# MICROANALYTICAL FLOW SYSTEM FOR THE SIMULTANEOUS DETERMINATION OF ACETIC ACID AND FREE SULFUR DIOXIDE IN WINES

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## Highlights

- An accurate and interference-free system based on a pH sensor and a gas-diffusion membrane for the determination of acetic acid and free $SO_2$.
- A microanalytical portable flow system for the on-site control of wine aging.
- An automated compact system easy to install in ageing barrels.


## Abstract

Free sulfur dioxide and volatile acidity are parameters related to the quality of wines. Traditional methods for their determination are tedious, time consuming and require analysis in decentralized laboratories, therefore corrective actions cannot be applied on time. This may be more complex in aging wine cellars, where hundreds of individual barrels containing almost finished wines should be monitored. To achieve this aim, a portable microanalytical flow system for the simultaneous detection of free $SO_2$ and acetic acid during the ageing of wines is proposed in this work. The miniaturized system is based on the use of a gas-diffusion membrane and a pH-ISFET, and can be easily installed in barrels. The system was optimized in the range of 5-60 mg $L^{-1}$ and 0.15-1.40 g $L^{-1}$ for $SO_2$ and acetic acid, respectively. It was validated with different sets of wine samples by comparing the results with standard methods, demonstrating a good agreement between methods.



**Keywords:** miniaturized analytical flow-system; permeable gas-diffusion membrane; pH-ISFET; monitoring of acetic acid and free sulfur dioxide; wine samples


## 1. Introduction

Volatile acidity and sulfur dioxide ($SO_2$) are two important parameters related to the quality of the wine measured during the winemaking process (Bakker, Bridle,

Bellworthy, Garcia-Viguera, Reader & Watkins, 1998, Ribereau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2006a). An excess of volatile acidity is mainly related to a high concentration of acetic acid, which can be produced in small concentration at the beginning of alcoholic fermentation (AF). In addition, during the malolactic fermentation (MLF) there is a slight increase of volatile acidity due to the metabolism of some heterofermentative lactic acid bacteria strains and the breakdown of citric acid and tartaric acid. Wine naturally has a volatile acidity of 0.15-0.70 g $L^{-1}$, expressed as acetic acid. This value tends to increase slightly during aging due to the bacterial activity. Acetic acid is not easily detectable for humans in wine if the concentration is below 0.7-0.8 g $L^{-1}$. Above this value, the smell becomes acid and the flavor deteriorates, becoming harsh and bitter on the finish. The maximum level of acetic acid is fixed in the European Community by the International Organization of Vine and Wine (OIV). This value varies depending on the wine class, 1.2 g $L^{-1}$ for most of wines, although some old wines can exceed this limit because they are subjected to a particular legislation (OIV, 2017).

Regarding sulfur dioxide, it is used to prevent the microbial spoilage, the oxidation and the color changes of wine due to undesirable reactions (Fazio & Warner, 1990). Nevertheless, the control of the $SO_2$ concentration in wine is essential to avoid associated negative effects in the health of allergic consumers (e.g., breathing difficulty, migraine or gastrointestinal disorder) (Adams, 1997). Moreover, disagreeable odor and bad taste of wines can be caused if the $SO_2$ concentration exceeds a set value fixed by the OIV in Europe (150 mg $L^{-1}$ and 400 mg $L^{-1}$ for red wines and sweet white wines, respectively).

Sulfur dioxide can be found in wine in two forms: free and bound (Eschenbruch, 1974). Free sulfur dioxide is the most active form for protecting wine against spoilage

and oxidation. Therefore, by controlling the level of free sulfur dioxide, winemakers promote the bacterial stability to guarantee the quality of the product (Ribereau-Gayon, Dubourdieu, Donèche & Lonvaud, 2006a).

There is an important interdependence of these two parameters (acetic acid and free sulfur dioxide) during the aging process of red wines in oak barrels. The aging process enables the extraction of wood flavors, aromas and the production of phenolic components that influence astringency and phenolic polymerization and, overall, this process allows the organoleptic improvement of wines (Ribéreau-Gayon, Glories, Maujean & Dubourdieu, 2006b). But throughout this aging period, there is a continuous need of microbial control carried out by the addition of free sulfur dioxide in wine. The prevention of unwanted yeast and bacterial activity (i.e *Brettanomyces, Pediococcus*, *Lactobacillus*, *Gluconobacter* and *Acetobacter*), that could cause an increase of acetic acid in wine, is carried out by maintaining appropriate levels of molecular $SO_2$. The molecular form corresponds to the undissociated form of the free $SO_2$ and its concentration depends on the total concentration and the pH of the wine. It is recommended to maintain a free $SO_2$ level of 20 – 40 mg $L^{-1}$ (Morata, 2019). For that reason, it is important to check regularly both, acetic acid and free $SO_2$, along ageing in barrels to avoid loss of organoleptic quality of wines.

Analytical methods used for free sulfur dioxide detection and defined by the OIV in Europe are the Paul's method (OIV, 2009a), the Ripper's method (OIV, 2009b) and the Beech and Thomas method (OIV, 2009c). Regarding the acetic acid, the steam distillation/titration method (OIV, 2015) and the enzymatic method through automated colorimetric analyzers (OIV, 2010) are used. These methods have limitations like low accuracy for colored samples (i.e. red wines), they are time-consuming, and have to be carried out in specialized laboratories by highly skilled personnel. Besides, the

enzymatic methods for acetic acid detection are not automated. Therefore, continuous monitoring of these parameters in the ageing barrels is not available. Other alternative methods described in the literature with more compact and simple equipment are those using electrochemical biosensors (Adeloju & Hussain, 2016, Li, et al., 2019, Sroysee, Ponlakhet, Chairam, Jarujamrus & Amatatongcha, 2016). However, they have problems of biofouling and they are affected by some interferences, so their implementation in portable systems for long-term monitoring is limited. Colorimetric methods combining gas-diffusion systems for avoiding the interferences of the sample matrix have also been described (Araujo, de Carvalho, Mota, de Araújo & Coelho, 2005, Bartroli, Escalada, Jimenez-Jorquera & Alonso, 1991). These methods are based on the acid/base and volatile nature of $SO_2$ and acetic acid, and their diffusion through a hydrophobic permeable membrane. Being the analyte separated from the sample, indirect methods of detection can be used as for example pH detection. The use of Ion Selective Field Effect Transistors (ISFETs) for on-line measurements has been described and their advantages (i.e. small size, fast response, long-term stability and easily integration in flow systems) highlighted (Alegret, Bartrolí, Jiménez, del Valle, Domínguez, Cabruja & Merlos, 1991, Gimenez-Gomez, P., et al., 2017).

Commercial analytical systems to determine these parameters in wines are based on titration or absorbance colorimetric methods (AntonPaar, 2020, Foodlab, 2015, GAB, 2012). They provide fast-response and high accuracy, but they require laboratory facilities, therefore they cannot be installed within the barrels in ageing wine cellars.

The aim of this work is to develop and validate a compact, automatic and rapid microanalytical flow system for the determination of free sulfur dioxide and acetic acid in winemaking industry. This system was planned to be placed in a wine barrel and therefore it needed some special requirements of miniaturization and reduction of

reagents/sample consumption. For this reason, a flow cell assembly fabricated with low-cost polymeric materials and integrating a gas-diffusion chamber and a pH-ISFET is proposed. The system will enable winemakers' automatic sampling, conditioning and analysis in the barrels, providing an accurate and fast determination method for free $SO_2$ and acetic acid. The flow system has been validated with wine samples by comparing the results with those obtained by the official methods for both parameters, showing a good agreement between them.

# 2. Materials and Methods

## 2.1. Reagents and solutions

All reagents are of high purity, analytical grade or equivalent and were purchased from Sigma-Aldrich, unless stated otherwise. All solutions were prepared using de-ionized water.

For the determination of acetic acid, stock solutions of 1 M acetic acid and 0.1 M potassium acetate ($CH_3COOK$) were prepared and renewed every 10 days. A solution of potassium acetate containing 0.01 M KCl used as acceptor solution was prepared every day from the stock solution of 0.1 M $CH_3COOK$. The calibration solutions containing different concentrations of acetic acid in a range of 0.15 to 1.40 g $L^{-1}$, were prepared every day from the 1 M stock solution, by diluting it in a background solution containing the main species present in wines: 12 % (v/v) ethanol absolute, 0.12 mM KNO3, 5.26 mM $KH_2PO_4$, 4.41 $MgSO_4$, 2.26 mM $CaCl_2$, 19.0 mM malic acid, 33.0 mM potassium bitartrate and 13.9 mM NaOH. This background solution was not used for the free $SO_2$ method because was demonstrated that these species did not interfere the pH-ISFET detection for the free $SO_2$ assays (Gimenez-Gomez, P., et al., 2017). A 0.6 M HCl solution was used for adjusting the pH of the samples.

For the free sulfur dioxide determination, a solution containing 0.1 M sodium hydroxide (NaOH), 5 % (v/v) glycerol and 0.1 M sodium sulfite ($Na_2SO_3$) was used as stock for preparing the acceptor solution and was renewed every 10 days (Bartroli, Escalada, Jorquera & Alonso, 1991). A solution containing $2\times10^{-5}$ M $Na_2SO_3$ and 0.02 M sodium chloride (NaCl) was used as acceptor solution. The calibration solutions, containing different concentrations of free $SO_2$ (5 – 60 mg $L^{-1}$), were prepared every day from a stock solution of 400 mg $L^{-1}$ of $SO_2$ diluted in a buffer solution containing 12 % (v/v) ethanol absolute (Panreac, Spain). The stock solution and the buffer solution were provided by the company Biosystems (Spain). These solutions enabled the stabilization of $SO_2$ during all the measurements. $2\times10^{-6}$ M HCl and 0.6 M HCl solutions were used for adjusting the pH of the acceptor solution to pH 6 and of the sample below pH 1, respectively.

## 2.2. Devices and flow system

For pH detection a 3×3 $mm^2$ ISFET chip fabricated with standard photolithographic techniques at the Instituto de Microelectrónica de Barcelona (IMB-CNM, CSIC) (Jimenez, Abramova & Baldi, 2006, Jimenez-Jorquera, Orozco & Baldi, 2010,) was used. This chip was wired-bonded and encapsulated on a printed circuit board (PCB) (Fig. S1, in the Supporting Information, SI) (Gutierrez, et al., 2010). A double junction Ag/AgCl reference electrode (RE) (Orion 92-02-00, Thermo Fisher Scientific Inc., Waltham, MA USA) was used. Potentiometric measurements were performed with a portable homemade multi-ISFET meter readout fabricated at the IMB-CNM (Gimenez-Gomez, Escudé-Pujol, Capdevila, Puig-Pujol, Jiménez-Jorquera & Gutiérrez-Capitán, 2016). This readout (21 cm×10 cm×3 cm) enables the real-time simultaneous measurement of six ISFETs by including the power supply unit, the digital part and the analog part in the same device. The pH- ISFET measurement was carried

out by applying 100 µA and 0.5 V between the drain and the source, and recording the ISFET gate potential (in mV). The visualization of the results was carried out by employing a virtual instrument programmed with LabView 2013 (National Instruments, Austin, USA).

