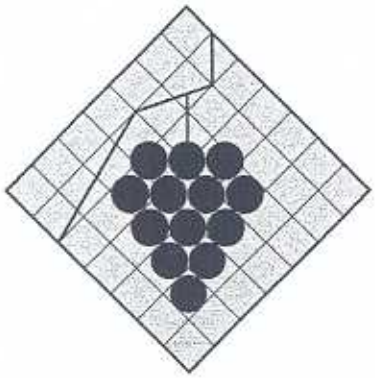


Rapid determination of free and total sulphur dioxide in wine

FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **ULA 1V (1GW)**

Principal Investigator: **Dr T J Cardwell**

Research Organisation: **La Trobe University**

Date: **January 1991**

FINAL PROJECT REPORT 1989/90

Project Title	Rapid Determination of Free and Total Sulfur Dioxide in Wine.
Project Number	ULA IV
Organisation	La Trobe University Department of Chemistry
Location	Bundoora, Victoria.
Supervisors	Drs.T.J.Cardwell, R.W.Cattrall and G.R.Scollary
Funding	$3,910
Time Span	1989-1990

Objective

* To develop a procedure, based on flow injection analysis, for the rapid determination of free and total sulfur dioxide in wines.
* To evaluate and compare existing commercial instrumentation with that developed in this project.

Research Results and Benifits

Before the start of this project, the Analytical Research group at La Trobe University had developed a new flow injection procedure for the determination of free and total sulfur dioxide in red and white wines. The values for free sulfur dioxide in red wines obtained by the new procedure, as with the Aspiration - oxidation procedure, are a combination of free plus pigment bound forms of SO_2. A spectrophotometric method is accepted by the Australian wine industry for the determination of the real free SO_2 in red wines. The spectral method was used for comparative purposes in the present project.

A new electrochemical procedure has been developed which yields results for SO_2 in red wines that correlate well with the data obtained by the spectral procedure. The method is extremely simple as it requires minimal pretreatment of the sample before analysis. An electrolyte at pH 1.8 is added to the wine sample before SO_2 is reduced at a glassy carbon electrode by AC voltammetry. As there is negligible interference from oxygen, there is no need to deoxygenate the sample before analysis which is the usual practice in polarographic methods. Voltammetric scans show that anthocyanins in red wines and anthocyanin-bound SO_2 do not interfere in the measurement of free SO_2.

On the average, 5-6 samples can be analysed per hour by AC voltammetry whereas it requires about 1 hour to do a spectrophotometric analysis. Furthermore, the electrochemical method gives better precision at levels of SO_2 below 4 ppm.

Difficulties encoutered

Although the project was successful in developing a rapid procedure for measurement of the true free SO_2 in red wines, attempts to adapt the new method to flow injection analysis were unsuccessful. The major obstacle to be overcome is the unavailability of commercial AC detectors for use in flow-based procedures. Additional experiments using a combination of coulometric and amperometric flow-cells set at different potentials in DC detection modes also failed to differentiae between free and pigment-bound SO_2.

Recommendation for future work

Future work should be directed towards the design and construction of an AC amperometric detector suitable for use with conventional flow-cells in liquid chromatography or continuous flow analytical techniques.

Publications

The AC voltammetric procedure developed in the project is described in detail in the following publication:

"Determination of Free Sulfur Dioxide in Red Wine by Alternating Current Voltammetry", T.J.Cardwell, R.W Cattrall, Guo Nan Chen, G.R.Scollary and I.C.Hamilton, Analyst, in press.
(A copy is attached to this report).

T.J.Cardwell

T.J.Cardwell
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DETERMINATION OF FREE SULFUR DIOXIDE IN RED WINE BY ALTERNATING CURRENT VOLTAMMETRY

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Abstract

Anthocyanins and oxygen interfere in the determination of " free SO_2 " in red wine by DC and DP voltammetry. However, the AC voltammetric behaviour of anthocyanins extracted from red wine shows that there is no interference in the determination of SO_2. Furthermore, there is minimal interference from oxygen which overcomes the need for deoxygenation and its associated loss of SO_2.

A phase selective second harmonic AC voltammetric procedure is described for the determination of " free SO_2 " in red wine. The results of the analysis of several red wine samples are compared with the spectrophotometric method accepted by the Australian wine industry.

Keywords: AC Voltammetry; red wine analysis; free SO_2 analysis; anthocyanin interference.

