Journal International des Sciences de la Vigne et du Vin, 1995, 29, N°3, 159-169

BIBLIOGRAPHIC STUDY OF POTENTIOMETRIC METHODS OF WINE AND MUST ANALYSIS

ÉTUDE BIBLIOGRAPHIQUE DES MÉTHODES POTENTIOMÉTRIQUES D'ANALYSE DU VIN ET DU MOÛT

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Abstract : In this paper, an overview of the application of potentiometry to wine and must analysis is presented. The large number of electrodes nowadays available with good characteristics for routine use, namely good selectivity and low response times, allowing measurements in a broad linear dependence range, with a relative low cost, simple sample pre-treatment requirements, no sensitivity to colour or turbidity of the samples, make potentiometry an attractive instrumental technique for wine and must analysis. Methods developed so far present good precision and the results show a fairly good agreement with those obtained by the application of reference methods.

Résumé : . Cet article présente les applications de la potentiométrie pour l'analyse du vin et du moût. Un grand nombre d'électrodes présentant de bonnes caractéristiques pour les déterminations de routine est aujourd'hui disponible. Leurs propriétés sont d'une bonne selectivité et d'un temps de réponse court, ce qui permet des mesures sur un grand intervalle de réponse linéaire. Par ailleurs, un faible coût relatif, un traitement simple des échantillons et une absence de sensibilité à la couleur et à la turbidité font de la potentiométrie une méthode instrumentale intéressante pour l'analyse du vin et du moût. Les méthodes déjà developpées montrent une bonne précision et les résultats sont en accord avec ceux obtenus par les méthodes de référence.

Mots clefs : potentiométrie, électrodes spécifiques, vin, moût, analyse
Key words : potentiometry, ion-selective electrodes, wine, must, analysis

INTRODUCTION

The use of potentiometry as an analytical tool was quite scarce until the sixties, due to the limited number of electrodes available. In fact, only the glass electrode sensitive to proton and second-kind electrodes, such as silver/silver chloride, presented suitable characteristics to be used in direct potentiometry. Others presented lack of specificity, low reproducibility and stability, and also high response times, making its use in routine laboratories unattractive. This situation has drastically changed in the late sixties due to the appearance of the fluoride ion-selective electrode and the commercialisation in the seventies of ion-selective electrodes sensitive to silver, chloride, bromide and iodide. Since then many other units responding to inorganic and organic species have been reported and commercialised, turning potentiometry into a powerful and common instrumental method of analysis in routine laboratories.

Several properties and characteristics make electrodes, namely ion-selective electrodes (ISEs) very attractive analytical tools : (a) they present a broad linear dependence range, usually of more than four orders of magnitude of concentration ; (b) the signal is not dependent on the colour or turbidity of the samples ; (c) simple sample treatment, usually only ionic

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strength adjustment is necessary ; (d) relatively inexpensive if compared with some other instrumental methods of analysis ; (e) non-destructive nature, thus allowing sequential measurements ; (f) they can be designed with different shapes and sizes to be used either in conventional batch analysis, or to be incorporated in flow systems ; (g) they can be used in routine work by relative unskilled personnel ; (h) they are adaptable to very small sample volumes and can be constructed as micro electrodes. For all these reasons, it can be concluded that these detectors are very versatile regarding its use and its insertion in different kinds of systems. This last feature turns them particularly interesting since they can be easily incorporated in semi-automatic or fully automatic systems. The combination of these advantages makes ISEs suitable for certain applications such as food analysis, but their use requires some precautions like ensuring that the ion to be measured is in an uncomplexed state, that major interferences have been eliminated and that pH and ionic strength have been adjusted.

There are now many publications on the application of these electrochemical sensors to food analysis. These applications were reviewed by COVENEY (1980), COMER (1978) and HAILER (1975). Other authors have reviewed more restricted areas like vegetables, fruit, juices and oils (MOODY and THOMAS, 1976), beet sugar industry (HENSCHEID et *al.*, 1971) dairy products (PÉREZ-OLMOS and LIMA, 1990), brewing industry (BUCKEE, 1975).

DETERMINATION OF ANIONIC SPECIES

The determination of bromide in wine using a bromide-selective electrode (Orion Model 94-35A) has been reported by GRAF et *al.* (1976). Samples of still and carbonated bottled wines have been analysed by the multiple standard addition method, after buffering the samples with phosphoric acid, potassium nitrate and copper sulphate solutions. The authors claim that the procedure is rapid, reliable and reproducible, if the electrodes are given proper care and maintenance, and sufficient time is allowed for stabilisation of the potential measurements. The results obtained for 20 samples have shown that the bromide content ranged from 0.6 mg dm^{-3} to 1.7 mg.dm^{-3}.

A method for chloride determination in wine using a chloride-selective electrode (IS-550-Cl^-) has been developed by BERNAL et *al.* (1983). A conditioning solution which consisted of a mixture of sodium nitrate, acetic acid and sodium hydroxide has been used in this work. The use of a multiple standard addition method has been recommended due to the ethanol interference. The results showed good agreement when compared with those obtained by the reference analytical method recommended by the O.I.V., seeing that the average percentage of difference between both methods has been 3.7 p. cent.

Del NOZAL et *al.* (1983) have suggested the use of the same electrode and conditioning solution for the determination of chloride in must. The most probable interferences were studied using a synthetic must being its composition based on the wine composition. Standard addition method was selected as analytical technique of measurement because of the interferent action of the tannic acid present in the sample.

