reduction between pH 1.1 to 2 (which corresponds to 200 and 50 μL of added acid) is less than 10 %, (see Fig S5A) therefore we can assume that the addition of 100 μL is robust and that a slight change of pH (within 0.3 units) does not significantly induce error in measurements.

Based on previous voltammetric experiments, the detection potential was optimized (Fig S5B). The viable range was determined between -0.6 V and -0.7 V . Reduction of SO_2 would not occur at less negative potentials and at more negative potentials hydrogen evolution took place at the SPE. The optimal potential was therefore determined as -0.65 V . Detection time was

kept at 240 s in order to decrease the capacitive current as much as possible and to keep a total analysis time within 5 mins. The optimal technique for SO₂ determination in wine was: the oxygen filter was biased at a potential of -0.3 V for 60 s before the start of the amperometric scan at SPE biased at -0.65 V for 240 s (with the oxygen filter still biased at -0.3 V).

Using this protocol, we followed the increase of an amperometric response with the concentration of sulphite in a model red wine matrix (Fig 4A). The reduction current increased linearly in the concentration range from 6 – 200 mg L⁻¹ SO₂ (Fig 4B). The calculated limit of quantification was 7.5 mg L⁻¹ in the case of this low-sulphite red wine sample from Fig 4B.

Next step of the investigation was to obtain a standard calibration curve for SO₂ in model matrices for both red and white wines. We investigated the reliability and repeatability of the amperometric response at different carbon SPEs. We obtained 4 similar calibration curves for the low-sulphite red wine samples measured within two days with 4 different SPEs (as working electrodes). The calculated average slope value was -0.0609 μA L mg⁻¹ with an error of 9.7 % (see Fig 4C). The same was repeated with low-sulphite white wine. The average slope in this case was -0.0789 μA L mg⁻¹ with an error of 4.0 % (see Fig 4D) and slightly better repeatability, probably due to the less complex matrix of white wines. These values were then used to calculate the unknown concentration of free SO₂ in 27 samples of white and red wines. In both cases, intercept values were not statistically different from 0 and therefore, only slope value was used for the following calculations.

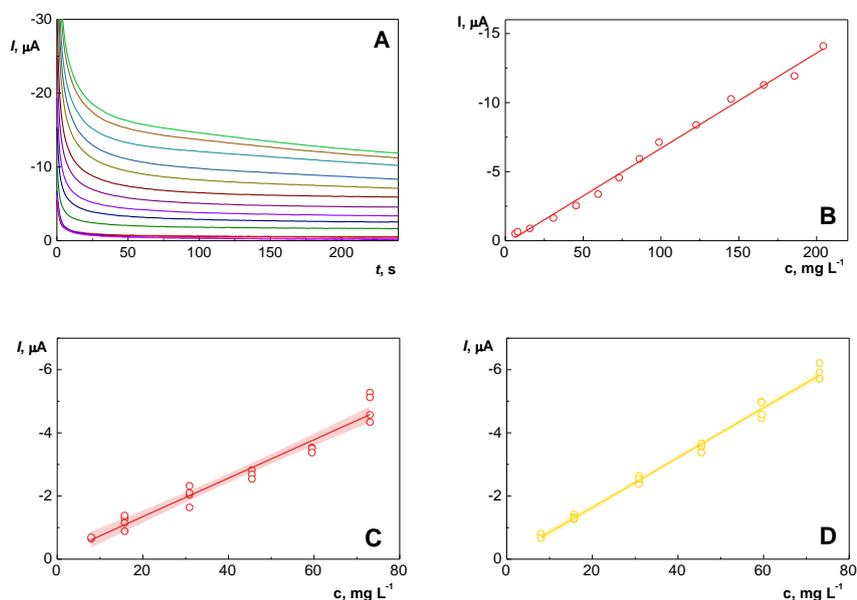


Fig.4: (A) Amperometric recordings of SO_2 ($c = (0) 6 - 200 \text{ mg L}^{-1}$) obtained on SPE in deoxygenated acidified low-sulphite red wine sample with the oxygen filter set at the potential -0.3 V . (B) Dependency of the current on the concentration of SO_2 . Typical calibration curves obtained on 4 different SPEs in (C) deoxygenated acidified low-sulphite white wine sample (pH 1.4) and (D) low-sulphite red wine sample (pH 1.6). Shaded areas correspond to 95% confidence interval of the obtained dependencies.

