A Cyclic Voltammetry Method Suitable for Characterizing Antioxidant Properties of Wine and Wine Phenolics

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Phenolic antioxidants are ranked by reducing strength and characterized for reversibility using cyclic voltammetry at a glassy carbon electrode. Phenolics with an *ortho*-diphenol group show a first oxidation peak close to 400 mV (vs. Ag/AgCl) in a model wine solution (12% ethanol, 0.033 M tartaric acid, adjusted to pH 3.6), with a linear concentration dependence below 0.01 mM. Dilution of white wines 10×, and red wines 400×, gave first oxidation peak currents in the 1.5 to 2.2 μA range and 1.9 to 3.4 μC of charge passed by 500 mV, producing values for the concentrations of phenolic antioxidants with low oxidation potentials in the wines. Further peaks in the cyclic voltammograms of the diluted wines correspond to classes of phenolics with higher oxidation potentials, providing a qualitative assessment of wine phenolics based on reducing strength.

Keywords: *Cyclic voltammetry; wine; phenolics; antioxidants*

INTRODUCTION

Interest in the antioxidant substances in wine originated in epidemiological studies that showed that wine-consuming populations were protected from high rates of heart disease (*1*, *2*). An explanation for this effect suggested that the phenolic substances were acting in vivo as antioxidants (*3*): a mode of action thought to delay atherosclerosis (*4*), and thus reduce coronary disease and consequently decrease mortality. This theory has been widely tested, and among other results, polyphenolics have been shown to be potent antioxidants in vitro (*5*). Many methods involving radicals have been proposed to test the antioxidant properties of biological samples, foods, extracts, and pure substances (*6*), but none provide quantitative information on the relative ease of oxidation, an important factor in antioxidant properties.

Compounds which are antioxidants by virtue of their ability to act as reductants in solution tend to be easily oxidized at inert electrodes. Cyclic voltammetry has also been applied to characterize a range of antioxidants, including phenolic acids and flavonoids (*7*−*16*), ascorbic acid (*17*), and synthetic antioxidants (*18*, *19*). Phenolics with *ortho*- or *para*-diphenol groups are typically found to have lower oxidation potentials than compounds with *meta*-diphenols or isolated phenols, and ascorbic acid has an even lower oxidation potential. However, in none of these studies were phenolics analyzed under the conditions found in wines, and the effect of the antioxidant concentration was rarely considered.

Methods using cyclic voltammetry have also been described to measure the reactive (low oxidation potential) fraction of reducing substances in a fluid. In the case of blood plasma, a low potential anodic wave at 400 mV (Ag/AgCl) has been ascribed to ascorbate and urate, and further waves have been ascribed to antioxidants with a higher oxidation potential (*20*). Extracts from edible plants have also been examined, with the area under the anodic peak(s) taken as a measure of the content of low-molecular-weight antioxidants (*21*). Another approach to monitoring the reducing strength of a solution at an inert electrode has been developed for wines by Mannino et al. (*22*), in which the current at 400 mV at a carbon electrode following flow-injection analysis of a wine diluted in 50 mM tartaric acid is used to quantify the "antioxidant power" of the more readily oxidizable phenolics.

In the present study, cyclic voltammetry is used to characterize a range of phenolic acids and flavonoids, ascorbic acid, and sodium metabisulfite, which make an important contribution to the antioxidant properties of wines. A model wine solution is used as the solvent to establish the oxidation potential that each phenolic exhibits in wine. An easily obtainable measure is then presented for diluted red and white wines, in which the cyclic voltammogram is used to distinguish classes of phenolic compounds by their relative propensity to be oxidized.

MATERIALS AND METHODS

Reference Standards. Caffeic acid, catechin, *p*-coumaric acid, epicatechin, ferulic acid, quercetin, *t*-resveratrol, rutin hydrate, tannic acid, and vanillic acid were purchased from Sigma. Other purchased chemicals included L-ascorbic acid (Scientific Supplies), morin (May & Baker), gallic acid monohydrate (Riedel-de Haën), malvin (Pfaltz and Bauer), and $Na_2S_2O_5$ (Scharlau). The chemical structures for these standards are given in Chart 1.

Cyclic Voltammetry. Cyclic voltammograms were recorded using Bioanalytical Systems (BAS) equipment at a 100A electrochemical analyzer with BAS C2 cell stand. The working electrode was a 3-mm glassy carbon disk electrode (BAS M-2012) which was cleaned by polishing with 3 μm alumina powder (PK-4 polishing kit) for 2 min between runs. A Ag/AgCl reference electrode (+207 mV versus SHE) was used in conjunction with a platinum counter electrode. Solutions of antioxidants, to final concentrations of 0.5, 0.05, and

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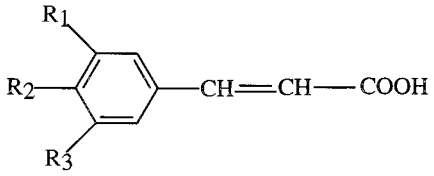
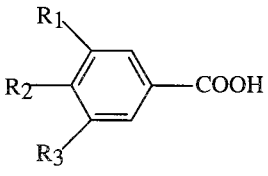
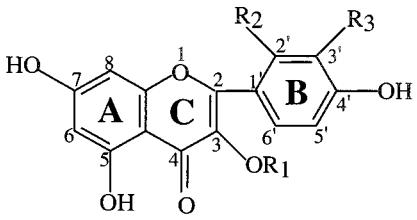
Chart 1. Chemical Structures for the Phenolic Antioxidants and for Ascorbic Acid