LIMA and RANGEL (1989) have determined the chloride content of 19 samples of different types of Portuguese wines, either current table wines or Port wine. Concentration was determined by potentiometric titration with silver cation in a flow injection analysis (FIA) system using a second kind tubular electrode of silver/silver sulphide. The samples were introduced in the system without any pre-treatment. The methodology developed was adequate for samples with chloride contents between 5-600 mg.dm^{-3}, at sampling rates of 60-120 samples per hour, with a coefficient of variation for 10 consecutive determinations less than 1 p. cent. There was a good agreement between the results obtained by this method and those obtained using the reference procedure. The relative standard deviation was about 2 p. cent.

FERREIRA et *al.* (1994) developed a flow injection system for the pseudo-titration of chlorides in wines, very similar to the previous one, but with a different kind of electrode. For this purpose, a tubular silver ion selective electrode based on a homogeneous crystalline membrane was constructed. Its operational characteristics were evaluated and compared with the corresponding conventionally shaped electrodes obtained with the same sensor. The results obtained for chloride determination in wine were in good agreement with those provided by reference procedures. The maximum relative deviations observed were about 6 p. cent. The sampling rate achieved with this system varied between 120 and 360 samples per hour, with no need for previous sample treatment.

The determination of fluoride content in wine by means of ion-selective electrodes was first described by MARTIN and BRUN (1969). These authors analysed 37 samples of wines, from different growing areas in France, by direct potentiometry and by standard addition methods. Adjustments of both pH and ionic strength were achieved by addition of phosphoric acid solution to the wine sample. The average fluoride content in the samples was 0.52 mg dm^{-3}.

MORENO et *al.* (1971) have determined the fluoride content of 25 samples of wines from Cataluña (Spain) by direct potentiometry, but in this case the samples were conditioned by adding a TISAB solution (total ionic strength adjustment buffer) in a ratio 1:1 (v/v). The function of this conditioning solution was to maintain the pH (5.0-5.5), to adjust the ionic strength and to avoid possible interferences of complexes formed by fluoride and certain cations. The average value of fluoride content in these wines resulted to be 0,58 mg.dm^{-3}. The results obtained showed good agreement when compared with those obtained by a volumetric method (after distillation).

De BAENST et *al.* (1973) have also conditioned the wine samples in a proportion 1:1 (v/v) with a TISAB solution, but in this case the chelating agent was different. The application of the standard addition method on 12 samples of different European wines have shown a mean concentration of 0.17 mg dm^{-3}.

POSTEL et *al.* (1975) used a fluoride selective electrode (Corning-EEL N° 476042) for the determination of this anion in wine. Both standard curve and standard addition method were tested, being the first one more problematic due to the interference of ethanol. The white wine samples presented values for fluoride ranging from 0.05 to 0.33 mg dm^{-3} and the red wines from 0.12 to 0.72 mg dm^{-3}.

Several samples of wine were analysed by WOLLER and HOLLBACH (1978) for the determination of F^- using a fluoride specific electrode. Most of the samples contained less than 0.5 mg dm^{-3}.

BERNAL et *al.* (1979) developed a method for the determination of fluoride in wines by an ion selective electrode. It involved prior determination of ethanol interference followed by fluoride analysis either by direct potentiometry or by a standard addition method. The second method gave better results and was applied to 57 samples of red, white and rose wines. The results ranged from 0.02 to 0.38 ppm.

HIDALGO et *al.* (1983) used the same conditions as MORENO et *al.* (1971) for fluoride determination in wine (from Jerez, Spain). These authors have studied both direct potentiometry and multiple standard addition as techniques of measurement. They obtained similar results of precision and accuracy, but taking into account that it is necessary to add to the standard solutions of the calibration curve an amount of ethanol close to the alcoholic grade of the wine samples. Average fluoride levels for dry sherry resulted to be 0.25 mg dm^{-3} and 0.70 mg dm^{-3} for cream sherry.

The determination of fluoride in must has been performed by Del NOZAL et *al.* (1983) using a fluoride selective electrode (Orion 94-09-F^-). First of all, they studied the influence of some organic acids, usually present in must, such as citric, oxalic, lactic, tartaric, malic, etc. and they have found no interference effects on the behaviour of the fluoride-selective electrode. This study was made on a synthetic must. The determination of fluoride in real must samples was done using the standard addition method.

GIL ARMENTIA et *al.* (1988) used the same conditions as MORENO et *al.* (1971) for fluoride determination in wine samples from Rioja, Spain. Comparative tests were conducted by direct, standard addition and Gran's methods. The standard addition method gave the best results. The F^- concentration values recorded decreased with increasing ethanol concentration, but the slope remained the same when compared to the calibration line. The values obtained using the standard addition method were less than 0.4 mg dm^{-3} in all 40 samples analysed.

ESCALADA et *al.* (1988) used a selective electrode (from Ingold) to determine the fluoride content of musts and wines. They used the standard addition method to eliminate the alcohol interference. The reproducibility obtained with this method was better than 2 p. cent.

ALCORTA et *al.* (1989) developed an ion-selective electrode method for measurement of fluoride in wines. A buffer of a composition not usually used for determination of fluoride in food was employed to eliminate interferences. Electrode response time and influence of alcohol were also determined. Fluoride concentration found in the analysed wines ranged from 0.09 to 0.8 mg dm^{-3}.

The fluoride content of wines from Méntrida has been determined by MARTINEZ RINCON and ABUIN CABEZA (1990), using a lanthanum trifluoride selective electrode (ORION RESEARCH). They employed the calibration curve method and the standard addition one. For both methods, the standards were prepared with an alcoholic level similar to the wine being analysed. Comparing the results, the standard addition method was found to be more appropriate than the direct method since the fluoride content of the wines was very small.

