Critical evaluation of potentiometric redox titrations in enology

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Abstract

Measurements of the zero current potential of a platinum electrode immersed in solutions of tanins or in wines of various origins, were performed during the additions of a solution of Ti(III), or of a solution of dichlorophenolindophenol (DCPIP), in order to obtain a global indication for the resistance to oxidation of some wines. The steady state intensity–potential curves on a platinum electrode highlight the occurrence of mixed potentials between the oxidation of ethanol or catechin and the reduction of oxygen present at very low concentrations, as well as the irreversibility of the redox system Ti(IV)/Ti(III). The adsorption of various species on the platinum and the slowness of the oxidation reactions by DCPIP exclude use of potentiometric titration theory. The shape of the potential–reagent volume curves depends on the nature of the solution and on the rate of reagent introduction. Generally, due to the slowness of the oxidation reactions with DCPIP it is impossible to find a linear relationship between the volume of titrant solution necessary to reach the inflexion point of the curves and the solution composition.

Keywords: Catechin; Dichlorophenolindophenol; Potentiometric titration; Titanium(III); Wines

1. Introduction

Research into the determination of the antioxidant properties of plants, fruit, food products and drugs has been increasing over recent years. This tendency is justified by the fact that the oxidation resistance of a product depends in particular of the concentration of the antioxidant compounds originally present or added, and also on the quantity of oxygen. This tendency is also related to the fact that oxidative stress is involved in the pathogenesis of several chronic diseases and that antioxidant compounds provide protection from the free radical effects.

Many redox reactions take place during the making and ageing of wines. In particular oxygen plays a fundamental role in color, aromatic degradation or early browning of white wines. As aromatic degradation occurs before chromatic degradation, oenologists are in the search of early indications to estimate the oxidation resistance of a wine. Thus, the determination of the antioxidant capacity related to the rate of oxygen uptake is also at the center of concern.

Many methods to evaluate the total antioxidant capacity of a compound have been proposed such as chemiluminescence, spin electronic resonance and various spectrophotometric methods possibly carried out on continuous flow [1].

The oenology world has adopted an additional method for many years, which consists in determining the zero current potential generally of a platinum electrode versus a reference electrode [2–6]. The evolution of the medium analyzed in the course of time and the possible fouling of the electrodes are at the origin of the drift of the potential value and hinder the interpretation of the measurements. Although it is not possible to deduce quantitative information about the composition of the solution, this method can provide information, for example, about the evolution of fermentation during wine making [7]. Because neither the studied sample nor the interface between platinum and wine are at equilibrium and because of the high number of redox systems present in the sample, it is impossible to propose an electrode reaction and to apply Nernst's equation. So the potential is improperly called redox potential. However, because of their simplicity zero

current potential determinations are still performed during wine making. More recently, other electrode materials have been suggested, such as platinized platinum, gold or vitreous carbon and various protocols were proposed to allow a faster stabilization of the potential [8–10].

In spite of these improvements, difficulties still remain for interpretation of the results. This is why relatively recent works to adapt electrochemical methods for the definition of a total antioxidant index have been proposed. For instance cyclic voltammetry allowed the identification and the quantitative analysis of some products considered as antioxidants such as polyphenols, tocopherols, gallic acid, thioacetic acid, rutin or catechin [11–14]. Comparison between the voltammograms obtained with these products and with wine samples diluted in a model wine solution, gives an idea of the total antioxidant capacity. But the complexity of the matrix is responsible for the adsorption of molecules on the electrodes, which causes an evolution of the electrode surface quality and poor reproducibility of measurements. Likewise, a spectroelectrochemical method based on constant current intensity oxidation of products generating a colored cation radical has been proposed. The introduction of an antioxidant in the solution results in delayed oxidation and the reaction consumes a greater quantity of electricity [15]. Some authors have also recommended the use of potentiometric titrations, using a platinum electrode and a reference electrode. Recently, for example, the potentiometric titration of white wines made it possible to claim that a correlation can be established between the potentiometric results and three parameters: the index of oxidative degradation obtained by sensory analyses, the consumption of dissolved oxygen and the concentration of substances responsible for bad flavours [16,17]. Once more potentiometric titrations were introduced in the world of oenology. In fact such methods enjoy periodical revivals.

The first titration paper appeared in 1932 [18]. A first phase of development is due to Ribereau-Gayon and Gardrat who, in 1957, carried out a series of potentiometric titrations of ascorbic acid, reductone, tanins, tannic acid, anthocyanins, oenine and red wines [19]. It was then necessary to wait until 1981 and Chapon's studies on beers [20], then those of Vivas in 1992 on catechin solutions, phenolic fractions of grape seeds and wines [21] for potentiometric titrations find a new interest. This is certainly related to the release on the market of automated apparatuses allowing the recording of the titration curve, the detection of the equivalent points by derivation of the titration curves and the modulation of the titrating reagent introduction rate according to the slope of the titration curve. For instance, Vivas uses derivative curves in order to distinguish some phenolic fractions contained in grape seeds. Moreover, oenological laboratories often have this equipment for titrations of the total acidity and the sulphur dioxide in wines.

Whatever the period, the authors use titanium(III) chloride as titrating reagent for the reduction and dichlorophenolindophenol as titrating reagent for the oxidation.

The usual protocol consists in initially performing the reduction of the oxidized compounds present in the sample by a titration with Ti(III) as titrating reagent. The total volume of Ti(III) added is known. Secondly, titration with DCPIP makes it possible to distinguish (i) the compounds originally present in a reduced form; (ii) the compounds reduced by Ti(III); (iii) the excess Ti(III) added. The ratio of the two equivalent volumes enables a resistance to oxidation factor ROX to be defined [16].