Sulfur dioxide is traditionally used as a preservative in winemaking to prevent oxidation and bacterial growth. Dissolved sulfur dioxide in wine always coexists with "free SO_2" and "bound SO_2 ". "Free SO_2" is an equilibrium mixture of S(IV) species consisting of sulfur dioxide, bisulfite and sulfite, and the relative concentration of each species is a function of the pH of the medium. "Bound SO_2", which occurs in many different forms, is mainly divided into carbonyl bound (eg. with acetaldehyde) and pigment bound (eg. with anthocyanin), the latter existing in red wine. Several methods have been recommended for the determination of "free SO_2" in white wines [1-8]. However, there is still no satisfactory method for determination of "free SO_2" in red wines, not only because of the low actual level of "free SO_2" which can be tolerated in red wines but also because of the interference of anthocyanins. An aspiration-oxidation method, which was recommended for adoption by the Association of Official Analytical Chemists (AOAC)[6], has been widely used for the determination of free and total SO_2 in the Australian wine industry[9]. This method has the major advantage that free sulfur dioxide can be determined accurately in white wines, but it is still very difficult to determine the real "free " sulfur dioxide in red wines. So called "free SO_2" in this method for red wines is actually the sum of free and pigment bound SO_2. A spectrophotometric method is accepted by the Australian wine industry for the determination of free sulfur dioxide in red wines[10,11].

Several procedures for the determination of sulfur dioxide have been based on electrochemical reduction at a dropping mercury

electrode[12-23]. In this paper, the reduction of sulfur dioxide and other wine constituents has been carried out on a glassy carbon electrode using DC, AC and pulse voltammetric techniques. A phase selective second harmonic AC voltammetric procedure is recommended for the determination of "free SO_2" in red wine and the results of these analyses are compared with those from the spectrophotometric method accepted by the Australian wine industry.

EXPERIMENTAL

Instrumentation

The polarograph used was the Metrohm Polarecord E506 with a three-electrode cell consisting of a saturated calomel reference electrode, a platinum auxiliary electrode and a glassy carbon working electrode.

Spectrophotometric analysis of the red wine samples was carried out using a Shimadzu Recording Spectrophotometer UV-240.

Reagents and solutions

A stock solution of sulfur dioxide (1000 mg l^{-1} SO_2) was prepared fresh daily from analytical reagent-grade sodium metabisulfite and then standardised against a standard iodine solution. The supporting electrolyte for all voltammetric measurements was made up by adding 0.2 M HCl to 0.2 M KCl solution until the pH was 1.8.

An anthocyanin concentrate from red wine was prepared by solvent extraction[10] of 650 ml of red wine successively with four 200 ml portions of iso-amyl alcohol, previously equilibrated with

10% v/v ethanol/water saturated with potassium hydrogen tartrate. The combined extracts (800 ml) were shaken with 400 ml of petroleum ether (boiling range 60-80 °C) plus 1 ml 1M hydrochloric acid, the lower aqueous phase containing most of the anthocyanin was separated. This was evaporated at 30 °C to remove traces of solvent and finally rediluted to 40 ml. Analysis of the concentrate was carried out by two-dimensional paper chromatography using a two phase solvent system consisting of butanol-acetic acid-water (4 : 1 : 5)[24]. Matching of R_f values showed that the extract contained chiefly malvidin-3-glucoside pigments with only trace amounts of other monomeric anthocyanins[10,24].

Procedures

Voltammetric procedure for standards.

A 20 ml aliquot of a sulfur dioxide standard solution was added to the electrochemical cell, 2.0 ml of the KCl-HCl electrolyte solution (pH 1.8) was added and the solution mixed. The surface of the working electrode was freshly polished with alumina powder before each scan. Phase-selective second-harmonic AC voltammograms were recorded by scanning the potential from -0.1 to -1.4 V (vs S.C.E.) at a scan rate of 16 mV s^{-1}. with 20 mV amplitude and zero phase angle. Peak heights were measured and a standard calibration curve was plotted.

Voltammetric procedure for wine samples

After addition of electrolyte to 20 ml of the wine sample as in the above procedure, the pH was adjusted to 1.8 by the dropwise

addition of 3M HCl and allowance made for the volume change in the final calculation. Peak heights were measured under the same conditions as the standards and the concentration of sulfur dioxide in the wine sample was evaluated from the calibration plot. A "blank" wine sample was obtained from a wine sample containing added electrolyte by bubbling nitrogen through it to remove the sulfur dioxide.

Spectrophotometric procedure:[10,11]

1. The wine spectrum was recorded in the region 400-550 nm using a 1 mm pathlength cell of volume 0.33 ml. The absorbance (A_0) was measured at 520 nm.