PÉREZ-OLMOS et *al.* (1990) have studied the utilisation of several TISAB solutions. One of them was the 0.75 mg dm^{-3} phosphoric acid solution and the others contained sodium chloride, acetic acid and different chelating agents such as EDTA and sodium citrate. After applying the standard addition method,

the first solution resulted to be the most efficient, in terms of precision and percentages of spike recovery. These authors analysed 50 samples of wine from Tenerife (Spain) obtaining an average fluoride content of 0.20 mg dm^{-3}.

The determination of fluorine in wines was described by DESCHREIDER and MEAUX (1973) by means of a specific ion electrode. The electrode was an Orion model 94-09A specific for F^-. The concentration of fluorine in the samples was calculated from the standard addition method, making one single addition. The method was found to be simple rapid and precise. The fluorine contents found in the analysed wines ranged from 0 to 0.425 mg dm^{-3}.

VECCHIO et *al.* (1981) have used an ion specific electrode for the determination of fluorine in Italian wines. The results ranged from 0.04 to 1.33 mg dm^{-3} for fluorine. Regional differences in F contents of wine were discussed, together with the relation of these results to literature data.

The determination of iodine contents in wine was studied by CAVA and CAVALLARO (1979) using a specific electrode. The determination was performed either directly on buffered wine samples or on extract from the ash of alkali-treated wine. Both determination agreed well with the thiosulphate method (recommended by AOAC).

VECCHIO et *al.* (1981), in the same work mentioned above, have used an ion specific electrode for the determination of iodine in Italian wines. The results ranged from 0.01 to 0.87 mg dm^{-3} for iodine.

The determination of nitrate in musts using a nitrate selective electrode was done by Del NOZAL et *al.* (1983). The procedure was similar to the one described above by the same authors for the determination of chloride. However, in this case some interferences were found, specially due to the presence of certain acids. The samples were then submitted to mineralization prior to analysis. The determination was done employing the standard addition technique.

BERNAL et *al.* (1988) have suggested that the use of a nitrate selective electrode could be a good option for the determination of nitrate in wine. They used as detector the electrode Philips IS-561-NO_3-. The multiple standard addition method was chosen for this determination since the alcoholic content of the wine samples affected the measurement. To avoid interferences and to adjust pH and ionic strength a TISAB solution, was added to the samples in a proportion 1:1 (v/v). The results obtained ranged from 8.6 mg dm^{-3} to 47.3 mg dm^{-3}.

The determination of sulphide in wine using a selective-electrode with a membrane of silver/silver sulphide has been proposed by KOVAC et *al.* (1987). The electrode had to be conditioned for an hour in a 10-4 mg dm^{-3} sodium sulphide solution before the analytical determination. Samples have been analysed, by direct potentiometry, adding 5 cm^3 of buffer solution to 45 cm^3 of wine. According to the authors, when the samples were preserved in a solution composed of sodium hydroxide (10.0 mg dm^{-3}), ascorbic acid (0.25 mg dm^{-3}) and salicylic acid (0.05 mg dm^{-3}), the reproducibility of the method was satisfactory.

The determination of sulphite in wines was done by JINGJING et *al.* (1992) by potentiometric titration. Iodide was added to titrate and react with SO_3^{2-} in a wine sample to form I- and SO_4^{2-}. The sample was pre-treated with H_2CO to release complexed SO_3^{2-}. The titration end point was detected by potentiometry. The consumption of I_2 in the titration was correlated with SO_3^{2-} concentration. Recoveries were of 90-110 p. cent.

DETERMINATION OF CATIONIC SPECIES

A calcium-selective electrode (Orion n° 92-20) has been used by CASANOVA (1974) for the determination of total calcium in wines. Organic complexing agents are responsible for part of the complexed calcium present in wine. These interferences were suppressed by re-dissolving the dry ashes of the sample in concentrated hydrochloric acid and then adjusting the pH to 6.5-7.0 with ammonium hydroxide. The concentration was determined by the double standard addition method. For calcium contents of 80-90 mol dm^{-3} the standard deviation of the method was of 4 mol dm^{-3}. These results were in good agreement with those obtained by other methods (oxalate precipitation on ashes and titration with permanganate, complexometric admixture on ashes by EDTA and atomic absorption spectrometry).

SCOLLARY (1987) developed a method for the determination of free and bound calcium in wine using an ion-selective electrode (ISE). Five red wines contained 57-85 mg dm^{-3} total Ca, of which 21-40 p. cent were free ; six white wines contained 28-124 mg dm^{-3} total Ca, of which 12-45 p. cent were free. The major experimental difficulty associated with the use of this kind of electrode in wine was the fact that its membrane slowly dissolved and loose its sensitivity.

Calcium was determined in musts and wines by ESCALADA et *al.* (1988) using direct potentiometry.

The proposed method required previous calcination, as recommended by O.I.V., but after that step the methodology simplified a lot the determination. Consequently, the potentiometric method had some advantages since it was simpler than the classic ones (redox titration with CaC_2O_2 and complexiметry) and much less expensive than atomic absorption spectrometry.

More recently, a discontinuous-flow analyser (DFA) was used for the determination of total calcium in wine, using as detector a flow cell with a coated-wire calcium-selective electrode (CARDWELL et *al.*, 1990). The method consisted of a titration with EGTA (ethyleneglycoltetraacetic acid). The wine samples and EGTA solutions were adjusted to pH 10 and 12 respectively. The wine samples were diluted 1:1 with the pH adjuster. It was found an excellent concordance in both accuracy and precision between the proposed method and the atomic absorption spectrometry adopted as reference technique. The relative standard deviation obtained was less than 1.8 p. cent.