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Determination of free SO_2 in red and white wines

The optimized amperometric method was used for the determination of free SO_2 in a total of 27 different wine samples (14 white and 13 red) produced in different regions of France (see Table S1). To note, standard addition method is not suitable for any on-site (or commercial) sensor due to the inherent instability of stock solutions of SO_2 . The second method used for determination of free SO_2 is already established iodometric titration method (using the protocols published by OIV⁷).

The content of free SO_2 measured by the two methods is summarized in Table 1. Most of the wines (with three exceptions) contained between 10 to 50 mg L^{-1} of free SO_2 . This was well within the range of the standard calibration curves built in Fig 4C and 4D. The comparison of free SO_2 content obtained by amperometry and iodometry gave an excellent correlation (Fig 5). A perfect agreement between the two methods would give a regression equation of $y = 1.0 x$. The obtained regression slopes were 0.982 for red wines and 1.022 for white wines. Correlation coefficients were greater than 0.95 in both cases. The t-test values (listed in Table 1) were all

lower than the critical value, $T_{crit} = 2.78$, indicating that there were no significant differences between the two analytical methods in all cases where the value could be calculated.

As can be seen on three examples of low sulphite wines, i.e. wines R1, R11, and R12, results obtained by amperometry were discarded by the rule related to the mathematical elimination of false positive, as described in Experimental Section. Values obtained by iodometry confirmed that the free SO_2 concentration is below LOQ of the amperometric method (see Table 1). The concentration dependency has a U-shape in the range of 0 – 12 mg L^{-1} SO_2 . The higher current values for blank ($c = 0 \text{ mg L}^{-1}$) probably corresponded to the charging current whose value was decreasing more rapidly compared to faradaic current corresponding to SO_2 reduction. Therefore, one current value only in this range could correspond to two concentrations and a procedure of eliminating false positive results was devised. The decrease of the current value during the amperometric scan of wines R1, R11, and R12 is greater compared to other wines (R4, R7, R9, R10, and W8) with SO_2 concentration around 10 mg L^{-1} . The critical value of 2% was established empirically comparing in total 8 wine samples with low sulphite content. The elimination of false positives this way is graphically illustrated in Fig. S6B (and Table S4) where 2% decrease clearly eliminates false positive readings.

During the tests with different wine samples, one significant advantage of the method presented here over iodometry was demonstrated. Presence of ascorbic acid as an interferent of iodometry can be seen on three white wines (W8, W9, and W11) and two red wines (R7 and R12). The concentration obtained after the first titration (in brackets in Table 1) was several times higher than the actual free SO_2 concentration only obtained after the second titration which estimated the amount of interferents. This was observed because iodine can oxidize numerous compounds that are present in wine such as ascorbic acid. There are some reports of adding sulphites and ascorbic acid together as preservatives into the wine and, therefore, a significant amount of ascorbic acid (30 – 400 mg L^{-1}) can be present in white wines^{41,42}. As an illustration, the addition of 36 mg L^{-1} of ascorbic acid increased the titration volume by about 0.6 mL (from ~1.1 to ~1.7 mL in a prepared model sample) (Fig S7A) but in the same red wine sample the amperometric method was not sensitive to ascorbic acid presence as it is not reducible. Indeed, the addition of the same amount of ascorbic acid (36 mg L^{-1}) did not increase the current response. The current before (-5.4 μA) only slightly decreased after the addition of ascorbic acid to -5.2 μA due to the dilution of the sample (Fig S7B). In these cases, a second titration determining the content of interferents is necessary in which free SO_2 is masked by the addition of aldehydes (formaldehyde) and the result is then deducted from the first titration. However, this brings more complexity and error into the analysis by iodometry.

