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RESEARCH PAPER

Determination of polyphenol content and colour index in wines through PEDOT-modified electrodes

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Abstract Poly(3,4-ethylenedioxythiophene)-modified electrodes have been used for the estimation of the polyphenolic content and of the colour index of different samples of wines. Synthetic wine solutions, prepared with different amount of oenocyanins, have been analysed spectrophotometrically and electrochemically in order to find a correlation between the total polyphenolic content or colour index and the current peak. The regression curves obtained have been used as external calibration lines for the analysis of several commercial wines, ranging from white to dark red wines. In this way, a rapid estimation of the total polyphenolic content and of the colour index may be accomplished from a single voltammetric measurement. Furthermore, principal component analysis has also been used to evaluate the effect of total polyphenolic content and colour index on the whole voltammetric signals within a selected potential range, both for the synthetic solutions and for the commercial products.

Keywords PEDOT-modified electrodes · Wines · Polyphenols · Colour index · Differential pulse voltammetry · Principal component analysis

Introduction

Polyphenols are naturally occurring compounds largely present in fruits, vegetables, cereals, as well as in beverages, which are essential for the growth and reproduction of plants and for protection against pathogens [1, 2]. Polyphenols are present in grapes and, consequently, in wines, being however also produced by yeast metabolism and extracted from the oak barrels in which wine is aged or stored. The polyphenol content contributes substantially to the quality of wines and affects the colour, stability, and behaviour in respect to ageing. Furthermore, the quantitative determination of the compounds belonging to this class can help identify variants of type and differences in winemaking and maturation processes [3–5]. Hence, it is of great importance to characterise a wine by its total phenolic content (TPP). The term TPP refers to the polyphenolic amount measured by spectrophotometric methods, especially by the so-called Folin-Ciocalteu method, which is based on the reaction of phenols with a colorimetric reagent suitable to induce absorption in the visible region of the spectrum [4, 6].

Colour index (CI) constitutes an additional discriminant parameter within the wide class of wines. CI, defined by Sudraud in 1958 [7, 8], is commonly used to define the colour of red wines in an objective way; it is calculated on the basis of the absorbance of the sample at the two wavelengths where the absorption maxima of green and blue colours (representing the red and yellow components of wine colour, respectively) are located. The CI, which varies widely depending on the origin and age of the wine, is conditioned by the presence of a specific class of polyphenols, namely anthocyanins. Recently, the interest of the research activity of academia and of food industry for anthocyanins has notably increased, since, besides being natural pigments for foodstuffs, they have been proved to possess beneficial properties for health [9, 10].

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Besides spectrophotometric methods, high-performance liquid chromatographic (HPLC) techniques are used in the quantification and identification of individual polyphenolic substances [11, 12]. However, HPLC-based procedures do not constitute an easy, rapid, or cheap approach; moreover, they require skilled personnel and cannot be performed in situ or with minimal pretreatment of the sample. These can constitute very critical points in those situations, such as in the course of harvest, in which the fast evaluation of the aromatic and polyphenolic potential of the grape is most often crucial for the final oenological result.

Therefore, the development of effective new devices and methods for the evaluation of polyphenolic content is urgent. They are requested to be suitable for online detection and for operating on small amounts of samples, possibly requiring minimal or no sample pretreatment. Electrochemical sensing can give important contributions to achieve these goals [13]. Cyclic voltammetry (CV) has been used to determine TPP and the concentration of some individual groups of polyphenols [14–17] also taking advantage of microdialysis online-coupled with amperometric systems [18]. Analytical methods based on differential pulse voltammetry (DPV) for the determination of polyphenol content in wines have been also proposed, using glassy carbon electrode nanocomposite electrode systems [19–22]. Chronoamperometry and chronocoulometry have been also used for the evaluation of antioxidant properties of red and white wines [23, 24]. Furthermore, in the frame of the analysis through 'electronic tongue', exploiting multivariate analysis and regression procedures, electroanalytical techniques have been used for the determination of polyphenolic substances in food and wines [25–27].

In this paper, we report the results achieved by using poly(3,4-ethylenedioxythiophene) (PEDOT)-modified electrodes for the estimation of TPP and of CI in different samples of wines. PEDOT coatings offer several advantages with respect to bare electrodes. Most notably, they minimise fouling effects on the electrode surface due to oxidation of polyphenols, which leads to the formation of a passivating polymer film on surfaces of conventional electrode materials. Moreover, PEDOT-modified electrodes work properly in aqueous media, which makes them attractive for direct analyses of food matrices, as already demonstrated in our previous studies [27–29].

