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Titrimetric method based on potentiometric titration to evaluate redox couples in wine and polyphenols

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Summary

Polyphenols are electroactive compounds, each molecule having reductive (Rd) and oxidative forms (Ox) due to the presence of several phenolic hydroxyls. Direct study of Rd/Ox forms was conducted using potentiometric titration in two steps: First, a complete reduction of the polyphenol Rd/Ox couples by titanium III chloride $TiCl_3$ (100 % Rd), and then, oxidative titration by dichlorophenolindophenol DCPIP (100 % Ox). The second curve represents the totality of polyphenol couples titration with, for each couple, a characteristic E_0 point representing the equilibrium between the concentration of Rd and Ox forms (Rd/Ox = 1). This value was typical for each polyphenol. We conducted several preliminary experiments with a model polyphenol (catechin) to establish the analysis conditions. The titration solution concentration was N/10 in HCl N and N/20 in water for $TiCl_3$ and DCPIP respectively. The concentration of this solution diminished very rapidly, *i.e.* within 24 h for $TiCl_3$ and DCPIP (under nitrogen and in darkness) and regularly needed a fresh preparation. To control the consistency and precision of the method we performed a few tests on pure products (*e.*g. hydroquinone/quihydrone, Fe III, Fe II, Cu II) permitting comparison between theoretical and experimental E_0 values. Our result indicated less than 5 % variation and curves with a high reproducibility.

K e y w o r d s : Wines, polyphenols, oxido-reduction, potentiometric titration, dichlorophenolindophenol, E_O.

Introduction

Polyphenols are electroactive compounds which are involved in many different oxidation or reduction reactions affecting the composition and quality of food and beverages (Weeny *et al.* 1974, Tanizawa *et al.* 1984, Mayer 1987, Lattanzio *et al.* 1989, Hirose *et al.* 1990). This property is due to phenolic hydroxyls that depend on the polymeric level of the molecules. Oxidation by enzymatic or chemical reactions induces polyphenol polymerization and intensifies browning (Waters 1964). On the basis of these enzyme oxidation properties, many investigations have contributed to the determination of polyphenols by amperometric biosensors (Pravda *et al.* 1995, Eggins *et al.* 1997).

When analyzing wine, rather than quantifying total polyphenols (Santos-Buelga and Williamson 2003, Vivas *et al.* 2003) it is more appropriate to evaluate each redox polyphenol group in order to follow the evolution of the wine during oxidation and to estimate its oxido-reductive capacity by a global index (Vasconcelos *et al.* 1999, Kilmartin *et al.* 2001). In research laboratories the oxidative status of molecules under different conditions (solution composition, pH, reagent concentration, metallic catalyst) is monitored. In enology, potentiometric titration of oxido-reductive compounds in wine was demonstrated by Ribereau-Gayon and Gardrat (1957). These authors showed that it is possible to quantify and measure the different redox polyphenols and their respective normal E_0 potential.

Potentiometric titration is a two-step process. First, the polyphenol or wine solution is completely reduced by a selected reducer. Thereafter, all redox couples are in a reduced form. Subsequently, the same solution is oxidized by a selected oxidizing agent. A second curve is obtained that displays all the redox compounds of a sample with the expression of each characteristic E_0 value.

Material and Methods

All solutions were prepared with ultrapure water from a Millipore Milli-Q system. HCl purum quality (Merck), alcohol (absolute, Merck Eurolab), L(+)-tartaric acid (>99 % purity, Acros Organics), NaOH (98 %, SDS) were used as hydroalcoholic solution for sample dilution before analysis (adjustment of pH at the initial pH of wine). The composition of the hydroalcoholic solution was 12 % vol. EtOH, 5 $g \cdot l^{-1}$ tartaric acid and NaOH to adjust the pH. (+)-catechin and (-)-epicatechin hydrates (Sigma Aldrich, 93.7 % purity, by H^1 NMR, Bruker DPX300) were used as wine polyphenol models in order to determine the method's parameters (1 $g \cdot l^{-1}$ in hydroalcoholic solution at pH 3.5). The phenols were two isomeric forms of flavan-3-ols. $TiCl_3$ (reducer agent, 10 % in HCl 20-30 %, Sigma Aldrich) was prepared in 1N HCl (Sigma Aldrich) and DCPIP (oxidizing agent, dichlorophenolindophenol sodium hydrate, Sigma Aldrich) in ultrapure water. The titrator was a DL50 (Mettler Toledo) with two interchangeable 10 ml burettes (DV910), a potentiometric electrode (DM140-SC) combined with a platinum ring electrode for redox titration in a range of 0-70 °C, with 3M KCl satu-