In all tested wine samples, we have not found any indication of some reducible species that could interfere with the free SO_2 analysis. In addition, we do not suspect that any compound usually present in wine⁴³ could significantly interfere with this analysis considering their normal concentrations in wine samples.

Table 1: Comparison of determination of free SO₂ in 21 different samples of white and red wines using the developed amperometric method and iodometry.

Wine	Amperometry free SO ₂ , mg L ⁻¹	Iodometry free SO ₂ , mg L ⁻¹	T-test (T _{crit} = 2.78)
W1	19.7 ±0.8	23.5 ±1.9	2.32
W2	34.3 ±1.4	30.3 ±2.1	1.36
W3	20.2 ±0.8	22.2 ±2.3	0.91
W4	25.5 ±1.0	28.7 ±2.0	1.57
W5	19.7 ±0.8	22.3 ±1.6	1.93
W6	44.2 ±1.8	45.7 ±3.9	0.20
W7	21.2 ±0.8	22.7 ±2.3	0.68
W8	18.7 ±0.7	13.3 ±3.4 (44) ^a	1.41
W9	26.0 ±1.0	24.6 ±3.9 (45) ^a	0.26
W10	23.6 ±0.9	24.0 ±1.6	0.24
W11	23.5 ±0.9	27.9 ±4.1	0.72
W12	23.3 ±0.9	20.0 ±2.2	1.51
W13	19.7 ±0.8	17.5 ±1.6	1.74
W14	25.2 ±1.0	- (64) ^b	-
R1	<LOQ ^c	1.8 ±1.2 ^d	-
R2	22.4 ±2.2	23.9 ±2.9	0.24
R3	26.7 ±2.6	26.5 ±4.8	0.01
R4	17.8 ±1.7	15.6 ±2.0	0.60
R5	21.6 ±2.1	16.9 ±2.0	0.94
R6	44.3 ±4.3	42.8 ±4.0	0.07
R7	11.7 ±1.1	10.6 ±13.1 (30) ^a	0.02
R8	20.9 ±2.0	25.6 ±3.6	0.64
R9	11.3 ±1.1	15.3 ±3.8	0.77
R10	16.5 ±1.6	19.3 ±3.2	0.55
R11	<LOQ ^c	4.0 ±1.2	-
R12	<LOQ ^c	3.3 ±2.5 (10) ^{a, d}	-
R13	17.4 ±1.7	21.2 ±2.2	1.01

^a – Wines with higher interferent concentration (probably ascorbic acid); value in italics in brackets denotes the concentration value obtained after the first titration. Second titration was carried out after the sample was treated with formaldehyde, see experimental section for details.

^b – Titration to determine interferents was not carried out. This was the first case where interferent (ascorbic acid) was observed.

^c – Value was discarded by evaluating the decrease of the current.

^d – Most likely below LOQ of iodometry.

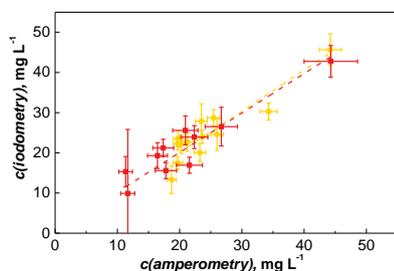


Fig 5: Correlation between measured concentration of free SO₂ in samples of white wines (yellow circles and error bars) and red wines (red squares and error bars) by amperometric detection and iodometric titration from Table 1. Dashed lines (yellow and red) represent a linear fit for white/red wine samples.