2. 5 µl Sodium metabisulfite solution was added to the above sample (giving approximately 2000 mg l^{-1} SO_2), mixed thoroughly by inversion and the spectrum recorded again after 1 min. The absorbance (A_1) of this solution was measured at 520 nm.

3. 20 µl Acetaldehyde solution was added to 2.0 ml fresh wine sample. After about 45 min. at 25 °C, the absorbance (A_2) was measured at 520 nm in a 1 mm cell.

Free SO_2 in the wine sample was calculated as follows:

$$\text{Free } SO_2 \text{ (mg } l^{-1}) = 3.84\,(A_2 - A_0)/(A_0 - A_1)$$

RESULTS AND DISCUSSION

Voltammetric behaviour of anthocyanins

The anthocyanins are red pigments widely distributed in red wine and they are known to exist as the mono- and diglucosides of common anthocyaninidins such as cyanidin, peonidin, delphinidin, petunidin and malvidin[24]. The sample of anthocyanin used in this work was extracted from a red wine and has been confirmed by paper chromatographic examination to contain chiefly malvidin-3-glucoside[10,24].

Several chemical properties of anthocyanins influence their structure and consequently their coloration which in turn affect the color of red wine. Under the weakly acid conditions in wines, anthocyanins exist in a red oxonium form which is in reversible equilibrium with a colorless pseudo-base, the position of the equilibrium being dependent on the pH as represented by the general reaction equation:

$$\underset{\text{red}}{[AnO]^+} + OH^- \text{ ===== } \underset{\text{colorless}}{[AnO]OH}$$

where AnO^+ designates the oxonium form of anthocyanins.

The reaction of anthocyanins with sulfur dioxide probably involves the combination of bisulfite anion (which predominates at wine pH) with the oxonium cation to form an anthocyanin-bisulfite addition complex. The general equation is

$$\underset{\text{red}}{[AnO]^+} + SO_3H^- \text{ ====== } \underset{\text{colorless}}{[AnO]SO_3H}$$

which again decreases the color by forming a colorless compound. This reaction is the foundation of the spectrophotometric procedure for the determination of free sulfur dioxide in red

wines.

DC and differential pulse voltammetric scans at a glassy carbon electrode show that sulfur dioxide and anthocyanin give overlapping peaks at -0.60 V and -0.46 V (vs S.C.E.), respectively. When sulfur dioxide is added to an anthocyanin solution, after about 30 mins. the SO_2 peak potential shifts to -0.56 V, which is very close to that for free SO_2. Hence, both anthocyanin and pigment-bound SO_2 are readily detected at a glassy carbon electrode and they would interfere with the determination of sulfur dioxide using the DC (or DP) voltammetric procedure.

The phase selective fundamental harmonic and phase selective second harmonic AC voltammograms of sulfur dioxide and anthocyanin are shown in Figs. 1 and 2, respectively. In both scans, sulfur dioxide gives a peak at -0.60 V and anthocyanin a peak at -0.52 V. Moreover the reduction current of anthocyanin is much smaller than that observed for sulfur dioxide, yet the concentration of anthocyanin used in these scans is of the order of 2100 mg l^{-1} (measured spectrophotometrically[11]), which is 2-10 times higher than the normal concentration of anthocyanins in red wine (200-1000 mg l^{-1})[25]. It should be noted that the anthocyanin bound SO_2 also gives a low reduction current compared to the free SO_2 peak and its peak potential is slightly more positive than the anthocyanin peak. Therefore, neither anthocyanin and anthocyanin-bound SO_2 would interfere in the determination of sulfur dioxide by phase-selective AC voltammetric techniques as the total concentration of the anthocyanin species in wines would be much lower than those

used in the anthocyanin samples in Figs 1 and 2.

Interference from oxygen

It is well known that dissolved oxygen is reduced during cathodic voltammetric scans and it is normal practice to remove the oxygen by passing nitrogen through the solution for about 10 mins. before the run. The electrochemical reduction of sulfur dioxide at a glassy carbon electrode is dependent on the pH of the solution giving the most sensitive signal at about pH 2. At this pH, calculations show that molecular sulfur dioxide represents about 50% of the total sulfur(IV) species present in solution. As nitrogen is bubbled through the solution to remove oxygen, molecular sulfur dioxide is easily removed from solution at low pH. If the pH of the solution is higher than pH 3, at which the molecular sulfur dioxide represents about 5% of the total, the signal will decrease after deoxygenation, but not to the same extent as at pH 2 or lower. Fig. 3 shows that oxygen interferes in the determination of sulfur dioxide using DP voltammetry at a glassy carbon electrode and a similar result is obtained using DC voltammetry.