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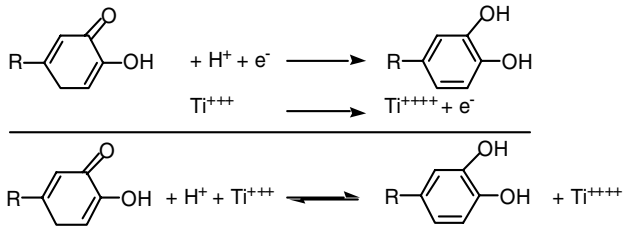
rated with AgCl reference electrolyte. The electrode was calibrated with a redox buffer solution and washed overnight in concentrated ammonia and a few min in an ultrasonic bath with water. The whole system was controlled by computer. Preparation of samples: 50 ml of hydroalcoholic solution of (+)-catechin, other polyphenols or 50 ml of wine diluted at 1/50 in the same solution for reduction. Titration conditions were: ml per 20 s for a reduction increment of 0.03 and ml per 30 s for an oxidation increment of 0.02. Titration started only after nitrogen saturation of the samples and all experiments were managed under a nitrogen atmosphere. Temperature: 20 °C ±1 °C.

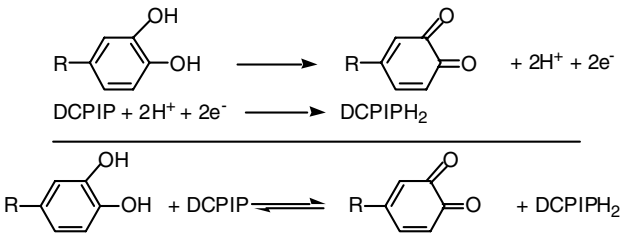
Results and Discussion

Choice of titration agents: A reducer and an oxidant were used for the potentiometric titration. The selected products did not affect the polyphenol structures and allowed a back-titration with no changes compared to the first titration. Several agents were tested: sodium hydrosulfite for the reduction was unstable in solution; for oxidation, hydrogen peroxide was very unstable; potassium permanganate is a stronger oxidant destroying polyphenols, and iodine causes the formation of strong complexes with polyphenols. Finally, we retained two agents: titanium III chloride ($TiCl_3$) as reducer and dichlorophenolindophenol (DCPIP) as oxidant.

For $TiCl_3$, solutions prepared at N/100 to N were tested during a titration of a (+)-catechin solution (1 $g \cdot l^{-1}$). At N/100, N/80 reductive power was too low, at N/50, N/30 the reduction was incomplete and slow (> 1 h), at N/10 the reduction went to completion in 15 min and consequently was selected for the experiment. In accordance with the following equation of reaction, 1 mole of $TiCl_3$ reduced one phenolic ketone in phenols:



For DCPIP, in order to respect the stoichiometry of the global reaction, the N/20 solution was retained. With these conditions, titration of (+)-catechin solution (1 $g \cdot l^{-1}$) was performed in 25 min. In accordance with the following equation of reaction, 1 mole of DCPIP oxidized 2 phenolic OH group:



Titration agents lifetime: Titration agents in solution lost their reductive or oxidative power when stored. To determine the lifetime of agents, we titrated a (+)-catechin hydroalcoholic solution (1 $g \cdot l^{-1}$) over time. The results collected in Fig. 1 show that for $TiCl_3$ and DCPIP the correct lifetime (*i.e.* without any change in the titration curves) was 24 h; the best preservation conditions were in a dark room at ambient temperature. After 24 h, some crystals were formed in the aqueous DCPIP solution. After 10 d, all the solutions were completely de-titrated.

