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Electrochemical Sensing of Total Antioxidant Capacity and Polyphenol Content in Wine Samples Using Amperometry Online-Coupled with Microdialysis

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ABSTRACT: This work describes the method for total antioxidant capacity (TAC) and/or total content of phenolics (TCP) analysis in wines using microdialysis online-coupled with amperometric detection using a carbon microfiber working electrode. The system was tested on 10 selected wine samples, and the results were compared with total reactive antioxidant potential (TRAP), oxygen radical absorbance capacity (ORAC), and chemiluminescent determination of total antioxidant capacity (CL-TAC) methods using Trolox and catechin as standards. Microdialysis online-coupled with amperometric detection gives similar results to the widely used cyclic voltammetry methodology and closely correlates with ORAC and TRAP. The problem of electrode fouling is overcome by the introduction of an electrochemical cleaning step (1−2 min at the potential of 0 V vs Ag/AgCl). Such a procedure is sufficient to fully regenerate the electrode response for both red and white wine samples as well as catechin/Trolox standards. The appropriate size of microdialysis probes enables easy automation of the electrochemical TAC/TCP measurement using 96-well microtitration plates.

KEYWORDS: *wine, antioxidant capacity, phenolics, ORAC, TRAP, CL-TAC, amperometry, microdialysis*

INTRODUCTION

Wine is one the most studied beverages due to its verified antioxidant potential and health benefits attributable to its high content of polyphenols. Wine polyphenolics involve both flavonoids and non-flavonoids and depend on the grape variety, vineyard location, cultivation system, climate, soil type, vine cultivation practices, harvesting time, production process, and aging.[1] Functional ingredients of grape seeds, skins, and musts include phenolics such as monomeric flavanols catechin and epicatechin; dimeric, trimeric, and polymeric proanthocyanidins; phenolic acids (gallic acid and ellagic acid); and anthocyanins.[2] Grape polyphenolics are very effective in preventing cancer and cardiovascular diseases. These substances were reported to exhibit in vivo and in vitro antioxidant activities[3−5] exceeding those of vitamins C and E.[6] Red wine is perhaps the beverage richest in polyphenols. From common polyphenol-containing drinks, the antioxidant activity expressed per serving is highest for red wine: 1 glass (150 mL) of red wine = 12 glasses of white wine = 2 cups (2 × 150 mL) of black tea and = 3.5 (3.5 × 500 mL) glasses of beer.[7] An extensive study of antioxidant capacities and phenolics levels of French wines was performed by Landrault et al.[8] The studied French red wines had high total levels of catechins (i.e., the sum of analyzed monomers and procyanidin dimers analyzed), on average, 177.72 mg/L; the levels of catechins in the white wines were lower, on average, 26.36 mg/L. This represents the ratio of 7.6 between polyphenol content in red and white wines. In keeping with these data, the measurements of antioxidant activities using various methods favor the red wines, which outperform the dry white wines.

The use of cyclic voltammetry (CV) to determine the content of easily oxidizable compounds (polyphenols) in wines was first suggested by Mannino et al.[9] An extensive and widely cited study discussing the electrochemical characteristics of individual phenolic compounds constituting wine as well as the CV protocols to determine the overall content of phenolics in wines was performed by Kilmartin et al.[10] A charge passed during electrooxidation of wine phenolics in wine sample diluted by "artificial wine solution" composed of ethanol 12% (v/v, final concentration) and tartaric acid (50 mM) is determined as an integral of anodic current on cyclic voltammogram in the range from −100 to 500 mV (Q_{500}) and compared to the response of gallic acid using the same protocol. The parameter Q_{500} correlates with total phenolics content determined using the Folin−Ciocalteu procedure. The Folin−Ciocalteu procedure is based on the assessment of reducing capacity of the phenolics relative to the reducing capacity of an equivalent of gallic acid. If gallic acid is replaced

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with catechin as a standard, a close correlation with methods commonly employed to evaluate the reducing capacity of wines (i.e., 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP) assay, and β-carotene bleaching method)[11] is achieved. Improvements in sensitivity and ease of electrochemical data evaluation were achieved by replacing CV with differential pulse voltammetry[12] or square wave voltammetry.[13] Different research groups focused on the replacement of the usually utilized glassy carbon electrode by those exhibiting less electrode fouling (e.g., electrodes modified with conductive films such as PEDOT[14] or carbon paste electrodes with added carbon nanotubes[15]) to enable several determinations without the need for electrode cleaning prior to each analysis. Contrary to this approach, electrode fouling was taken advantage of to determine caffeic acid and its derivatives in wine.[16] Caffeic acid is identified as one of the main causes of electrode passivation due to the formation of poly(caffeic) acid redox active film at potentials as low as 0.35 V versus saturated calomel electrode (SCE).