On-site testing

The proposed method was subsequently tested on-site in the winery in Alsace region of France to evaluate the portability, robustness, and easy-to-use aspect of the whole procedure. The analysis was successfully performed by the winemaker himself. Evaluation of the measurement was automated by exporting the current values and using macro in Excell which instantly calculated the concentration of free SO₂. Results are summarized in the supplementary material (Tab S5). Sample 1 was later analysed in the laboratory which confirmed the accuracy of the analysis with iodometry and it corresponds to the wine W13 (see Tab 1). The second sample was taken from the wine tank and it is an example of a sample for which an on-site analysis with a portable sensor is highly preferred. This is also an example of a sample for which this sensor is mostly intended to.

Conclusions

In this work we presented an amperometric sensor for the determination of free SO₂ in wine. The design of the sensor included the oxygen filter in order to remove oxygen interference in the vicinity of the sensing electrode. This removed a necessity to purge a solution before the analysis which is practically impossible for SO₂ solutions considering the fast escape of molecular SO₂ during purging.

The proposed method is intended for the on-site analysis of wines. The required sample preparation only includes the addition of the hydrochloric acid (as the only chemical necessary) immediately followed by the detection step. Analysis of one sample including preparation and evaluation of the result can be performed in less than 10 minutes. The amperometric sensing technique is technically simple to construct with straightforward calculation of a concentration from a current reading compared to voltammetric techniques, where an automatic peak

evaluation process must be integrated into the sensor. It is built on commercially available and low-cost screen-printed electrodes that proved to give a repeatable performance and provide a standard calibration for the determination of free SO₂ in red and white wines. So far, electrochemical sensors based on the reduction of SO₂ used only standard additions to determine SO₂ content in real wine samples. This is practically impossible to carry out on-site due to the inherent instability of SO₂ standard solutions. Using the standard calibration for SO₂ determination completely eliminates this issue and greatly improves practical application of the sensor.

The presented sensor provided a linear response up to 200 mg L⁻¹ with a limit of quantification at 7.5 mg L⁻¹. The application of the sensor for the determination of free SO₂ in wine was proven by determining the quantity of free SO₂ in 27 red and white wine samples. Results obtained by amperometric sensing method were comparable to the values obtained by the standard method of iodometry recommended by OIV. Additionally, the amperometric method did not suffer from any interferences in all of the measured wines. On the other hand, higher content of ascorbic acid can give a false overestimation of free SO₂ content by iodometry. The presented sensor was successfully tested on-site with a winemaker who carried out the analysis correctly. Therefore, we propose this method as an alternative to currently used Ripper method based on iodometry that can be potentially used for the determination of SO₂ content in other foods and drinks directly at the point of interest by producers.

Associated content

The following content is available as supporting information: Wine tested in the studies; Optimisation of the electrochemical cell design; Optimisation of the SO₂ sensing parameters; Determination of free SO₂ in red and white wine.

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References

- (1) Vally, H.; Misso, N. L. A.; Madan, V. Clinical Effects of Sulphite Additives. *Clin. Exp. Allergy* **2009**, *39* (11), 1643–1651. <https://doi.org/10.1111/j.1365-2222.2009.03362.x>.
- (2) Garcia-Fuentes, A.; Wirtz, S.; Vos, E.; Verhagen, H. Short Review of Sulphites as Food Additives. *Eur. J. Nutr. Food Saf.* **2015**, *5* (2), 113–120. <https://doi.org/10.9734/ejnfs/2015/11557>.
- (3) OIV. *Resolution OIV-OENO 631-2020: Review of Practices for the Reduction of SO₂ Doses Used in Winemaking*; Paris, 2020.
- (4) Commission of the European Communities. Commission Regulation (EC) No