A first step of the present report has consisted in defining the operative conditions in which a good correlation between electrochemical signals and spectrophotometric data used for the determination of CI and TPP is achieved. For this purpose, solutions of synthetic wines suitable to mimic real samples as to the species of interest were prepared using a red grape skin extract powder (oenocyanin—OC). In the Asian market, OC powders from black rice are commonly used as correctors for wine colours, since they are particularly rich of anthocyanins, which are the substances mainly responsible for red wine's colour. OC from grape skin extract is actually a mixture of different compounds, including anthocyanins, tannins, and other organic acids that are naturally present in wines; we profitably used it in the preparation of solutions simulating wines. Secondly, we verified whether linear regression models could be used in external calibration procedures for a rapid electrochemical estimation of CI and TTP in commercial wine samples. The aim is to avoid the use of the standard addiction method which is used in the cited literature [17, 21, 22], improving the rapidity of the analysis. DPV has been adopted as the voltammetric technique, in order to improve the sensitivity of the responses, enhancing the faradic component of the signal over the capacitive one.

Finally, principal component analysis (PCA) [30] showed to be effective in achieving a visual representation of remarkable trends, within the dataset collected, with respect to the TPP and CI parameters. This was proved by testing both the synthetic and the commercial products. Following this approach, each DPV signal is considered as a sort of fingerprint of the analysed sample, supposed to bring the significant chemical information for the envisaged purpose [27, 31, 32].

Experimental

Reagents and chemicals

All chemicals were of reagent grade. 3,4-Ethylenedioxythiophene (EDOT), $LiClO_4$, and anhydrous CH_3CN, packaged under nitrogen, were from Sigma-Aldrich. Gallic acid monohydrate was from Carlo Erba. Monobasic and dibasic potassium phosphate salts (Carlo Erba) were used to prepare aqueous phosphate buffer solutions (0.1 M PB, pH 7.0). Tartaric acid (Riedel de Haen) and NaOH (Sigma-Aldrich) were used to prepare aqueous tartrate buffer solutions (0.1 M TB, pH 3.0). Ultra-pure deionised Milli-Q water (18 MΩ cm resistivity) was used.

The OC powder from red grapes (mainly Ancellotta variety) was supplied by a local winery. The main free anthocyanins present revealed by HPLC analysis are malvidin (31.62 %), delphinidin (14.30 %), petunidin (12.05 %), peonidin (7.13 %) and cyanidin (3.38 %).

Wine samples

Variable amounts OC were added to a 0.033 M tartaric acid/12 % *v*/*v* ethanol solution, in order to prepare 7 synthetic wine samples at final OC concentration ranging from 500 to 40,000 ppm, indicated in the following as wine: 524, 1016, 3000, 3996, 5174, 20530, and 40640, respectively.

Fifteen different types of commercial wines have been used for this study: a white wine (WhiteW), two rosé wines

(RosèW_1, RosèW_2), seven red wines (RedW_1, RedW_2, RedW_3, RedW_4, RedW_5, RedW_6, RedW_7) and five dark red wines (DRedW_1, DRedW_2, DRedW_3, DRedW_4, DRedW_5). All studied samples were dry red wines of national origin, and they were made by one or more of the following grape varieties, present in different percentage: Ancellotta, Lambrusco Salamino, Lambrusco Grasparossa, Lambrusco Marani, Lambrusco Maestri, Lambrusco Puglia, Scorza Rossa and Trebbiano Emilia.

All samples were stored at 8 °C in the dark, and analyses were performed shortly after opening the relevant containers.

Electroanalytical apparatus and procedures

The electrochemical experiments were carried out with an Autolab PGSTAT12 electrochemical instrument (Ecochemie), using a single-compartment, three-electrode cell. A 3-mm-diameter Pt disk (Metrohm) was used as the working electrode. An Ag/AgCl electrode (3 M KCl, Amel) and a glassy carbon rod (Metrohm) were the reference and the auxiliary electrode, respectively. Before each electrochemical deposition of PEDOT, the surface of the working electrode was polished with 0.05 μm alumina powder to a mirror finish, dipped in an ultrasonic bath for 10 min and then rinsed with doubly distilled water.

According to the procedure reported in ref. [29], the polymer electrode coating was prepared by direct electrochemical polymerisation–deposition, carried out in a solution containing 10 mM EDOT and 0.1 M $LiClO_4$ supporting electrolyte, CH_3CN de-aerated solvent. Electropolymerisation was performed using the potentiostatic method. A potential of +1.20 V was applied until a charge of 1.5 mC was spent; the procedure was then terminated by holding the potential at −0.80 V for 30 s, inducing partial de-doping of the coating. The potential value for growing the polymer was chosen at the onset of the EDOT oxidation peak. After electrochemical deposition, the status of the polymer film was stabilised through ten subsequent CV scans in the potential range from −0.50 to +0.50 V at a rate of 0.05 V s^{-1} in a monomer-free, 0.1 M PB solution.

The DPV waveform used for the quantitative estimations consisted of 10 mV potential impulse, 4 mV potential step, 0.15 s impulse time and 0.6 s time interval between two subsequent potential pulses. The potential range was from −0.30 to +0.70 V. Before each scan, the electrode was kept at −0.30 V for 30 s. Such an electrode pretreatment allowed us to achieve repeatability of the responses. Therefore, the overall time for each DPV scans was of 180 s.