It is surprising that a lot of publications periodically appear which are related to a simple method such as potentiometric titration, which should be used in routine in analytical laboratories. Such a situation undoubtedly indicates some difficulties of implementation of the method or interpretation of the results.

The goal of this work is not to propose a new analytical method for the quantitative titration of oxidized and reduced compounds in wines but to discuss, on thermodynamic and electrochemical kinetic basis some of the difficulties correlated to the use of Ti(III) and DCPIP in the redox titration of wines.

In the first part, we use the determination of the concentrations of the solutions of Ti(III) and DCPIP by potentiometric titration to discuss the precautions necessary for the implementation of the experiments. In the second part we present the titration of catechin chosen as a model of polyphenols. The third part is devoted to present typical results obtained with tanins and wines and to discuss the limits of the method.

2. Experimental

2.1. Apparatus

Spectrophotometric measurements were performed on a Hewlett-Packard HP 8453.

Titration curves were obtained using an automatic potentiometric Metrohm Titrino 716 titrator equipped with a platinum (99.99% purity) electrode and a Ag/AgCl/3M KCl reference electrode.

Known volumes of solutions were introduced into the titration vessel under inert atmosphere using a Metrohm Liquino 711 pump.

Voltammetric work was performed using an Autolab voltammetric system controlled with a PC equipped with GPES Metrohm software. The three-electrode potentiostatic system was composed of a platinum-rotating disc as working electrode, a glassy carbon auxiliary electrode and a saturated calomel reference electrode.

All the potentials were expressed versus Ag/AgCl/3M KCl reference electrode in the following, except in the voltammetric experiments.

2.2. Chemicals and solutions

Titanium(III) chloride (30% wt solution) in 2 mol L^{-1} hydrochloric acid, 2,6-dichlorophenolindophenol salt hydrate

and 0.05 mol L^{-1} iodine solution were from Acros, L-tartaric acid and ethyl alcohol from Prolabo, catechin from Sigma. Nitrogen R was purchased from Linde.

Grape seed tanins, grape skin tanins and oak tanins of unknown composition were kindly given by the Institut Technique de la Vigne et du Vin, Lisle sur Tarn (France).

All wine samples were kindly given by Oenodev Compagny Maumusson, Laguian (France).

All experiments were performed using a model wine solution containing 12% (v/v) ethanol, 0.033 mol L^{-1} tartaric acid with added NaOH to give a pH of 3.6.

DCPIP solution preparation: the model wine solution containing DCPIP powder was stirred during 30 min, then filtered through Whatman 0.2 μm cellulose nitrate membranes.

2.3. Procedures

For potentiometric titrations, the sample volume used was 20 mL. The sample and the titrating solutions were deaerated by bubbling with nitrogen for 20 min. Unless otherwise specified all titrations were performed under nitrogen atmosphere. The titrating solution was introduced by 30 μL volume increments at 30 mL/min rate. Introduction was automatic when the rate of the platinum electrode potential variation was under 0.83 mV s^{-1}. The titration was stopped either when an inflexion point was detected on the titration curve or according to two criterias: a predetermined value of the total volume of titrating solution or a predetermined value of the final potential.

Voltammetric measurements were performed using sample volumes of 30 mL deaerated for 20 min. The working electrode was a rotating platinum disk. Voltammetric experiments were performed using a 40 rad s^{-1} electrode rotation rate and linear sweep potential rate of 8 mV s^{-1}. Dissolved oxygen was removed by bubbling N_2 through the solution for 20 min before the measurements performed with a N_2 flow on the liquid surface.

In order to simplify writing, the two-electron reduction product of DCPIP is called DCPIP red and the two-electron oxidation product of the catechin is called catechin ox.

A potentiometric titration includes a reaction of the kind $Red_1 + Ox_2 = Red_2 + Ox_1$. The standard potentials of the two redox systems (1 and 2) are sufficiently different so that the reaction is spontaneous and complete. The determination of the unknown concentration with 1% accuracy implies that the difference of the standard potentials be higher than $0.240/n$ V, where n is the number of moles of electrons exchanged per mole of compound. Moreover, the titration reaction must be fast enough for the solution to be at equilibrium when the measurements are performed.

The composition of the solution is correlated to the measurement of the zero current potential of an electrode made either of carbon or of inert metals such as gold or platinum. This potential is recorded versus the added volume of titrating reagent. When the measurement is performed, both the titration reaction and the electrode–solution interface must be at equilibrium. So the rate of establishment of a steady state potential, which depends on the electrochemical behaviour of the various redox systems on the chosen electrode materials, must be higher than the rate of the introduction of the titrating reagent.

In the case where the sample contains several species or species having several oxidation numbers, one can consider a rather precise titration if the difference between the standard potentials of two successive systems is higher than 200 mV.

Changing out a potentiometric redox titration to determine the antioxidant capacity of a wine sample requires the use of a titrating reagent which is neither able to oxidize ethanol nor to take part in reactions of nucleophilic addition on aromatic compounds. This excludes the majority of oxidants traditionally used in analytical chemistry during potentiometric titrations such as MnO_4^-, $Cr_2O_7^{2-}$ and I_3^-. Dichlorophenolindophenol is a mild oxidizing reagent frequently used to carry out oxidation reactions with biological compounds. The standard apparent potential (pH 3.6) is equal to 0.24 V/Ag/AgCl [22].