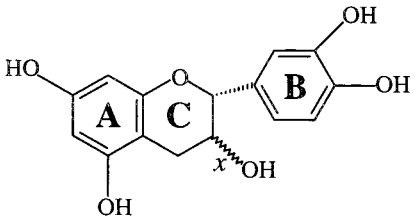
Caffeic acid: $R_1 = R_2 = OH$; $R_3 = H$
Ferulic acid: $R_1 = H$; $R_2 = OH$; $R_3 = OCH_3$
p-Coumaric acid: $R_2 = OH$; $R_1 = R_3 = H$



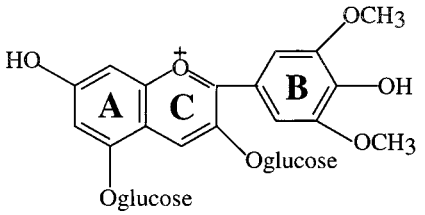
Gallic acid: $R_1 = R_2 = R_3 = OH$
Vanillic acid: $R_1 = H$; $R_2 = OH$; $R_3 = OCH_3$



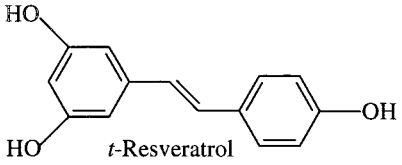
Quercetin: $R_1 = R_2 = H$; $R_3 = OH$
Morin: $R_1 = R_3 = H$; $R_2 = OH$
Rutin: R_1 = rutinose; $R_2 = H$; $R_3 = OH$



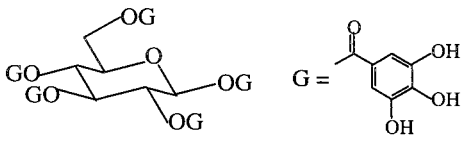
Catechin: bond *x* ——
Epicatechin: bond *x* ·····



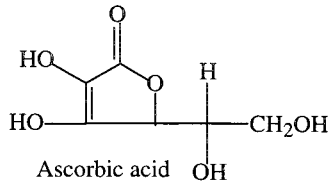
Malvin



t-Resveratrol



Penta-galloyl glucose (in Tannic acid)



Ascorbic acid

0.01 mM, were dissolved in a model wine solution consisting of 12% (v/v) ethanol, 0.033 M L-tartaric acid (Panreac), and added NaOH to give a pH of 3.6, of which 10 mL was added to the cell. In the case of tannic acid, which is a mixture of mainly gallate derivatives including penta-galloyl gallate, solutions were made up using the molar mass of gallic acid.

The initial cyclic voltammograms were recorded soon after the glassy carbon electrode was inserted into the solution, to minimize adsorption of the antioxidant onto the electrode surface prior to the first run. The scan rate was 100 mV s^{-1}, and the initial scan was taken from −100 mV to 100 mV past the first anodic peak, and was repeated 3−5 times in sequence. Further scans were taken to 1200 mV to look for the presence of subsequent redox processes at more positive potentials. Background cyclic voltammograms were taken in the model wine solution and were recorded on the same day as the standards or the wine in question. The voltammograms were analyzed for peak potentials (all reported vs. Ag/AgCl) and currents as described below.

Wines. Cyclic voltammograms of two white wines (Sauvignon Blanc, Esk Valley, N. Z., 1999, pH 3.4, and Sauvignon Blanc, Montana, N. Z., 1984, pH 3.6) and two red wines (Pinot Noir, Coopers Creek, N. Z., 1998, pH 3.7, and Cabernet Sauvignon, Penfolds, Australia, 1991, pH 3.5) were taken with the glassy carbon electrode directly in the wine. The wines were then diluted in the model wine solution to determine the linear concentration range. In each case a scan was taken to 500 mV, by which point a peak was recorded because of the more reactive antioxidants (repeated 3 times). Further scans were taken to 1200 mV to examine the effect of compounds with higher oxidation potentials. The level of total phenols in the wines was also determined using the Folin-Ciocalteau reagent calibrated against gallic acid (*23*).

RESULTS AND DISCUSSION

Oxidation Potentials of the Antioxidant Standards. The behavior of a range of phenolic antioxidants commonly found in wines, along with ascorbic acid and sodium metabisulfite, at concentrations of 0.01, 0.05, and 0.5 mM were examined at the glassy carbon electrode. The initial reaction often had the following form:

$$R \rightleftharpoons O + 2H^+ + 2e^- \quad (1)$$

where R is the antioxidant (reductant) and O is the product formed which can itself act as an oxidant if the process is reversible. Electrochemical parameters were extracted from the cyclic voltammograms to describe the oxidation and the reverse reduction process (*24*), and the results are summarized in Table 1. The peak potentials showed excellent reproducibility, with standard deviations of repeat runs typically less than 3 mV.

Table 1. Electrochemical Characteristics of the First Anodic Peak of Diluted Wines and 0.5, 0.05, and 0.01 mM Antioxidants at a 3-mm Glassy Carbon Electrode at 100 mV s^{-1} in 12% Ethanol, 0.05 M Tartaric Acid, and Added NaOH to Give pH 3.6 ± 0.2