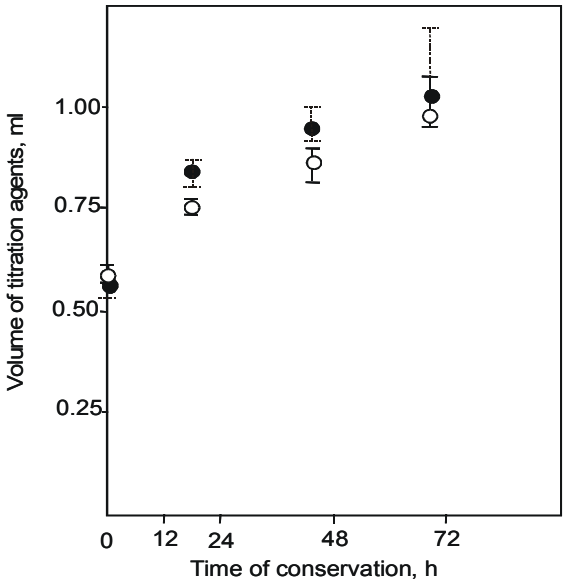


Fig. 1: Titration agents as function of time of conservation at room temperature in the dark. White circles corresponding to $TiCl_3$, black circles to DCPIP. Mean values of three replicates.

Repeatibility of the method: A repetition of (+)-catechin titration yielded satisfactory results (Tab. 1). Under our conditions, for a pure product, the maximum variability of the curves was < 5 % when replications were made in succession; however, when replicate titration was performed several days later, variability was higher, but no more than 8 %. Statistical results were similar for wine: < 7 % variability for successive replications and < 10 % after some time.

Table 1

Precision of titration assay for (+)-catechin solutions. EHmin, minimim of EH at the end of reduction; EHmax, maximum of EH at the end of oxidation. Vmax, total volume of titration solutions for a complete titration

	Reduction[a]		Oxidation[b]	
	EHmin (mV)	Vmax (ml)	EHmax (mV)	Vmax (ml)
Average (n = 6)	-328	0.825	348	1.02
Standard deviation	5.01	0.04	2.38	0.08
Confidence interval (for a 5 %)	4.01	0.03	1.9	0.06

[a] $TiCl_3$, [b] DCPIP.

Importance of pH value: Before analysis, the samples need to be diluted in order to shorten analysis time and to limit the volume of titration agents required. To reproduce a composition similar to wine, we chose an hydroalcoholic medium. The pH values of this preparation significantly affected the curve profiles obtained with (+)-catechin (Fig. 2). Particularly, with increasing pH we noted a faster reduction, mainly at pH 4.5. For average wine values (3.5-4.0), there was little variation. The difference was greater for oxidation than for reduction, even in the pH range of wine. Normal E_0 potential was not affected. To standardize the conditions of the assay, polyphenols were all analyzed at pH 3.5. For wines, the dilution medium was prepared at the same pH as the titrated wine.

Comparison of theoretical and experimental E_0 values: The normal E_0 potential was typically that of constant redox couples, some of which have been reported in literature (Atkins 1990). E_0 was calculated in accordance with the Nernst law (Vivas *et al.* 1996). Fig. 3 shows experimental E_0 determination of (+)-catechin and (-)-epicatechin, which were different (275 and 171 mV, respectively). It is interesting to note that the epicatechin, with the lower E_0 value, is more oxidized than catechin (Freitas *et al.* 1996). In Tab. 2, we show three examples: two mineral redox couples and one organic couple, a simple phenol quinhydrone/hydroquinone indicating high agreement between theoretical and measured E_0 values.