A robust and portable flow system assembly containing the pH-ISFET, the RE and the gas-diffusion chamber was designed and fabricated with two low-cost polymers: poly (methyl methacrylate) (PMMA) and double-sided pressure-sensitive-adhesive (PSA). The polymers were fast-mechanized by using a $CO_2$-laser writer (Epilog Mini 24, Epilog Laser, United States). The flow cell sized 3 cm width, 7 cm length and 2 cm height, and was formed by two structures with different layers of PMMA and PSA (Fig. 1). The bottom structure was formed by 3 PMMA layers bonded together with 175-µm thick PSA layers. The structure allowed the position of the microfluidic threads used as sample inlet and outlet for the bottom side of the 150-µL gas-diffusion chamber (5 mm width×10 mm length×3 mm height). A space for hosting the pH-ISFET was also defined in this structure. Regarding the top structure, it was formed by 5 PMMA layers also bonded with PSA layers of 175 µm. This contained another 150-µL gas-diffusion chamber with the same dimensions as before, and a 7-µL cell for the pH-ISFET. The flow channels (1 mm of diameter) were defined and connected to the top gas-diffusion chamber, the pH-ISFET cell and the RE cell. In the top of the structure were defined the microfluidic threads used as acceptor inlet for the top side of the gas-diffusion chamber, and also the thread used as waste outlet of the system. Both fluidic PMMA/PSA structures were fixed with 8 screws (1 mm diameter) to allow easy assembly and disassembly of the system. O-ring junctions were used for the pH-ISFET, the RE and the fluidic inlets and outlets in order to avoid the fluidic leakage. A hydrophobic polyvinylidene fluoride (PVDF, VHP09050 Durapore®, 0.22 µm pore size, from Merck

Millipore, Germany) gas-permeable membrane (5 mm width×10 mm length) was placed between the two symmetrical gas-diffusion chambers for separating the analyte from the sample. The suitability of this membrane for gas-diffusion processes has been studied previously (Gimenez-Gomez, P., et al., 2017). Two peristaltic pumps (403U/VM3, Watson Marlow, UK) and Teflon pump tubes with an internal diameter of 1.0 mm (Teknokroma, Barcelona, Spain) were used for driving the solutions.

A scheme of the fluidic design of the proposed device is depicted in Fig. 1c. This flow system was the same for both parameters, but for $SO_2$ detection, an extra channel with HCl for conditioning the acceptor solution was added.

## 2.3. Detection methodology

The detection methodology for both species is based on the use of the gas-diffusion membrane to separate the analyte from the sample. This methodology exploits the acid/basic characteristics of the analytes and their volatile gaseous state in their acidic forms. In both cases, the diffusion ratio depends on the gas concentration, the gradient and the contact time between both sides of the membrane, as well as on the pH of the solutions. The main advantage of this method is the removing of matrix interferences, resulting in a rapid detection method with low-reagent consumption.

### 2.3.1. Acetic acid determination.

The acetic acid/acetate equilibrium as function of pH is shown in Fig. S2a (in the SI). The detection of the acetic acid is based on the conversion of all the acetate present in the sample to its gas form (acetic acid) with the acidification of the sample to pH below 1. Then, the acetic acid diffuses through the membrane and it is recollected by the acceptor solution at around pH 6, converting all the acetic acid to $CH_3COO^-$ plus protons. If more acetic acid diffuses, the acceptor solution becomes more acidic. This

pH change is detected by the pH-ISFET and correlated with the concentration of acetic acid in the sample.

As is shown in Fig. 1c, for acetic acid measurement, the sample or calibration solution is injected in the bottom side of the cell and mixed 1:1 in volume with a 0.6 M HCl solution to adjust the pH below 1 and convert all to acetic acid. The flow rate is 0.5 mL $min^{-1}$ (0.25 mL $min^{-1}$ each channel). The calibration solutions are performed in the range from 0.15 to 1.40 g $L^{-1}$ of acetic acid. At the top side of the gas-diffusion membrane, the acceptor solution was flowed at 0.5 mL $min^{-1}$.

The hydrodynamic conditions of the system and their effect on the diffusion rate of the acetic acid were evaluated. Firstly, the study was carried out under continuous flow conditions. For that, the acceptor ($1\times10^{-4}$ potassium acetate and 0.01 M KCl) and the acidified sample (or calibration solution) with 0.6 M HCl were pumped during 7 min. Secondly, stop flow conditions were evaluated by using a colorimetric method with a bromothymol blue acid/base colorimetric indicator and an image recorder. A $10^{-4}$ M potassium acetate acceptor solution with an excess of the colorimetric indicator and a sample solution with a 1 g $L^{-1}$ of acetic acid was used. Both solutions were pumped and kept in contact in both sides of the membrane during 10 min. The acceptor solution chamber was recorded on video to evaluate diffusion process of the acetic acid under stop flow conditions.

The chemical parameters of the acceptor solution were also optimized. For that, three potassium acetate acceptor solutions ($10^{-2}$ M, $10^{-3}$ M and $10^{-4}$ M) containing 0.01 M KCl were checked with the hydrodynamics conditions previously optimized. The pH of the acceptor solution in a range from 3 to 7 was also evaluated.

### 2.3.2. Free $SO_2$ determination.

The different forms of the sulfur dioxide (free and bounded) depend on the pH (Fig. S2b, in the SI). As for the acetic acid detection, the determination of free $SO_2$ is based on the conversion of all the bisulfite in the sample to its $SO_2$ gas form by acidifying the sample to pH below 1. Then, $SO_2$ diffuses through the gas-diffusion membrane and it is recollected by an acceptor solution adjusted to a pH value where $HSO_3^-$ is the predominant specie (pH 6), converting all the $SO_2$ to $HSO_3^-$ plus protons. The same as in the case of acetic acid, if more $SO_2$ diffuses, the acceptor solution becomes more acidic. This pH change is detected by the pH-ISFET and the signal variation (mV) is correlated with the concentration of free $SO_2$ in the sample.

As is shown in Fig. 1c, for free $SO_2$ measurement, the sample (or calibration solution) is pumped and mixed 1:1 in volume with a 0.6 M HCl solution with a total flow of 0.5 mL $min^{-1}$ (0.25 mL $min^{-1}$ each channel) for adjusting the pH below 1. The acceptor solution is mixed 1:1 with $2\times10^{-6}$ M HCl to get pH 6 with a total flow of 0.5 mL $min^{-1}$ (0.25 mL $min^{-1}$ each channel). Most of the chemical parameters and the hydrodynamic conditions for the free $SO_2$ determination are used from a previous work of our group (Gimenez-Gomez et al., 2017). These conditions were re-evaluated by comparing the results obtained under continuous flow conditions during 10 min with those obtained by flowing the solutions during 5 min and then stopping the flow during 2 min to enhance the diffusion in the chamber. Once selected the diffusion process, the optimal measurement time under these conditions was also studied. For this optimization, calibration solutions containing 5, 30 and 60 mg $L^{-1}$ of free $SO_2$ were used.

### 2.3.3. Acetic acid and free $SO_2$ determination in wine samples.

Several sets of wines samples were used in order to test the feasibility of the developed microanalytical flow system. Firstly, two commercial wines (a table red

wine, *Don Simón*, and a white wine with Apellation of Origin Penedés, Spain, *Sumarroca*) were analyzed by spiking them with different concentrations of acetic acid (0.00, 0.15, 0.50 and 1.00 g $L^{-1}$) and free $SO_2$ (0, 5, 10, 15 and 60 mg $L^{-1}$). The percentage of recovery was calculated for each case

After that, the proposed system was validated by comparing the obtained results of a set of samples with the official methods of the OIV for the free $SO_2$ and the acetic acid. These analysis were carried out by the Institut Català de la Vinya i el Vi (IRTA–INCAVI), which is an accredited laboratory (ISO 17025) in Catalonia. For the free $SO_2$ detection, the wines were analyzed by the Paul method (OIV, 2009a), by purging the free $SO_2$ from the wine sample at low temperature (10 ºC) with a stream of air (or nitrogen). Then, the $SO_2$ was fixed and oxidized by bubbling through a dilute and neutral $H_2O_2$ solution. Finally, the formed $SO_2$ was determined by titration with a standard NaOH solution. For the acetic acid detection, the enzymatic method based on the reaction of acid in presence of the coenzyme A (CoA) and adenosine triphosphate (ATP), catalyzed by the acetyl–CoA synthetase (ACS) was used. The formed acetyl–CoA (acetyl coenzyme A) reacted with oxaloacetate (catalyzed by the L-malic acid) to produce reduced nicotinamide adenine dinucleotide (NADH). Finally, the NADH concentration was determined by absorbance at 340 nm, and stoichiometrically related to the acetic acid concentration in the sample (Megazyme, 2017, OIV, 2015). The IRTA–INCAVI validates regularly both methods with a weekly analysis of real samples duplicates. The accuracy of the method is determined by duplicate monthly analysis of a standard solution and by the periodically measurement of uncertainty at 95 % calculated from the results of intercomparison analysis with other accredited laboratories. The standard solution used is prepared in the laboratory by making additions in a wine matrix without $SO_2$ or acetic acid, depending on the analyte to determine. All the

accredited range is considered by changing the concentration every month. Therefore, the interval of the standard methods includes the conditions of maximum variability (different equipment, different people, different day), and the formula is directly dependent on the concentration of $SO_2$ or acetic acid. Compared to the analysis that can be performed in laboratories without accreditation, this method supposes a significant advantage because it provides greater reliability of the results.

The first validation set was analyzed at the IRTA–INCAVI facilities with the standard method and then transported to our institute. Five white wines (W1 to W5), two red wines (R1 and R2) and two rosé wines (RO1 and RO2) were analyzed for this first validation set. The samples W1, R1 and RO1 were also spiked with 0.7 g $L^{-1}$ of acetic acid to evaluate the potential interference of acetic acid concentrations for the free sulfur dioxide detection. The second validation set was analyzed at the IRTA-INCAVI laboratories simultaneously with the proposed flow system in order to avoid the evolution of the wine and the change in concentrations during the shipment and storage. Four white wines (W6 to W9), five red wines (R3 to R7) and three rosé wines (RO3 to RO5) were used in this validation. Although rosé wines are not aged in barrels, they were used to validate the proposed flow system in all types of wines.

The procedure used for the analysis of the wine samples was as follows: firstly, the microanalytical flow system was calibrated with three $SO_2$ calibration solutions (5 mg $L^{-1}$, 30 mg $L^{-1}$ and 60 mg $L^{-1}$) and acetic acid solutions (0. 15 g $L^{-1}$, 0.70 g $L^{-1}$ and 1.40 g $L^{-1}$). Then, wine samples were consecutively analyzed and the recorded signals were interpolated in the calibration plots to obtain the experimental results, which were compared to those obtained by the standard method by the IRTA-INCAVI staff.

## 3. Results and Discussion

### 3.1. Optimization of the system for acetic acid determination.

First tests for acetic acid determination were carried out under continuous flow conditions. The signal of each sample was recorded by the pH-ISFET, and the mean signal value of the last 20 s was used to represent the calibration plot (data not shown). The results showed a nonlinear fitting and an unstable signal indicating that under these conditions there was not an efficient diffusion of the acetic acid from the sample to the acceptor solution.