It is well known that the electrochemical reduction of oxygen is a slow charge transfer process, so AC voltammetry may be suitable for the determination of sulfur dioxide in the presence of oxygen. The phase selective second harmonic AC voltammograms in Fig. 4 show that there is negligible interference from oxygen in the determination of sulfur dioxide in solutions where their concentrations are similar. As the levels of oxygen in bottled wines are much lower than that used in Fig 4,[27] it is possible to determine sulfur dioxide in wine samples by this method without

interference from oxygen. Furthermore, one avoids the loss of sulfur dioxide from solution which would accompany the normal deoxygenation step as there is no need to deoxygenate the solution before doing the scan.

Optimization of experimental parameters

Condition of the electrode surface.

In the analysis of sulfur dioxide in wine samples, the reproducibility of the method is very dependent on the condition of the surface of the glassy carbon elecrode. In repeated voltammetric scans, the height of the SO_2 peak was found to decrease unless the electrode surface was polished before each scan. Electrochemical pre-treatment of the electrode was found to be ineffective in conditioning of the surface before each scan. In general, precision was of the order of 5% rsd (n = 5) on a polished electrode at levels of SO_2 below 4 mg l^{-1}

Effects of pH.

As in the case of mercury electrodes in polarography[14,17-19], the electrochemical reduction of sulfur dioxide at a glassy carbon electrode is dependent on the pH of the solution. No reduction wave is observed in neutral or alkaline solutions, but a well-defined wave is recorded at pH < 5. The most sensitive measurements can be made in the range pH 1.0-2.3 with the maximum signal being achieved at pH 1.8.

It is interesting to note that the pH of the electrolyte not only affects the peak height but also the difference in the potentials of the sulfur dioxide and oxygen peaks, mainly because of a shift in the sulfur dioxide peak to more negative potentials in the pH range 2.0-5.0. Fig. 5 indicates that the largest peak potential

difference is achieved at pH 1.8 for both techniques and that natural wine pH (3.0-3.5) is not ideal for performing the voltammetric analysis. Therefore, it was considered to be essential to adjust the wine to pH 1.8 for all later experiments.

Amplitude Effects.

In the presence of oxygen, the ideal amplitudes were found to be 15-20mV since the peak height ratio and the peak-to-peak separation of the oxygen and sulfur dioxide peaks were optimal. At amplitudes above 20mV, the resolution of the two peaks is inferior. Hence, an amplitude of 20mV was chosen for all subsequent work.

Selection of phase angle.

In phase-sensitive AC techniques, it should be possible to discriminate against the charging current by careful choice of the phase angle. For a fast electron transfer AC electrode process, if measurements are carried out at a phase angle of 0° relative to the applied voltage, then the charging current will be theoretically zero. As expected, voltammograms for the sulfur dioxide system at phase angles greater than 0° tended to be less sensitive. Hence, a phase angle of 0° was chosen for all subsequent wine analyses.

Interference of other components in wine

The voltammetric behaviour of some of the more frequently occurring compounds in wine such as glucose, malic acid, benzoic acid, glycerol, proline, α-ketoglutaric acid, ascorbic acid,

succinic acid, acetaldehyde, fructose, gallic acid, pyruvic acid and arabinose were examined in the reduction mode. None of these constituents gave reduction waves in the potential range 0.0 to -1.2V.

Ascorbic acid and gallic acid (a natural component and a model phenolic compound) are easily oxidized in solution by oxygen and it is usually considered that these compounds are present in their oxidized forms in wine[26-27]. In order to investigate the possible interference of the oxidized forms of these acids in the analysis of sulfur dioxide, complete oxidation of the acids was carried out by controlled potential coulometry of individual solutions of ascorbic and gallic acids in KCl/HCl electrolyte (pH 1.8) using a large surface area carbon felt electrode maintained at +1.00V (vs SCE) for 20 mins in a stirred solution. The current was recorded during the electrolysis to determine when the oxidation was essentially 99% complete. No reduction waves were detected in the range 0.0 to -1.4 V for the voltammetric scans of these solutions at a glassy carbon electrode indicating that the oxidized forms of these two acids would not interfere in the analysis of SO_2 by cathodic AC voltammetry.