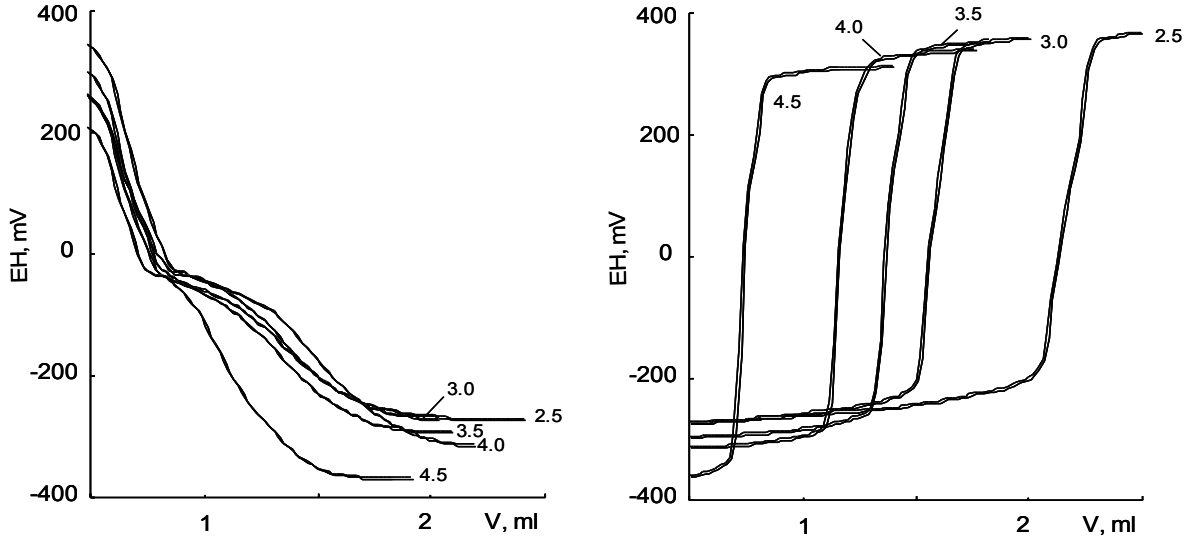


Fig. 2: Influence of pH on the titration curves in reduction (left graph) and oxidation (right graph) of (+)-catechin in hydroalcoholic media. pH values ranged from 2.5 to 4.5. V (ml) is the volume of titration agent ($TiCl_3$ N/10 for reduction, DCPIP N/20 for oxidation), EH (mV) is the oxidoreduction potential.