E in mV (Ag/AgCl)	conc. (mM)	$E_{p,a}$	ΔE_p	$E_{p,a} - E_{p/2}$	E_{mid}	$(E_{p,a} + E_{p/2})/2$	$I_{p,a}$ (μA)	Q (μC) to 500 mV
				white wines				
1999 Sauv. Blanc	(10 x dil.)	408	43	54	386	381	1.96 ± 0.08	3.12 ± 0.17
1984 Sauv. Blanc	(10 x dil.)	404	51	49	379	380	1.67 ± 0.10	1.92 ± 0.19
				red wines				
1998 Pinot Noir	(400 x dil.)	396	68	58	362	367	2.22 ± 0.14	3.40 ± 0.18
1991 Cab. Sauv.	(400 x dil.)	393	66	55	360	366	1.53 ± 0.04	2.20 ± 0.18
ascorbic acid	0.5	242	[a]	66		209	12.0	21.7[b]
	0.05	215		53		188	1.24	2.3[b]
	0.01	211		50		186	0.25	0.49[b]
quercetin	0.5[c]	376	28	43	362	355	3.1	3.2[d]
	0.05[c]	378	18	37	369	360	2.3	1.9[d]
	0.01	377	13	29	370	362	0.98	0.67[d]
epicatechin	0.5	415	101	55	365	388	11.2	14.0
	0.05	380	45	37	357	361	4.3	4.1
	0.01	372	21	31	362	357	2.0	1.7
catechin	0.5	455	142	73	384	419	10.7	12.7
	0.05	389	40	38	369	370	3.4	3.3
	0.01	379	19	29	369	364	1.18	0.91
gallic acid	0.5	418		47		395	23.0	26.1
	0.05	391		32		375	3.3	3.5
	0.01	386		31		370	0.74	0.95
morin	0.5	419		58		390	11.6	15.2
	0.05	398		44		376	3.9	4.8
	0.01	396		31		380	2.4	1.9
tannic acid	0.5	429		53		403	9.7	11.4
	0.05	416		42		395	6.6	7.0
	0.01	405		36		387	1.45	1.43
caffeic acid	0.5	435	100	52	385	409	20.7	22.2
	0.05	416	31	37	400	397	3.6	3.1
	0.01	410	17	35	401	392	1.46	1.6
rutin	0.5	469	78	48	430	445	15.4	12.5
	0.05	471	33	37	454	452	4.3	3.0
	0.01	470	19	33	460	453	2.7	1.6
ferulic acid	0.5	639		100		598	12.4	
malvin	0.5	677		130		612	10.0	
t-resveratrol	0.5[c]	667		79		627	3.1	
vanillic acid	0.5	764		73		723	16.0	
p-coumaric acid	0.5	804		112		748	13.9	
$Na_2S_2O_5$	0.5	970		190		875	18.5	

[a] In places where no values are given a cathodic peak was not seen and the oxidation was largely irreversible. [b] Charge to 400 mV. [c] Not fully dissolved. [d] Charge to 450 mV.

The anodic peak current ($I_{p,a}$), expected to be proportional to the concentration of the antioxidants, showed greater variability with a relative standard deviation of up to 10% due to variations in the surface of the carbon electrode after abrasive cleaning.

The result for catechin is illustrated in Figure 1 where the various potential values are defined. When the scan was taken just to 500 mV a reversible process was observed due to the oxidation of the *ortho*-diphenol group on the B-ring of catechin in a 2-electron process to an *ortho*-quinone. Estimates of the formal potential $E^{\circ\prime}$, which quantifies the reducing power of the antioxidant, were given by the potential (E_{mid}) mid-way between $E_{p,a}$ and $E_{p,c}$, a better estimate when the anodic peak was very broad, and by the potential halfway between $E_{p/2}$ and $E_{p,a}$, which can be used when no return cathodic peak is produced (as for ascorbic, gallic, and ferulic acids in Figure 1), and which was used to rank the antioxidants in order of decreasing reducing strength in Table 1. For epicatechin, catechin, caffeic acid, and rutin, the two estimates of the formal potential (E_{mid} and $(E_{p/2} + E_{p,a})/2$) were closer together upon dilution, particularly at 0.05 mM (all within 4 mV).

From Table 1 it can be seen that ascorbic acid will react some 170 mV earlier than a group of phenolics with formal potentials in the 360 to 420 mV range. These phenolic acids and flavonoids contain an easily oxidizable *ortho*-diphenol group, and include caffeic, gallic, and tannic acids, catechin, and epicatechin, with rutin appearing at about 450 mV. The values quoted in Table 1 are consistent with previous literature studies, taking into account the expected 59 mV per pH unit shift in the potentials (*10*, *11*, *13*, *15*, *25*, *26*). Tests in phosphate buffers from pH 3 to 7 confirmed that the formal potential varied by 56 mV per pH unit for catechin and by 66 mV per pH unit for caffeic acid.

The one compound with a low oxidation potential but no *ortho*-diphenol group was morin, which otherwise has a structure similar to that of quercetin (Chart 1). In both cases the hydroxy substitution at C-3 of the C-ring of the flavonol imparts a unique electrochemical behavior due to the electron-donating abilities of the ketone at

