

## USE OF A MEMBRANELESS EXTRACTION MODULE FOR THE VOLTAMMETRIC DETERMINATION OF TOTAL SULFITES IN WINE

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Received December 7, 2009

Accepted February 5, 2010

Published online July 8, 2010

A recently developed membraneless extraction module (MLEM) for sample preparation aiming the analysis of volatile and semi-volatile compounds is applied in the voltammetric analysis of total sulfites in wine. Square-wave voltammetry (SWV) is very advantageous in this case because sulfur dioxide (SO<sub>2</sub>) is instrumentally directly detected. The developed method shows good repeatability (RSD lower than 5%) and linearity (between 20 to 220 mg l<sup>-1</sup>) as well as suitable limits of detection (6 mg l<sup>-1</sup>) and quantification (19 mg l<sup>-1</sup>). The proposed method was also compared with the reference methodology (an iodimetry, the Ripper method) showing no significant differences in the obtained results.

**Keywords:** Square-wave voltammetry (SWV); Membraneless extraction module (MLEM); Wine; Sulfur dioxide (SO<sub>2</sub>); Sulfites; Electroanalysis.

Sulfites are widely used as preservatives in the food industry. They are well established anti-oxidants of dual action – by direct reaction and by inhibiting some enzymes that promote oxidation. Hence sulfites minimize product degradation to air exposure and also avoid common food browning. Moreover sulfites have an important anti-microbial effect and also act as a flavor stabilizer since they react with and hinder some strong taste, texture and color off-flavors<sup>1</sup>. Therefore, its use is important in several foodstuffs, including wine, to obtain a long high-standard shelf life. However at high levels an unpleasant aroma/taste is produced and may also become a health hazard.

Sulfites have been associated with allergic reactions and food intolerance symptoms such as difficult breathing, wheezing and hives, as well as gastrointestinal distress<sup>2</sup>. Studies show that consumers with asthma believe that

sulfites in wine trigger asthmatic responses<sup>3</sup>, however such effect appears to be overestimated by results obtained in human trials<sup>4</sup>. Most probably, co-factors and other components in wine also play an important role in induced asthma; nevertheless more studies have to be performed to confirm these assumptions<sup>5</sup>. On the other hand, sulfites appear not to have an influence in wine-caused migraines<sup>6</sup> and may even help to explain the so-called "French paradox" since they may be anti-atherogenic<sup>7</sup>.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an acceptable daily intake (ADI) of 0.7 mg kg<sup>-1</sup> bw (expressed in terms of SO<sub>2</sub>), and, though without sufficient data to estimate the problem magnitude, such level of intake appears to be reached by a significant percentage of consumers<sup>8</sup>. Furthermore, studies consistently point to wine as a major source of sulfite consumption<sup>9-11</sup>. In the European Union, the expression "contains sulfites" must be labeled for beverages containing above 10 mg l<sup>-1</sup> of sulfites<sup>12</sup>. Considering all mentioned, and also that the current legal limits are not too far from the values usually found in wines, the large relevance of sulfite analysis is unquestionable.

Among a whole variety of methodologies proposed for the determination of free and total SO<sub>2</sub> in wine, the most widely used are based on the classical Monier-Williams procedure<sup>13</sup>, where sulfites are extracted with an inert gas, made react with H<sub>2</sub>O<sub>2</sub> producing H<sub>2</sub>SO<sub>4</sub> and then titrated with NaOH; and the classic Ripper method<sup>14</sup> that consists on direct iodimetric titrations, a simple and commonly used method, although prone to interferences<sup>15</sup>. Nevertheless, voltammetric-based methodologies appear to be the most accurate ones<sup>16-21</sup>; this is explained by a certain selectivity and by the direct detection, i.e., no derivatization or other side reaction is needed. Electrochemical techniques are also user-friendly and cheap<sup>1</sup>. The major drawback when using voltammetry is the advisable previous analyte extraction that can be, among others, achieved by using a gas generating and purging device, pervaporation<sup>22</sup> or gas diffusion<sup>21,23-32</sup>.

In this work, we apply a recently developed membraneless extraction module (MLEM) (Fig. 1)<sup>33</sup>. The extraction process is based on the same principles of gas diffusion and pervaporation, however, it does not require a membrane. This module has a lower chamber, where the sample lays while the volatile compounds diffuse to the headspace. Inside the module, there is a suspended small reactor, where a small volume of an acceptor solution is placed. After a defined period of time of extraction, the acceptor solution, now containing the analyte, is collected and taken to be instrumentally measured.