The possibility to automate an analytical technique is crucial for its application in routine analytical practice. Whereas spectrophotometric, fluorometric, and chemiluminescence-based assays for the determination of phenolics content in wines have been automated, electrochemical instruments using a 96-well microtitration plate format are not available. The difficulty in automating wine analysis by CV lies in the size of conventional three-electrode systems and especially in the necessity to mechanically clean (usually by hand polishing) the working electrode prior to each analysis.

In this work we propose a method of wine analysis using microdialysis-coupled amperometry in which electrochemically conditioned (pretreated) cylindrical carbon fiber microelectrodes (CFEs) are used as sensing electrodes. Besides the suitable size of CFEs, favorable properties of their artificially oxidized surface ensure that the introduction of an electrochemical cleaning step prior to the analysis of individual wine samples is sufficient to overcome the problems with electrode inactivation. The results obtained using amperometry are compared with automated methods (TRAP, ORAC, and chemiluminescent determination of total antioxidant capacity (CL-TAC)) available in our laboratories.

MATERIALS AND METHODS

Catechin, horseradish peroxidase, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), and fluorescein disodium salt were purchased from Sigma-Aldrich (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), luminol, hydrogen peroxide, and tartaric acid were Fluka (Deisenhofen, Germany) compounds. Sodium chloride was obtained from LachNer, Czech Republic. Samples of wines were purchased from the local supermarket. After opening, aliquots of wines were poured into plastic test tubes leaving no air above the wine sample and stored in a light-tight box in a refrigerator. Data concerning the wine samples used are summarized in Table 1.

CFE Fabrication and Electrochemical Measurements. Both CFE pretreatment and amperometric assay were performed using a Nanoampere electrochemical workstation (L-Chem, Czech Republic). A polyacrylonitrile-based carbon fiber (7−8 μm Courtaulds XA-S, type α, Courtaulds Ltd., UK) was obtained from a local distributor (Havel Composites Inc., Czech Republic). The procedure for CFE fabrication is as follows: Carbon fiber is glued using conductive silver epoxy (Epotek H20E, Polytec, Germany) onto a copper wire, and the junction is then cured at 150 °C for 10 min. The fiber with the attached copper contact is fitted into a glass capillary; about 10 mm of the fiber is left protruding from its tapered end. Both ends of the capillary are sealed using epoxy resin (CHS Epoxy 1200, Sindat Pilsen, Czech Republic). Prior to use, the protruding fiber is cut to the length of about 5 mm by a lancet, and the fiber end of the electrode is briefly sonicated in dichloromethane to remove grease. The prepared microelectrode is subjected to electrochemical pretreatment performed by cycling the electrode in 1% (w/w) NaCl solution between 0 and 2.9 V (vs Ag/AgCl) for 20 s, 50 Hz sine wave, followed by 5 s at constant potential −0.8 V and 5 s at 1.5 V.

Table 1. Wine Samples

sample	wine	provenience	year	alcohol content (%)	packaging (mL)
		Red Wines			
1	Ruby Cabernet dry	California, USA	2009	13.0	250
2	Cabernet Sauvignon dry	Valle Central, Chile	2010	12.5	250
3	Cabernet Sauvignon dry	Valle Central, Chile	2010	12.5	750
4	Cabernet Sauvignon dry	Venezia, Italy	2010	11.5	750
		Rosé Wines			
5	Zinfandel rosé	California, USA	2009	10.5	250
6	Cinsault-Grenache	Pays D'oc, France	2010	12.0	250
		White Wines			
7	Chardonnay dry	Slovakia	2010	11.0	750
8	Chardonnay dry	Valle Central, Chile	2009	14.0	250
9	Chardonnay dry	California, USA	2009	13.0	750
10	Chardonnay dry	Australia	2009	12.5	750