Although some common metal ions are known to be reduced in the potential range used in the voltammetric scans presented in this paper, there is no interference from Cu^{2+}, Fe^{2+} and Fe^{3+} up to 5 mg l^{-1} or from Cd^{2+} and Pb^{2+} up to 1 mg l^{-1}.

Analysis of Red Wine samples.

In principle, the fundamental and the second harmonic AC techniques could be used for the determination of free sulfur

dioxide in wine samples. Only the results of the second harmonic procedure are presented in this paper because of better discrimination against oxygen.

Using this procedure under the specified conditions, the calibration plot for sulfur dioxide is linear in the concentration range 0-20 mg l^{-1}. The analyses of 15 red wine samples were performed and a comparison of these results with the spectrophotometric method (Fig. 6) gave a correlation coefficient of 0.97.

The AC voltammetric procedure is recommended as an alternative to the spectrophotometric procedure because of the time saved in performing an analysis. On the average, 5 - 6 samples hr^{-1} can be analysed by AC voltammetry including the time required to polish the electrode, whereas it requires 1 hr to perform each analysis by spectrophotometry. At concentrations of sulfur dioxide below 4 mg l^{-1}, the precision in the AC technique (~ 5%) is better than that achieved in the spectral method and the correlation between the data from the two methods is acceptable. Furthermore, the spectrophotometric procedure is subject to significant error because the calculations rely on the use of absorbance differences which are experimentally quite small due to the low levels of free sulfur dioxide in red wines.

Acknowledgement

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FIGURE LEGENDS:

Fig. 1 First Harmonic AC voltammograms of anthocyanin and sulfur dioxide at a glassy carbon electrode.

Conditions: KCl-HCl electrolyte, pH 1.8; scan rate, 16 mV s^{-1}; amplitude, 20 mV; phase angle, 0 °; frequency, 75 Hz.

Solution identities: 1. Background; 2. 10 mg l^{-1} SO_2 solution; 3. Anthocyanin solution; 4. Anthocyanin + added SO_2(10 mg l^{-1}); 5. Solution 4 run 30 min. later.

Fig.2 Second Harmonic AC voltammograms of anthocyanin and sulfur dioxide solutions.

Frequency, 37.5 Hz; Other conditions and solution identities as in Fig.1.

Fig.3 Differential Pulse voltammogram of SO_2 in the presence of oxygen at a glassy carbon electrode.

Electrolyte: as in Fig.1; scan rate, 16 mV s^{-1}; pulse amplitude, 30 mV.

1. Background electrolyte without deoxygenation;
2. Sulfur dioxide solution (10 mg l^{-1}) without deoxygenation;
3. Background electrolyte after degassing for 10 min.

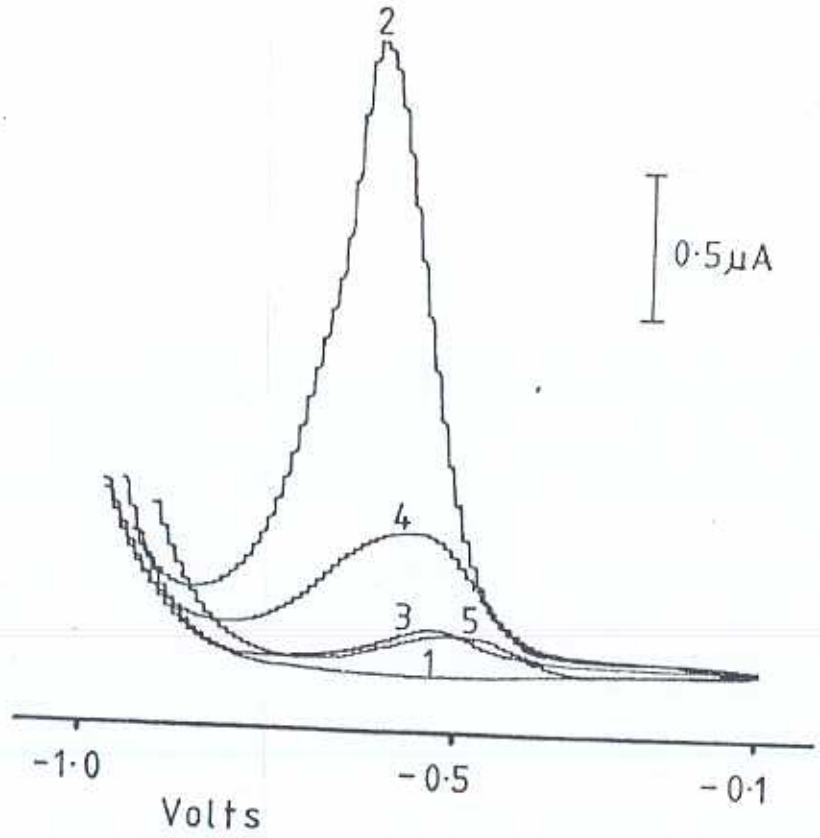
Fig.4. Second Harmonic AC voltammogram of SO_2 at a glassy carbon electrode in the presence of oxygen.