Research on the polarographic reduction of  $\text{SO}_2$  in aqueous solution is archaic and filled with some disagreement<sup>34</sup>. Relative concentrations of  $\text{SO}_2$ , bisulfite ( $\text{HSO}_3^-$ ) and sulfite ( $\text{SO}_3^{2-}$ ) in solution are highly dependent on the pH:  $\text{p}K_1$  (1) and  $\text{p}K_2$  (2), at 298 K, are generally accepted to be 1.86 (ref.<sup>35</sup>) and 7.20 (ref.<sup>36</sup>), respectively.



This means that  $\text{SO}_2$  is dominant, with a sulfur species fraction above 0.9, only below pH 1 and  $\text{HSO}_3^-$  is dominant in the pH range from 3 to 6. However, according to Tolmachev and Scherson<sup>37</sup> even at such pH,  $\text{HSO}_3^-$  becomes  $\text{SO}_2$  at the mercury drop surface previously to the electroanalytical determination and suggest the following reaction at the electrode:



Other possible reactions at the electrode can be found in literature<sup>34,38,39</sup>, nevertheless even if it is not clear which is the electrochemical mechanism, it is unquestionable that the hanging mercury drop electrode (HMDE) is very sensible, reproducible and accurate.

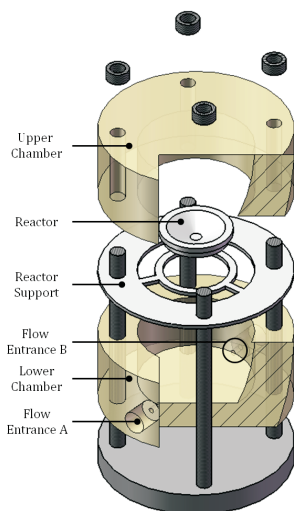


FIG. 1  
MLEM drawing, exploded view

## EXPERIMENTAL

Voltammetric measurements were performed on a Metrohm 663 VA voltammetric stand (Herisau, Switzerland) equipped with an HMDE, drop size of  $0.024 \text{ mm}^3$ , with glassy carbon auxiliary electrode and reference  $\text{AgCl}|\text{Ag}$  ( $3 \text{ M KCl}$ ) electrode, potential step of measurement was  $2.5 \text{ mV}$  and amplitude  $25 \text{ mV}$ . Measurements were conducted without any accumulation time and without stirring. The start and end measurement potentials, with the exception of the cyclic voltammetry (CV), were  $-400$  and  $-850 \text{ mV}$ , respectively. The system was connected to an Autolab PGSTAT 10 voltammetric system (Eco Chemie, Utrecht, The Netherlands). All measurements were made at room temperature.

All reagents used were of analytical grade and were used without further purification. Ultra-pure water from a Simplicity 185 water purification system (Millipore, Billerica, USA) was used. The supporting electrolyte consisted of  $0.1 \text{ M}$  acetate buffer pH 4.0, sodium acetate was purchased from Merck (Darmstadt, Germany). pH of the buffer was adjusted with  $6 \text{ M}$  HCl from Merck.  $\text{Na}_2\text{SO}_3$  (Sigma-Aldrich, St. Louis, USA) was used as a source of  $\text{SO}_2$ . Wine samples were purchased in local supermarkets.

The extraction system is depicted in Fig. 2. The extraction time started to be counted in the moment when the lower chamber was filled with  $5 \text{ ml}$  ( $2.5 \text{ ml}$  of sample and  $2.5 \text{ ml}$  of acid), since both flow rates were  $2.5 \text{ ml min}^{-1}$ , this occurred just after  $1 \text{ min}$ . The wine sample was acidified inside the module with  $3.6 \text{ M}$   $\text{H}_2\text{SO}_4$  (Merck) to  $\text{pH} < 1$ , in order to turn all sulfites into  $\text{SO}_2$ . The produced  $\text{SO}_2$  fills the MLEM headspace, and then, a portion of this compound is collected and fixated by the acid-base reaction with the acetate buffer present in the reactor, hence  $\text{SO}_2$  becomes  $\text{HSO}_3^-$ . That is how the extraction, on a simplified manner, works.