The reducing reagent we chose was titanium(III) which is often used in the field of oenology. The standard apparent potential of the system TiO^{2+}/Ti^{3+} (pH 3.6) is equal to −0.57 V/Ag/AgCl [23].

3. Results and discussion

3.1. Discussion of the experimental protocol

3.1.1. Determination of the concentrations of the two titrating solutions

The calibration of the solution of Ti(III) was carried out by using the solution as a reagent in a potentiometric titration of an I_3^- sample of known concentration. A classical titration curve is obtained. The volume at the equivalent point corresponds to the mass balance: 2 Ti(III) for 1 I_3^-. At the solution pH the following reaction: $I_3^- + 2H_2O + 2Ti^{3+} = 3I^- + 2TiO^{2+} + 4H^+$ may be proposed.

In the following the concentration of the DCPIP solutions was systematically determined by potentiometric titration using a solution of Ti(III) of known concentration. The low solubility of the DCPIP and the slowness of the dissolution reaction in aqueous medium in fact make it difficult to prepare a solution of precise concentration. Spectrophotometric assay could be performed, but there is a great diversity for the values of the extinction coefficient in references [24–27], for the same value of the pH. It was verified that the concentration of the DCPIP is identical to that obtained by a potentiometric titration with a freshly prepared ascorbic acid solution of known concentration.

The titration reaction with Ti(III) can be written: $2\,Ti^{3+} + DCPIPox = 2\,TiO^{2+} + DCPIPred + 2H^+$.

Some remarks can be made about these above results. The values of the potential obtained before the equivalent

point are close to the theoretical values calculated according to Nernst's equation applied to the system I_3^-/I^-. On the other hand, the experimental values of the potential obtained at the end of titration (close to −0.40 V) is not in agreement with the thermodynamic data relative to the system TiO^{2+}/Ti^{3+} which would give a potential close to −0.55 V. Such a result may be correlated with the existence of a mixed potential.

In the second titration, the values of the potential at the end of the titration are in close agreement with those calculated by Nernst's equation using a 0.43 V standard potential for the DCPIP redox system.

3.1.2. Influence of dissolved oxygen on the potential measurement

The influence of dissolved oxygen concentration on the potential of a platinum electrode immersed in a Ti(III) solution was studied. Three experiments were carried out with the same volume of model wine solution, saturated with N_2 or saturated with air. Additions of volumes of a Ti(III) solution were carried out while the potential of a platinum electrode was measured.

Fig. 1a, the initial solution was saturated with O_2 under atmospheric pressure. It was vigorously stirred during the introduction of Ti(III) with an increment of volume of 30 μL. The monotonously decreasing curve shows that the solution always contained O_2. So, the rate of mass transfer for O_2 at the gas–liquid interface was of the same order of magnitude as the chemical reaction rate between O_2 and Ti(III).

Fig. 1b, to make the interfacial gas transfer reaction negligible, the solution was very moderately stirred and Ti(III) was added in increments of 250 μL. The experimental curve obtained has the usual shape of a redox titration curve. The end point volume corresponds to a concentration of 0.20×10^{-3} mol L^{-1} for dissolved oxygen, near to the solubility calculated according to the following reaction: $4Ti^{3+} + O_2 + 2H_2O = 4TiO^{2+} + 4H^+$.

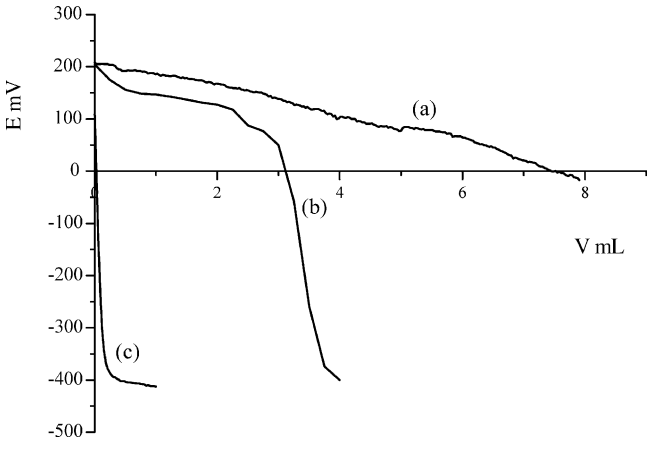

Fig. 1. Evolution of zero current potential of the platinum electrode during introduction of a Ti(III) solution in various oxygen concentrations. Model wine solution volume: 20 mL. Ti(III) concentration: 4.8×10^{-3} mol L^{-1}. (a) Volume increment 30 μL, strongly stirred solution. (b) Volume increment: 250 μL, gently stirred solution. (c) Initially deaerated solution and N_2 bubbling during Ti(III) introduction.

Note that this mass balance was not obtained in [16].

Fig. 1c, the model wine solution was deaerated for 20 min by bubbling nitrogen and no reaction took place between Ti(III) and O_2 present in the sample at very low concentrations.

These results show that the use of a Ti(III) solution requires good deaeration not only of the analyzed solution, but also of the titrating solutions, with bubbling nitrogen in both solutions during the titration.

The initial zero current potential varied from 209 to 109 mV according to whether the solution was saturated with O_2 or with N_2. The final potential was identical to that obtained in the titration of iodine (−0.40 V). This is another case where it is not possible to give an account of these experimental values on the basis of thermodynamic data. For example the pressure of O_2 calculated with the Nernst's law in the case of an initial potential of 0.209 V would be about 10^{-38} bar.