The free, or ionised, calcium concentration in white wines have also been determined by CARDWELL et *al.* (1991) using a calcium ISE. In this work it was also investigated the ratio of the free to bound calcium in wine. The effect of the dilution on this ratio was studied to determine whether a routine analytical procedure for the direct measurement of the total calcium concentration could be established without the dry-ashing pretreatment. The estimation of the total calcium concentration, based on the value obtained for free calcium from the 10-fold dilution seemed to be adequate for the analysed samples. The method could not be applied to red wines because the slope of the electrode was significantly affected after soaking 10 days in a sample of red wine.

The complexing, or binding, of potassium ions in dealcoholized wines was studied by BERTRAND et *al.* (1978) with a potassium electrode (Orion Model 90-01). Potassium concentration values obtained with the electrode were considerably lower than those obtained by flame photometry, but the two measurements converged when the wine was diluted or treated with charcoal. Those observations were interpreted as evidence of binding of potassium by sulphate, tartrate and bitartrate ions, pigments and other materials removed by charcoal.

A potentiometric method for the determination of potassium in wine using an ion selective electrode (membrane-active valinomycine/EM-K-01) was developed by PARFENT'EVA et *al.* (1982). The procedure involved standard addition of KCl to the wine samples. Good agreement was achieved between this method and the flame photometric one. The difference between them did not exceed 15 p. cent.

Some manufacturers have recommended the utilisation of selective electrodes for measuring potassium in wine. ORION has developed a procedure in which the samples were diluted 1/10 with de-ionised water, and the potassium level was determined directly from a calibration curve prepared from aqueous potassium standard solutions. Recalibration every two hours has been recommended (ORION, 1979). The firm PHILIPS (1987) has suggested that sample addition technique of measurement is preferable to direct potentiometry for the determination of potassium in Sherry, since its high alcohol content would interfere with the electrode characteristics and reduce the life-time of the plastic membranes. In this method 0.4 cm^{-3} of sherry samples was added to 100 ml of a 1 mol dm^{-3} standard potassium solution. This 250 fold dilution reduces the alcohol to a level where the electrode is unaffected and allows rapid and routine analysis.

ESCALADA et *al.* (1988) made the determination of potassium in musts and wines by direct potentiometry. The precision obtained was better than 2 p. cent and the advantages were the same as mentioned above for calcium.

The concentration of potassium in sixty-three samples of red, rose and white wines, was determined by PÉREZ-OLMOS and ECHEVARRÍA (1989), using a potassium selective electrode (Orion model 93-19). For this determination, the samples, previously diluted 1/10 with deionized water, were analysed by the multiple standard addition method. The equipment used for this purpose was the Autochemistry System Orion 960. The precision of the analytical procedure was evaluated by analysing 11 samples of the same wine. This wine had an average concentration of 1 001 $mg.dm^{-3}$ with a relative standard deviation of 0.78 p. cent. The percentages of recovery ranged from 98.1 p. cent to 99.0 p. cent. Values ranging from 10.0 to 30.0 mg l^{-1} were found for the concentration of potassium in the sixty-three samples of wine. Regression analysis proved that a good agreement exist between this technique and the recommended procedure (flame emission photometry).

A flow injection potentiometric method for the simultaneous determination of potassium and calcium in wines was proposed by ILCHEVA et *al.* (1990). Use was made of a two-channel flow injection system with serial indicator electrodes (with a flow-through configuration) and a common reference one at the end of the line. The two indicator electrodes were made by Radiometer. The sampling rate achieved in this case was of 30-60 determinations per hour. The reproducibility of the results was better than 2 p. cent and the relative deviation as compared to other methods was

not more than 4 p. cent. No preliminary treatment of the wine samples was required.

ORION (1976) recommended a direct potentiometric method for the determination of sodium in wine using a sodium-selective electrode. The samples were previously diluted 1/10 with de-ionised water, and the pH adjusted by means of an ammonium chloride/ammonium hydroxide buffer.

PÉREZ-OLMOS and ECHEVARRÍA (1989) have determined the concentration of sodium in different samples of wine using the same analytical technique as mentioned above for potassium. The sodium selective electrode used was an Orion model 97-11. In this case, the samples were diluted 1/10 with a 20 p. cent v/v triethanolamine solution. For a sample of wine, with an average sodium concentration of 36.4 mol dm^{-3} the relative standard deviation was of 1.67 p. cent and the average percentage of spiked recovery was 98.6 p. cent. When the results were compared with those obtained by flame emission spectrometry, a good agreement was found.

DETERMINATION OF GASEOUS SPECIES

McWILLIAMS and OUGH (1974) have used an ammonia gas-sensing probe from Orion to analyse must and wine and measure ammonia uptake during fermentation. Direct potentiometry and standard addition were selected as analytical techniques of measurement. For comparison purposes the reference method was distillation-titration after ion-exchange. The standard addition method showed better relative standard deviation (1.6 p. cent) than direct potentiometry (7.9 p. cent), and both of them were better than the reference method (10.8 p. cent). The values of percentage of spiked recovery of added ammonia were 98.6 p. cent for direct potentiometry and 101.5 p. cent for the standard addition method. These authors have demonstrated that the presence of ethylamine in wine gave significant errors in the potentiometric determination.

BERNAL et *al.* (1986) have determined the ammonia concentration in wine using an ammonia gas-sensing probe (Philips IS-570 NH3). The procedure used a standard additions technique without any previous treatment of the sample. Direct potentiometry was also tested, but with no good results since alcohol was an important interference. However, when the standards were prepared with the appropriate alcoholic level, the direct measurement could be performed. The difference between the results obtained by application of the recommended procedure and the Dimotaki-Kouraku method, adopted as reference method, was no higher than 2 p. cent.