For the electroanalytical investigations, both synthetic and commercial wine samples were properly diluted in the TB solution, pH = 3.0.

Spectrophotometric measurements

Spectrophotometric measurements have been carried out with a PerkinElmer Lambda 650 spectrophotometer. The Folin-Ciocalteu method [4, 6] has been followed for the determination of TPP in both synthetic and commercial wines. The overall pretreatment of samples requires more than 30 min. Gallic acid was used as the standard for the construction of the spectrophotometric calibration curve; indeed, TPP is expressed in *gallic acid equivalent* units.

The CI [7, 8] is calculated starting from the absorbance of the diluted sample at $\lambda = 420$ nm (A420) and $\lambda = 520$ nm (A520), and it is then computed as:

$$\mathrm{CI} = (\mathrm{A420} + \mathrm{A520}) \times (\text{dilution factor})$$

Multivariate analysis

Considering the overall number of analysed diluted samples and the number of replicates, 18 electrochemical signals recorded on synthetic wine samples and 41 electrochemical signals of commercial wine samples were evaluated using PCA [30]. The DPV responses collected on both diluted synthetic and commercial wine samples were organised into two separate data matrices of dimensions given by (18 samples × 243 current values at the different potentials) and by (41 samples × 243 current values at the different potentials), respectively. The potential range explored was from −0.30 to +0.70 V.

PCA was first applied to the whole dataset, i.e. considering the two data matrices altogether, in order to verify whether the electrochemical signals measured on the synthetic wines were similar to those measured on the commercial wines. Then, a PCA model was calculated separately for each data matrix, in order to verify to which extent the major part of the variation of the electrochemical signals (accounted for, in terms of variance, by the principal components) was ascribable to CI and to TPP.

Before calculating the PCA models, the synthetic sample dataset was preprocessed by first-order derivative [33] and mean centering. The choice of this preprocessing procedure is the result of a selection for best effectiveness carried out on the occasion of a previous work of us, made on DPV signals measured on analogous samples [27]. Since the DPV signals collected on commercial wine samples were rather noisy, signal smoothing [33] was also performed, in advance to first-order derivative and mean centering.

The PCA models were calculated using the PLS Toolbox (ver. 7.5, Eigenvector Research Inc., USA) running into the Matlab 7.12 environment (The Mathworks Inc., Natick, MA, USA).

Results and discussion

Spectrophotometric measurements

In Table 1, the results obtained in the analysis of both synthetic and commercial wines are reported.

In the case of synthetic wines, TPP and CI values are linearly dependent on the OC concentration; the relevant r^2 values resulted equal to 0.99_9.

DPV measurements in synthetic wines

In the preliminary part of this study, DPV measurements have been performed in solutions of synthetic wines containing different amounts of OC. In this way, a better simulation of the polyphenolic content of commercial wines has been obtained, with respect to what performed usually in the literature, in which a single polyphenolic substance is added in model wine solutions.

As it is evident from Fig. 1, at variance with a bare Pt electrode, a well-defined anodic peak with a maximum at +0.40 V followed by a very broad shoulder is detected when using a PEDOT-modified electrode in synthetic wine 5174. In accordance to the literature [15, 16, 34], the cyclic voltammograms of red wines usually exhibit two or three anodic peaks, attributable to the oxidation of the cathecol, gallate phenolic and methoxy groups of tannins and anthocyanins, respectively. The presence of one defined peak followed by lower oxidation peaks is also reported in the voltammograms of a red wine extract [34].

Qualitatively similar voltammograms are recorded on any examined synthetic samples.

Table 1 TPP and CI values determined spectrophotometrically for synthetic and commercial wines

Synthetic wine	TPP (ppm)	CI	Commercial wine	TPP (ppm)	CI
524	112	0.5	Rosè_W1	565	1.3
1016	205	0.9	Red_W1	1760	4.0
3000	590	3.8	Rosè_W2	1147	2.8
3996	716	5.2	Red_W2	3836	14.7
5174	951	7.6	DRed_W1	7172	36.2
20530	5222	31.8	Red_W3	2416	4.7
40640	9718	59.3	Red_W4	1873	5.3
			Red_W5	2674	7.7
			Red_W6	1823	5.3
			Red_W7	2295	8.9
			DRed_W2	5021	28.2
			DRed_W3	5609	31.5
			DRed_W4	7087	48.4
			DRed_W5	6335	41.4

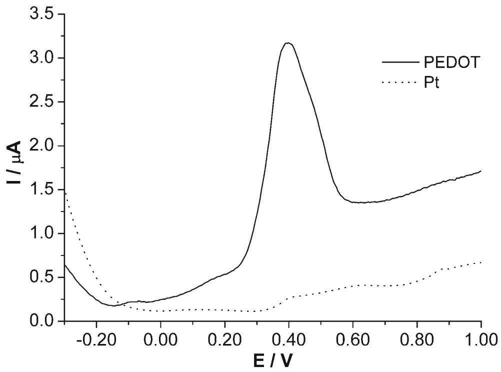


Fig. 1 DPV signals of (**a**) PEDOT-modified electrode (*solid line*) and (**b**) Pt bare electrode (*dotted line*), in synthetic wine sample 5174