Therefore, stop flow conditions were checked to improve the response. For that, an experiment using colorimetric detection to visualize the diffusion of acetic acid through the membrane was performed. As can be seen in the Fig. S3 (in the SI), the initial color of the solution containing the bromothymol blue (at t = 0 min) was blue (pH above 8), indicating that the diffusion of the acetic acid has not initiated. After 5 min of contact, the color of the acceptor solution became green, indicating that a fraction of the acetic acid from the sample was diffused through the membrane and mixed with the acceptor solution producing a decreasing pH (around 6.5−7). Finally, after 10 min, the acceptor solution became totally yellow (pH < 6), meaning that all the acetic acid from the sample passed through the membrane and was collected by the acceptor solution. Therefore, the optimized time for stop flow conditions was fixed at 10 min for each sample for next experiments.

Three different concentrations of potassium acetate ($10^{-2}$ M, $10^{-3}$ M and $10^{-4}$ M) in the acceptor solution were evaluated. This solution and the acidified sample were flowed during 5 min. Then, the flow was stopped to let the acetic acid to diffuse through the gas-diffusion membrane during 10 min. Afterwards, the acceptor solution was flowed to the pH-ISFET cell by pumping during 10 s at 0.5 mL $min^{-1}$, and the signal

339 was recorded during 2 min in stop flow conditions. The mean value of the last 15 s was
340 used for the calibration plots in a range from 0.15 to 1.40 g $L^{-1}$ (Fig. S4, in the SI). A
341 linear response was obtained for the $10^{-3}$ M and $10^{-4}$ M potassium acetate, but for $10^{-2}$
342 M the linear fitting was poor (r = 0.870). The sensitivity obtained for $10^{-3}$ M potassium
343 acetate (-34.32 ± 1.15 mV $dec^{-1}$) was twice higher than that obtained for $10^{-4}$ M (-18.45
344 ± 2.10 mV $dec^{-1}$). The stability of consecutive measurements was also better for $10^{-3}$ M
345 (RSD 5.8%) than for $10^{-4}$ M (RSD 10.6%). Therefore, the optimal concentration of
346 potassium acetate in the acceptor solution was fixed to $10^{-3}$ M.

347 The pH of the acceptor solution for the acetic acid determination was also
348 evaluated and optimized. As shown in Fig. S2a (in the SI), acetic acid and acetate
349 coexist in the pH range from 3 to 7. A freshly prepared solution containing $10^{-3}$ M
350 potassium acetate and 0.01 M KCl had a pH value around 6, where acetate is the
351 predominant specie. Two adjusted pH solutions were tested, pH 5.5 and 7.0. From
352 results shown in Fig. S5 (in the SI), the non-adjusted acceptor solution presented a
353 sensitivity of -34.6 ± 1.1 mV $dec^{-1}$ (RSD 4.8%). Results obtained when the pH was
354 adjusted showed a decrease in the sensitivity (-23.9 ± 4.1 mV $dec^{-1}$ and -28.4 ± 3.0 mV
355 $dec^{-1}$, for pH 5.5 and 7, respectively). Besides, adjusted solutions showed a high
356 unstable signal with time, showing a RSD above 12% and 18% for the pH 7 and 5.5,
357 respectively. Therefore, a solution containing $10^{-3}$ M potassium acetate and 0.01 M KCl
358 without pH adjustment was used as acceptor solution for next assays.

359 The fluidic system was evaluated for acetic acid determination using the
360 optimized conditions described above (see Fig S.6, in the SI, for more details). The
361 acceptor solution ($10^{-3}$ M $CH_3COOK$ and 0.01 M KCl) and the sample solution (pH
362 below 1, acidified with 0.6 M HCl) were pumped during 5 min at 0.5 mL $min^{-1}$ (Fig
363 S.6i, in the SI). The flow was stopped during 10 min (Fig S.6ii, in the SI), and then, the

acceptor solution was flowed during 10 s at 0.5 mL $min^{-1}$ to reach the pH-ISFET chamber. Finally, the signal was recorded during two minutes (Fig S.6iii, in the SI) for each concentration of acetic acid in the range from 0.15 to 1.4 g $L^{-1}$ (Fig. 2a). Each sample was measured by triplicated. A differential method was used for plotting the calibration curves, which avoided the periodic use of control solutions. The analytical signal was the difference between the mean value of the last 15 s of the sample solution and the baseline (Fig. 2b). A linear variation of the potential in mV with the increasing concentration of acetic acid was observed, with a sensitivity of -32.4 ± 1.8 mV $dec^{-1}$ (r = 0.999). The limit of detection (LOD) and the limit of quantification (LOQ), calculated according to the IUPAC criterion for potentiometric sensors (Inczèdy, Lengyel & Ure, 1998), were 0.04 g $L^{-1}$ and 0.12 g $L^{-1}$, respectively.

### 3.2. Optimization of the system for free $SO_2$ determination.

Most of the parameters were already optimized in a previous study (Gimenez-Gomez, P., et al., 2017). A solution containing $2\times10^{-5}$ M $Na_2SO_3$ and 0.02 M sodium chloride (NaCl) was used as acceptor solution, which pH was adjusted with $2\times10^{-6}$ M HCl. To convert all the sulfites species of the sample into $SO_2$ gas, a 0.6 M HCl solution was used for adjusting its pH below 1. Results obtained from the comparative study of continuous and stop flow conditions are shown in Fig. S7 (in the SI). The determination of free $SO_2$ was more efficient under continuous flow conditions (-56.0 ± 0.4 mV $dec^{-1}$; r = 0.999) than under stop flow conditions (-50.3 ± 0.6 mV $dec^{-1}$; r = 0.993) due to the higher drift of the baseline under later conditions. Moreover, the determination was much faster under continuous flow conditions, therefore this methodology was chosen for next experiments. The flow rate for each channel was selected at 0.25 mL $min^{-1}$. The measurement time for one sample was optimized taking into account the stabilization of the signal thus indicating that the diffusion of the $SO_2$ has been completed. For that,

triplicate calibrations were recorded in the range from 5 to 60 mg $L^{-1}$ of free $SO_2$ (Fig. 3a).

The signal recorded by the pH-ISFET was quite stable after 8 min for the background solution (0 mg $L^{-1}$) and after 6 min for the calibration solutions (5, 30 or 60 mg $L^{-1}$). The use of the background solution after each assay guaranteed the perfect cleaning of the flow system as shown by the recovery of the base line and provided also the correction of drift. Therefore, these time conditions were fixed for the next experiments. The analytical signal used was the difference between the mean value of the last 15 s for each sample (or calibration solution) and for the background solution (Fig. 3b). A linear variation of the potential with the increasing concentration of $SO_2$ was observed, with a sensitivity of -56.1 ± 0.5 mV $dec^{-1}$ (r = 0.997). The LOD and the LOQ, calculated as before (Inczèdy, Lengyel & Ure, 1998), were 1.5 mg $L^{-1}$ and 4.5 mg $L^{-1}$, respectively. The variation of potential for the whole concentration range corresponded to more than 1 pH unit decreasing which is in agreement with theoretical values under these conditions.

## 3.3. Analysis of wine samples.

### 3.3.1. Determination of acetic acid.

The acetic acid system was evaluated with two commercial wines and nine wines samples provided by the IRTA-INCAVI. The content of acetic acid in commercial wines was unknown, therefore solutions containing a fixed volume of wine spiked with different concentrations of acetic acid were measured. The results obtained for the two commercial wines (the red wine *Don Simón*, and the white wine *Sumarroca*) are shown in Table S1 (in the SI). Theoretical values corresponded to the sum of the initial experimental value calculated for the sample without spiking acetic acid and each

spiked concentration for this sample. According to the results, *Don Simón* red wine had an acetic acid concentration around 0.56 g $L^{-1}$. This value had a perceptible deviation (RSD 18%) due to the drift of the baseline. However, wine samples with spiked acetic acid provided better results, with a RSD below 5%. The recovery values are above 98% for 0.50 and 1.00 g $L^{-1}$ spiked samples and 94% for 0.15 g $L^{-1}$ spiked samples. Regarding the *Sumarroca* white wine, it had an acetic acid concentration of 0.41 g $L^{-1}$. Unlike the red wine *Don Simón*, the white wine *Sumarroca* presented a low relative deviation (RSD 7%), meaning a more stable baseline. For the spiked samples, the RSD was below 6% in all the cases. The recovery values for samples with an acetic acid spiked concentration of 0.15 and 0.50 g $L^{-1}$ were above 90%, while the recovery for the sample spiked with 1.00 g $L^{-1}$ was poorer (84%).

Results obtained from the test carried out with the set of wine samples provided by IRTA-INCAVI are shown in Table S2 (in the SI). The repeatability of the proposed flow system is quite good, showing a RSD below 10% for all the samples. Values obtained with the flow system were lower (around 14−20% for all the samples) than the obtained with the enzymatic method. This underestimation could be attributed to the nature of samples provided by the IRTA-INCAVI. They had not completed the fermentation process; therefore the concentration of acetic acid could have changed due to evaporation and spontaneous evolution of the sample from their first analysis carried out at the IRTA-INCAVI facilities.

### 3.3.2. Determination of free $SO_2$.

The free $SO_2$ determination system was evaluated with two commercial wines and nine wine samples provided by the IRTA-INCAVI laboratory. At first, the system

436 was calibrated in a range from 5 mg $L^{-1}$ to 60 mg $L^{-1}$. Then, each wine sample was
437 evaluated by triplicate and the recorded signal was interpolated in the calibration plot.

438 The same methodology used for acetic acid was applied here for commercial
439 wines: wine samples were spiked with 5, 10, 15 and 40 g $L^{-1}$ of $SO_2$. The results are
440 shown in Table S3 a (in the SI) for the red wine *Don Simón* and the white wine
441 *Sumarroca*. Theoretical free $SO_2$ concentration corresponds to the sum of the initial
442 concentration of wine calculated experimentally and the 70 % of added $SO_2$, assuming
443 that the other 30 % is bounded to aldehydes, ketones, sugars or acids present in wines
444 (Ribereau-Gayon, Dubourdieu, Donèche & Lonvaud, 2006a). According to the results
445 for the red wine *Don Simón*, this had a $SO_2$ concentration of around 23 mg $L^{-1}$ (RSD
446 8%). The recovery values obtained for the spiked samples were quite satisfactory
447 (above 90% in all cases) and stable (RSD below 7% in all the cases). From the results
448 for the *Sumarroca* white wine, the calculated initial concentration of the sample was 21
449 mg $L^{-1}$, with a RSD of 9%. The percentage of recovered acetic acid was above 95% for
450 all the cases, except for the 40 mg $L^{-1}$ addition (92 % of recovered $SO_2$). The RSD was
451 below 8% for all the spiked samples.