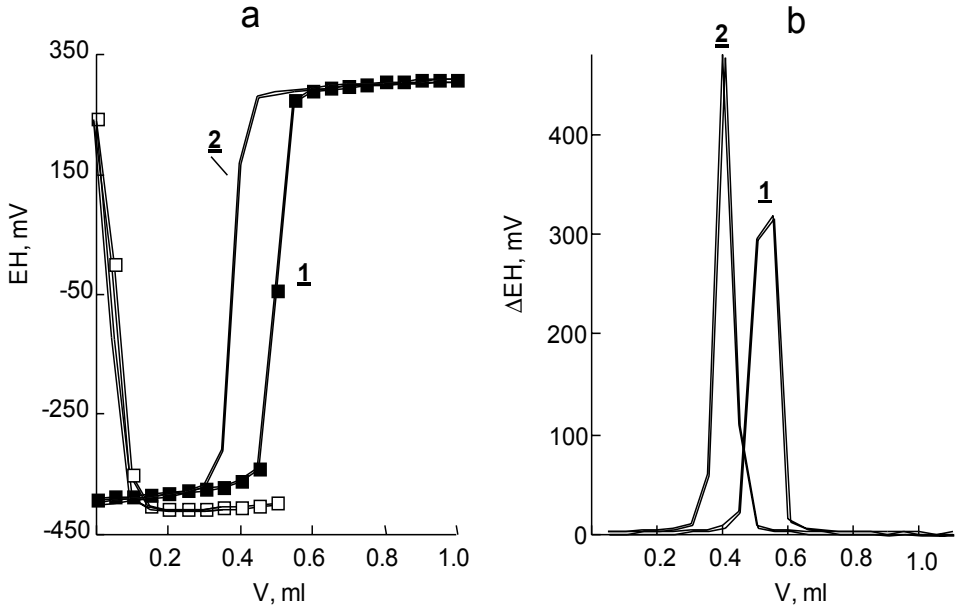


Fig. 3: Potentiometric titration of (+)-catechin (1) and (-)-epicatechin (2). **a**: Characteristic curve (white symbols: reduction, black symbols: oxidation). **b**: Titrogramme obtained by calculation of ΔEH. E_0 of (+)-catechin and (-)-epicatechin were 275 and 171 mV, respectively.

Table 2

Values of normal potentials E_0 obtained by theoretical calculation and experimental measurements

	Eo		Variation
	Theoretical	Experimental	%
Fe^{++}/Fe^{+++}[a]	770	710 ± 15	7.8
Cu^{+}/Cu^{++}[a]	150	160 ± 5	6.7
Quinhydrone/ hydroquinone[b]	699.5	713 ± 24	1.9

[a] titration in distilled water.
[b] titration in hydroalcoholic solution at pH 3.5.

Application to alcoholic beverages and vinegar: The method was applied to different samples of wines, vinegar and brandies; satisfactory results were obtained in all cases (Fig. 4). The method was also tested on different polyphenol sources and pure molecules; it proved to have a general validity.

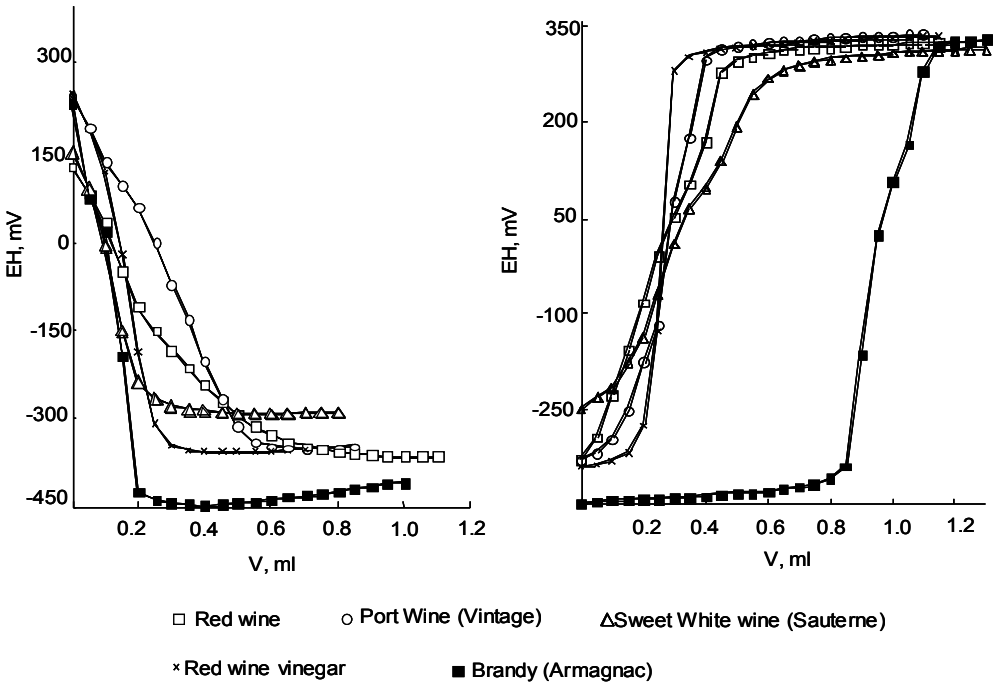


Fig. 4: Potentiometric titration curves for wines, brandy and red wine vinegar. On the left: reduction, on the right: oxidation. pH of dilution solutions was adjusted to the pH of samples.

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