From preliminary measurements, we could ascertain that wine samples should be considerably diluted in order to record an electrochemical signal with intensity linearly increasing with the polyphenolic content. Actually, either polyphenolic substances themselves or their oxidation products can be strongly adsorbed on PEDOT, inducing a sort of saturation of the electrode surface, which becomes insensitive to the oxidation of species beyond a certain concentration limit [29]. As a consequence, the sequence of signal intensities follows the trend of a Langmuir-like isotherm, reaching constant values at the highest explored concentrations. Then, a suitable dilution of the real samples is necessary in order to obtain reliable quantitative information. In order to remove the effect of the dilution on the polyphenol acid–base dissociation processes, all the electrochemical measurements have been performed in TB solution, pH = 3.0, i.e. at a value at which all the acidic functionalities are present under protonated form.

In Fig. 2, some DPV responses of PEDOT-modified electrodes in solutions of differently diluted sample 5174 are reported. A well-defined peak centred at 0.42 V is well visible in all the DPV curves. Also, in this case, measurements have been performed also reaching up to +1.0 V, but as expected any additional peak was observed. At varying dilution extent, the current peak height varies correspondingly; no other modification in the DPV profile is detectable. Obviously, taking

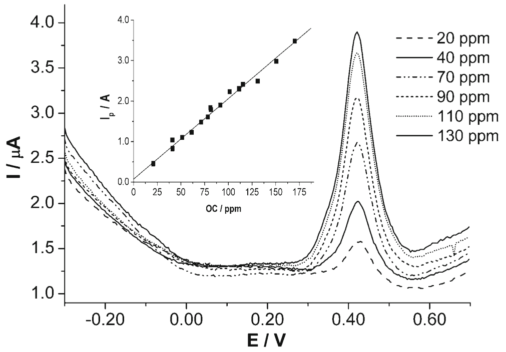


Fig. 2 DPV responses of selected solutions at different concentrations of OC at PEDOT-modified electrodes in 0.1 M TB, pH = 3.0. *Inset*: calibration curve

Table 2 Electrochemical and spectrophotometric TPP and CI values determined for some synthetic wines. The relevant recovery *R* is also reported in the table, accounting for the agreement between the electrochemical and the spectrophotometric data

Wine	$\mathrm{TPP}_{\mathrm{spectro}}$ (ppm)	$\mathrm{TPP}_{\mathrm{electro}}$ (ppm)	R_{TPP}	$\mathrm{CI}_{\mathrm{spectro}}$	$\mathrm{CI}_{\mathrm{electro}}$	R_{CI}
524	112	119	1.1	0.5	0.9	1.9
1016	205	225	1.1	0.9	1.7	1.8
3000	590	566	1.0	3.8	4.4	1.2
3996	716	735	1.0	5.2	5.7	1.1
20,530	5222	4591	0.9	31.8	34.9	1.1
40,640	9718	8311	0.9	59.3	64.6	1.1

into account the dilution factor, it is possible to compute the OC content in any diluted solutions, allowing the construction of a calibration curve.

A linear regression model between i_{p} intensity and OC concentration, reported in the insert of Fig. 2, has been then computed (20 points overall, 95 % confidence):

$$i_{\mathrm{p}}/A = (1.991 \pm 0.094)\cdot 10^{-8}[\mathrm{OC}] + (7.2 \pm 8.5)\cdot 10^{-8} \quad (1)$$

The high uncertainty in the intercept value does not reflect on the values found, due to the region of the calibration line used for the analysis of the unknown samples.

The limit of linearity found for the calibration curve corresponds to an OC concentration of 170 ppm. Each point of the curve has been obtained with a different PEDOT film; the low value of $s_{x/y}$, i.e. the standard deviation of the regression, is equal to $8.755 \cdot 10^{-8}$ indicating the high reproducibility degree of the method.

Since all the synthetic wine samples have been spectrophotometrically characterised to determine their CI and TPP (see 'Experimental' section), and these two parameters are dependent on the OC content, it is possible to find the regression models relating i_{p} to TPP and i_{p} to CI for the diluted samples:

$$i_{\mathrm{p}}/A = (1.08 \pm 0.05)\cdot 10^{-7}\mathrm{TPP} + (4.7 \pm 9.3)\cdot 10^{-8} \quad (2)$$

$$i_{\mathrm{p}}/A = (1.37 \pm 0.09)\cdot 10^{-5}\mathrm{CI} + (9.2 \pm 13.0)\cdot 10^{-8} \quad (3)$$

As to the uncertainty in the intercept values, the consideration made for Eq (1) still holds.

Using Eqs. (2) and(3), it is possible, by a DPV measurement in a diluted wine sample, to find the TPP and CI of the diluted solutions and, considering the dilution factor, to compute the TPP and CI in the original sample.