452 Another test to validate the microanalytical flow system for the free $SO_2$
453 detection was carried out with a set of red, white and rosé wine samples provided by
454 IRTA-INCAVI laboratory. Results were compared with those obtained with the
455 accredited Paul method (Table S4 in the SI). The repeatability of the flow system
456 method was good, with a RSD below 9% for all samples. Regarding the comparison of
457 values obtained for both methods, the error in percentage was in the range from 5% to
458 35%, with a predominant overestimation of free sulfur dioxide for the flow system.
459 Nevertheless, it is important to notice that all values of the flow system method were
460 within the confidence interval of the standard method. The highest differences (from

22% to 35%) were obtained for the three white wines samples (W1, W2 and W3) with a concentration of free $SO_2$ below 12 mg $L^{-1}$ according to standard methods and from 12 to 15 mg $L^{-1}$ according to the flow method. This overestimation could be attributed to the low concentration of free $SO_2$ of the sample, whose differences resulted in higher relative errors.

On the other hand, the samples containing highest concentrations of acetic acid (W1, R1 and R2, see Table S2) did not showed high relative errors between methods. That means that the analysis of free $SO_2$ was not affected by the presence of high acetic acid concentrations. The acceptor solution containing $HSO_3^-$ is only affected by the presence of $SO_2$ coming from the sample after its acidification. For white wines with a concentration of free $SO_2$ above 20 mg $L^{-1}$, the difference between methods was lower (below 13%). The difference between methods was from 0% to 5% and from 14% to 18% for red and rosé wines, respectively. There was a dispersion in the accuracy (negative and positive) in rosé and red samples, which means that the difference between both methods was not due to a systematic error. Besides, in samples spiked with 0.7 g $L^{-1}$ of acetic acid after the $SO_2$ analysis (samples R1, W5 and RO2), the value of free $SO_2$ changed below 5% for all samples, meaning that the acetic acid did not interfere in the $SO_2$ detection in the proposed system. Despite these results, as in the case of the acetic acid determination, these samples were measured by the IRTA-INCAVI staff in their facilities, and then the samples were transported to the IMB-CNM laboratory for the determination with the microanalytical flow system. During this time, samples could have evolved, causing a change in the concentration of $SO_2$ from the initial measurements, therefore some differences between methods could be attributed to this fact.

### 3.3.3. Simultaneous determination of acetic acid and free $SO_2$.

After the individual analysis of the acetic acid and the free $SO_2$, a new set of wine samples were analyzed to validate the system for the simultaneous detection of both analytes against the official methods. All these analyses were carried out at the IRTA-INCAVI laboratories in order to compare the results between methods in real time then avoiding differences between methods caused by the evolution of the sample with time. Once the system was calibrated for both species, 12 wine samples were tested for acetic acid and free $SO_2$ consecutively.

Results of the comparative analysis are shown in Table 1 and Table 2 for acetic acid and free $SO_2$, respectively. Acetic acid values presented a high dispersion of results. For white wines the errors were high for the two sparkling wine samples (W6 and W9). This overvalue was due to the presence of carbonic acid that interferes in the analysis due to the diffusion of this gas through the gas-diffusion membrane producing a higher pH decreasing. A severe de-aeration method to remove the excess of carbonic acid is not feasible in this case since free $SO_2$ and acetic acid could also be lost. Therefore, the current method is not suitable for sparkling wines. Nevertheless, considering that a low number of white wines follow the process of ageing and these are never sparkling wines, we cannot consider this interference for the proposed application in this work. However, for the two other white wine samples (W7 and W8), the correlation between both methods was high. For red wine samples, values were in good agreement with the obtained by the standard method with an error below 12% in all the cases. The different signal of relative error indicated that there was not a systematic error associated. For rosé wine samples, the accuracy between methods was high for two of the three samples (RO3 and RO5), but the different signal meant also that there was not a systematic error associated. It could be associated to some matrix effect due to the composition of the rosé wines, affecting the diffusion of the acetic acid.

511 Regarding the values obtained for the free $SO_2$ for white wines samples, the
512 dispersion of errors is also high. For W8 sample, the error is high, maybe due to the low
513 concentration of free $SO_2$ in this sample. For sparkling white wines (W6 and W9), the
514 positive error indicated a slight effect of carbonic gas as interfering, as was discussed
515 above for the acetic acid detection. Concerning the results obtained for red wine
516 samples, the results obtained with the flow system were similar to those obtained with
517 the standard method, with low differences for samples R4, R5, R6 and R7. The sample
518 R3 showed a slightly higher difference, but the experimental value obtained with the
519 flow system was within the confidence interval of the IRTA-INCAVI measurements,
520 demonstrating the good agreement between methods for this case too. It is worthwhile
521 to notice that for red wine samples with low concentration of free $SO_2$ (R7, 4 mg $L^{-1}$),
522 the values for the flow system were similar to the standard ones, demonstrating the
523 suitability of the method for the entire range of red wines. For rosé wines, the difference
524 between both methods was also low for RO3 and RO4. However, if we consider the
525 confidence interval the value is quite similar for RO5 in both methods. Therefore, we
526 can conclude that in general and mostly for low concentrations, the values for the flow
527 system are in agreement or even higher than that for standard method. That suggests
528 that the diffusion of the $SO_2$ is not affected in this case by the composition of the
529 sample.

530 The data obtained with the microanalytical flow system, for each type of wine,
531 was plotted versus that obtained with the standard method (Fig. 4). The differences
532 between both methods were analyzed by the least squares method (Table S5, in the SI).
533 Regarding the acetic acid, samples W6, W9 and RO5 were not included in the analysis,
534 thus only the red wines were analyzed by the least square method. The slope obtained
535 was acceptable (0.71 ± 0.06), with an intercept close to zero (0.12 ± 0.03 g $L^{-1}$). The

positive value of the intercept was due to a certain overestimation of the values for the flow system for low concentrations of acetic acid.

Regarding the free $SO_2$, data from W8 was removed for the analysis. There was a good agreement between both methods for white wine samples. The slope was above 1 (1.4 ± 0.1), due to the slightly overestimation of the value calculated by the flow system. Although it is important to notice again that all values of the flow system method were within the confidence interval of the standard method. From the analysis of white wine samples could be concluded that the developed method in its current form was accurate enough to be applied in white samples not containing very low concentration of free $SO_2$. For red wine samples, the slope was very close to the ideality (1.0 ± 0.1), and the intercept was almost zero (0.2 ± 1.0 mg $L^{-1}$), demonstrating the great competence of the developed system. For rosé wines, results are also good. The slope and the intercept were close to one and zero, respectively (1.0 ± 0.12 and -1.0 ± 2.0 mg $L^{-1}$). The uncertainty intervals were through the ideal value, indicating a no-systematic deviation between methods.

### 3.4. Hydrodynamic parameters for the aging in barrels.

Regarding the hydrodynamic performance of the flow system, only one gas-diffusion membrane was used during the complete validation of the system, demonstrating the robustness of this membrane as was observed in our previous work for the free and total $SO_2$ determination (Gimenez-Gomez, P., et al., 2017). Volumes of solutions consumed for each assay of the process were as follows: To calibrate the system, the volume of the reagents used is below 10.5 ml in all the cases. Then, 1.5 ml of each wine sample and below 3.5 ml of all the involved reagents were consumed for each assay. In the case of acetic acid, below 15 ml of all the reagents were consumed to calibrate the system. Then, 1.25 ml of wine sample and reagents were consumed per

assay. The volumes used of each solution were chosen for injecting three times the volume of the new solution in comparison to the volume within the microfluidic channels. In this way, the perfect renovation of the fluidic microchannels with next solutions was guaranteed, avoiding the memory effect for the sensor from previous assays by removing any trace of $SO_2$ or acetic acid coming from them.

From these numbers, and considering one assay per day to control the concentration of free $SO_2$ and acetic acid within the barrel, if 1 L containers are used for each reagent/solution, the microanalytical flow system can work up to three months without any user intervention. Taking into account that wine ageing in winemaking industry normally takes up to 18 months depending of the wine, it will be necessary to substitute some of the solution containers six times at most during the full process, without manipulating the barrels and avoiding risks for the wine quality. The quality of the wine is also maintained because the proposed method needs to take up low volumes of samples from the barrel, avoiding the refill of the barrels of current systems. Therefore, this new microanalytical flow system for the simultaneous determination of acetic acid and free $SO_2$ can be a suitable and a feasible tool for controlling excessive oxidation processes or detection of spoilage microorganism metabolism in red and white wines during ageing in barrels, preventing aroma and flavor deviations.

## 4. Conclusions

A new microanalytical flow system for monitoring free $SO_2$ and acetic acid concentration in wines samples has been developed and evaluated. This system incorporates a permeable gas-diffusion membrane to separate the analyte from the sample avoiding interferences and uses as detector a pH-ISFET. Profiting the acid/basic characteristics of the analytes and the volatile nature of the acidic form, indirect detection of pH is carried out. The system was optimized in a range from 5 mg $L^{-1}$ to 60

586 mg $L^{-1}$ and from 0.15 g $L^{-1}$ to 1.40 g $L^{-1}$, for free $SO_2$ and acetic acid, respectively, in
587 accordance with the expected values in the winemaking industry. This was validated
588 with several set of wine samples, and comparing the results with those obtained with
589 standard methods. Values for acetic acid showed quite good correlation for red wine
590 between both methods. For white wines, the correlation between methods was also
591 good, except for samples containing carbonic acid, which interfered the detection and
592 caused a high overestimation of the acetic acid value, as was also observed for the free
593 $SO_2$ detection. For rosé wines, in general, experimental results showed higher
594 differences in comparison with the standard method. This fact could be attributed to the
595 composition of rosé wines affecting the diffusion rate of the acetic acid. Regarding free
596 $SO_2$ determination, the correlation between methods was quite good for white wines,
597 except for those samples with low concentration of free $SO_2$ (according to the standard
598 method). In that case the flow system overestimated the concentration given by the
599 standard method. For red and rosé wines the correlation between methods was quite
600 good, demonstrating the good agreement between the compared methods.

601 Therefore, the results in this work demonstrated the high potential of the
602 developed microanalytical flow system for monitoring acetic acid and free $SO_2$ in the
603 field of the winemaking industry, mostly in red wines. The accuracy of the proposed
604 flow system is appropriate for the considered application: the periodic control of both
605 parameters in aging barrels for red and some white wines, allowing corrective actions to
606 be carried out in real time if it were necessary. The size of the system allows its easy
607 integration within ageing barrels, enabling the calibration and wine measurements in an
608 automated mode. Besides, the use of pH-ISFET provides added advantages for the
609 proposed application, like low cost, reproducibility and robustness, making the system
610 fabrication and maintenance costs effectively low. The low volume required for each

analysis enables a long-term autonomy of the device. Thereupon, this proposed approach could be a promising alternative to traditional methods of wine control in the winemaking industry, especially for the monitoring of red wines in barrels, which are the most common variety of wines aged in barrels.

## Credit authorship contribution statement

**Pablo Giménez-Gómez:** Management, Coordination, Investigation, Methodology, Experimental, Data curation, Supervision, Writing - review & editing. **Manuel Gutiérrez-Capitán:** Supervision, Draft writing. **Juan Manuel Ríos:** Methodology, Coordination, Experimental. **Fina Capdevila:** Methodology, Experimental, Wine samples, Draft writing. **Anna Puig-Pujol:** Methodology, Experimental, Wine samples, Draft writing. **Cecilia Jiménez-Jorquera:** Supervision, Draft writing, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

The following is Supplementary data to this article:…….