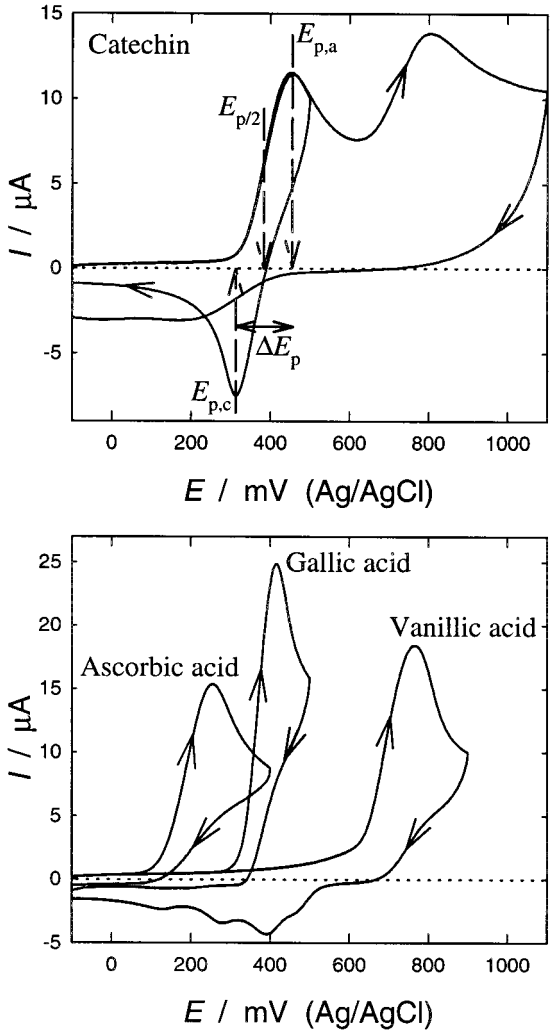


Figure 1. Cyclic voltammograms of 0.5 mM ascorbic acid, gallic acid, and vanillic acid taken to 100 mV past the first anodic peak, and catechin taken to 1100 mV, measured at 100 mV s^{-1} at a 3-mm glassy carbon electrode in the model wine solution (12% ethanol, 0.05 M tartaric acid, and added NaOH to pH 3.6).

C-4 and the oxygen within the ring (*8*). The oxidation of morin, however, was quite irreversible, as is usually seen with the oxidation of isolated phenol groups. Having a rutinose group attached to the C-3 position in rutin removes this irreversibility, but also gives rutin a higher oxidation potential than morin, despite the presence of the more easily oxidized *ortho*-diphenol group.

For a process entirely under diffusion control, $|E_{p,a} - E_{p/2}| = 28$ mV at 25 °C, with larger values, such as the 73 mV value for 0.5 mM catechin (Table 1), indicating a lower degree of reversibility at the electrode. A further indicator of the quasi-reversibility of catechin oxidation at this concentration is the difference in the peak potentials (ΔE_p) equal to 142 mV for 0.5 mM catechin, much larger than the theoretical value for a reversible system of 29 mV at 25 °C. Upon dilution $|E_{p,a} - E_{p/2}|$ and ΔE_p decreased for the phenolics as the peaks became sharper, showing that low concentrations are required to ensure a near reversible response. The exceptionally small peak separation (ΔE_p) for quercetin has been associated with subsequent chemical reactions for the oxidation product of quercetin (*7*, *14*). A different type of irreversibility occurred with the likes of ascorbic and gallic acids (Figure 1), where the return cathodic peak was largely absent, indicating that the oxidation product undergoes a further chemical reaction or is not reduced at the glassy carbon electrode (*15*).

The formal potential often quoted for the one-electron oxidation of the ascorbate monoanion at pH 7 is 282 mV (SHE) (*27*). Taking into account the pK_{a1} value of 4.04, this is equivalent to 189 mV (Ag/AgCl) at pH 3.6, which is in good agreement with the measure provided by the current work. The low formal potential for ascorbic acid has a number of consequences when it is added to stabilize a white wine against browning (*28*). It can be expected to react more readily than the phenolic antioxidants, and so it will be the least stable. It also has a formal potential sufficiently low to reduce the oxidized forms of the phenolics (*29*), effectively regenerating the other antioxidants, in a manner similar to the interaction established between ascorbic acid and vitamin E (*27*).

A much broader peak was obtained with sodium metabisulfite. The current began to rise from 400 mV, but the peak itself was not reached until 970 mV. This is despite a much lower formal potential calculated using thermodynamic free energy data (*30*), for the expected oxidation to sulfate:

$$HSO_3^- + H_2O \rightarrow SO_4^{2-} + 2e^- + 3H^+ \qquad (2)$$

This process has a standard potential ($E°$) of 105 mV (SHE), for $\Delta G° = -20.2$ kJ mol^{-1}, which is equivalent at pH 3.6 to a formal potential of −420 mV (Ag/AgCl). The fact that the oxidation of HSO_3^- is not seen at lower potentials can be ascribed to energy barriers at the glassy carbon electrode commonly seen for sulfur containing compounds. When a drop of acetaldehyde was added to the solution, an amount sufficient to fix all of the metabisulfite present, the oxidation peak was completely lost, showing that only the free form is electroactive at the carbon electrode. Metabisulfite remains a strong reducing agent with roles as a preservative in wines, but will not interfere with peaks in cyclic voltammograms of wines due to phenolic antioxidants.

The other phenolics which had significantly higher formal potentials, ferulic acid, *t*-resveratrol, malvin (expected to produce a response typical of the malvidins which make up the major part of the anthocyanins found in red wines), and vanillic and *p*-coumaric acids (Table 1), all lacked an *ortho*-diphenol and relied on oxidation of an isolated phenol group, sometimes adjacent to a methoxy group. These compounds are therefore expected to be less active as antioxidants where reducing ability is the key requirement. The oxidation of these phenolics, which could be due to processes involving one or two electrons (*8*), produced broad peaks and was largely irreversible. Some small cathodic peaks were seen at times, as for vanillic acid in Figure 1, at potentials more negative than those expected for a reversible reduction. These cathodic currents are due to the reduction of various oxidation products, some of which may remain adsorbed on the carbon electrode as an electrode film. In these cases it was essential to clean the electrode by abrading with alumina to recover the full anodic response on subsequent scans.

With several phenolics that showed a first anodic peak in the 370 to 470 mV range, a second oxidation process was observed at higher potentials, close to 800 mV and in the vicinity of the main peak of vanillic acid.