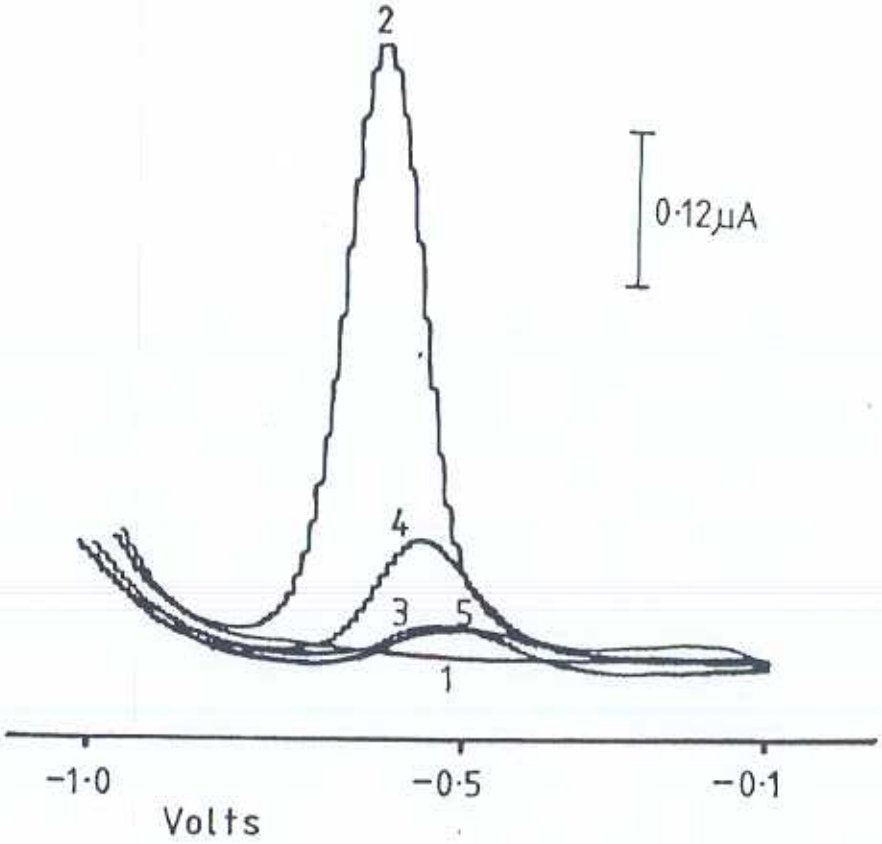
Conditions: as in Fig.2. Solution identities: as in Fig.3.

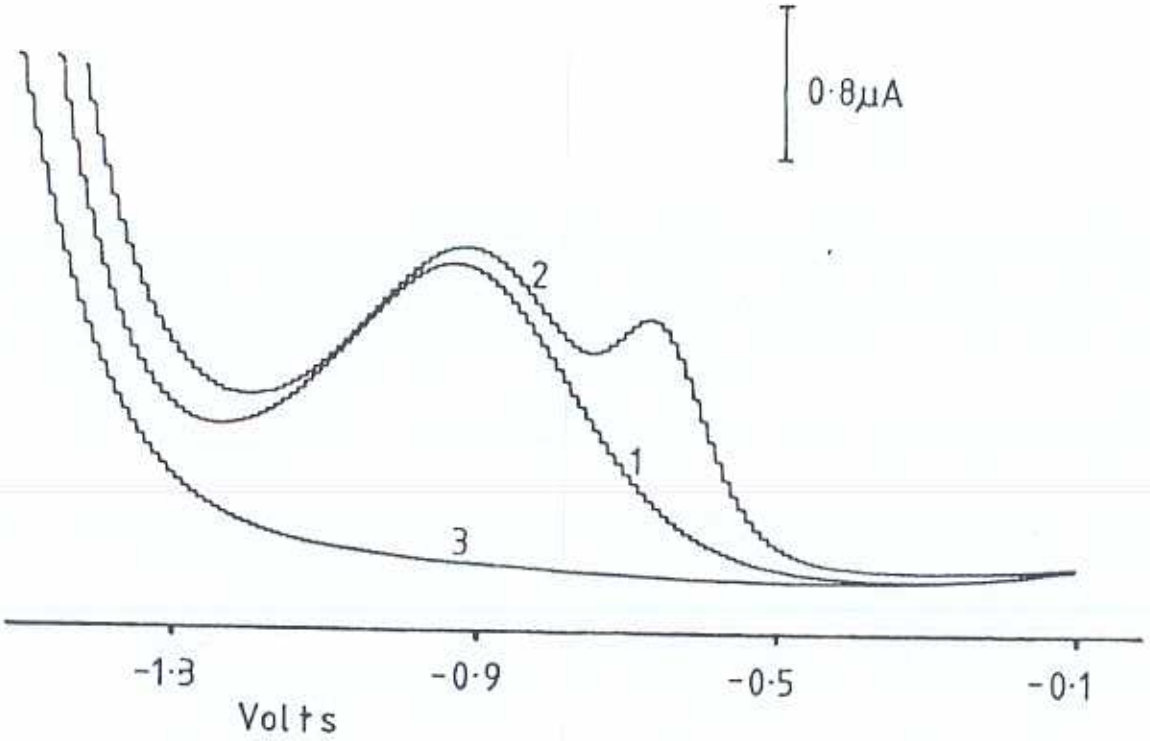
Fig.5. Effect of pH on the peak potential difference (ΔE) of the SO_2 and oxygen AC voltammetric peaks.