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729 **Table 1.** Values of acetic acid concentration obtained with the microanalytical flow
730 system and with the standard accredited potentiometric method for 12 wine samples.
731 The confidence intervals are calculated with a level of 95% for the standard method.

| Wine[1] | Acetic acid concentration (g $L^{-1}$) | | |
|---|---|---|---|
| | Microanalytical flow system | Standard method[2] | Relative error % |
| W6[a] | 0.52 | 0.18 ± 0.02 | 190 |
| W7 | 0.31 | 0.29 ± 0.04 | 7 |
| W8 | 0.17 | 0.17 ± 0.02 | 1 |
| W9[a] | 1.54 | 0.23 ± 0.03 | 569 |
| R3 | 0.44 | 0.47 ± 0.06 | -7 |
| R4 | 0.30 | 0.26 ± 0.03 | 12 |
| R5 | 0.64 | 0.70 ± 0.09 | -8 |
| R6 | 0.32 | 0.26 ± 0.03 | 10 |
| R7 | 0.73 | 0.70 ± 0.09 | 4 |
| RO3 | 0.37 | 0.29 ± 0.04 | 26 |
| RO4 | 0.25 | 0.24 ± 0.03 | 2 |
| RO5 | 0.20 | 0.35 ± 0.04 | -43 |

732 [1]*W: white wine; R: red wine; RO: rosé wine*
733 [2]*Confidence interval calculated by IRTA-INCAVI from the results of inter-comparison*
734 *analysis*
735 [a]*sparkling*
736
737

738 **Table 2.** Values of free $SO_2$ concentration obtained with the microanalytical flow
739 system and with the standard accredited Paul method for 12 wine samples. The
740 confidence intervals are calculated with a level of 95% for the standard method.

| Wine[1] | Free $SO_2$ concentration (mg $L^{-1}$) | | |
|---|---|---|---|
| | **Microanalytical flow system** | **Standard method[2]** | **Relative error %** |
| W6[a] | 25 | 22 ± 7 | 14 |
| W7 | 18 | 19 ± 6 | -5 |
| W8 | 22 | 5 ± 2 | 340 |
| W9[a] | 56 | 46 ± 14 | 22 |
| R3 | 13 | 11 ± 4 | 18 |
| R4 | 15 | 15 ± 5 | 0 |
| R5 | 26 | 25 ± 8 | 4 |
| R6 | 14 | 15 ± 5 | -7 |
| R7 | 4 | 4 ± 2 | 0 |
| RO3 | 5 | 5 ± 2 | 0 |
| RO4 | 17 | 19 ± 6 | -11 |
| RO5 | 3 | 6 ± 2 | -50 |

741 [1]*W: white wine; R: red wine; RO: rosé wine*
742 [2]*Confidence interval calculated by IRTA-INCAVI from the results of inter-comparison*
743 *analysis*
744 [a]*sparkling*
745
746

## Figure Captions

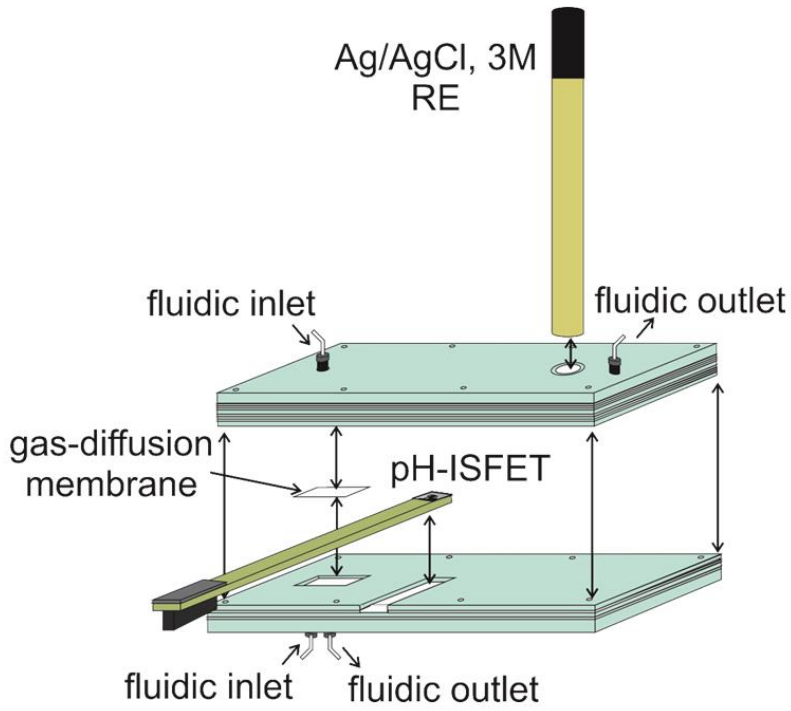
**Fig. 1.** Design of the flow system. (a) Scheme of the flow chamber for free $SO_2$ and acetic acid determination with the top and bottom structures; (b) Picture of the assembled analytical device. (c) Scheme of the fluidic performance of the microanalytical device. (*) The channel containing $2 \times 10^{-6}$ HCl is only used for the free $SO_2$ detection.

**Fig. 2.** Analytical response for acetic acid. (a) pH-ISFET recording and (b) the obtained calibration curve for 0, 0.15, 0.20, 0.40, 0.70 and 1.40 g $L^{-1}$ acetic acid solutions. The numbers inside the plot (a) represent the concentration of acetic acid (g $L^{-1}$). Standard deviation of triplicated calibrations is drawn as error bars.

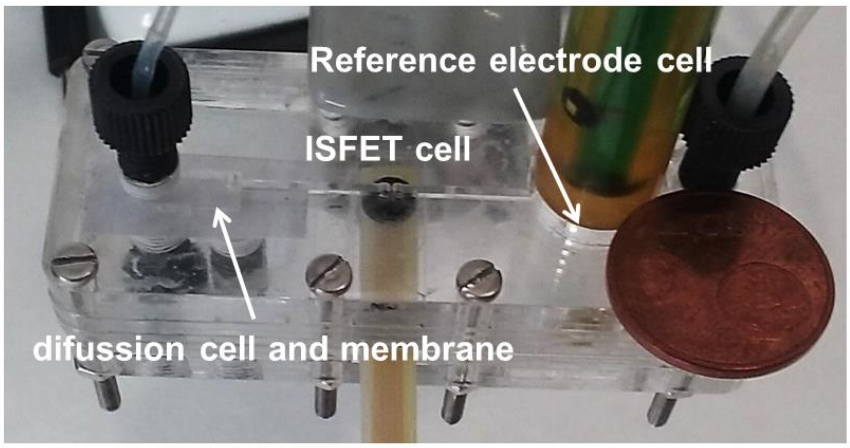
**Fig. 3.** Analytical response for free $SO_2$. (a) pH ISFET recording and b) the obtained calibration curve for 0, 5, 30 and 60 mg $L^{-1}$ $SO_2$ solutions. The numbers inside the plot (a) represent the concentration of free $SO_2$ (mg $L^{-1}$). Standard deviation of triplicated calibrations is drawn as error bars.

**Fig. 4.** Plot representing the data of the standard method vs. the microanalytical flow system for free $SO_2$ determination. The dotted line corresponds to the ideal correlation between methods. Red, green and blue filled squares correspond to red, rosé and white wine samples, respectively.

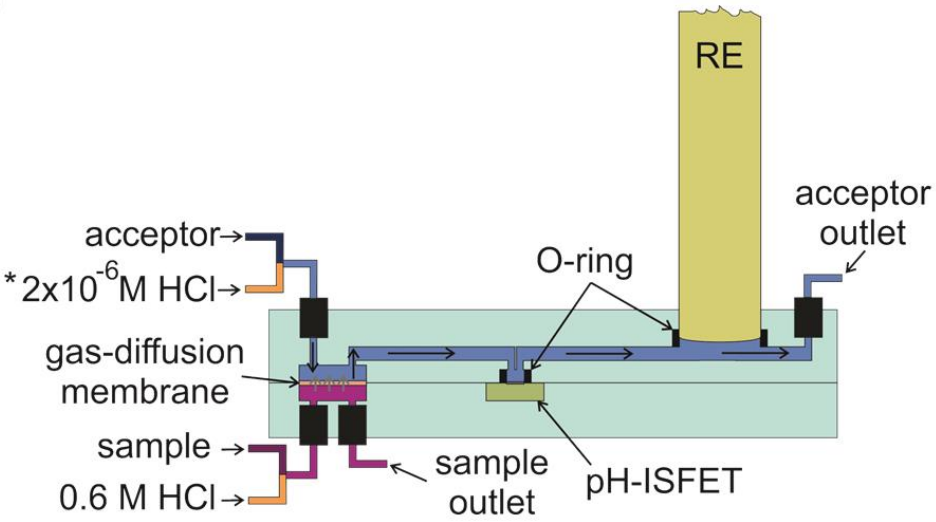
a)


Ag/AgCl, 3M
RE
fluidic inlet
fluidic outlet
gas-diffusion
membrane
pH-ISFET
fluidic inlet
fluidic outlet

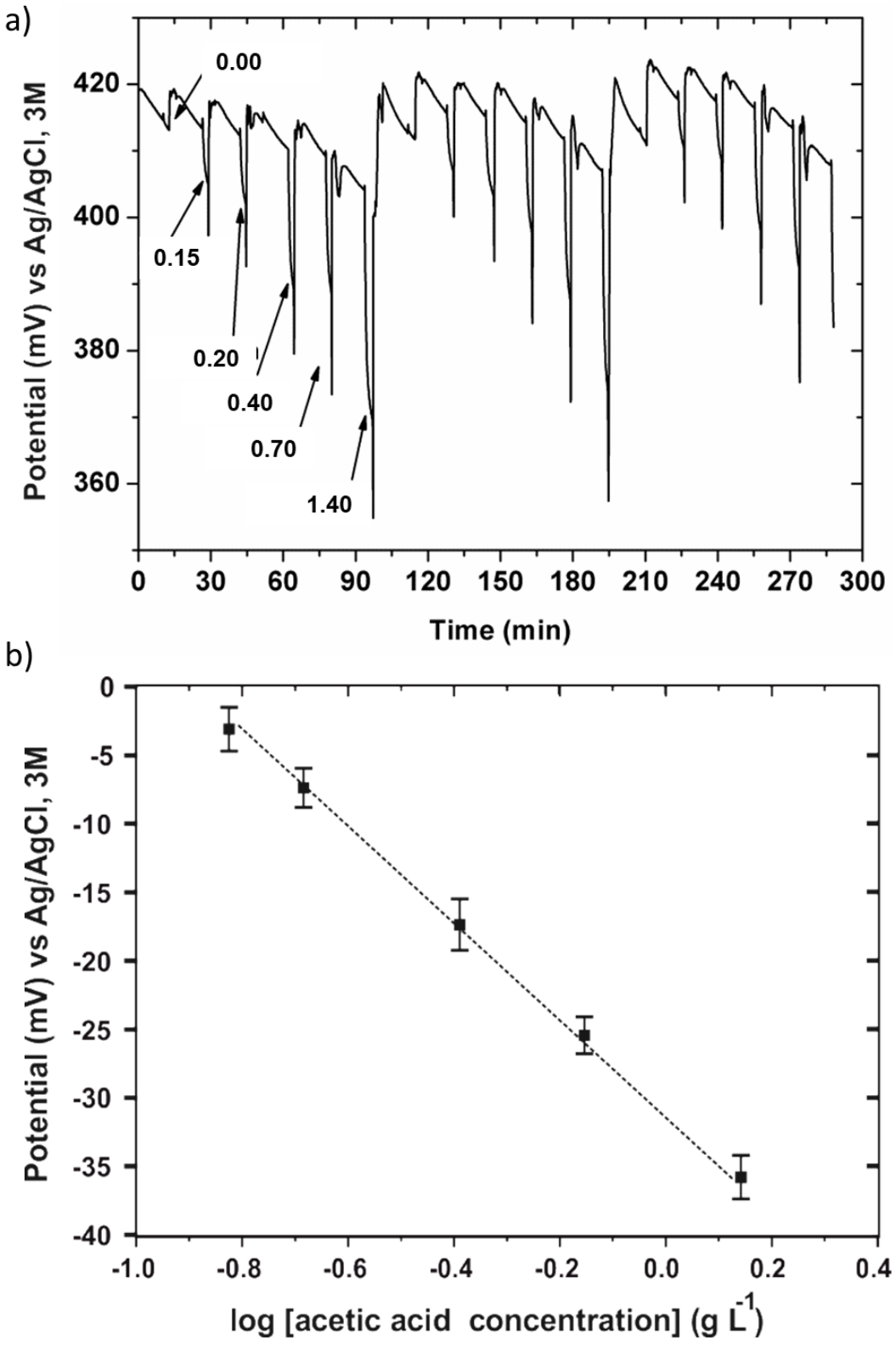
b)