3.1.3. Mixed potentials

In order to try to justify the values of the zero-current potential, steady state intensity–potential curves were obtained using a rotating disc platinum electrode. The experiments were performed with a model wine solution initially saturated with O_2. The potential range extends between the initial potential of −0.42 V and the final potential of 1.10 V. The results are presented in Fig. 2. Curve (a) is obtained when the solution contains dissolved oxygen and curves (b–d) were drawn after various times of deaeration of the solution obtained by a constant flow of nitrogen on the liquid surface, curve (e) was obtained after N_2 bubbling for 20 min.

The zero current potentials of curves (a–d) are in agreement with the evolution of the potential in Fig. 1a and b as long as the solution contains O_2.

Using N_2 as an inert gas it was not possible to obtain solutions completely free of dissolved oxygen. But when the solution contained only very low oxygen concentrations as a consequence of bubbling N_2 for 20 min, the application of the initial cathodic potential of −0.42 V allowed the electrochemical reduction of O_2 at the platinum/solution interface. Moreover, at this potential and at this pH the other cathode reaction product is adsorbed hydrogen, which is oxidized during the anodic sweep between the potentials of −0.42 and −0.20 V.

Beyond −0.10 V, the curve corresponds to the beginning of the oxidation of ethanol in agreement with references [28–30]. Curve (2f) represents the total curve of ethanol oxidation in the deaerated solution. Curves (a–d) in Fig. 2 are global curves resulting from the addition of curve (f) and the theoretical curves of reduction of O_2. The zero current potential now belongs to two different redox systems and cannot be calculated by Nernst's law.

The addition of Ti(III) and Ti(IV) into the model wine solution does not modify the steady state intensity–potential

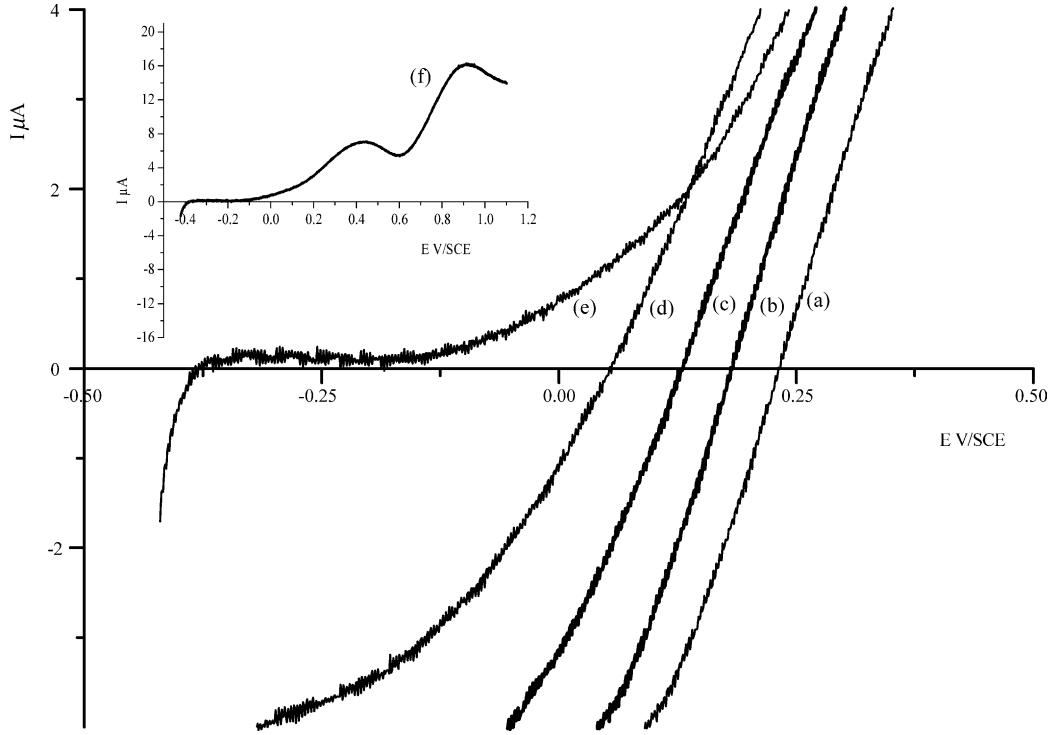

Fig. 2. Steady state intensity–potential curves for a model wine solution (20 mL) containing various oxygen concentrations. Working electrode: rotating platinum disk. Rotation rate: 40 rad s^{-1}. Potential sweep rate: 8 mV s^{-1}. Curves (a–e): the only parts between +4 and −4 µA are shown. N_2 bubbling time: (a) 0 min, (b) 3 min, (c) 6 min, (d) 9 min, (e) 20 min. Curve (f): global intensity–potential curve for a deaerated model wine solution.

curve (f) of Fig. 2. This indicates that the system Ti(IV)/Ti(III) is highly irreversible under the experimental conditions selected. These results are in conformity with the electrochemical behaviour of this system already studied on vitreous carbon [31] and on mercury [32]. So the potential of −0.40 V obtained at the end of the titration by Ti(III) is correlated with the system H^+/H adsorbed on platinum, the adsorbed hydrogen coming from the reaction between the proton and Ti(III). This is why the potential in the second part of the titration reduction does not obey Nernst's law applied to Ti(IV)/Ti(III).

Hydrogen adsorption is also responsible for non-reproducible values of the platinum electrode potentials obtained before titration. Indeed several consecutive titrations by Ti(III) are accompanied by a systematic decrease of the initial potential, even after washing the electrode.