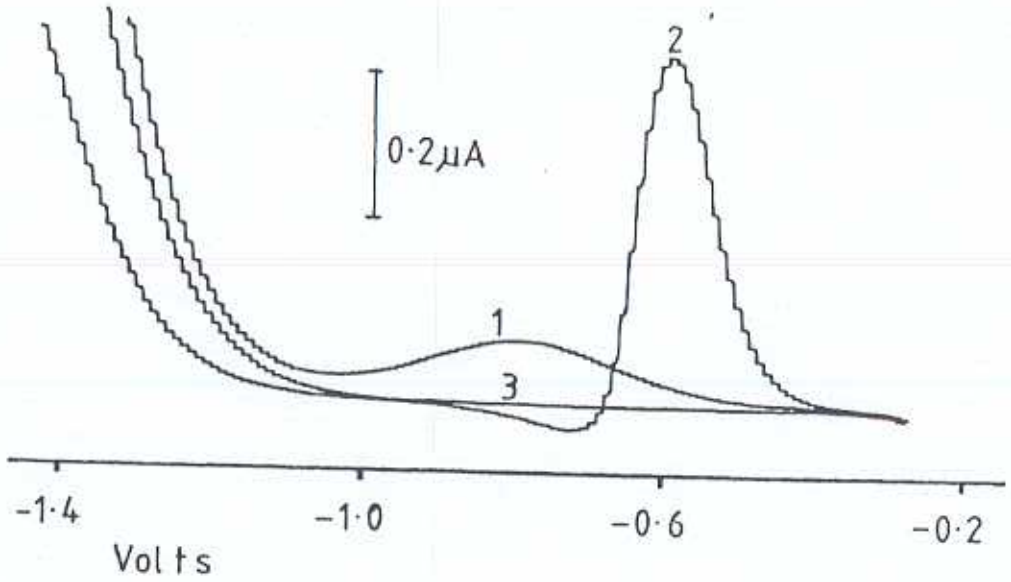
1. First Harmonic AC data; 2. Second Harmonic AC data.

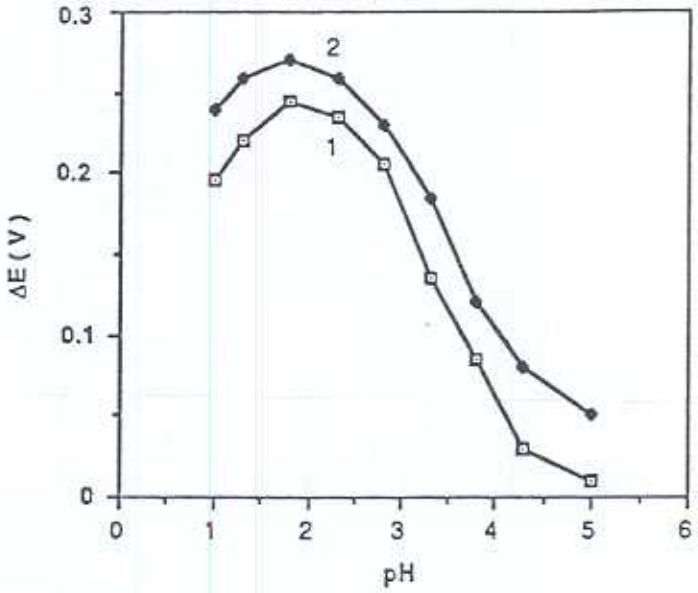
Fig.6. Correlation of AC voltammetric and spectrophotometric data for "free SO_2" in 15 red wines.