LONVAUD-FUNEL and RIBÉREAU-GAYON (1976) used a specially-made carbon dioxide gas-sensing probe, located in a thermostatically controlled sample cell, for measuring carbon dioxide in wines. The probe had a teflon membrane and an external reference electrode. Wines were analysed after dilution with de-ionised water, and an aliquot mixed in the sample cell with a pH 3 buffer solution. In case of sparkling wines, the samples were first cooled to -5 °C. The proposed technique was compared with manometric and titrimetric methods being preferable for its simplicity and precision. Since in the manometric method, the instrument read-out was calibrated in partial pressures of carbon dioxide (in mmHg), using gaseous mixtures of carbon and nitrogen dioxide, the calibration procedure turned to be time-consuming.

The firm ORION (1976) has suggested a potentiometric method for determining carbon dioxide in carbonated beverages and wines. When the sample was collected, it was immediately turned basic, to prevent escape of carbon dioxide to the atmosphere. After dilution, the sample was acidified by means of a buffer solution and its pH adjusted to 4,5. Carbon dioxide concentration was determined by direct potentiometry.

ESCALADA et *al.* (1988) have recommended a similar procedure in which the samples were previously cooled. Acidification was achieved with the same buffer solution recommended by ORION. The measurements of potential needed five minutes for stabilisation. The relative standard deviation of the method resulted to be inferior to 5 p. cent.

LINARES et *al.* (1989) proposed a method for the simultaneous determination of carbon dioxide and sulphur dioxide in wine. They developed a flow injection analysis system incorporating a flow-through gas-diffusion unit. The analytes were sensed by two detectors in series, a potentiometric detector (glass-calomel microelectrode) responding to both analytes and a photometric detector for SO_2 only. The usefulness of the method was tested by applying it to samples of fruity wines and the results were compared with those obtained with the standard EEC recommended method. The reproducibility was generally 7 p. cent or better, with a sampling frequency of about 25 h^{-1}.

GARCÍA et *al.* (1983) have developed a procedure for the determination of total nitrogen contents in wines. All the nitrogen compounds were transformed into ammonium ion by applying the Kjeldahl digestion process. This ion was converted, once again, to

free ammonia by alkalinization at pH 12. The destilation stage of the Kjeldahl method was omitted. The determination was carried out by direct potentiometry using an ammonia gas-sensing probe (Orion model 95-10). This procedure showed good precision since the mean relative standard deviation, for all samples analysed, was 2.5 p. cent. Accuracy was also good, since the results obtained differed only 4.4 p. cent from those obtained by application of the volumetric standard method. In this work, it was also proposed some modification to the Kjeldahl digestion method turning it quicker. The results obtained presented an acceptable precision and accuracy being advantageous since they reduce the time needed to perform the determinations.

The concentration of H_2S formed from elemental sulphur was followed during fermentation by SCHUETZ and KUNKEE (1977) using an ion electrode specific for S^{2-}. This method allowed H_2S to be measured throughout the fermentation in a more precise way than by chemical methods previously used. The reduction of the elemental sulphur was apparently a nonenzymic chemical reaction caused by certain reducing compounds formed in the yeast cell during fermentation.

The total sulphur dioxide concentration in wine and beer has been carried out by BAILEY (1975) using a sulphur dioxide gas-sensing probe. The total time for each analysis was less than 10 minutes. The probe responded fast enough to be used together with an autoanalyzer type system, and samples at the rate of 30 per hour could be determined when the concentrations were around 100 mg dm^{-3}.

The determination of free and total SO_2 by direct potentiometry has also been proposed by Orion. Standards solutions of sodium sulphite containing glycerine and recalibration every two hours were recommended (ORION, 1975).

Studies on the use of the Orion 95-64 ion-selective electrode for direct potentiometric determination of free and total SO_2 in wines were performed by BINDER et *al.* (1975). Acid was added to liberate the bounded SO_2 in order to make the total determination. Equations for the calculation of the results are also presented. Comparison of this method with other methods of analysis (acidimetric and complexometric) showed similar results. However, its accuracy was found to be lower.

JENNINGS et *al.* (1978) have compared the gas sensing probe method with a Monier-Williams distillation and with an automated para-rosaniline spectrophotometric methods for the analysis of SO_2 in wine. Because of the tendency of the probe to show long-term drift, standard addition, as analytical technique of measurement, was preferable to direct potentiometry in terms of accuracy. The probe results were more variable than those from the other methods. The authors have found that the inner electrolyte solution of the probe was unstable and could cause erratic behaviour. For that reason it was recommended to change this solution weekly. Besides, red wines had a particularly deleterious effect and membranes had to be replaced after each series of analysis. The diffusion of alcohol vapour across the membrane probably caused dilution of the electrolyte solution. To overcome this problem, HANSEN et *al.* (1974) recommended the preparation of this solution in 20 p. cent ethanol.

SPEEDING and STEWART (1980) used a sulphur dioxide gas sensing membrane probe (Orion model 95-64) to determine the concentration of free sulphur dioxide in wines at their natural pH. Standards contained approximately 10 p. cent of ethanol. For each of the standards they studied the relationship between pH and sulphur dioxide electrode potential (which was linear for the concentrations in question). From the plots mentioned above, they built another plot of electrode potential versus concentration. In this last graph they could read the sample concentration after measuring the corresponding electrode potential. The sulphur dioxide electrode was able to determine the sulphur concentration to within 5 p. cent of the known addition for white wine.

A biosensor based on a glass electrode was developed by NAKAMURA et *al.* (1993) to determine free sulphur dioxide in wine. The biosensor consisted of a microbial membrane of Thiobacillus thiooxidans JMC7814 and a flat glass electrode. A porous gas-permeable membrane was incorporated in the biosensor system to separate free sulphur dioxide from bound sulphur dioxide and to avoid buffering from the sample solution. The biosensor yielded a linear relationship between pH decrease and sulphur dioxide concentration. Limit of detection for sulphur dioxide was 5.0 mg dm^{-3}, and the life-time of microbial membrane was approximately 30 days at 4°C. The relative standards were 7.41 p. cent for red wine and 5.00 p. cent for white wine.