CFE and Microdialysis Online Connection. For the purpose of amperometric measurement of wines, a microdialysis system was coupled to voltammetry using CFE as a working electrode. The microdialysis setup consisted of Pye-Unicam Philips isocratic pump (model PU4015), a pulse damper, a flow splitter, a microdialysis probe (MAB 11.8.10, 6 kDa cutoff polyethylenesulfone membrane, active length = 10 mm, outer diameter = 0.5 mm; Microbiotech/se AB, Stockholm, Sweden), and connecting tubing (PEEK). The microdialysis probe was mounted into a custom-made positioning device allowing the semipermeable membrane to be inserted into the wells of the microtitration plate (96-plate format) containing the measured solution (wine or standard). A quartz capillary (inner diameter = 80 μm) was inserted behind the microdialysis probe outlet. Interconnections were made using standard HPLC fittings. CFE with a fiber length of 5 mm was partially introduced (∼4 mm) into the outlet of the aforementioned quartz capillary. Both the capillary and the CFE (by means of its glass body) were mounted to a piece (∼30 × 30 mm) of a printed circuit board and fixed firmly to avoid any accidental positional changes. A three-electrode arrangement was used: the auxiliary large-surface electrode was realized by the copper foil of a printed circuit board, and an Ag/AgCl reference mini electrode (L-Chem, Czech Republic) was placed in close proximity to the end of the capillary (Figure 1). The interconnection of the electrodes was mediated by the perfusion solution emulating a real wine sample ("artificial wine solution" composed of 12% (v/v) EtOH and 0.05 M tartaric acid). The pH of the solution was adjusted with 1 M NaOH to the value required (3.6 or 7.4). All solutions were prepared using ultrapure (18.2 MΩ cm^{-1}) water from a Millipore Milli-Q system.

TRAP Assay. The luminol-enhanced chemiluminescence (CL) was used to follow up the peroxyl radical reaction as documented previously.[18] The CL signal is driven by the production of luminol-derived radicals from the thermal decomposition of AAPH. The reaction mixture contained 160 μL of sodium phosphate-buffered saline (PBS 10^{-1} mol/L, pH 7.4), 16.7 μL of 10^{-2} mol/L luminol in 10^{-1} mol/L borate buffer (pH 10.0), and 6.7 μL of the examined sample. The reaction mixture was incubated at 37 °C in the

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Figure 1. (A) Scheme of the arrangement used for electrochemical assay of antioxidant capacity of wine (AUX, auxiliary electrode; REF, reference electrode; W, working (carbon fiber) electrode). (B) Photograph of the apparatus (1, HPLC pump; 2, pulse damper; 3, guard column; 4, flow splitter; 5, microdialysis probe; 6, microplate positioner; 7, electrode system; 8, potentiostat). (C) Photograph of the electrode system layout (a piece of white paper was inserted below the carbon fiber electrode to make the fiber−capillary connection visible; 1, CFE; 2, carbon fiber inserted into fused silica capillary; 3, reference electrode; 4, copper foil auxiliary electrode).

temperature-controlled luminometer for 10 min. Thereafter, 16.7 μL of 4×10^{-1} mol/L AAPH (prepared in 10^{-1} mol/L PBS) was added. A known final concentration (8.0 nM) of Trolox standard was used as a reference antioxidant instead of the sample. The TRAP value is determined from the duration of the time period (T_{sample}) during which the sample quenched the CL signal due to present antioxidants. The T_{sample} is compared to T_{Trolox}, and the TRAP value is calculated according to the equation

$$\text{TRAP} = 2.0[\text{Trolox}]T_{sample}/fT_{Trolox}$$

where 2.0 is the stoichiometric factor of Trolox (the number of peroxyl radicals trapped per molecule of Trolox) and f is the dilution of the sample. The TRAP assay was conducted on a Luminometer Orion II (Berthold Detection System, Pforzheim, Germany). TRAP values were expressed as micromoles of Trolox equivalents per liter of wine sample.