Reference electrode cell
ISFET cell
difussion cell and membrane

c)


RE
acceptor
outlet
acceptor→
*2x10-6M HCl→
O-ring
gas-diffusion
membrane
sample →
0.6 M HCl→
sample
outlet
pH-ISFET

770

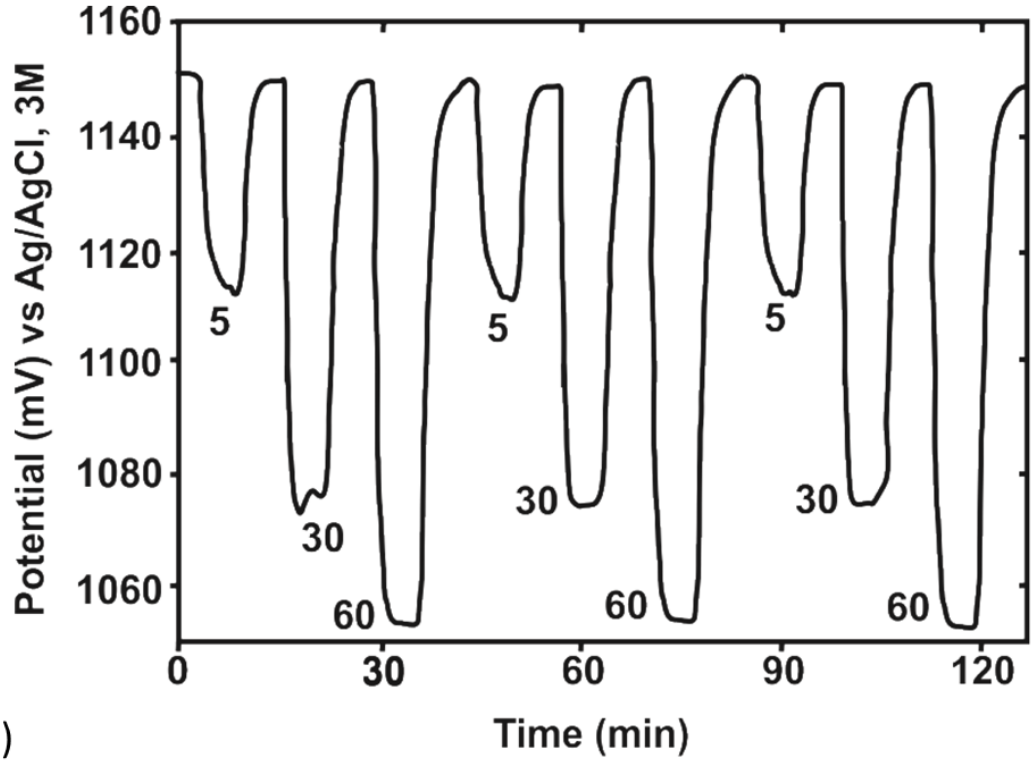

a)
Potential (mV) vs Ag/AgCl, 3M
Time (min)
0.00
0.15
0.20
0.40
0.70
1.40
b)
Potential (mV) vs Ag/AgCl, 3M
log [acetic acid concentration] (g L-1)

771

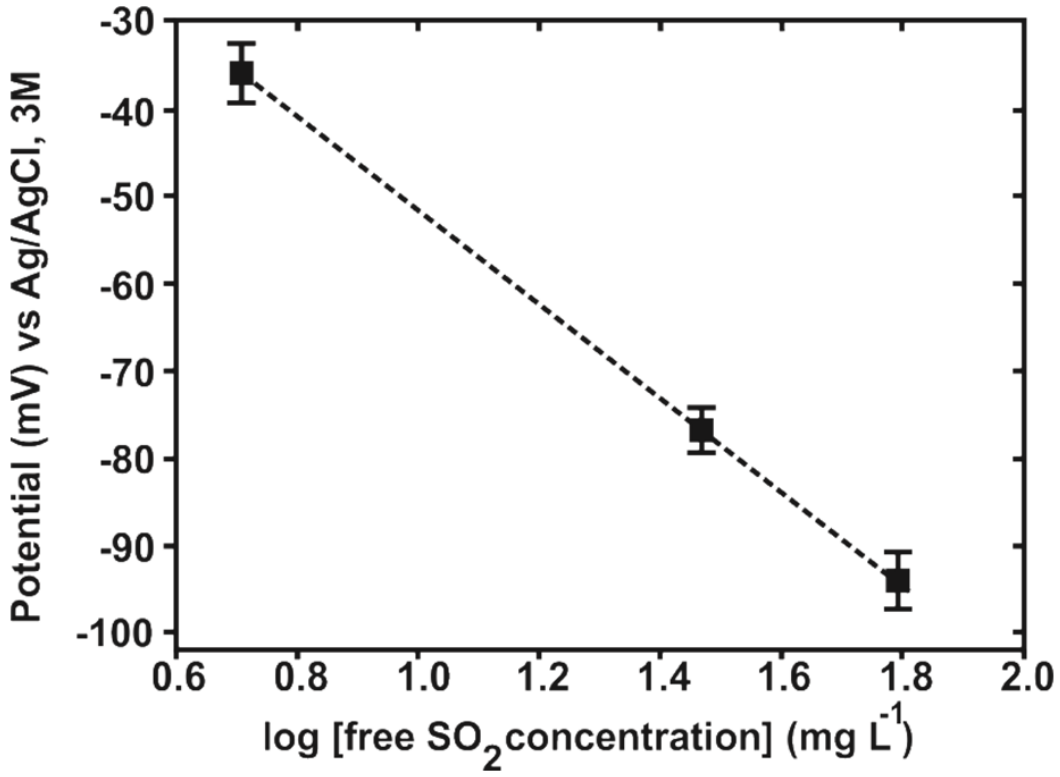
772

773

a)




b)

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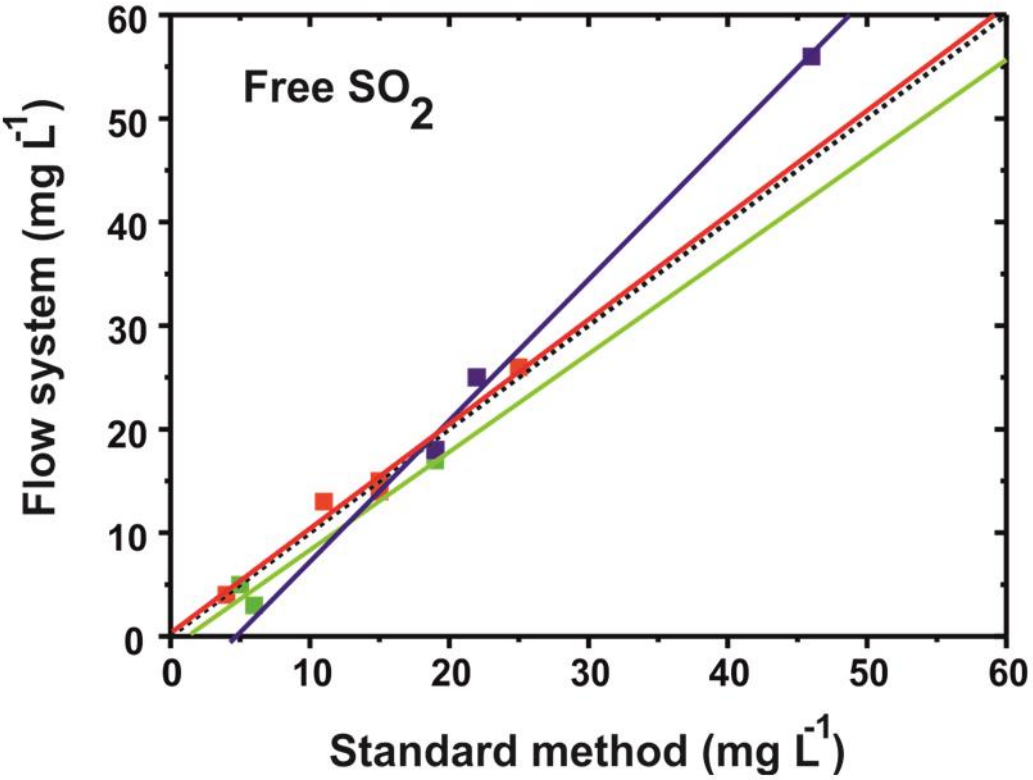
Free SO2
Flow system (mg L-1)
Standard method (mg L-1)
0
10
20
30
40
50
60