OTHER DETERMINATIONS

LONVAUD-FUNEL and RIBÉREAU-GAYON (1973) made use of a CO_2 electrode for determining the activity of malic enzyme of lactic acid bacteria in wine. In preliminary studies, the amount of CO_2 produced were shown to be in agreement with the amounts of malate used and of lactate formed.

DESCHREIDER and MEAUX (1974b) developed a potentiometric method for the determination of boron in wines. They used a fluoroborate-specific electrode. After degassing and/or evaporation of alcohol, the sample was passed through a strongly acid cation-exchange resin (Dowex 50 W) to separate interfering cations. The boron present in the samples was then converted to BF42- and this anion was determined potentiometrically. The percentage of recovery attained was from 91.8 to 104 and accuracy was found to be around 5 p. cent. The wines examined contained 2.36-8.75 ppm.

LOPATINA and RUBINSKI (1985) developed a simple and rapid method for the determination of inverted sugars in wine. The method was based on the interaction between glucose, fructose and boric acid. The wine samples must have an inverted sugar content in the range of 5-20 p. cent. A glass electrode was used as the detector and its potential directly related with the inverted sugar concentration.

LOPES et *al.* (1995) developed a FIA system for the determination of reducing sugars in Port wine. The methodology involves a reaction between reducing sugar and picric acid, being the decrease in the picrate concentration monitored with a tubular picrate ion-selective electrode. This electrode was also developed by the authors. The standards were composed of a mixture of fructose (60 p. cent) and glucose (40 p. cent), corresponding to the usual relative sugar percentages in Port wine, and ethanol in a concentration of 20 p. cent (concentration usually found in Port wines). A linear dependence between peak width, at a fixed potential, and the logarithm of sugar concentration in the range 25-200 g l^{-1} was obtained. The proposed system allowed wine samples injection with no previous treatment and presented a sampling rate of 50 determinations per hour and a relative standard deviation less than 4p. cent.

Water soluble tanning and colouring agents were determined in red wine by STUDENSKAYA and KOSTITSYNA (1974) by potentiometric titration. The authors studied the conditions of compensated and non-compensated potentiometric titration of tanning agents by potassium permanganate. Use of a graphite electrode permitted the use of the non-compensated method without recourse to titration curves. Tanning and colouring agent concentration less than 1 p. cent could be determined with a high degree of accuracy and reproducibility.

CONCLUSIONS

From the literature revised in this work it becomes clear the potentialities of using potentiometric measurements in wine and must analysis. In spite of the wine complex matrix, the analytical procedures developed are rapid and simple to operate, require a minimum sample size and inexpensive equipment, and are usually precise, reproducible and accurate.

Regarding the number of ion-selective electrodes nowadays available it is to be expected that other analytes could be measured in wines and musts. Another important trend will surely be the incorporation of potentiometic detectors in flow injection systems, allowing to automatically carry out direct or even titration measurements. This only became feasible to be achieved in a robust way when electrodes with a suitable configuration, namely tubular ones, were developed. Additionally, as the hydrodynamic characteristics of the sample plug are not altered by the potentiometric unit, the bolus can be led to another detector and perform multicomponent analysis.

Acknowledgements :

The authors wish to thank financial support from Department of Education, Universities and Research of the Basque Government (Spain), Project PGV 92-37. This work was also supported by an European Union programme (AAIR3 - CT - 94 - 2468). T. I. M. S. Lopes also acknowledges grant from JNICT (BD/2096/92-IF).

REFERENCES

ALCORTA J. C., ELIAS I., LIMA J. L. F. C. and PÉREZ-OLMOS E. R., 1989. Determinação de fluoreto em vinhos e cervejas com eléctrodos Selectivos de Iões. *Rev. Port. Farm.*, **39**, n°4, 35-40.

BAILEY P. L., 1975. The measurement of sulphur dioxide in food with a gas-sensing membrane probe. *J. Sci. Fd. Agric.*, **26**, 558.

BERNAL J. L., Del NOZAL M. J. and PARDO R., 1979. Determinación de fluoruros en vinos. *Rev. Agroquím. Tecnol. Aliment.*, **19**, n°4, 541-548.

BERNAL J. L., Del NOZAL M. J., ALLER A. J. and DEBAN L., 1983. Nota. Determinación de cloruro en vinos con un electrodo selectivo. *Rev. Agroquím. Tecnol. Aliment.*, **23**, n°1, 137-142.

BERNAL J. L., Del NOZAL M. J., DEBAN L. and TORREMOCHA I., 1986. Determination of ammonia in wine and milk with an ammonia gas-sensing probe. *Analyst*, **111**, 631-634.

BERNAL J. L., Del NOZAL M. J., CALVO A., TASCÓN M. L. and DEBAN L., 1988. Determinacion de nitratos en vinos con un electrodo selectivo de nitratos. *X Jornadas de Viticultura y Enología de Tierra de Barros*, Almendralejo, Spain.

BERTRAND G. L., CARROLL W. R. and FOLTYN E. M., 1978. Tartrate stability of wines. I. Potassium complexes with pigments, sulfate and tartrate ions. *Am. J. Enol. Vitic.*, **29**, n°1, 25-29.

BINDER A., EBEL S., KAAL M. and THRON T., 1975. Quantitative determination of SO_2 in wine by direct potentiometry. *Deutsche Lebensmittel Rundschau*, **71**, n°7, 246-249.