ORAC Assay. The ORAC assay measures the antioxidant scavenging function against peroxyl radical induced by AAPH at 37 °C.[18] Fluorescein is used as a fluorescent probe. The loss of fluorescein fluorescence is indicative of the extent of its reaction with the peroxyl radical. The total reaction mixture volume was 200 μL, and all

solutions were prepared in a phosphate buffer (75 mM, pH 7.4). One hundred and seventy microliters of fluorescein solution (60 nM final concentration) and 10 μL of the sample were placed in a microplate well and incubated at 37 °C directly in the Infinite M200 (Tecan, Austria) for 10 min. After the incubation, the reaction was started by adding 20 μL of AAPH (51.5 mM final concentration). Fluorescence was recorded every minute, and the microplate was automatically shaken prior to each reading. Blank readings using phosphate buffer instead of the antioxidant and calibration solutions of Trolox (12.5–100 μM) were also carried out in each assay. The final ORAC values were calculated using a regression equation between Trolox concentration and the net area under the curve (AUC). The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. ORAC values were expressed as micromoles of Trolox equivalents per liter of wine sample.

CL-TAC Assay. CL-TAC assay measurements were performed on a Luminoskan Ascent (Thermolabsystems, Finland) using a modified[17] protocol.[19,20] Wine sample or standard (20 μL) was mixed in the microplate well together with the horseradish peroxidase solution (20 μL, 25 KU/mL) before the start of the measurement. One hundred and sixty microliters of the chemiluminescent mixture containing hydrogen peroxide (0.1 mM) and luminol (0.040 mM) in phosphate buffer (pH 7.4) was injected using the autosampling device of the luminometer to trigger the luminol chemiluminescence reaction. In the absence of antioxidant species in the sample, the light emission occurs immediately after the injection of the CL mixture into the well. If antioxidant species are present in the sample, the light signal is inhibited for a certain period of time and then increases abruptly to values close to those observed in the absence of antioxidants. The time period (in seconds) between the injection and the return to maximum emission is a function of the antioxidant capacity of the examined sample. CL-TAC values were expressed as micromoles of Trolox equivalents per liter of wine sample.

RESULTS AND DISCUSSION

Among the electrochemical methods for the assessment of TAC/TCP in wines cyclic voltammetry with a glassy carbon

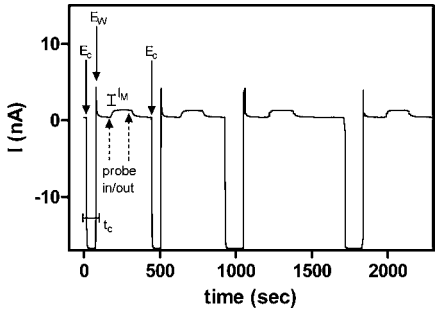

Figure 2. Amperometric analysis of wine. Four analyses of red wine (sample 1) are performed with 60 s (first two analyses) and 120 s cleaning steps to illustrate the reproducibility of the method. E_c = cleaning potential (0 V), E_w = working potential (800 mV), I_M = measured current due to oxidizable compounds in the wine. The flow rate of the perfusion solution was 30 μL min^{-1}, pH 7.4. Times at which the microdialysis probe is inserted into and removed from the wine sample are indicated by dashed arrows.

working electrode has been widely publicized. A glassy carbon electrode is, however, less compatible as far as coupling microdialysis with electrochemical sensing is concerned. We employed the CFE as a working electrode because its convenient size enables insertion directly into the microdialysis flow path (0.2 mm diameter flexible tubing). The tiny dimensions of carbon fibers constituting the active part of CFEs (8 μm in diameter, 5 mm length) govern the shapes of

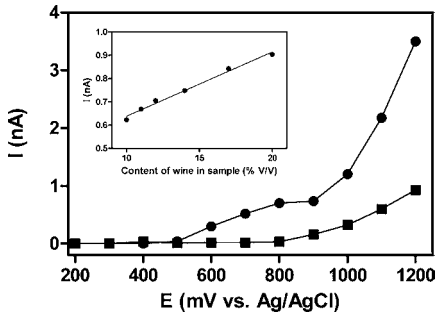

Figure 3. Hydrodynamic voltammograms constructed from amperometric responses of red wine (sample 1, diluted to 20% (v/v), circles), and sodium sulfite (1 mM, squares), determined using different E_w values. Other parameters of the analyses are identical with those described in Figure 2. (Inset) Calibration curve for the red wine at 800 mV.