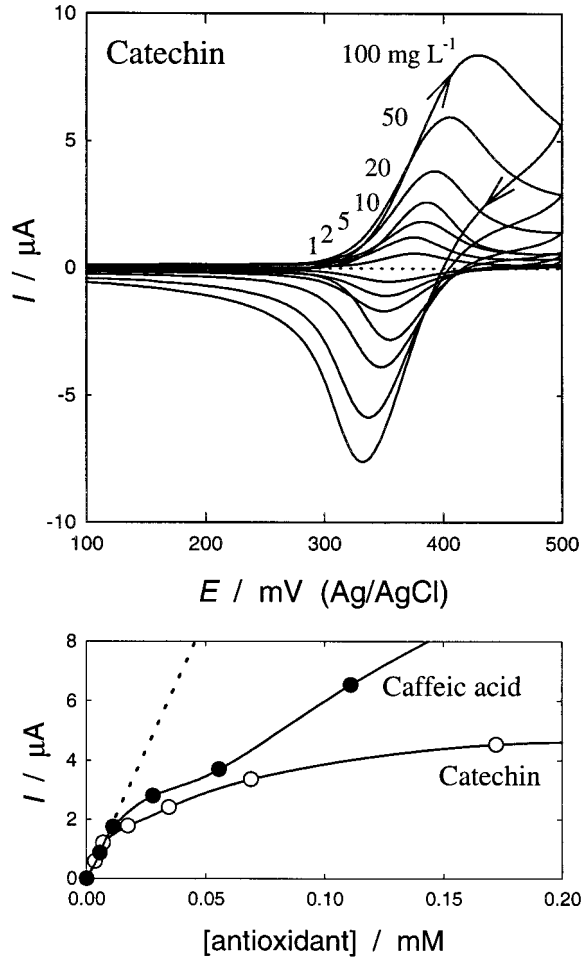


Figure 2. Cyclic voltammograms (background subtracted) of catechin diluted in the model wine solution measured at 100 mV s^{-1} at a 3-mm glassy carbon electrode, along with peak current as a function of concentration for both catechin and caffeic acid.

This was seen with catechin (Figure 1) and epicatechin (due to the *meta*-diphenol groups on the A-ring), quercetin (oxidation of the hydroxyl on the C-ring), and gallic acid (due to a third phenol group adjacent to the *ortho*-diphenol already oxidized). By this point contamination of the electrode by oxidation products can make this peak difficult to analyze.

Current Response and Calibration Curves. Samples of catechin and caffeic acid were diluted in the model wine solution to determine the range over which the current was directly proportional to the concentration. A plot of peak current against concentration showed that although caffeic acid had a peak current twice that of catechin at 0.5 mM, the current response was closer at lower concentrations (Figure 2). A linear concentration range was obtained only for currents less than 2 μA. The nonlinear response at higher concentrations held for each of the antioxidants except ascorbic acid, which showed a linear increase of current with concentration from 0.01 to 0.5 mM. The ratio of $I_{p,a}$ over $I_{p,c}$ for catechin (and epicatechin) went from about 0.7 at 0.5 mM to 1.0 in the more dilute solutions, a further sign of increased reversibility. This ratio also increased for the other reversible peaks (caffeic acid reaching 0.8 and rutin 0.9).

An average peak current of 3.8 ($\pm$ 0.4) μA for the 0.05 mM solutions, and of 1.7 ($\pm$ 0.7) μA for the 0.01 M solutions, was obtained for the 6 phenolic compounds with a first oxidation peak at potentials less than 500 mV (excluding quercetin and tannic acid). The difference in the current response between the antioxidants can be ascribed to differences in the diffusion coefficients (D_o), and to energy barriers at the electrode which may be enhanced as the surface becomes contaminated by oxidation products. At 0.5 mM gallic acid displayed the highest peak current, but at 0.01 mM it displayed the lowest peak current, showing that it had a lower absolute current response but maintained that response more effectively at higher concentrations. A calibration curve involving gallic acid can thus be used to provide a gallic acid equivalent measure for complex mixtures such as wine, in a similar manner in which it is used in the Folin-Ciocalteau procedure for total phenols.

The charge passed (Q) for a scan to 500 mV was also recorded (Table 1), or to 400 mV for ascorbic acid and 450 mV for quercetin (a second peak was seen with quercetin by 500 mV). The charge passed by gallic acid standards can be used as a further measure of the response of the more reactive phenolic antioxidants in wines. This measure is more suitable than the peak current for samples such as wine where the anodic peak is broadened due to the response of several antioxidants with slightly different oxidation potentials.

Cyclic Voltammetry of White Wines. The antioxidants present in a wine can be expected to be successively oxidized at a glassy carbon electrode as their formal potentials are approached. The current produced will generate a peak as the antioxidants in close vicinity to the electrode are depleted by oxidation, and the current continues only as the reactant diffuses in from the bulk solution. Antioxidants present in smaller amounts may appear only as a shoulder on some larger feature.

A cyclic voltammogram taken in a 1999 Sauvignon Blanc white wine showed an anodic current peaking at around 12 μA by 400 mV and at 25 μA by 900 mV (Figure 3). This can be compared to the background current generated in the model wine solution used as a blank. The current generated in the blank run is due to the oxidation of ethanol, which is particularly significant beyond 1000 mV. This background current is substantially larger on platinum or gold electrodes, but remains relatively low on the glassy carbon electrode.