- 118/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. *Off. J. Eur. Union* **2006**, L 364/5 (364), 5–24.
- (5) *OIV Resolution OENO 09/1998*; International Organisation of Vine and Wine, 1998.
 - (6) Stockley, C.; Paschke-Kratzin, A.; Teissedre, P.-L.; Restani, P.; Tejedor, N. G.; Quini, C. *OIV Collective Expertise Document SO₂ and Wine: A Review*; OIV publications: Paris, 2021.
 - (7) Method OIV-MA-AS323-04B : R2009. In *Compendium of international methods of wine and must analysis*; OIV, Ed.; Paris, 2021.
 - (8) Method OIV-MA-AS323-04A. In *Compendium of international methods of wine and must analysis*; OIV, Ed.; Paris, 2021.
 - (9) Metrohm. *Titration Application Note T-211*; 2019.
 - (10) Vinmetrica SC-100A Sulfite (SO₂) Analyzer Kit <https://vinmetrica.com/product/vinmetricas-sc-100-sulfite-analyzer/> (accessed 2021 -09 -27).
 - (11) Free Sulfite Test Strips <https://bartovation.com/product/water-quality/sulfite-test-analytical-strips-vial-10-500-ppm/> (accessed 2021 -09 -27).
 - (12) Sentia Free SO₂ Test Strips - Universal Biosensors <https://www.universalbiosensors.com/products/sentia/sentia-products/sentia-free-so2-test-strips/> (accessed 2022 -03 -15).
 - (13) Carrascon, V.; Ontañón, I.; Bueno, M.; Ferreira, V. Gas Chromatography-Mass Spectrometry Strategies for the Accurate and Sensitive Speciation of Sulfur Dioxide in Wine. *J. Chromatogr. A* **2017**, 1504, 27–34. <https://doi.org/10.1016/j.chroma.2017.05.012>.
 - (14) Wang, X. W.; Liu, J. F.; Wang, X. Y.; Shao, B.; Liu, L. P.; Zhang, J. Direct Quantification of Total Sulfur Dioxide in Wine Using Triple Quadrupole ICP-MS. *Anal. Methods* **2015**, 7 (7), 3224–3228. <https://doi.org/10.1039/c4ay02863e>.
 - (15) Deng, Z.; Chen, X.; Wang, Y.; Fang, E.; Zhang, Z.; Chen, X. Headspace Thin-Film Microextraction Coupled with Surface-Enhanced Raman Scattering as a Facile Method for Reproducible and Specific Detection of Sulfur Dioxide in Wine. *Anal. Chem.* **2015**, 87 (1), 633–640. <https://doi.org/10.1021/ac503341g>.
 - (16) Gómez-Otero, E.; Costas, M.; Lavilla, I.; Bendicho, C. Ultrasensitive, Simple and Solvent-Free Micro-Assay for Determining Sulphite Preservatives (E220-228) in Foods by HS-SDME and UV-Vis Micro-Spectrophotometry Microextraction Techniques. *Anal. Bioanal. Chem.* **2014**, 406 (8), 2133–2140. <https://doi.org/10.1007/s00216-013-7293-3>.
 - (17) Danchana, K.; Clavijo, S.; Cerdà, V. Conductometric Determination of Sulfur Dioxide in Wine Using a Multipumping System Coupled to a Gas-Diffusion Cell. *Anal. Lett.* **2019**, 52 (9), 1363–1378. <https://doi.org/10.1080/00032719.2018.1539742>.
 - (18) Toniolo, R.; Pizzariello, A.; Susmel, S.; Dossi, N.; Bontempelli, G. A Sensor Based on Electrodes Supported on Ion-Exchange Membranes for the Flow-Injection Monitoring of Sulphur Dioxide in Wines and Grape Juices. *Talanta* **2010**, 80 (5), 1809–1815. <https://doi.org/10.1016/J.TALANTA.2009.10.024>.
 - (19) Giménez-Gómez, P.; Gutiérrez-Capitán, M.; Ríos, J. M.; Capdevila, F.; Puig-Pujol, A.; Jiménez-Jorquera, C. Microanalytical Flow System for the Simultaneous Determination of Acetic Acid and Free Sulfur Dioxide in Wines. *Food Chem.* **2021**, 346, 128891. <https://doi.org/10.1016/J.FOODCHEM.2020.128891>.
 - (20) Arroyo, M. J.; Orbe-Payá, I. de; Ortega-Muñoz, M.; Vilar-Tenorio, J.; Gallego, D.; Mohr, G. J.; Capitán-Vallvey, L. F.; Erenas, M. M. Capillary Microfluidic Platform for Sulfite Determination in Wines. *Sensors Actuators B Chem.* **2022**, 359, 131549. <https://doi.org/10.1016/J.SNB.2022.131549>.