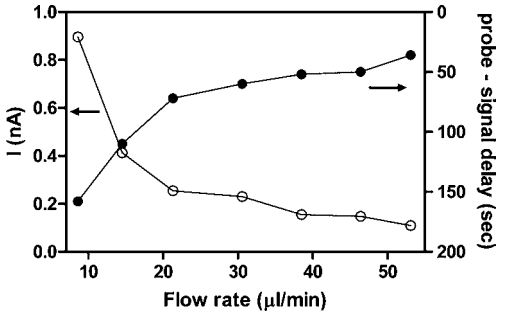

Figure 4. Dependence of amperometric current and signal delay between probe insertion and detector response on flow rate determined for red wine (sample 1, diluted to 20% (v/v). Applied potential = 800 mV vs Ag/AgCl.

cyclic voltammograms (waves rather than peaks) due to prevailing radial diffusion mode of transport of the electroactive substances onto the electrode surface. Owing to the absence of well-resolved CV peaks, these electrodes are less suited to the characterization of wines using CV. On the other hand, cylindrical CFEs are advantageous for amperometry, and for this reason the constant potential amperometry technique was selected as the electrochemical technique in this work. The sensitivity and response time are improved in amperometric measurements with microelectrodes due to enhanced Faradayic to non-Faradayic current ratio caused by radial diffusion mass transfer of the analyte to the surface of the microelectrode. Additionally, microelectrodes exhibit lower sensitivity of the electrode response to irregularities in stirring (in the case of batch electrochemical experiment) or irregularities in flow (when used in a flow system) during amperometric measurements compared to conventional millimeter-sized electrodes.[21] Amperometric determination of easily oxidizable species (i.e., wine antioxidants such as polyphenols) in a flowing stream is based on subjecting the electrode to a sufficient constant anodic potential. Antioxidants in wine sample capable of being oxidized at a potential equal to or lower than the set potential contribute to the measured current, which is therefore proportional to the concentration of oxidizable species in the analyzed sample. For this reason, amperometric measurement provides similar information as the integral under the CV peak. Figure 2 shows the typical amperometric measurement of a wine sample.

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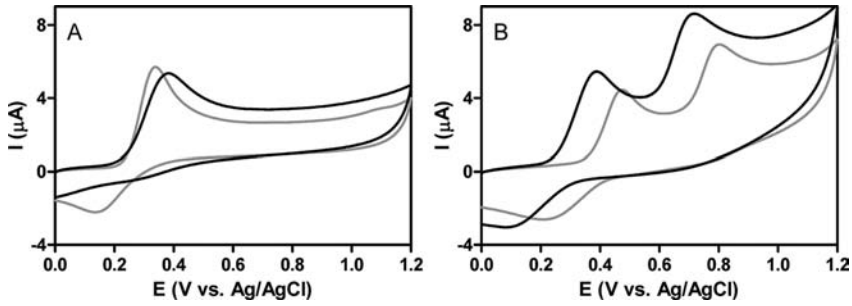

Figure 5. Cyclic voltammograms of 0.2 M Trolox (A) and catechin (B) at pH 7.4 (black) and 3.6 (gray) measured in artificial wine solution at 100 mV s^{-1} using a glassy carbon electrode.

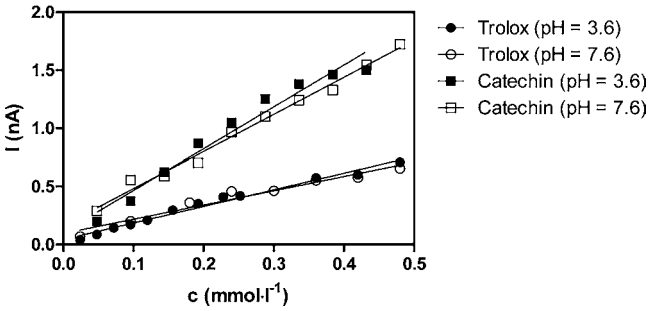

Figure 6. Calibration lines of catechin and Trolox at pH 3.6 and 7.4. E_w = 800 mV vs Ag/AgCl.