777

**INDEX OF SUPPLEMENTARY INFORMATION**

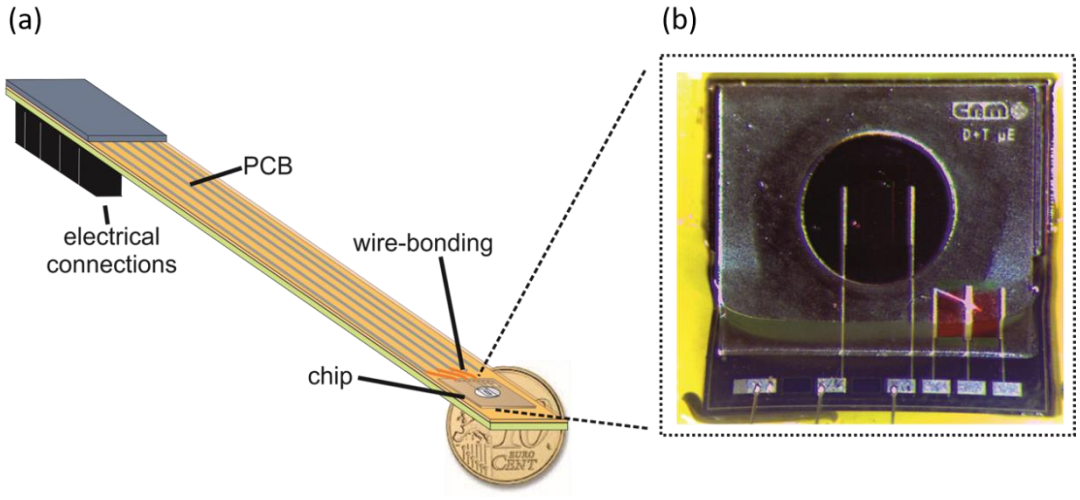


**Fig. S1.** (a) Drawing of the encapsulated pH-ISFET on a PCB and (b) picture detailing the pH-ISFET chip.

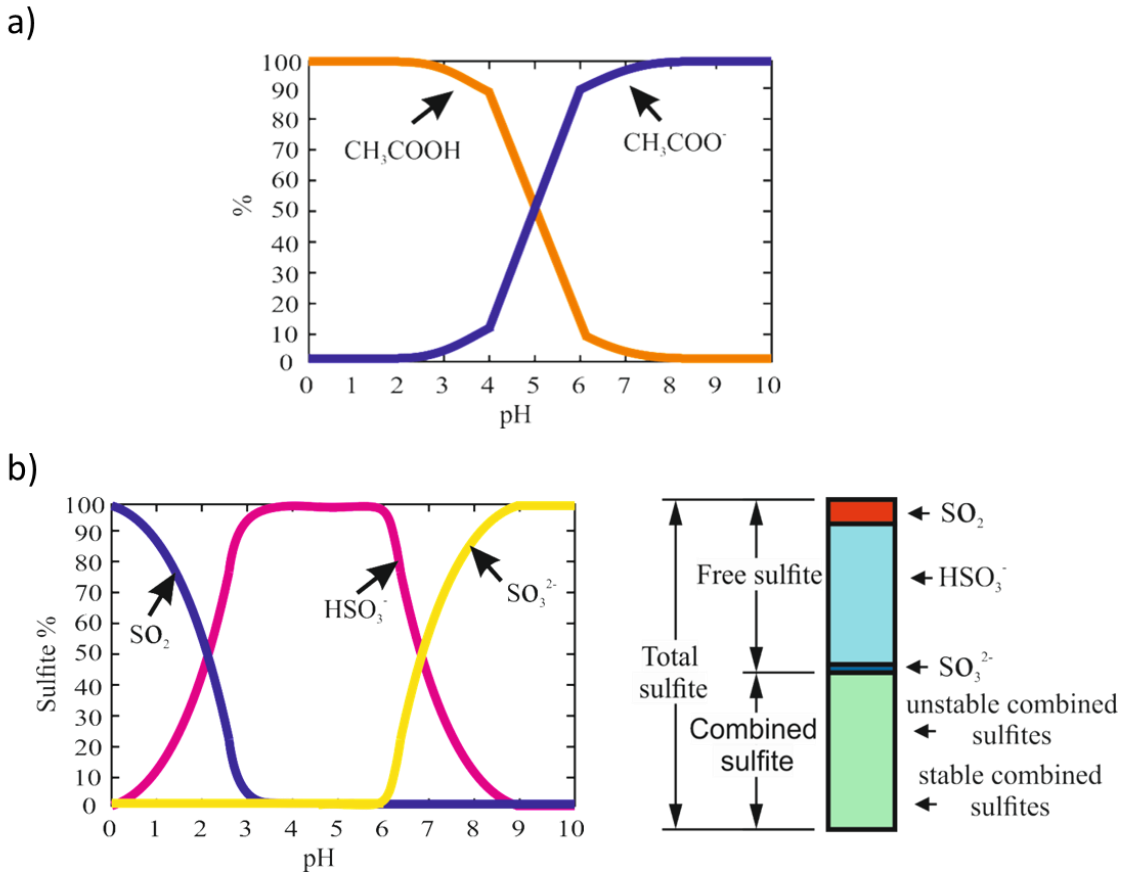


**Fig. S2.** (a) Percentage of the chemical forms of sulfite as function of pH and scheme of the different chemical forms of sulfite in real wine. (b) Percentage of acetic acid and acetate as function of pH.

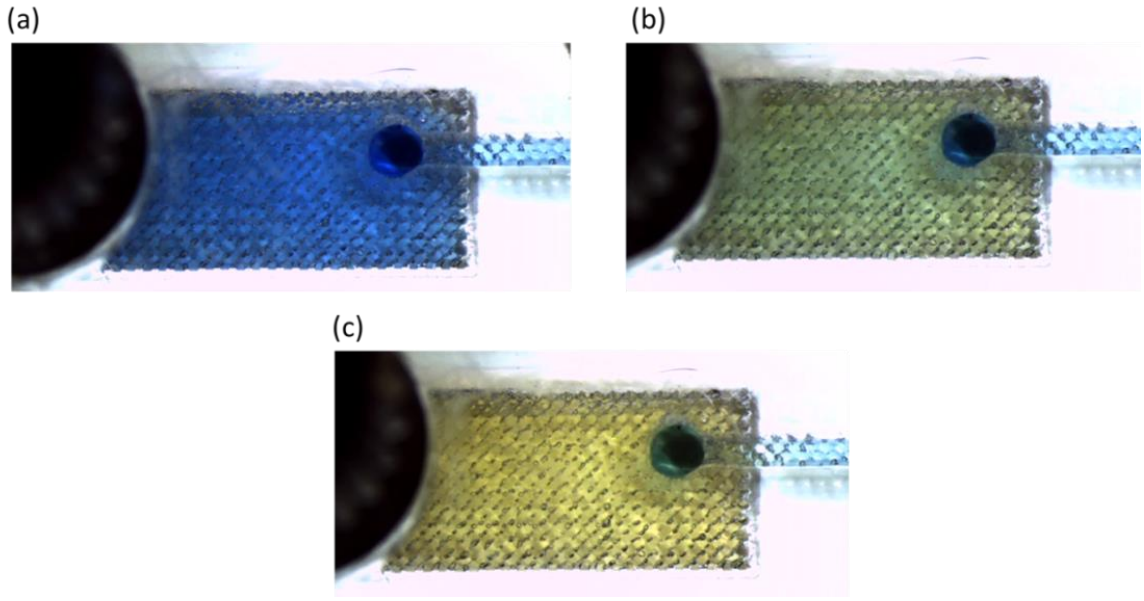


**Fig. S3.** Pictures of the acceptor solution chamber recorded on video in presence of bromothymol blue, a colorimetric pH indicator: (a) at the beginning of the test; (b) after 5 min and (c) after 10 min of contact between a solution containing 1 g $L^{-1}$ of acetic acid and the acceptor solution.

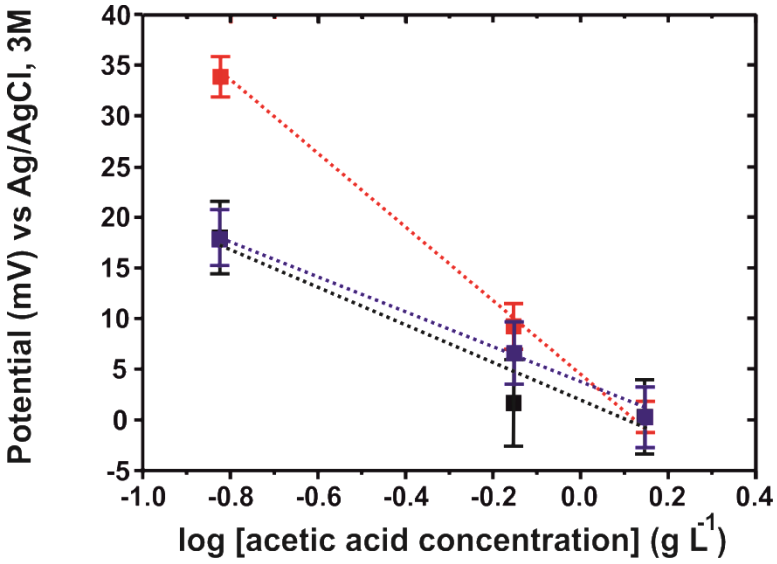


**Fig. S4.** Calibration curves obtained for the determination of 0.15, 0.70 and 1.40 g $L^{-1}$ acetic acid using an acceptor solution with $10^{-2}$ (black color), $10^{-3}$ (red color) and $10^{-4}$ M (blue color) $CH_3COOK$ concentrations. Standard deviation of triplicated calibrations in mV is drawn as error bars in the calibration plots.

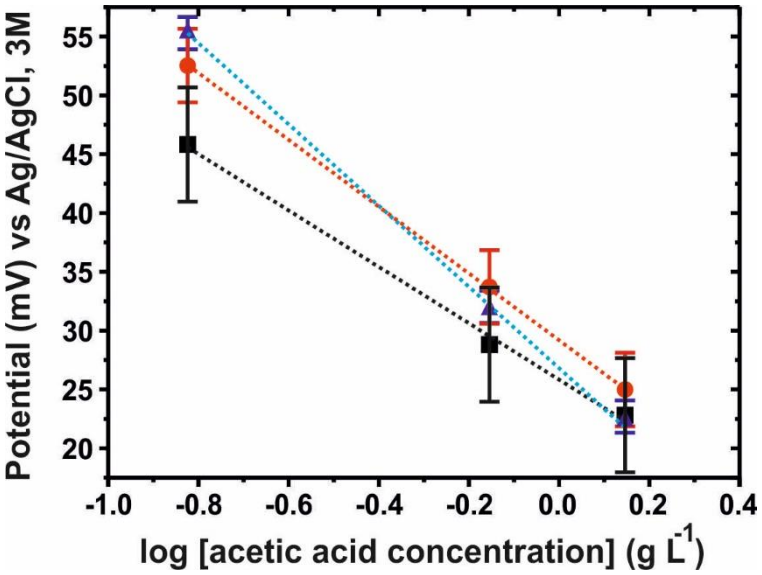


**Fig. S5.** Calibration curves obtained for the determination of 0.15, 0.70 and 1.4 g $L^{-1}$ acetic acid in presence of an acceptor solution without pH adjustment (blue color), with pH adjusted at 5.5 (black color) and with pH adjusted at 7 (red color). Standard deviation of triplicated calibrations in mV is drawn as error bars in the calibration plots.

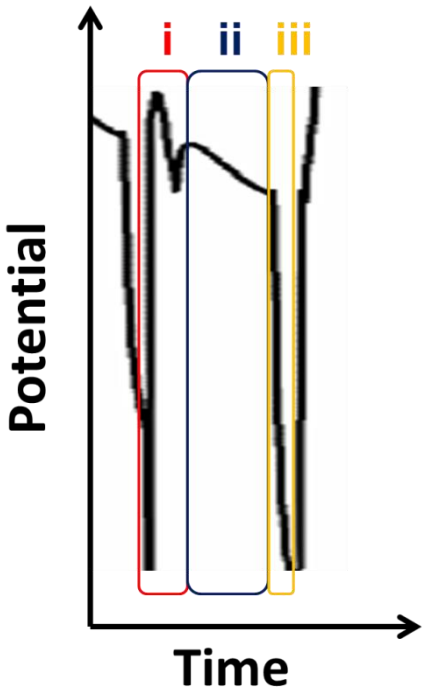


**Fig. S6.** Detail of one analytical response obtained during the acetic acid determination. The letters inside the plot represent the signal recorded in the three consecutive steps of the experimental procedure: (i) the acceptor and the acidified sample flowed during 5 min; (ii) the diffusion through the membrane in stop-flow during 10 min; and (iii) the signal recorded during 2 min in stop flow after flowing the acceptor solution to the pH-ISFET during 10 s.