- (21) Couto, R. A. S.; Lima, J. L. F. C.; Quinaz, M. B. Recent Developments, Characteristics and Potential Applications of Screen-Printed Electrodes in Pharmaceutical and Biological Analysis. *Talanta* **2016**, *146*, 801–814. <https://doi.org/https://doi.org/10.1016/j.talanta.2015.06.011>.
- (22) Isaac, A.; Davis, J.; Livingstone, C.; Wain, A. J.; Compton, R. G. Electroanalytical Methods for the Determination of Sulfite in Food and Beverages. *TrAC - Trends Anal. Chem.* **2006**, *25* (6), 589–598. <https://doi.org/10.1016/j.trac.2006.04.001>.
- (23) Pisoschi, A. M.; Pop, A.; Gajaila, I.; Iordache, F.; Dobre, R.; Cazimir, I.; Serban, A. I. Analytical Methods Applied to the Assay of Sulfur-Containing Preserving Agents. *Microchem. J.* **2020**, *155*. <https://doi.org/10.1016/J.MICROC.2020.104681>.
- (24) Isaac, A.; Wain, A. J.; Compton, R. G.; Livingstone, C.; Davis, J. A Novel Electroreduction Strategy for the Determination of Sulfite. *Analyst* **2005**, *130* (10), 1343–1344. <https://doi.org/10.1039/b509721e>.
- (25) Streeter, I.; Wain, A. J.; Davis, J.; Compton, R. G. Cathodic Reduction of Bisulfite and Sulfur Dioxide in Aqueous Solutions on Copper Electrodes: An Electrochemical ESR Study. *J. Phys. Chem. B* **2005**, *109* (39), 18500–18506. <https://doi.org/10.1021/jp058119n>.
- (26) Silva, E. M.; Takeuchi, R. M.; Santos, A. L. Carbon Nanotubes for Voltammetric Determination of Sulphite in Some Beverages. *Food Chem.* **2015**, *173*, 25–32. <https://doi.org/10.1016/j.foodchem.2014.10.106>.
- (27) Ramos, R. M.; Gonçalves, L. M.; Vyskočil, V.; Rodrigues, J. A. Free Sulphite Determination in Wine Using Screen-Printed Carbon Electrodes with Prior Gas-Diffusion Microextraction. *Electrochem. commun.* **2016**, *63*, 52–55. <https://doi.org/10.1016/j.elecom.2015.12.010>.
- (28) Gonçalves, L. M.; Pacheco, J. G.; Magalhães, P. J.; Rodrigues, J. A.; Barros, A. A. Determination of Free and Total Sulfites in Wine Using an Automatic Flow Injection Analysis System with Voltammetric Detection. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* **2010**, *27* (2), 175–180. <https://doi.org/10.1080/19440040903261547>.
- (29) Ramos, R. M.; Brandão, P. F.; Gonçalves, L. M.; Vyskočil, V.; Rodrigues, J. A. Electrochemical Sensing of Total Sulphites in Beer Using Non-Modified Screen-Printed Carbon Electrodes. *J. Inst. Brew.* **2017**, *123* (1), 45–48. <https://doi.org/10.1002/jib.402>.
- (30) Bezerra Martins, A.; Lobato, A.; Tasić, N.; Perez-Sanz, F. J.; Vidinha, P.; Paixão, T. R. L. C.; Moreira Gonçalves, L. Laser-Pyrolyzed Electrochemical Paper-Based Analytical Sensor for Sulphite Analysis. *Electrochem. commun.* **2019**, *107*, 106541. <https://doi.org/10.1016/j.elecom.2019.106541>.
- (31) Yilmaz, Ü. T.; Somer, G. Determination of Trace Sulfite by Direct and Indirect Methods Using Differential Pulse Polarography. *Anal. Chim. Acta* **2007**, *603* (1), 30–35. <https://doi.org/10.1016/j.aca.2007.09.035>.
- (32) Prasertying, P.; Ninlapath, T.; Jantawong, N.; Wongpakdee, T.; Sonsa-ard, T.; Uraisin, K.; Saetear, P.; Wilairat, P.; Nacapricha, D. Disposable Microchamber with a Microfluidic Paper-Based Lid for Generation and Membrane Separation of SO₂ Gas Employing an In Situ Electrochemical Gas Sensor for Quantifying Sulfite in Wine. *Anal. Chem.* **2022**. <https://doi.org/10.1021/acs.analchem.2c00496>.
- (33) Le, T. X. H.; Etienne, M.; Lapique, F.; Hehn, A.; Vilà, N.; Walcarius, A. Local Removal of Oxygen for NAD(P)⁺ Detection in Aerated Solutions. *Electrochim. Acta* **2020**, *353*, 136546. <https://doi.org/10.1016/j.electacta.2020.136546>.
- (34) Etienne, M.; Le, T. X. H.; Nasir, T.; Herzog, G. Electrochemical Filter to Remove Oxygen Interference Locally, Rapidly, and Temporarily for Sensing Applications.