The current generated at 250 to 500 mV can be ascribed to phenolic antioxidants with low formal potentials, and to ascorbic acid where this has been added to the wine. The value of the current can be used to quantify the antioxidants which contribute to the peak at 400 mV, but only once the wine has been diluted to bring it into the range in which the current is a linear function of concentration. A 10-fold dilution was found to be sufficient to reach the linear region for white wines (Figure 4), or be just at the high end, and still produce a peak current (about 2 μA) which could be measured repeatedly with a relative standard deviation of around 5%.

In the undiluted wine an anodic peak of over 20 μA is expected if all the more reactive antioxidants close to the electrode were oxidized; instead a large proportion remain to be oxidized during the reverse scan, swamping the cathodic current from the reduction of oxidized products. Although the anodic peak for the undiluted wine appears to be completely irreversible, a cathodic peak emerged with the diluted wine, showing that the

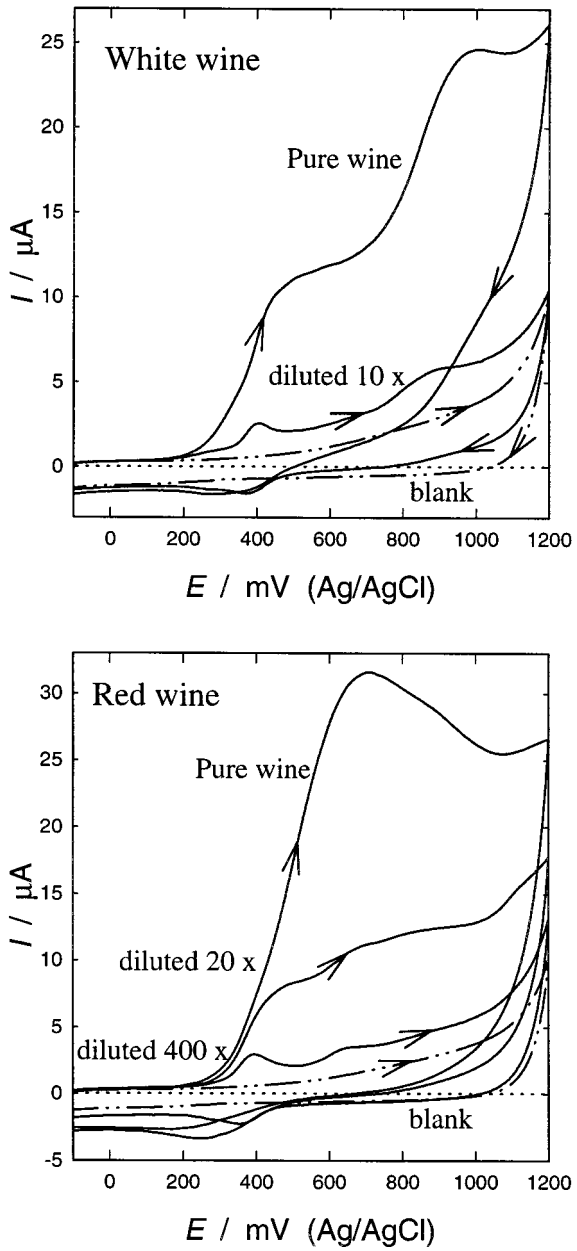


Figure 3. Cyclic voltammograms taken to 1200 mV for a white Sauvignon Blanc and a red Pinot Noir wine measured at 100 mV s^{-1} at a 3-mm glassy carbon electrode, both undiluted and diluted in a model wine solution (12% ethanol, 0.05 M tartaric acid, and added NaOH to pH 3.6).

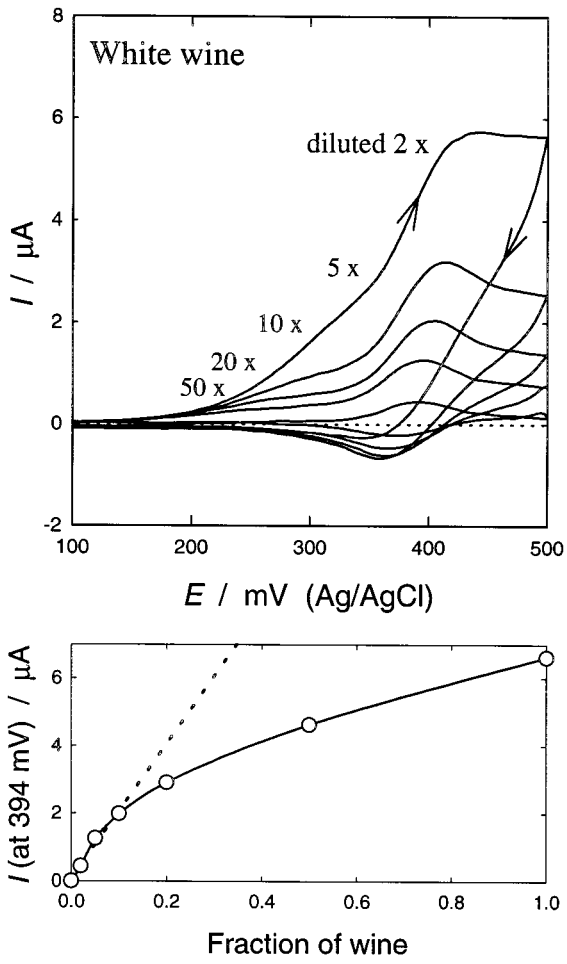


Figure 4. Cyclic voltammograms (background subtracted) taken to 500 mV of a white Sauvignon Blanc wine diluted in the model wine solution, measured at 100 mV s^{-1} at a 3-mm glassy carbon electrode, along with the current at 394 mV as a function of wine fraction.

oxidation of a certain fraction of the antioxidants was at least quasi-reversible (Figure 4). This was also shown by the increased value of the ratio of $I_{p,c}/I_{p,a}$ to 0.30 for the wine diluted 10×, and to 0.49 for the wine diluted 50×. The peak separation (ΔE_p) also decreased from 57 mV for the wine diluted 5×, to 43 mV for the wine diluted 10×, and to 22 mV for the wine diluted 50×, as the reversibility increased.