3.2. Potentiometric titrations of catechin solutions

To our knowledge the standard apparent potential for catechin is not known and cannot be deduced from the electrochemical studies recently published [12,14]. Moreover, there is some doubt about the reaction between catechin and DCPIP. However, the reactivity of the molecule can provide some information. The catechin molecule is frequently chosen as a model of polyphenol compounds because of its abundance in e.g. fruits, teas and wine and of its very important antioxidant properties. Moreover, it is well known that the catechin present in wines reacts with oxygen to give orthoquinone and hydrogen peroxide [33]. This last reaction indicates that the standard apparent potential is lower than 0.29 V/Ag/AgCl. It is also possible to deduce from HPLC experiments with electrochemical detection performed on a glassy carbon electrode that this potential is lower than 0.23 V/Ag/AgCl at pH 3.6 [34,35]. These values seem to be inconsistent with cyclic voltammetry experiments [12]. The electron transfer between the catechin molecule and the glassy carbon electrode is slow and polymerisation reactions are coupled to heterogeneous electron transfer [36]. As a consequence, the standard apparent potential may not be deduced from the difference between the anodic and cathodic potential peaks [12]. To verify the occurrence of a spontaneous reaction between catechin and DCPIP two kinds of experiments were performed. The first was based on spectrophotometric measurements. A model wine solution-containing DCPIP was introduced in a spectrophotometer cell and absorbance measurements performed over time at 518 nm, the wavelength corresponding to a maximum of absorbance of DCPIP in this medium. During the first 45 min the absorbance variation was 10% without catechin in solution correlated to the DCPIP instability at this pH. When catechin was introduced into the solution, absorbance decreased by 50% during the same time.

The second experiment was based on chronoamperometric measurements performed with a vitrous carbon-rotating anode. The electrode, held at a DCPIPred oxidation potential (0.30 V), was immersed in a model wine solution containing DCPIPox. When a catechin sample was added, the current

intensity increased with time, indicating that a reaction between catechin and DCPIPox is coupled to the electrochemical oxidation of the DCPIPred formed. These experiments indicate a spontaneous slow reaction between catechin and DCPIP predicting a very difficult redox titration of catechin in contrast to Vivas' results [21].

Nevertheless, we attempted the redox titration of catechin by the oxidized form of DCPIP. But, the potential taken by a platinum electrode immersed in a deaerated model wine solution, containing catechin (3.2×10^{-6} mol L^{-1}) was equal to 0.15 V, and the addition of DCPIP solution resulted in the fast increase of this potential without the appearance of an inflexion point. This indicates that the initial potential is too high for a potential jump to be detected. It is possible to explain the high value of the initial potential using the steady state intensity–potential curve obtained during catechin oxidation on a rotating disc platinum electrode. By steady state voltammetry it was shown that ethanol electrooxidation occurs at potentials slightly lower than that necessary for catechin oxidation. As a consequence, the value of 0.15 V corresponds to a mixed potential between alcohol oxidation and oxygen reduction and is not influenced by the presence of catechin.

In order to eliminate the last traces of dissolved oxygen and to decrease the initial potential value, the catechin titration was carried out in two steps. First of all the catechin was titrated using a Ti(III) solution; the curve is the same as that obtained with the model wine solution. This indicates that the catechin present in the sample is entirely in the reduced form. Titration by DCPIP was then carried out and the results are shown in Fig. 3. The standard potentials of the two redox systems are different enough to expect two inflexion points, but curve (3a) shows only one inflexion point whereas the solution contains both Ti(III) and catechin. The repeatability of the titrations was about 2%. Curve (3b) corresponds to titration by the same solution of DCPIP of a model wine solution containing the same quantity of Ti(III). Taking into account a reaction between catechin and DCPIP occurring mole by mole, the difference of volumes at the equivalent points corresponds to only 0.2% of catechin reacted with the DCPIP. This small percentage is the consequence of the slow reaction between catechin and DCPIP. The electrode potential rapidly indicates the presence of DCPIP in solution although catechin has not yet been oxidized. So, strictly speaking, the word titration is inappropriate to characterize the above experiments.

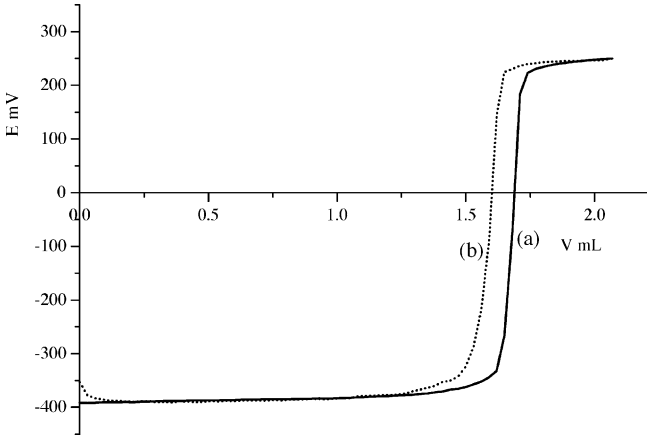

Fig. 3. Potentiometric titration curve of Ti(III) by DCPIP in presence of catechin. Model wine solution volume: 20 mL. DCPIP concentration solution: 3.1×10^{-3} mol L^{-1}. (a) Ti(III) initial quantity: 1.0×10^{-5} mol and catechin 30.6 mg. (b) Ti(III) initial quantity: 1.0×10^{-5} mol.

The potential decrease is correlated to the decrease of the concentration of the DCPIP oxidized form. Such a slow reaction rate is also confirmed by spectrophotometric measurements. Unfortunately due to the very different initial experimental conditions for the two kinds of experiments, it is not possible to compare the two reaction rates.