BUCKEE G. K., 1975. Brewing analysis involving the use of ion-selective electrodes. *J. Sci. Fd. Agric.*, **26**, 557-558.

CARDWELL T. J., CATTRALL R. W., CROSS G. J. and MRZLJAK R. I., 1990. Determination of calcium in waters, milk and wine by discontinuous flow analysis. *Analyst*, **115**, 1235-1237.

CARDWELL T. J., CATTRALL R. W., MRZLJAK R. I., SWEENEY T., ROBINS L. M. and SCOLLARY G. R., 1991. Determination of ionized and total calcium in white wine using a calcium ion-selective électrode. *Electroanalysis*, **3**, 573-576.

CASANOVA A., 1974. L'ionométrie. Dosage du calcium dans les vins. *Ann. Technol. agric.*, **23**, n°4, 403-410.

CAVA L. and CAVALLARO A., 1979. Determination of iodine contents in wine. *Bolletino dei Chimici dei Laboratori Provinciali*, **5**, n°1, 140-147.

COMER J.,1978. Chapt. 7. In *Developments in Food Analysis*, (vol.1), R. D. King (Ed), Applied Science Publishers, London.

COVENEY L. V., 1980. *Scientific and technical survey*, n°118. The British Food Manufacturing Industries Research Association, Leatherhead.

De BAENST G., MERTENS J., VAN DEN WINKEL P. and MASSART D. L., 1973. Détermination de l'ion fluorure dans le vin, par potentiométrie. *J. Pharm. Belg.*, **28**, n°2, 188-194.

Del NOZAL M. J., BERNAL J. L. and MARTÍN M. M. P., 1983. Determinacion espectrofotometrica y potenciometrica de elementos minerales en mosto de uva. *Anal. Bromatol.*, **35**, n°1, 51-65.

DESCHREIDER A. R. and MEAUX R., 1973. Determination of fluorine in wines and natural mineral waters by means of specific ion electrode. *Rev. Ferment. Ind. Aliment.*, **28**, n°4, 150-156.

DESCHREIDER A. R. and MEAUX R., 1974b. Potentiometric determination of boron in wines and in mineral waters. *Rev. Ferment. Ind. Aliment.*, **29**, n°3, 75-80.

ESCALADA M., FERNÁNDEZ-FERNÁNDEZ A., FERNÁNDEZ-ALBALATE M. J. and PAULIS J. M., 1988. Aplicaciones de los electrodos selectivos de iones (ISE) en el control analitico de mostos y Vinos. Proceedings of « *3e Encontro Galego-Português de Química, Comunicacion 2.3* », Vigo, Spain.

FERREIRA I. M. P. L. V. O., LIMA J. L. F. C. and RANGEL A. O. S. S., 1994. Flow injection titration of chloride in food products with a silver tubular electrode based on an homogeneous crystalline membrane. *Food Chemistry*, **50**, 211-217.

GARCIA C., HIDALGO J. L. and PÉREZ-BUSTAMENTE J. A., 1983. Determinación de nitrógeno total en vinos por potenciometría con un electrodo sensible al gas amoníaco. *Afinidad*, **40**, 41-46.

GIL-ARMENTIA J. M., ARRANZ J. F., BARRIO R. J. and ARRANZ A., 1988. Inorganic compounds in Rioja wine. I. Fluorides. *Anal. Bromatol.*, **40** N°1, 71-77.

GRAF J. E., VAUGHN T. E. and KIPP W. H., 1976. Analysis of bromide ion in wine by ion selective electrode. *Journal of the AOAC*, **59**, n°1, 53-55.

HAILER G., 1975. Ion sensitive electrodes in food chemistry. *Dt. Lebensmitt Rdsch.*, **71**, 208-209.

HANSEN E., FILHO H. and RUZICKA J., 1974. Determination of the hydrogen sulphide content of wine by means of the « air-gap » electrodes. *Anal. Chim. Acta*, **71**, 225-227.

HENSCHEID T., SCHOENROCK K. and BERGER P., 1971. Application of ion-selective electrodes in beet sugar industry. *J. Am. Soc. Sugar Beet Technol.*, **16**, 482-495.

HIDALGO J. L., PASTOR M. and PÉREZ-BUSTAMANTE J. A., 1983. Determinacion de fluoruro en vinos del Marco de Jerez. *Anal. Bromatol.*, **35**, 67-77.

ILCHEVA L., YANAKIEV R., VASILEVA V. and IBEKWE N., 1990. Multicomponent flow injection analysis : determination of potassium and calcium in wine. *Food Chemistry*, **38**, 105-112.

JENNINGS N., BUNTON N. G., CROSBY N. T. and ALLISTON T. G., 1978. A comparison of three methods for the determination of sulphur dioxide in food and drink. *J. Ass. Publ. Analysts*, **16**, 59-70.

JINGJING Y., DONGMEI L., LING Q., SHIQUE L. and DEFANG C., 1992. Determination of sulfite in foods by potentiometric titration. *Shipin Yu Fajiao Gongye*, 80, n°3, 60-66.

KOVAC J., FARKAS J., HUDEROVA L. and TRISKA V., 1987. Determination of sulphide in wine by ion-selective electrode. *Vinohrad, Bratislava*, **25**, 230-232.

LIMA J. L. F. C., and RANGEL A. O. S. S., 1989. Chloride pseudotitration in wines by FIA with a Ag2S/Ag tubular electrode as detector. *J. Food Comp. Anal.*, **2**, 356-363.

LINARES P., LUQUE DE CASTRO M. D. and VALCÁRCEL M., 1989. Simultaneous determination of carbon dioxide and sulphur dioxide in wine by gas-diffusion/flow injection analysis. *Analytica Chimica Acta*, **225**, 443-448.