To this aim, before considering the commercial wine samples, we performed tests on synthetic wines. We determined the TPP and CI both by the spectrophotometric ($\mathrm{TPP}_{\mathrm{spectro}}$, $\mathrm{CI}_{\mathrm{spectro}}$) and the electrochemical ($\mathrm{TPP}_{\mathrm{electro}}$, $\mathrm{CI}_{\mathrm{electro}}$) methods obtaining the results reported in Table 2.

The comparison between the TPP values obtained electrochemically and those obtained spectrophotometrically is definitely satisfactory, as evidenced by the recovery, *R*, expressing the ratio between the values obtained by electrochemical and spectrophotometric methods, respectively [35]. A good accordance between $\mathrm{CI}_{\mathrm{spectro}}$ and $\mathrm{CI}_{\mathrm{electro}}$ is also evident, except for those samples with very low CI values.

DPV measurements in commercial wines

In the second part of the study, the regression models previously computed (Eqs. (2) and (3)) have been used for the electrochemical determination of the TPP and CI in a number of commercial wines. It is important to evidence that the use of an external calibration curve allows the accomplishment of the analysis in a very short time. This represents a big advantage with respect to electrochemical procedures requiring the application of the more time-consuming method based on standard additions, which is proposed in the literature [17, 21, 22].

The commercial wines analysed are of different varieties and therefore require different dilution levels in order to be suitable for electrochemical determinations. After preliminary tests, 25-fold dilution for white wines, 50-fold dilution for rosé wines, 100-fold dilution for red wines and 250-fold dilution for very red wines were find to constitute the best choice in order that the current values measured for the unknown sample fall within the linearity range of the calibration curves relative to TPP and CI. Typical voltammetric responses of properly diluted white, rosé, red and dark red wines, respectively, are reported in Fig. 3.

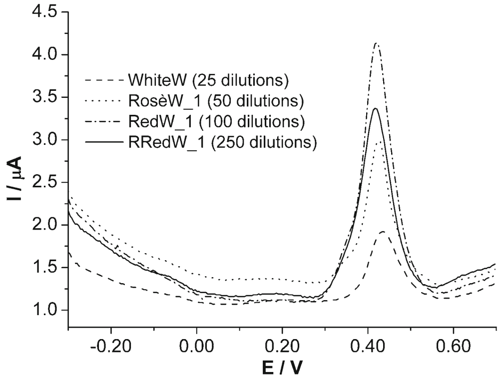


Fig. 3 DPV curves for differently diluted wines of various types, recorded on PEDOT-modified electrodes, in 0.1 M TB, pH = 3.0

Table 3 Electrochemical and spectrophotometric TPP and CI values for commercial wines. The relevant recovery *R* is also reported

Wine	$TPP_{spectro}$ (ppm)	$TPP_{electro}$ (ppm)	R_{TPP}	$CI_{spectro}$	$CI_{electro}$	R_{CI}
Rosè_W1	565	613	1.1	1.3	4.7	3.6
Red_W1	1760	1564	0.9	4.0	12.2	3.0
Rosè_W2	1147	800	0.7	2.8	6.2	2.2
Red_W2	3836	2565	0.7	14.7	20.1	1.4
DRed_W1	7172	4992	0.7	36.2	39.0	1.1
Red_W3	2416	1842	0.8	4.7	14.4	3.0
Red_W4	1873	1319	0.7	5.3	10.3	1.9
Red_W5	2674	2237	0.8	7.7	17.5	2.2
Red_W6	1823	1376	0.8	5.3	10.6	2.2
Red_W7	2295	1648	0.7	8.9	12.8	1.4
DRed_W2	5021	3233	0.7	28.2	25.4	0.9
DRed_W3	5609	4209	0.8	31.5	33.1	1.0
DRed_W4	7087	6508	0.9	48.4	51.1	1.0
DRed_W5	6335	5400	0.9	41.4	42.3	1.0

As expected, the DPV profiles of white wine samples show some significant differences with respect to those of the other wines examined. In particular, the peak current is located at a more anodic potential value and, despite the smaller value of the dilution factor, the current intensity is notably lower; this is ascribed to the absence of anthocyanins in white wines. For these reasons, this wine is not considered in the following.

Comparing the DPV profiles of rosé and red wines, some subtle differences are also noted that are attributable to the different composition of the various wine varieties. In this respect, it is not surprising that the DPV profile of RedW_1 and DRedW_1 wine produced from the same grape variety chosen for the extraction of the OC, i.e. Ancellotta, is very similar to that of the synthetic wine.

From the voltammograms of all the diluted commercial wines analysed, we evidence that in all cases only one current signal is present, located at a potential value very close to that recorded in synthetic wine samples. We think that the presence of one single peak, despite the variability of wines analysed and then of the variability of polyphenolic substances present, is a direct consequence of the low pH of the solution used for dilutions.