3.3. Potentiometric titrations of tanin solutions

In the following, the experimental protocol consists of initially adding Ti(III), then carrying out a reoxidation with DCPIP solution. The Ti(III) solution was added either by increments of volumes with recording of the electrode potential after each addition or all of a sudden.

3.3.1. Grape skin and oak tannins

A known quantity of Ti(III) was added to the model wine solution containing grape skin tanins, titration by a DCPIP solution was then carried out. The results are shown in Fig. 4. The first part of the curve presents an inflexion point. Then the evolution of the potential was identical to that observed during the titrations by DCPIP already presented. For example given curve seems to present two potential jumps, but this phenomenon is not reproducible and for other titrations only one inflexion point is present. When 14.5 mL of DCPIP are added, the introduction of the titrating reagent is stopped and a decrease of the potential is observed. This

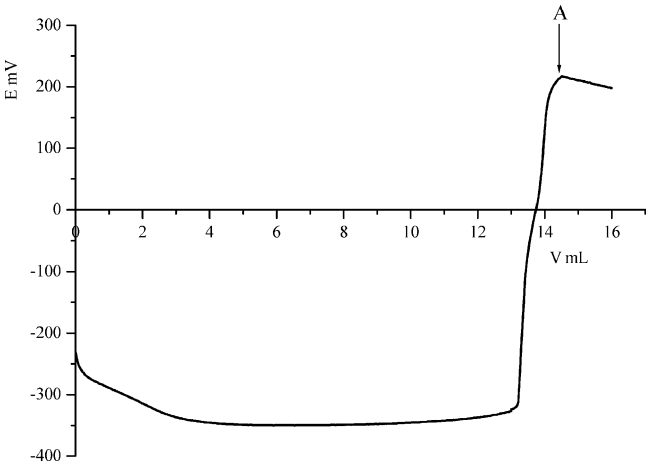

Fig. 4. Evolution of the zero current potential of a platinum electrode during introduction of DCPIP solution. Model wine solution volume: 20 mL. Grape skin tanins 27.0 mg. Ti(III) added: 2.0×10^{-5} mol. DCPIP solution: 1.1×10^{-3} mol L^{-1}. At point A DCPIP introduction is stopped and the potential is measured vs. time for 2 min.

phenomenon is also due to the slowness of the oxidation reaction between reduced molecules present in the sample and DCPIP.

To explain the first part of the curve shown in Fig. 4, the electrode potential is recorded versus time after the addition of Ti(III) and the zero current chronopotentiometric curve is shown in Fig. 5. Such an evolution can be explained by the presence in the tanins of compounds strongly adsorbed on the platinum surface, which complicates the establishment of equilibrium at the electrode–solution interface. Moreover, such behaviour indicates a slow reaction between the oxidized adsorbed molecules and Ti(III). An important consequence of such a reaction is the necessity to wait for sufficient time until the electrode potential is stabilized towards −0.36 V before beginning the introduction of DCPIP for the oxidation titration.

Similar results are obtained with oak tanins.

3.3.2. Grape seed tanins

The addition of Ti(III) in an model wine solution containing the grape seed tanins involved a very fast decrease of the potential with stabilization at a value lower than −0.40 V. In contrast a titration performed with a Ti(III) solution leads to a curve with a potential jump as shown in Fig. 6. The quantity of Ti(III) introduced at the equivalent point n_{Ti} (expressed in mol) is correlated with the mass of tanins m_t (expressed in mg) present in the sample of 20 mL of model wine solution. The correlation law R1 is: $n_{Ti} = 1.5 \times 10^{-7} m_t + 2.6 \times 10^{-8}$ with a determination coefficient equal to 0.9965. The quantity of Ti(III) introduced until the equivalent point is used to reduce molecules present in the oxidized form in the tanins. These molecules react rapidly with Ti(III) and the linear relationship obtained indicates that they were homogeneously distributed in the tanin powder.

Fig. 7 corresponds to the titration of a model wine solution containing grape seed tanins, after the addition of a known quantity of Ti(III), by a solution of oxidized DCPIP. Two inflexion points appear. Note that the DCPIP volume at the first inflexion point is proportional to the quantity of Ti(III) added in excess during the previous reduction titration by Ti(III). The quantity of DCPIP present in the volume added between the two potential jumps n_{DCPIP} is correlated to the mass of tanin (mg) by the following law R2: $n_{DCPIP} = 8.2 \times 10^{-8} m_t + 4.5 \times 10^{-7}$ with a determination coefficient of 0.9968.

Considering R1 and R2, the quantity of Ti(III) necessary for the reduction is twice the quantity of DCPIP necessary for the oxidation reactions. According to the mass balance for the reaction between Ti(III) and DCPIP, the total quantity of DCPIP used for reoxidation corresponds to the total quantity of Ti(III) initially introduced. This shows that the DCPIP does not react significantly with the reduced molecules present in the tanins, during the time involved for the measurement.

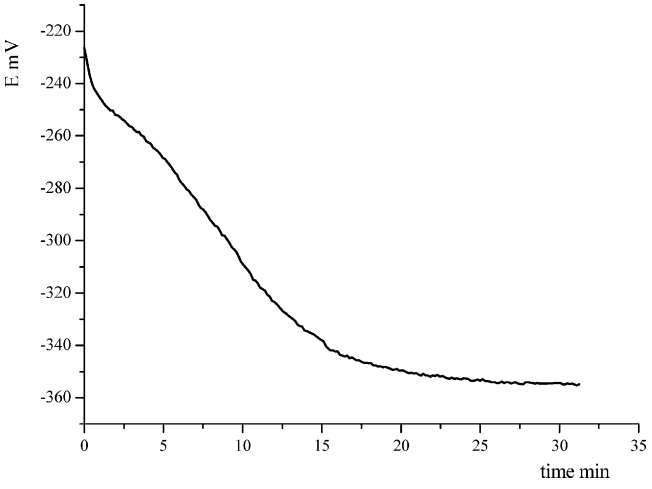

Fig. 5. Zero current chronopotentiometric curve. Model wine solution volume: 20 mL. Grape skin tanins 26.9 mg. 3.0×10^{-5} mol of Ti(III) are introduced at zero time.