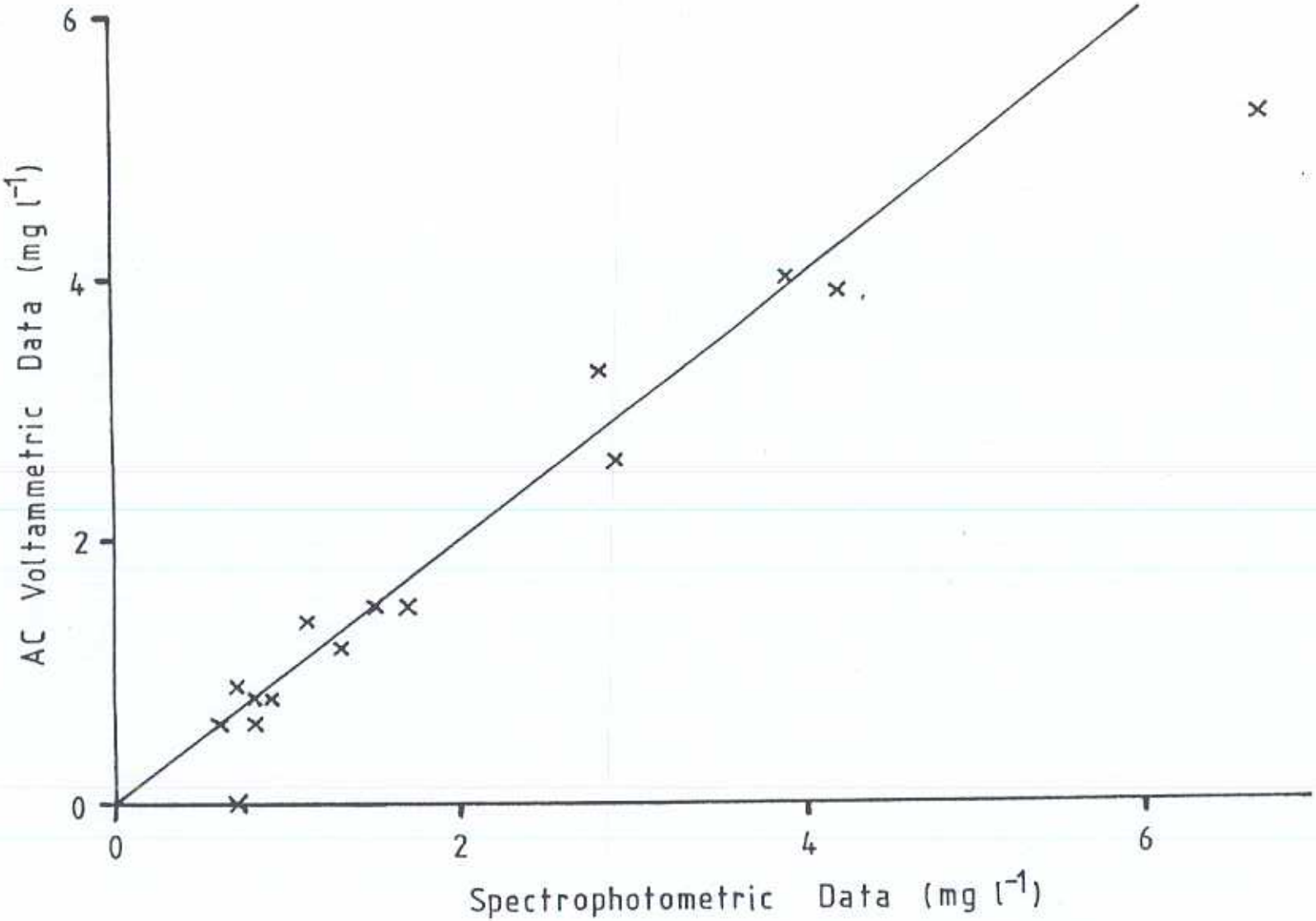

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Volts


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Volts


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pH


AC Voltammetric Data (mg l^{-1})
Spectrophotometric Data (mg l^{-1})
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