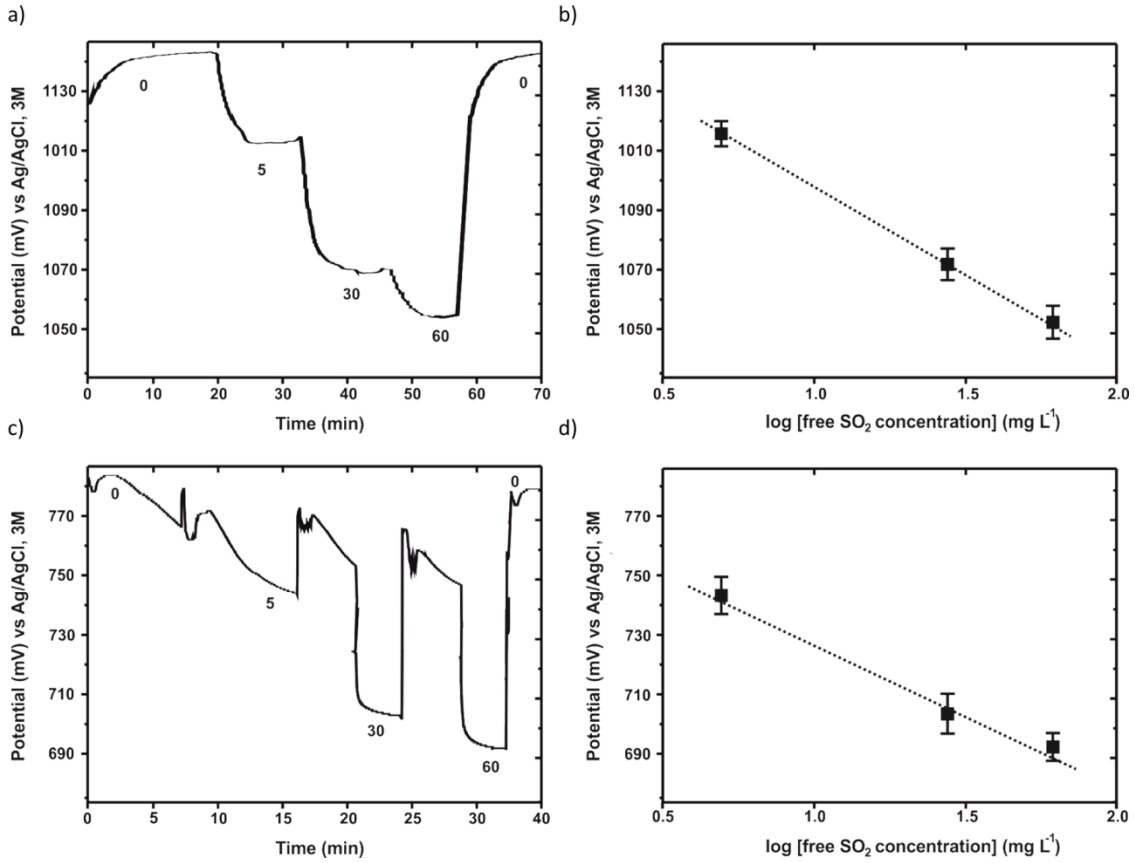


**Fig. S7.** a) Recording of the pH-ISFET signal (mV) under continuous flow conditions and (b) the obtained calibration plot for 0, 5, 30 and 60 mg $L^{-1}$ of free $SO_2$ . c) Recording of the pH-ISFET signal (mV) under stop flow conditions and (d) the obtained calibration plot for 0, 5, 30 and 60 mg $L^{-1}$ of free $SO_2$ . The numbers depicted in (a) and (c) plots represent the concentration of free $SO_2$ (mg $L^{-1}$) injected in the microanalytical flow system. Standard deviation of triplicated calibrations in mV is drawn as error bars in the calibration plots.

**Table S1.** Values of acetic acid concentration obtained with the microanalytical flow system for the red *Don Simón* and the white *Sumarroca* wine samples. Theoretical values correspond to the sum of the experimental value calculated from the sample without spiking acetic acid and that of each spiked sample. The standard deviation for the flow system is represented in brackets (n=3).

| **Wine** | **Acetic acid concentration (g $L^{-1}$)** | | **% Recovery*** |
|---|---|---|---|
| | **Flow system (n=3)** | **Theoretical** | |
| *Don Simón* | 0.56 (0.10) | 0.56 | - |
| *Don Simón* + 0.15 g $L^{-1}$ | 0.67 (0.03) | 0.71 | 94 |
| *Don Simón* + 0.50 g $L^{-1}$ | 1.04 (0.02) | 1.06 | 98 |
| *Don Simón* + 1.00 g $L^{-1}$ | 1.56 (0.04) | 1.56 | 100 |
| *Sumarroca* | 0.41 (0.03) | 0.41 | - |
| *Sumarroca* + 0.15 g $L^{-1}$ | 0.54 (0.01) | 0.56 | 96 |
| *Sumarroca* + 0.50 g $L^{-1}$ | 0.83 (0.05) | 0.91 | 91 |
| *Sumarroca* + 1.00 g $L^{-1}$ | 1.18 (0.05) | 1.41 | 84 |

* *100 × (experimental value/theoretical value)*

**Table S2.** Values of acetic acid concentration obtained with the microanalytical flow system and the standard method for several wine samples. The standard deviation for the microanalytical flow system (n = 3) is represented in brackets. The confidence intervals are calculated with a level of 95% for the standard method.

| Wine[1] | Free acetic acid concentration (g $L^{-1}$) | | |
|---|---|---|---|
| | **Microanalytical flow system (n=3)** | **Standard method[2]** | **Relative error %** |
| W1 | 0.99 (0.05) | 1.20 ± 0.15 | -19 |
| W2 | 0.15 (0.01) | 0.18 ± 0.02 | -14 |
| W3 | 0.18 (0.02) | 0.21 ± 0.03 | -16 |
| W4 | 0.11 (0.01) | 0.13 ± 0.02 | -14 |
| W5 | 0.09 (0.01) | 0.12 ± 0.01 | -20 |
| R1 | 0.31 (0.03) | 0.37 ± 0.05 | -15 |
| R2 | 0.45 (0.04) | 0.52 ± 0.06 | -14 |
| RO1 | 0.18 (0.01) | 0.21 ± 0.03 | -15 |
| RO2 | 0.15 (0.01) | 0.18 ± 0.02 | -16 |

[1]W: white wine; R: red wine; RO: Rosé wine.
[2]Confidence interval calculated by IRTA-INCAVI from the results of inter-comparison analysis.

**Table S3.** Values of free $SO_2$ concentration obtained with the microanalytical flow system for the *Don Simón* red and the *Sumarroca* white wine samples. Theoretical values correspond to the sum of the concentration of wine calculated experimentally with the flow system plus the 70 % of added $SO_2$, assuming that the other 30 % is bounded to aldehydes, ketones, sugars or acids present in wines. The standard deviation for the microanalytical flow system is represented in brackets (n = 3).

| **Wine** | **Free $SO_2$ concentration (mg $L^{-1}$)** | | **% Recovery** |
|---|---|---|---|
| | **Flow system (n=3)** | **Theoretical** | |
| *Don Simón* | 23 (2) | 23 | - |
| *Don Simón* + 5 mg $L^{-1}$ | 27 (2) | 27 | 102 |
| *Don Simón* + 10 mg $L^{-1}$ | 27 (2) | 30 | 90 |
| *Don Simón* + 15 mg $L^{-1}$ | 31 (1) | 34 | 91 |
| *Don Simón* + 40 mg $L^{-1}$ | 47 (2) | 51 | 92 |
| *Sumarroca* | 21 (2) | 21 | - |
| *Sumarroca* + 5 mg $L^{-1}$ | 24 (2) | 25 | 98 |
| *Sumarroca* + 10 mg $L^{-1}$ | 27 (2) | 28 | 96 |
| *Sumarroca* + 15 mg $L^{-1}$ | 30 (1) | 32 | 95 |
| *Sumarroca* + 40 mg $L^{-1}$ | 45 (2) | 49 | 92 |

**Table S4.** Values of free $SO_2$ concentration obtained with the microanalytical flow system and the standard method for several wine samples. The standard deviation for the microanalytical flow system (n = 3) is represented in brackets. The confidence intervals are calculated with a level of 95% for the standard method.

| Wine[1] | Free $SO_2$ concentration (mg $L^{-1}$) | | | Volatile acidity (g $L^{-1}$)[3] |
|---|---|---|---|---|
| | Microanalytical flow system (n=3) | Standard method[2] | Relative error % | |
| W1 | 15 (0) | 12 ± 4 | 22 | 1.20 ± 0.15 |
| W2 | 12 (0) | 9 ± 3 | 35 | 0.18 ± 0.02 |
| W3 | 15 (0) | 12 ± 4 | 28 | 0.21 ± 0.03 |
| W4 | 18 (1) | 21 ± 4 | -13 | 0.13 ± 0.02 |
| W5 | 25 (2) | 27 ± 8 | -7 | 0.12 ± 0.01 |
| R1 | 35 (1) | 33 ± 12 | 5 | 0.37 ± 0.05 |
| R2 | 16 (1) | 16 ± 5 | 0 | 0.52 ± 0.06 |
| RO1 | 19 (1) | 23 ± 7 | -18 | 0.21 ± 0.03 |
| RO2 | 15 (0) | 13 ± 4 | 14 | 0.18 ± 0.02 |
| R1 * | 36 (2) | – | 10 | – |
| W5 * | 26 (2) | – | -4 | – |
| RO2 * | 15 (1) | – | 15 | – |

[1]W: white wine; R: red wine; RO: Rosé wine.
[2]Confidence interval calculated by IRTA-INCAVI from the results of inter-comparison analysis.
[3] Standard method applied by the IRTA-INCAVI.
[*]Spiked with 0.7 g $L^{-1}$ acetic acid.

**Table S5.** Least squares parameters obtained comparing the microanalytical flow system and the standard method for acetic acid and free $SO_2$ determination. The uncertainty intervals are calculated at the 95% confidence level.

| **Wine*** | **Acetic acid** | | |
|---|---|---|---|
| | **Slope** | **Intercept (g $L^{-1}$)** | **r** |
| Red wine | 0.71 ± 0.06 | 0.12 ± 0.03 | 0.98 |
| **Wine*** | **Free $SO_2$** | | |
| | **Slope** | **Intercept (mg $L^{-1}$)** | **r** |
| White wine | 1.4 ± 0.1 | -7.0 ± 3.0 | 0.99 |
| Red wine | 1.0 ± 0.1 | 0.2 ± 1.0 | 0.98 |
| Rosé wine | 1.0 ± 0.2 | -1.0 ± 2.0 | 0.93 |

* W8 not included in the analysis of free $SO_2$