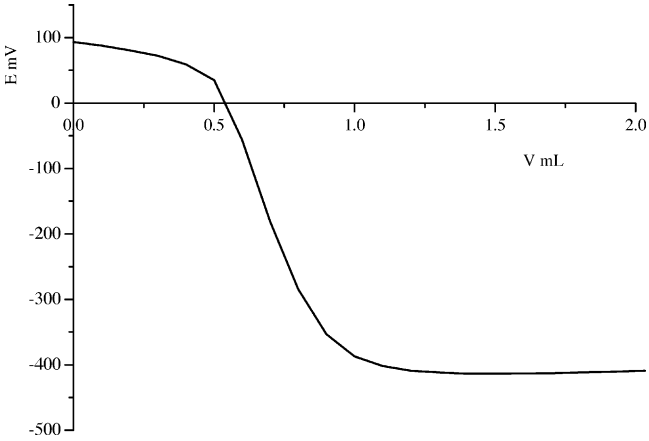

Fig. 6. Evolution of the zero current potential of a platinum electrode during introduction of Ti(III) solution. Model wine solution volume: 20 mL. Grape seed tanins 48.5 mg. Ti(III): 1.1×10^{-2} mol L^{-1}.

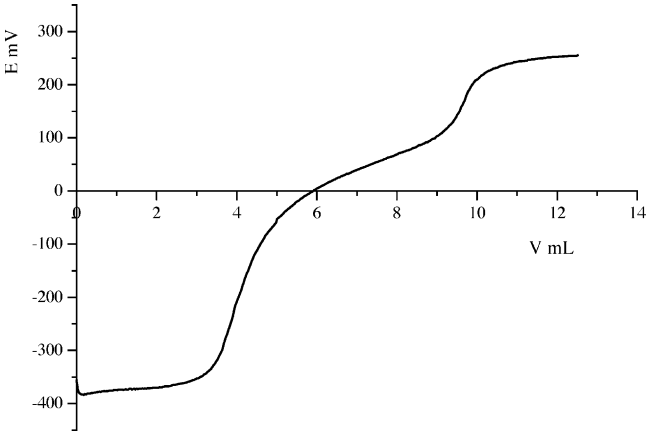

Fig. 7. Evolution of the zero current potential of a platinum electrode during introduction of DCPIP solution. Model wine solution volume: 20 mL. Grape seed tanins 56.6 mg. Ti(III) initially added: 1.7×10^{-5} mol. DCPIP concentration: 0.91×10^{-3} mol L^{-1}.

3.4. Potentiometric titrations of some wines

An example of the titration curve of a sample of wine made from Tannat grapes by a Ti(III) solution is presented in Fig. 8 curve (a). A first decrease in the reduction potential appears until about −100 mV, which corresponds to the reduction reaction with the adsorbed compounds at the platinum–solution interface. The potential did not reach values lower than −350 mV previously found in all the presented examples. Then a potential jump clearly appears. When the potential stabilized, addition of the titrating reagent was stopped (point B) and the electrode potential increased slowly. A slow reduction of some compounds present in the wine took place and some Ti(III) appeared in solution whereas not all the reducible compounds were reduced. This reaction continued and the Ti(III) quantity decreased regularly with time.

Titration by a DCPIP solution was carried out with the same wine sample, following the reduction titration by Ti(III) (curve b). The curve shows two potential jumps. When the potential variation became weak, DCPIP introduction was stopped (point C). Then the potential decreased with time, again indicating that the oxidation reaction with DCPIP was slow. The quantity of DCPIP corresponding to the second potential jump is lower than the theoretical quantity necessary to oxidize all the Ti(III) introduced. Such a situation indicates that quantitative analysis does not seem to be possible. This conclusion is in accordance with the results presented by Ferreira [16,17]. As a matter of fact in Ferreira's work the DCPIP volumes obtained for a wine sample V_0, for a wine containing n_1 moles of ascorbic acid V_1 and for a wine containing $2n_1$ moles of ascorbic acid V_2 does not verify the relation $V_2 - V_0 = 2V_1 - V_0$. The DCPIP quantities are proportional to the quantities of ascorbic acid, but V_0 does not appear to be a constant.

The same phenomena occurred with the sample of Pinot. To show the difficulty to determine reduced compound concentrations in the wine, the DCPIP reoxidation curves were obtained after addition of different volumes from the same solution from Ti(III) (volumes V_1 and $V_2 > V_1$). The curves show two potential jumps not very well defined. The total volume of titrating reagent corresponding to the second potential jump is lower than the volume necessary to react with all Ti(III) added. The difference of volumes at the first equivalent point is lower than the volume necessary to react with the Ti(III) present in the $(V_2 - V_1)$ volume. So the volume of DCPIP to reach the first potential jump is not correlated with the quantity of Ti(III) in solution before the beginning of titration.

Many red wine samples were titrated by Ti(III) solution. The volumes of Ti(III) necessary to reach the equivalent point are indicated in the following table as well as the total polyphenol index determined spectrophotometrically.