We then evaluated i_p to use in Eqs. (2) and (3), for the electrochemical evaluation of TPP and CI, respectively ($TPP_{electro}$ and $CI_{electro}$). The final results, together with the TPP and CI values estimated by the spectrophotometric procedure ($TPP_{spectro}$ and $CI_{spectro}$), are reported in Table 3.

In order to better visualise the results, the $TPP_{electro}$ vs. $TPP_{spectro}$ and $CI_{electro}$ vs. $CI_{spectro}$ values have been reported in subpanels a and b of Fig. 4, respectively.

A good linear correlation is achieved for both $TPP_{electro}$ vs. $TPP_{spectro}$ (slope at 95 % confidence interval = (0.79 ± 0.12); $r^2 = 0.973$) and $CI_{electro}$ vs. $CI_{spectro}$ (slope at 95 % confidence interval = (0.86 ± 0.10); $r^2 = 0.982$).

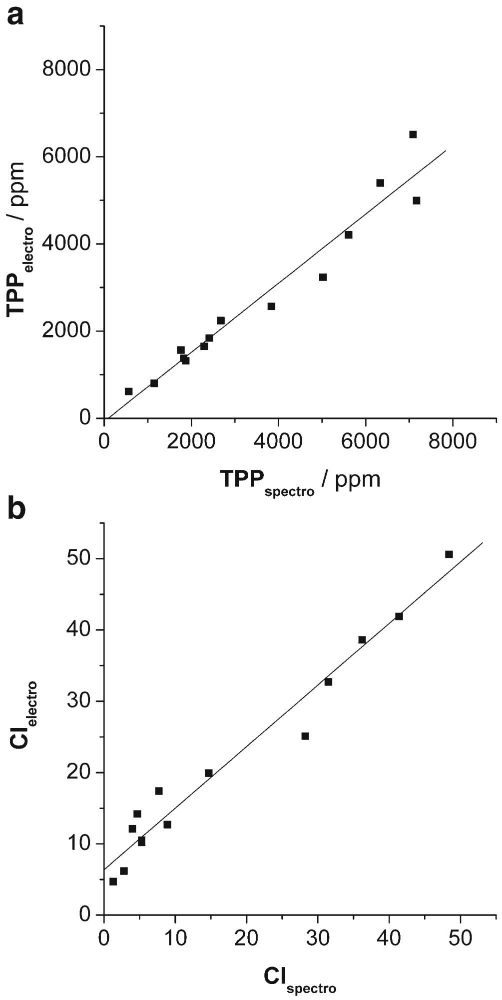


Fig. 4 Plot of (**a**) $TPP_{electro}$ vs. $TPP_{spectro}$ and of (**b**) $CI_{electro}$ vs. $CI_{spectro}$ for the 14 investigated wine samples

The slope value close to 0.9 means that TPP determined with electrochemical procedure are only slightly underestimated with respect the relevant values determined spectrophotometrically. The determination of $TPP_{electro}$ leads to satisfactory results, suggesting that the regression Eq. 2 is actually suitable for the electrochemical estimation of TPP in commercial wines, even of different varieties. It is important to notice that good predictions are obtained, although, as a consequence of the different varieties of wines analysed, the presence of various compounds differently reacting at the electrode and differently interfering with the target analytes can alter the sensitivity of the voltammetric analysis, which results are biased by the actual nature of the single matrices.

On the other hand, less satisfactory results were obtained in the estimation of CI by the electrochemical method. In particular, $CI_{electro}$ is usually higher than $CI_{spectro}$ in wine samples exhibiting low CI values, while a very good accordance is found in the case of dark red wines. As already mentioned before, dark wines are mainly made from Ancellotta grapes, i.e. the same grape variety of the OC powder used in this study. Reasonably, the current peak in the DPV signals is sufficiently informative for the estimation of CI of wines similar to those used for the calibration lines, while this is not true when considering wine of other varieties. In this case, probably other substances responsible of colour are not sufficiently deconvolved in the DPV signals, so that the measure of i_p of the overall current signal does not give exhaustive information. The choice of higher pH for the dilution solution may in principle favour the electrochemical discrimination of the several anthocyanins which are responsible of the colour. As it is well known, some varieties of wines possess high concentrations of malvidin, which is expected to be oxidised at potentials a little higher than the catechol-containing cyanidin anthocyanins. Other varieties present a very different mix of anthocyanins, amongst which delphinidin forms that, along with any 'gallocatechin' groups on the tannins, are expected to be oxidised easier [16, 36]. On the other hand, the solution pH strongly influences all these oxidation processes.

Principal component analysis

PCA was used to explore the electrochemical signals as a whole, i.e. to evaluate the information brought by all the current values measured in the potential range from −0.30 to +0.70 V. The aim of data exploration was twofold. Firstly, PCA was used to evaluate whether the overall shape of the electrochemical signals measured on the synthetic wines is similar to the shape of the signals measured on the commercial wine samples. Secondly, this technique was also employed to estimate whether the effect of CI and of TPP on the measured signals is predominant with respect to all the remainder sources of variation, like, e.g. contribution of other electroactive species and of instrumental noise.