- Anal. Chem.* **2020**, 92 (11), 7425–7429. <https://doi.org/10.1021/acs.analchem.0c00395>.
- (35) Rotureau, E.; Gajdar, J.; Herzog, G.; Waldvogel, Y.; Pinheiro, J. P.; Etienne, M. Electroanalytical Metal Sensor with Built-in Oxygen Filter. *Anal. Chim. Acta* **2021**, 1167, 338544. <https://doi.org/10.1016/J.ACA.2021.338544>.
- (36) Le, T. X. H.; Gajdar, J.; Vila, N.; Celzard, A.; Fierro, V.; Walcarius, A.; Lopicque, F.; Etienne, M. Improved Productivity of NAD⁺ Reduction under Forced Convection in Aerated Solutions. *ChemElectroChem* **2021**. <https://doi.org/10.1002/CELC.202101225>.
- (37) Fernández, B.; Lobo, L.; Pereiro, R. Buffer Solutions. In *Encyclopedia of Analytical Science Volume 1*; Worsfold, P., Townshend, A., Poole, C., Miró, M., Eds.; Elsevier: Amsterdam, 2019; p 341.
- (38) Kar, A. Iodometric Assays. In *Pharmaceutical Drug Analysis*; New Age International: New Delhi, 2005; pp 138–140.
- (39) Miller J. N.; Miller J. C. *Statistics and Chemometrics for Analytical Chemistry*; Pearson Education Ltd.: Harlow, 2005.
- (40) Yoo, E. H.; Lee, S. Y. Glucose Biosensors: An Overview of Use in Clinical Practice. *Sensors* **2010**, 10 (5), 4558–4576. <https://doi.org/10.3390/s100504558>.
- (41) Barril, C.; Rutledge, D. N.; Scollary, G. R.; Clark, A. C. Ascorbic Acid and White Wine Production: A Review of Beneficial versus Detrimental Impacts. *Aust. J. Grape Wine Res.* **2016**, 22 (2), 169–181. <https://doi.org/10.1111/ajgw.12207>.
- (42) Wallington, N.; Clark, A. C.; Prenzler, P. D.; Barril, C.; Scollary, G. R. The Decay of Ascorbic Acid in a Model Wine System at Low Oxygen Concentration. *Food Chem.* **2013**, 141 (3), 3139–3146. <https://doi.org/10.1016/j.foodchem.2013.05.024>.
- (43) Butnariu, M.; Butu, A. Qualitative and Quantitative Chemical Composition of Wine. In *Quality Control in the Beverage Industry, volume 17*; Grumezescu, A. M., Holban, A. M., Eds.; Elsevier: Duxford, 2019; pp 385–417.

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