Wine	Volume (mL)	Total polyphenol index	Volume of Ti(III) (mL)
Pinot	20	59	0.57
Sirah	20	64	1.02
Tannat	20	100	1.47
Tannat/cabernet	20	Not determined	0.89

Although the volume of Ti(III) required increases for wines with high polyphenol content, there is not a linear correlation.

The variation of the quantity of polyphenol can also be obtained by dilution of the wine with the model wine solution. The volume of Ti(III) at the equivalent point varies proportionally with the volume of wine introduced into the sample, with however a significant uncertainty. An example of curves is represented in Fig. 9. The correlation found between the

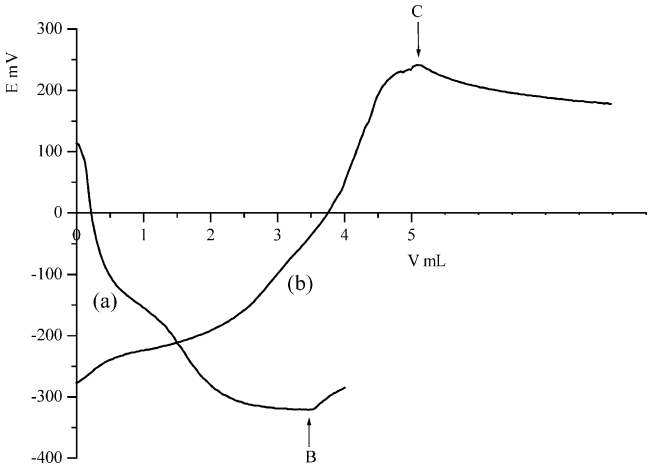

Fig. 8. Evolution of the zero current potential of a platinum electrode during successive introductions of Ti(III) and DCPIP solutions. Tannat wine sample: 20 mL. Curve (a): introduction of Ti(III) solution 2.0×10^{-2} mol L^{-1}. At point B, Ti(III) introduction is stopped and the potential is measured vs. time during 2 min. Curve (b), introduction in the precedent solution of DCPIP 2.8×10^{-3} mol L^{-1}. At point C, DCPIP introduction is stopped and the potential is measured vs. time during 15 min.

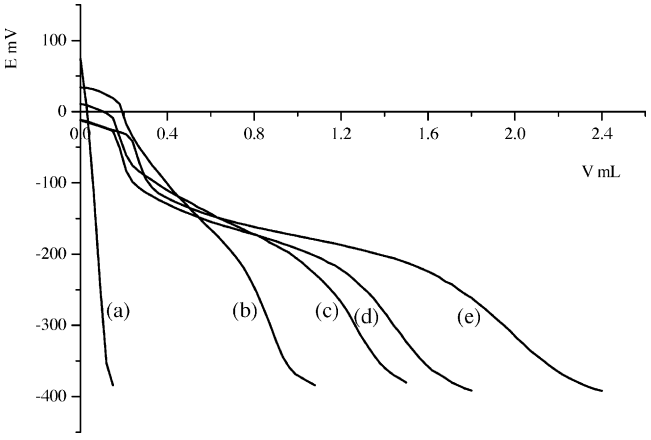

Fig. 9. Evolution of the zero current potential of a platinum electrode during introduction of Ti(III) solution into a red wine sample diluted in model wine solution. Ti(III) concentration: 1.0×10^{-2} mol L^{-1}. The total volume is equal to 20 mL. Wine volume: curve (a) 0 mL, curve (b) 5 mL, curve (c) 10 mL, curve (d) 15 mL, curve (e) 20 mL.

volume of Ti(III) to the equivalent point and the volume of wine is: $V_{Ti(III)} = 0.49\,V_{wine} + 3.05$ with a determination coefficient of 0.9761.

The titration reactions of the wines in oxidation are too slow for the quantitative determinations of concentration of reduced compounds to be made. Nevertheless, although the steady state is never reached equivalent points are detected. The reactant volumes may give some comparative indications from one wine to another, if the same parameters for titration are used. The main parameters are rate of introduction of reagent, the quantity of Ti(III) added, the latency between the reduction by Ti(III) and reoxidation by DCPIP.

4. Conclusion

Among the many experimental curves giving the evolution of the electrode potential versus the volumes of reagent introduced, a low number are true titration curves. They are essentially the curves obtained during the titration of solutions of Ti(III) by iodine and the DCPIP, which are useful for the calibration of the titrating solutions used in the study. Ti(III) gets rid of all traces of oxygen still present in solution after deaeration by bubbling with nitrogen, which results in lowering the zero-current potential of the electrode because of the modification of the redox system at the interface. Ti(III) is responsible for the appearance, on the platinum electrode, of adsorbed hydrogen atoms produced by the reduction of protons at the interface. The presence of Ti(III) in solution results in stable potentials due to the reversibility of the $H^+/H_{adsorbed}$ system. The lowering of the initial potential makes it possible to use a large range of potentials to visualize potential jumps for oxidation titrations. Ti(III) acts as a reducing reagent for compounds present in the studied tanin solutions and red wines. The reduction reaction rate is fast in the case of the tanins and there is proportionality between the tanin mass and the volume of Ti(III) at the equivalent point. The reaction rate is slower in the case of the wines and can no longer be considered a titration. The products formed during this reduction reaction belong to a redox system whose apparent standard potential is sufficiently low so that the DCPIP plays the role of an oxidant. The oxidation reaction by DCPIP is fast with the tannins but slow with the wines. But in general potentiometry is not a suitable method for the determination of oxidation resistance capacity of wines.

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