A Minipulls II peristaltic pump was used (Gilson, Middleton, USA) for the sample flow. Tygon tubing from Gilson with different internal diameters was used in the pump head. Polytetrafluoroethylene (PTFE) tubing with  $0.8 \text{ mm}$  of i.d. was used for connections with the home-made Perspex MLEM (Fig. 1)<sup>33</sup>. The MLEM temperature control was performed with a dry heating block Thermobloc TD 150 P3 from Falc Instruments (Lurano, Italy).

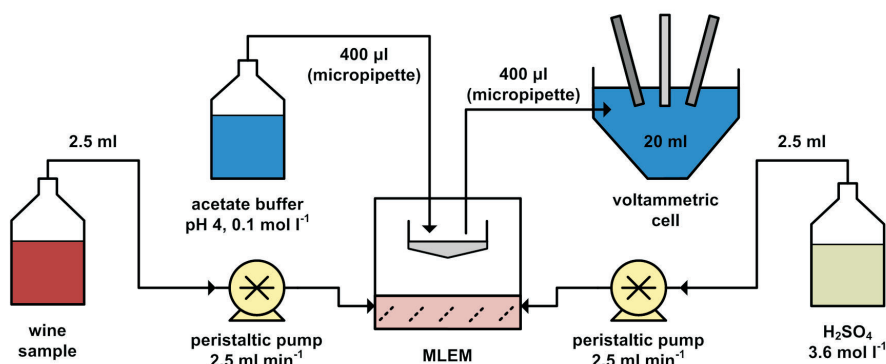


FIG. 2

Extraction scheme. The wine sample has previously endured an alkaline pre-treatment; the supporting electrolyte at the voltammetric cell was a  $0.1 \text{ M}$  acetate buffer pH 4

For the determination of total sulfites, an alkaline pre-treatment was essential to break the adducts ( $\text{SO}_2$  bounded to several aldehydes and ketones) formed in wine. For that purpose, 10 g of NaOH (Merck) were added per 1 l of sample. The sample was homogenized and left to rest for about 20 min.

The Ripper method was performed according to the Portuguese regulation<sup>40</sup>, described as follows: 50 ml of wine were placed in an Erlenmeyer flask, 8 ml of 4 M NaOH were added, and the sample was left to rest for about 5 min; 10 ml of 10%  $\text{H}_2\text{SO}_4$  (v/v) were added (starch (Merck), 4 g  $\text{l}^{-1}$ , was used as an indicator) and the titration started immediately with 0.05 M iodine, which was prepared by mixing excess KI (Sigma–Aldrich) with  $\text{KIO}_3$  (Merck) in acidic conditions. After reaching the endpoint, 20 ml of 4 M NaOH were added. After 5 min, 200 ml of cold water and then 30 ml of 10%  $\text{H}_2\text{SO}_4$  (v/v) were added, and the sample was again immediately titrated.

## RESULTS

Preliminary voltammetric studies were performed by CV (Fig. 3) confirming literature data<sup>16,26,38,39</sup>. At the studied pH, the reaction was quasi-reversible (although  $I_p$  was proportional to  $v^{1/2}$ ,  $E_p$  was independent of  $v$  and  $I_{p,a}/I_{p,c}$  was equal to 1, the difference between  $E_{p,a}$  and  $E_{p,c}$  was not equal to  $57.0/n$  mV), with oxidation and reduction peaks between  $-0.3$  and  $-0.8$  V (ref.<sup>39</sup>). Then optimizing studies were performed to choose the best measurement frequency (Fig. 4), it ended up being 350 Hz, a compromise between peak intensity and peak definition. This frequency of analysis was used in all following experiments.