The peak current of 1.96 (± 0.08) μA, for the wine diluted 10×, is equivalent, in the undiluted wine, to 0.28 mM, or 52 mg/L gallic acid equivalents (GAE). However, the peak was broader ($|E_{p,a} - E_{p/2}| = 54$ mV), than that for the individual phenolic standards, due to the combined effect of several compounds with slightly different formal potentials. Using the charge passed to 500 mV of 3.12 (± 0.17) μC, a second value of 78 mg/L GAE was obtained, which is a better estimate of the concentration of the more reactive antioxidants in the white wine. For this wine a value of 322 mg/L GAE was also obtained by the Folin-Ciocalteau procedure. We have found that the cyclic voltammetry measure is generally 4−5 times lower than the Folin-Ciocalteau value for white wines, because of a high proportion of phenolics contributing to the total phenols measure which are not oxidized by 500 mV.

A second peak was seen at 888 mV in cyclic voltammograms of the 1999 white wine diluted 10×. This is seen more clearly in Figure 5 where the current due to the blank model wine solution has been subtracted from the oxidation curve to 1000 mV; the accumulated charge has also been plotted as a function of potential. The current from 700 to 1000 mV can be ascribed to compounds such as vanillic and *p*-coumaric acids, but will also be due to the second oxidation of the flavonoids present in the wine. Antioxidants with formal potentials close to 600 mV, such as ferulic acid, are expected to contribute to the current seen between the two peaks, which was not large enough to form its own peak in the case of the white wine.

The voltammogram for the 1999 Sauvignon Blanc is typical of a range of white wines we have tested, but differences are also observed. The voltammogram for a second, older white wine has been included in Figure 5. In this case the first anodic peak was sharper, and the peak current and charge to 500 mV (Table 1) now produced similar values of 44 and 41 mg/L GAE

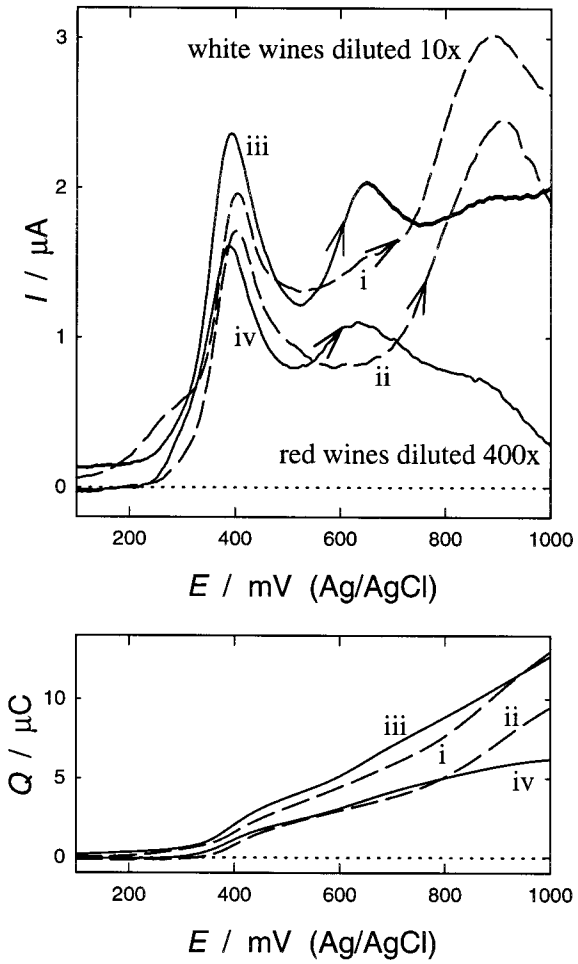


Figure 5. Linear voltammograms taken to 1000 mV for white wines diluted 10 times: (i) 1999 Sauvignon Blanc, (ii) 1984 Sauvignon Blanc; and red wines diluted 400 times: (iii) 1998 Pinot Noir, (iv) 1991 Cabernet Sauvignon; measured at 100 mV s^{-1} at a 3-mm glassy carbon electrode, having subtracted the current due to the blank model wine solution. For each solution a plot of anodic charge passed versus potential is also presented.