In order to compare the signals measured on the synthetic wine samples with those measured on the commercial wine samples, a PCA model was calculated on the whole dataset, composed by all the electrochemical signals measured on the samples belonging to both the categories. Two principal components (PCs) were selected, accounting for 93 % of the total dataset variance, which is quite a high value. On commercial wines, the PC1 vs. PC2 score plot should show two distinct clusters, each one comprising the signals obtained on one of the two categories. Conversely, from the PC1 vs. PC2 score plot reported in Fig. 5, it is evident that the clusters corresponding to the synthetic and to the commercial samples are severely overlapped to each other, suggesting a first

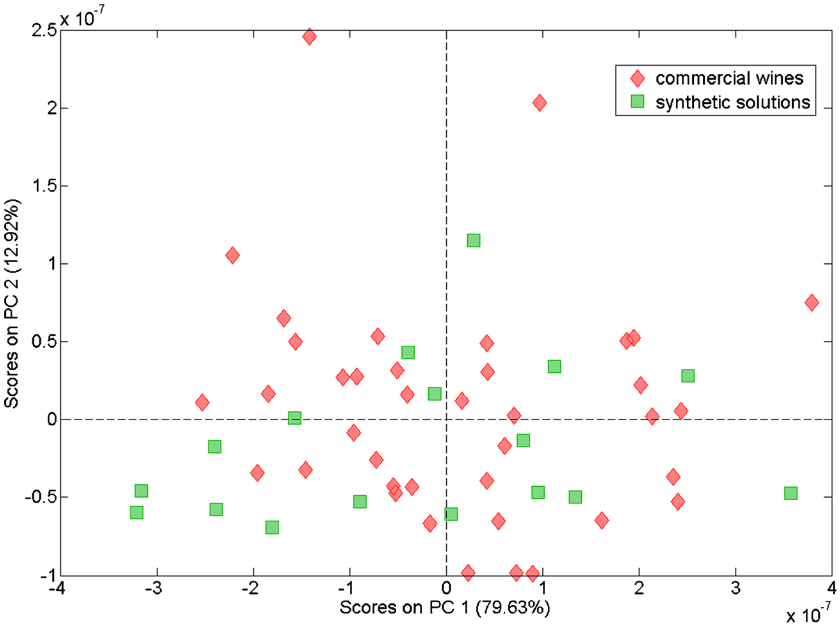
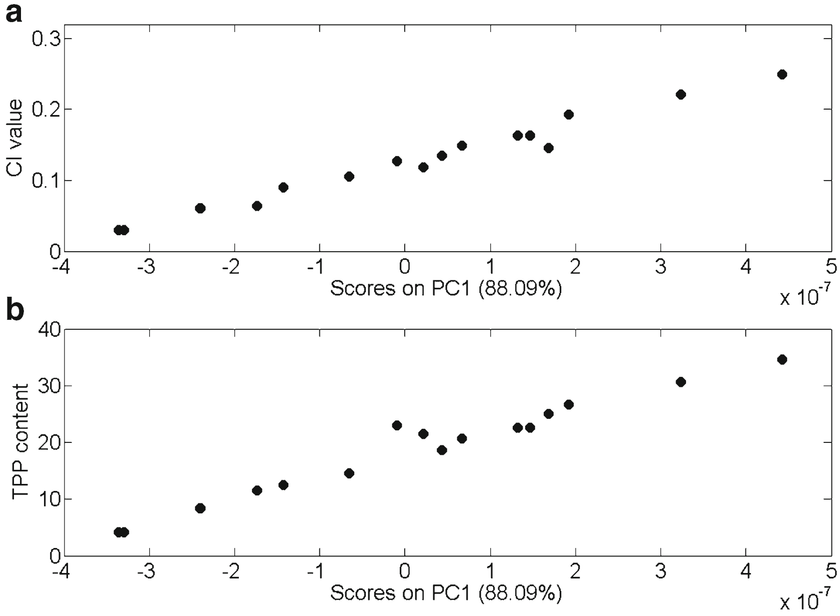


Fig. 5 PC1 vs. PC2 score plot obtained by PCA on all wine samples analysed. Synthetic and real samples are differently coloured

Fig. 6 PCA on synthetic wines: PC1 score values vs. CI value (**a**) and vs. TPP content (**b**)