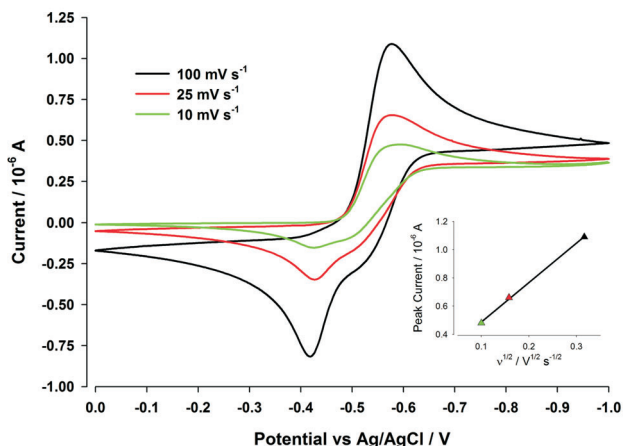


FIG. 3

Cyclic voltammetry of a  $\text{SO}_2$  standard solution ( $70 \text{ mg l}^{-1}$ ) with scan rates of 10, 25 and  $100 \text{ mV s}^{-1}$ . Inset: Variation of reduction peak current with square root of scan rate

Since the extraction is based on the analyte volatilization, temperature is obviously a very important parameter to be controlled. Best results were obtained at 40 °C (data not shown). This temperature was a compromise between increased volatility with temperature and kinetically favoring of

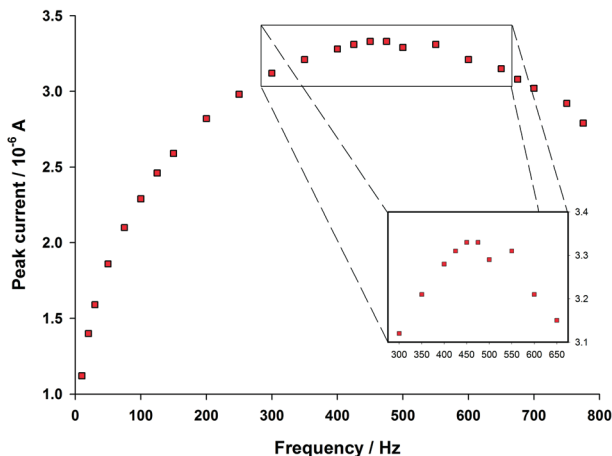


FIG. 4  
Voltammetric signal variation of a standard  $\text{SO}_2$  solution ( $70 \text{ mg l}^{-1}$ ) with frequency

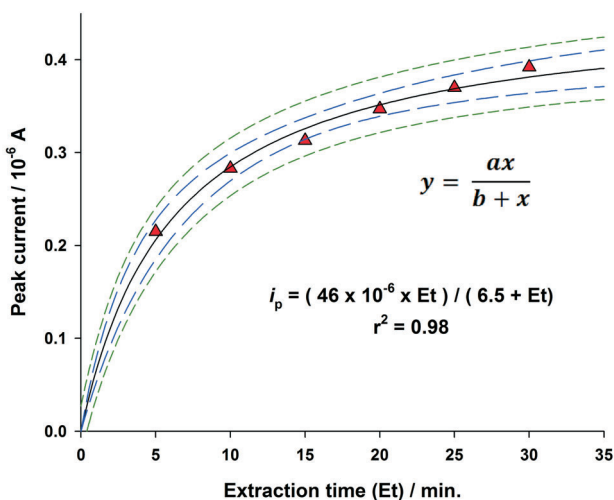


FIG. 5  
Signal variation of a standard  $\text{SO}_2$  solution ( $100 \text{ mg l}^{-1}$ ) with the time of extraction. A hyperbola is presented along with its 95% confidence and prediction bands

oxidative reactions and also to avoid, due to possible bad isolation, the escape of  $\text{SO}_2$  at higher temperatures. The MLEM was kept thermostated at  $40^\circ\text{C}$  in all following experiments.

Then, extraction time was tested from 5 to 30 min (Fig. 5). A hyperbola, a typically used function for situations of saturation, could be used to represent the obtained results.

The method features were evaluated using a wine sample with standard additions of  $\text{SO}_2$ . The figures of merit in terms of linearity and repeatability, and limits of detection and quantification are summarized in Table I. These results were obtained within 5 min of extraction.