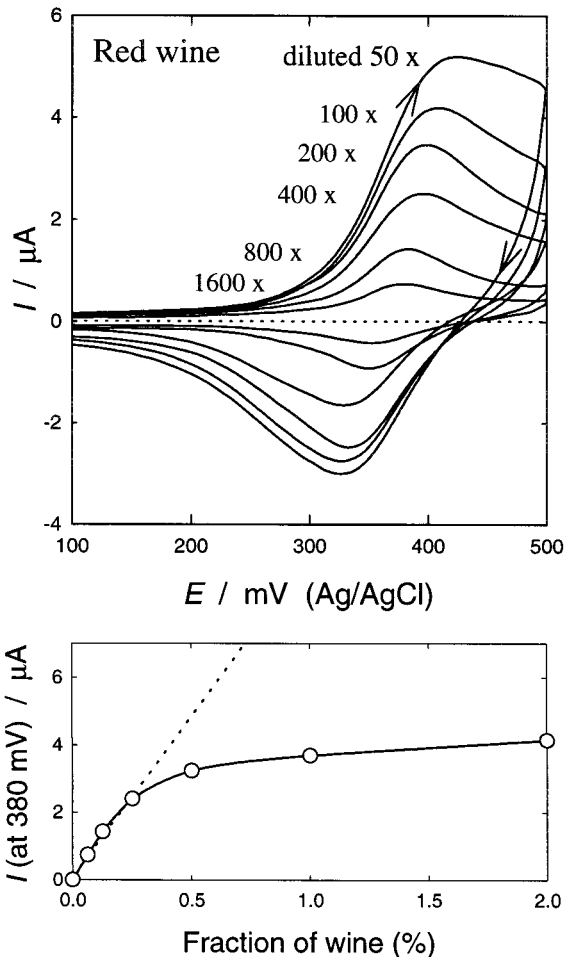


Figure 6. Cyclic voltammograms (background subtracted) taken to 500 mV of a red Pinot Noir wine diluted in the model wine solution, measured at 100 mV s^{-1} at a 3-mm glassy carbon electrode, along with the current at 380 mV as a function of wine fraction.

for the low oxidation potential antioxidants. This 1984 Sauvignon Blanc wine also had a lower Folin-Ciocalteau value of 269 mg/L GAE total phenols.

Cyclic Voltammetry of Red Wines. A cyclic voltammogram taken of the 1998 Pinot Noir red wine produced a large feature from which little information could be extracted (Figure 3). It was only after diluting the wine 400 times that the linear concentration range was reached (Figure 6), consistent with the much higher level of phenolic antioxidants present in the red wine. Once again the peak separation (ΔE_p) decreased from 99 mV for the wine diluted 50×, to 68 mV for the wine diluted 400×, and to 28 mV for the wine diluted 1600×, as the reversibility increased. On the other hand, the return cathodic peak was significantly larger for the red than for the white wine, with the ratio of $I_{p,c}/I_{p,a}$ lying between 0.56 and 0.71 over the 50× to 1600× dilution range, consistent with the diluted red wine containing a higher proportion of reversible compounds such as catechin and caffeic acid. At the same time the peak current leveled off more rapidly for the red wine compared to the white wine once the linear concentration range to about 2 μA was passed (Figure 6). This may be due to a greater tendency of the compounds present in the red wine to contaminate the carbon electrode.

The anodic current peak of 2.22 (± 0.14) μA is equivalent to 12.8 mM, or 2400 mg/L GAE, for the undiluted red wine. Alternatively, using the charge passed of 3.40 (± 0.18) μC (appropriate for the relatively broad peak), a concentration of 3580 mg/L GAE is given, over 40 times the values obtained for the white wines. These are comparable in size to the value of 2400 mg/L GAE given by the Folin-Ciocalteau procedure, although the charge passed to 500 mV produced a somewhat larger value. However, for an older 1991 Cabernet Sauvignon wine (Figure 5), the peak current and charge to 500 mV (Table 1) produced values of 1605 and 1945 mg/L GAE respectively, in contrast to the higher Folin-Ciocalteau value of 3342 mg/L GAE. It must be emphasized that cyclic voltammetry and the Folin-Ciocalteau procedure respond differently to different phenolic antioxidants, and that the charge to 500 mV only represents the most powerful reducing agents.

Unlike the white wine, the red wine showed a peak at 651 mV, which can be seen more clearly after subtracting the background due to the model wine solution (Figure 5). This peak can be associated with the malvidin anthocyanins present in the red wine; malvin, which has the same phenolic backbone as the malvidins, showed a peak potential of 677 mV. This peak was much reduced in the older 1991 red wine,

consistent with the expected decline in levels of anthocyanins with age. Ferulic acid and *t*-resveratrol would also contribute to this peak. However, the peak at 893 mV was much more limited for the red wines, corresponding to a lower proportion of higher oxidation potential compounds which produced a significant peak at this point in the diluted white wines.

Concluding Comments. Cyclic voltammetry can be used effectively to characterize the reducing ability and reversibility of antioxidant standards and the reducing constituents of wine. The technique provides a measure that is obtained in just a few minutes at little cost after the equipment is purchased. The test can be carried out for biological samples or food extracts at any pH of interest, and the solvent composition can vary over a wide range.

The best electrode for this purpose is glassy carbon which minimizes interferences from ethanol which oxidizes at inert metal electrodes such as platinum and gold. The measured oxidation potential varies predictably with pH enabling related measures taken at different pH levels to be readily compared. The concentration used for the analysis is critical to its success, and in the present case a peak current of 2 μA or less was a good indication that adequate dilution had been achieved. The area under the curve, namely the charge passed to 500 mV, is a better estimate of the concentration of phenols with low oxidation potentials, than the peak current itself.

The easily oxidized *ortho*-diphenols yield a low potential peak at around 400 mV, the anthocyanins in red wines yield a peak at 650 mV, and harder-to-oxidize functional groups produce higher potential peaks, providing facile discrimination between these types of substrates. The magnitude of the response is not identical on a molar basis for dissimilar compounds, and thus, quantitation of all reducing compounds is qualitative in mixtures such as wine. On the other hand, sulfur containing antioxidants are not readily oxidized at a carbon electrode and their contribution may be missed, but this is not a problem for wines and many other foods where the level of sulfur containing antioxidants is low. The method is also not sensitive to compounds that exert an antioxidant effect by other mechanisms, such as metal chelation.

The application of cyclic voltammetry may be useful in identifying wines or other foods for enhanced health benefits, but more importantly there is much promise in characterizing wines for their antioxidant protection and monitoring the development and aging of wine. We are currently pursuing these possibilities.

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