LONVAUD-FUNEL A. and RIBÉREAU-GAYON P., 1973. Use of a CO_2-electrode for determining the activity of malic enzyme of lactic acid bacteria in wine. *Comptes-rendus hebdomadaires des séances de l'Académie des Sciences*, D, 276, n°15, 2329-2331.

LONVAUD-FUNEL A. and RIBÉREAU-GAYON P., 1976. Le gaz carbonique des vins. I- Dosage à l'aide d'une électrode spécifique. Comparaison avec les méthodes traditionelles. *Connaissance Vigne Vin*, **10**, 391-407.

LOPATINA M. B. and RUBINSKII O. E., 1985. Potentiometric determination of invert sugar in wines. *Izvestiya Vysshikh Uchebnykh Zavedenii, Pishchevaya Tekhologiya*, n°4, 104-107.

LOPES T. I. M. S., RANGEL A. O. S. S., LIMA J. L. F. C. and MONTENEGRO C. B. S. M., 1995. Construction and use of a tubular picrate ion selective electrode for the reducing sugar. FIA Determination in Port wine. *Anal. Chim. Acta*, **308**, 122-128.

MARTIN C. and BRUN S., 1969. Le dosage du fluor dans les vins. *Trav. Soc. Pharm. de Montpellier*, **29**, 161-167.

MARTINEZ RINCON M. C. and ABUIN CABEZA L. M., 1990. Flúor en vinos de denominación de origem méntrida. *An. Real Acad. Farm.*, **56**, 279-282.

McWILLIAM D. J. and OUGH C. S., 1974. Measurement of ammonia in musts and wines using a selective electrode. *Amer. J. Enol. Viticult.*, **25**, n°2, 67-71.

MOODY G. J. and THOMAS J. D. R., 1976. The role of ion-selective electrodes in studies on vegetation, vegetables, fruit juices and oils. *J. Sci. Fd. Agric.*, **27**, 43-50.

MORENO F., De la TORRE M. C. and SERRAT M., 1971. Un electrodo especifico para la determinación de pequeñas cantidades de ion fluoruro. *Circ. Farm.*, **29**, 315-331.

NAKAMURA K., SAEGUSA K. KUROSAWA H. and AMANO Y., 1993. Determination of free sulfur dioxide in wine by using a biosensor based on a glass electrode. *Biosc. Biotechnol. and Biochem.*, **57**, n°3, 379-382.

ORION RESEARCH, 1975. *Sulphur dioxide electode 95-64, free and total sulphur dioxide in wines, Instruction Manual*. Cambridge, Mass.

ORION RESEARCH, 1976. *Carbon dioxide electrode 95-02, carbon dioxide in carbonated beverages and wines, Instruction Manual*, Cambridge Mass.

ORION RESEARCH, 1979. *Potassium in wine and grape juice, Methods Manual 93 Series Electrodes*, Cambridge Mass.

PARFENT'EVA T. L., TUR'YAN Y. and OVCHINNIKOVA S. A., 1982. Determination of potassium in wine by means of an ion selective electrode. *Izvestiya Vysshikh Uchebnykh Zavedenii, Pishchevaya Tekhnologiya*, n°6, 108-111.

PÉREZ-OLMOS R. and ECHEVARRÍA J., 1989. Comparative study of ion-selective electrodes versus flame emission photometry techniques for the determination of sodium and potassium in spanish wines. *Food Chemistry*, **32**, 201-207.

PÉREZ-OLMOS R., HARDISSON A., ELIAS J., RIOS R. and MARTIN M., 1990. Determination of fluoride in Tenerife wines. Comparative study of conditioning solutions. *Belg. J. Food. Chem. Biotechnol.*, **45**, 208-213.

PÉREZ-OLMOS R. and LIMA J. L. F. C., 1990. Aplicación de los electrodos selectivos de iones al analisis de productos lacteos. *Industria Farmaceutica*, **5**, 93-103.

PHILIPS ANALYTICAL, 1987. *Determination of potassium in sherry by sample addition, ion selective measurement* (3rd Ed.), Cambridge.

POSTEL W., GOERG A. and PRASCH E., 1975. Determination of fluoride in wine with an ion-selective electrode. *Wein-Wissenschaft*, **30**, n°6, 320-326.

SCHUETZ M. and KUNKEE R. E., 1977. Formation of hydrogen sulfide from elemental sulfur during fermentation by wine yeast. *Am. J. Enol. Vitic.*, **28**, n°3, 137-144.

SCOLLARY G., 1987. Free and bounded calcium content in wine : possible monitoring of protein haze formation. *Australian Grapegrower & Winemaker*, n°278, 25-26.

SPEDDING D. J. and STEWART G. M., 1980. Use of a sulphur dioxide gas sensing membrane probe in wines and juices at their natural pH. *Analyst*, **105**, 1182-1187.

STUDENSKAYA L. S. and KOSTITSYNA V. I., 1974. Determination of tanning and colouring agents in foods by potentiometric titration. *Izvestiya Vysshikh Uchebnykh Zavedenii, Pishchevaya Tekhnologiya*, n°4, 147-149.

VECCHIO A., FINOLI C., GERMANI S. and CERUTTI G., 1981. Genuine italian wines. III. Iodine and fluorine. *Tecnologie Alimentari*, **4**, n°5, 10-14.

WOLLER R. and HOLLBACH B., 1978. Chemical analysis of fluoride in grapes, grape juice and wine. *Wein-Wissenschaft*, **33**, n°1, 71-76.

Manuscrit reçu le 29 mars 1995,
accepté pour publication le 28 août 1995