TABLE I  
Method features,  $I = f([\text{SO}_2])$ , using an extraction time of 5 min ( $n = 5$ )

Linearity range	RSD	$r^2$	LOD	LOQ
20–220 $\text{mg l}^{-1}$	4%	0.9987	5.7 $\text{mg l}^{-1}$	19 $\text{mg l}^{-1}$

The determination of sulfite concentration in the different wine samples by the developed methodology was compared with the reference Ripper method<sup>14</sup>. The Ripper method was performed according to the Portuguese regulation<sup>40</sup>, based on a procedure from the Organisation Internationale de

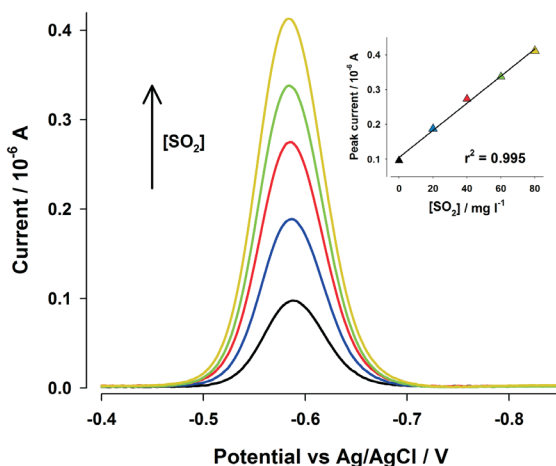


FIG. 6

Voltammograms of extracts, obtained using the MLEM, of a wine sample with different standard additions of  $\text{SO}_2$ , carried out by SWV with a HMDE; extraction time 5 min. Inset: A graph plotted with the voltammogram peak heights

la Vigne et du Vin<sup>41</sup>. At least 3 replicates were done in all samples by both methods. Due to the possible matrix effect, standard additions were used to quantify the content of SO<sub>2</sub> in the different wine samples, however, considering obtained linearity (Fig. 6), one single addition proved to be enough. According to results displayed in Fig. 7, no significant differences were found between both methodologies.

## DISCUSSION AND CONCLUSIONS

The analytical procedure developed was characterized by a good precision and a wide linear application range (Table I). Good agreement was also observed between the proposed new methodology and the one currently used in wine quality laboratory control (Fig. 7).

According to data given in Table I, LOD and LOQ were adequate to the analysis of total sulfites in wine. Still, Fig. 5 shows that longer periods of extraction lead to an increase in sensitivity to an expected saturation point. Therefore, results in Table I were obtained with only 5 min of extraction; if, e.g., 25 min were used instead, the LOD was noticeably lower. This is not needed at wine analysis, however this could be very useful for analysis of other matrices that have lower sulfite levels, like beer or juices.

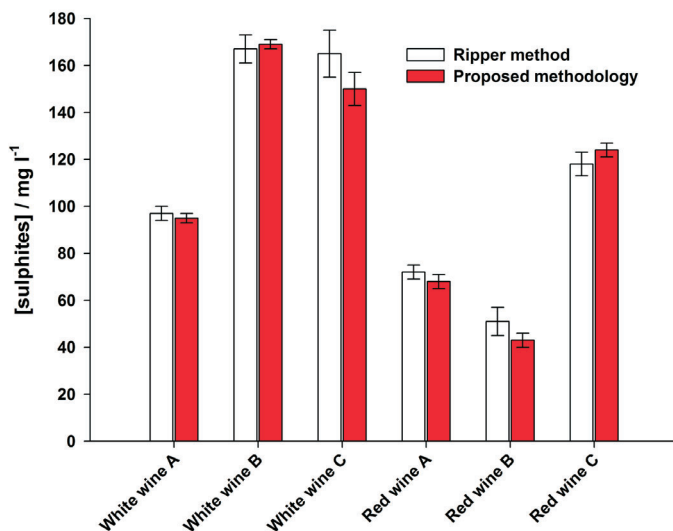


FIG. 7

Comparison of the proposed methodology and the reference Ripper method



To conclude, in this article, a recently developed MLEM for volatiles and semi-volatiles<sup>33</sup> was applied in the voltammetric analysis of sulfites. The developed system shows some advantages when compared to other analytical methodologies, mainly because voltammetry measures directly SO<sub>2</sub> which means a more accurate result. It is especially advantageous for red wines when compared to all the iodimetric procedures since the titration endpoint is of a redish tone (starch as an indicator). It should also be mentioned that it has some potential to become automated, which may be of a great interest for a routine food laboratory control<sup>21</sup>.

L. M. Gonçalves (SFRH/BD/36791/2007) and J. G. Pacheco (SFRH/BD/30279/2006) wish to acknowledge Portuguese Fundação para a Ciência e Tecnologia (FCT) for their Ph.D. studentships.

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