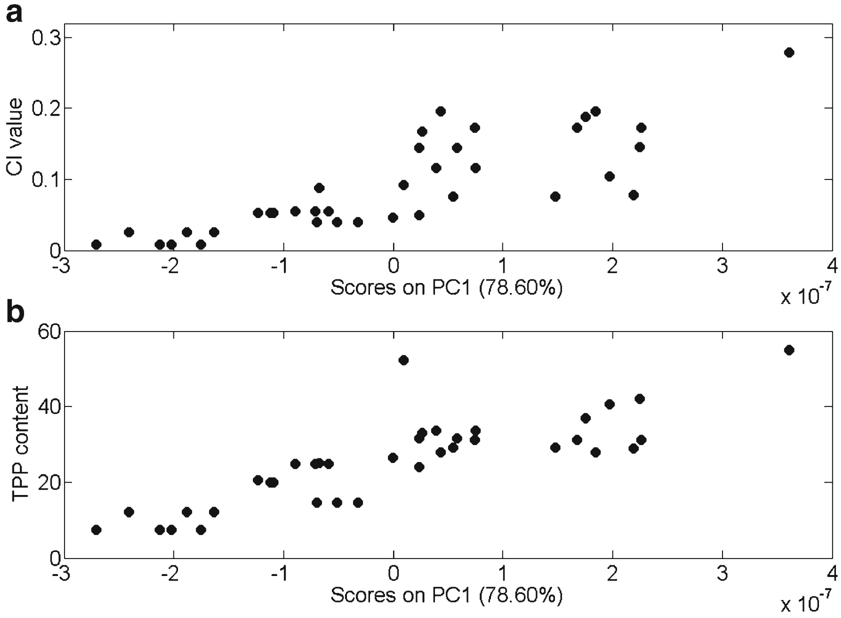


interesting conclusion, i.e. that synthetic samples can be profitably used to simulate real samples.

Regarding the estimates of CI and of TPP on the basis of the collected signals, a spontaneous attempt to overcome the possibility that different electroactive species concur to the value of the peak current consists in considering more than one single point of the voltammogram, namely i_p. A solution could lie in considering all the current values in the potential interval of the signal, in order to account for intensity and shape of the response at the same time. In other words, each single signal is implicitly related to the analytical quantity through a regression model, which involves the contribution of all the current values as independent variables.

However, following the direction addressed by such an approach through the development of a reliable multivariate regression model (e.g. by means of partial least squares regression, PLSR [23]), would have required a much wider number of samples in order to achieve sound results. Therefore, we chose to limit ourselves to a first step on this direction that consisted in verifying the possible presence of a common trend for all the variables of the electrochemical signal, attributable to the contribution of TPP and CI. A possible efficient approach seemed to us to evaluate the PCs which, as linear combinations of the current values, are suitable to account for highest spread of the ensemble of samples. Once ascertained that the major contribution to the variation, i.e. of the variance

Fig. 7 PCA on commercial wine samples: PC1 score values vs. CI value (**a**) and vs. TPP content (**b**)



of the electrochemical signals is due to CI and TPP, the location of the samples along the first PC(s), i.e. the PC score values, should reflect the corresponding change of the values of these two parameters. Thus, PCA was applied first to the synthetic wines and then to the commercial wine datasets, and the PC score values were graphically compared to the corresponding values of CI and of TPP.

As for the synthetic wines dataset, Fig. 6a, b reports the values of the score vector of PC1 (explaining 88 % of the total variance) vs. the corresponding TPP and CI values, respectively. Though a proper regression function is not proposed, it is evident that in both cases the trends expected are found. Both these figures show that, for each sample, the value of CI (Fig. 6a) and the content of TPP (Fig. 6b) vary according to the corresponding PC1 score value. Therefore, the major part of the variation of the preprocessed DPV signals, which is accounted for by PC1, is due to the variation of the oenocyanin content, which in turn determines the values of TPP and of CI.

The same approach was then used to analyse the dataset of the commercial wine samples: a PCA model was calculated on the DPV signals measured on these samples, and the values of the PC1 score vector (explaining 79 % of the total variance) were plotted vs. the relevant TPP and CI values, as reported in Fig. 7a, b, respectively. In both these figures, samples with low TPP and CI values have generally negative PC1 score values, while samples with high TPP and CI values have generally positive PC1 score values. However, this trend is less evident than for the synthetic sample dataset. The inspection of further PCs did not show the presence of significant correlations with TPP and CI. From Fig. 7, it is evident that, in contrast to what was observed for synthetic wines, TPP and CI are not strictly correlated to each other. This is obviously a consequence of the fact that very different varieties of wines were analysed, where the ratio between TPP content and CI is not constant.

Conclusions

The proposed electrochemical method allows a rapid evaluation of the TPP content of commercial wines thorough the execution of a single DPV measurement on the properly diluted samples, without the addition of any external reagents.

The very short time required by the electrochemical measurements (around 180 s), together with the minimal pretreatment of the sample that is required, makes the proposed electrochemical procedure a promising alternative to conventional spectrophotometric methods. Moreover, from a single DPV measurement, two distinct parameters, i.e. TPP and CI, can be estimated in dark red wines.

In order to improve the performance of the proposed method, especially with respect to the CI determination, measurements in diluted solutions at different pH are in progress in our lab. Moreover, the results of the analyses through PCs suggest the possibility to apply multivariate techniques to the DPV signals, in order to further improve the efficiency of the calibration models. To this aim, the availability of bigger datasets may allow us to gain most effective predictions of the parameters of interest, exploiting the information content brought by the whole DPV curve or by portions of it, that could be selected by means of proper feature selection methods.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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