The perfusion solution is allowed to flow to the CFE-based electrochemical detector through the microdialysis probe exposed to ambient air. Following flow stabilization (10 min), the CFE is subjected to a cleaning potential of 0 V (E_c) for the time period of t_c (60 s). Subsequently, the potential is set to the working potential E_w (800 mV). After reacquisition, the microdialysis probe is inserted into the wine sample (diluted 1:10 with artificial wine solution). The membrane permselective properties (6 kDa cutoff) ensure that the wine dialysate is virtually free from macromolecules. On the other hand, low molecular weight compounds diffuse freely into the probe, and after a certain lag time the sample zone reaches the electrode surface, causing the current I_M to respond depending on the wine oxidizable species content. When the measurement of I_M is complete, the probe is removed from the wine sample, making the current return to its baseline, and the system is ready for the next analysis.

Optimization of the Amperometric Technique for Wine Analysis. *Choice of the Working Potential.* The hydrodynamic voltammogram of red wine (sample 1, Figure 3), constructed from the amperograms measured at different working potentials using the method outlined in the previous section, suggests that the oxidizable species present in wine can be divided into two groups. This is in agreement with studies utilizing glassy carbon electrodes.[10] However, a positive shift in the potentials at which the oxidation of wine occurs is observed. This phenomenon results from the increased overvoltage on the artificially oxidized carbon fiber surface. The oxidation wave starts approximately at 400 mV and reaches a limiting current between 800 and 900 mV. At potentials higher than 900 mV another group of species becomes oxidizable and a current rise is observed again. From the hydrodynamic voltammogram (Figure 3, squares) it follows that sodium sulfite (a common wine preservative added during winemaking) contributes significantly to the amperometric current at potentials exceeding 900 mV. The concentration of sodium sulfite used to determine its hydrodynamic voltammogram in Figure 3 was selected to be 1 mM, that is, a 33% higher concentration than the recommended dose (80 mg/L or 0.75 mM) and ca. 50% lower than the maximum permitted concentration according to EU regulations (200 mg/L). At potentials greater than 1200 mV severe problems with electrode fouling were observed.

Table 2. Antioxidant Capacities of Wine Samples (Expressed as Micromoles of Trolox Equivalents (TE) per Liter of Wine) Determined Using the Proposed Amperometric Method and Standard Methods (TRAP, ORAC, and CL-TAC)

	amperometric						
	pH 3.6		pH 7.4				
sample	nA	μmol TE/L	nA	μmol TE/L	TRAP (μmol TE/L)	ORAC (μmol TE/L)	CL-TAC (μmol TE/L)
Red Wines							
1	0.83 ± 0.024	2710 ± 77.1	0.84 ± 0.013	2743 ± 43.1	25286 ± 573	25953 ± 1181	4792 ± 858
2	0.69 ± 0.047	2253 ± 153.5	0.79 ± 0.011	2580 ± 35.7	16549 ± 987	22446 ± 2083	6547 ± 153
3	0.65 ± 0.018	2123 ± 58.8	0.75 ± 0.009	2449 ± 28.3	18091 ± 1529	19496 ± 943	6897 ± 503
4	0.73 ± 0.038	2384 ± 123.7	0.70 ± 0.012	2286 ± 37.6	15831 ± 358	20421 ± 1613	5728 ± 783
Rosé Wines							
5	0.53 ± 0.040	1731 ± 131.6	0.27 ± 0.010	882 ± 31.7	4699 ± 310	4567 ± 280	441 ± 88.5
6	0.06 ± 0.021	196 ± 69.9	0.08 ± 0.011	261 ± 36.0	3973 ± 551	4567 ± 353	447 ± 49.1
White Wines							
7	0.03 ± 0.012	98 ± 39.2	0.04 ± 0.007	130 ± 22.5	2596 ± 229	2342 ± 229	257 ± 36.5
8	0.40 ± 0.008	1306 ± 25.5	0.21 ± 0.009	686 ± 29.7	5063 ± 218	5037 ± 424	272 ± 52.2
9	0.31 ± 0.048	1012 ± 156.4	0.19 ± 0.005	620 ± 17.3	4707 ± 176	3909 ± 360	472 ± 31.5
10	0.11 ± 0.006	359 ± 19.9	0.09 ± 0.005	294 ± 14.7	4916 ± 72	4043 ± 197	348 ± 39.3

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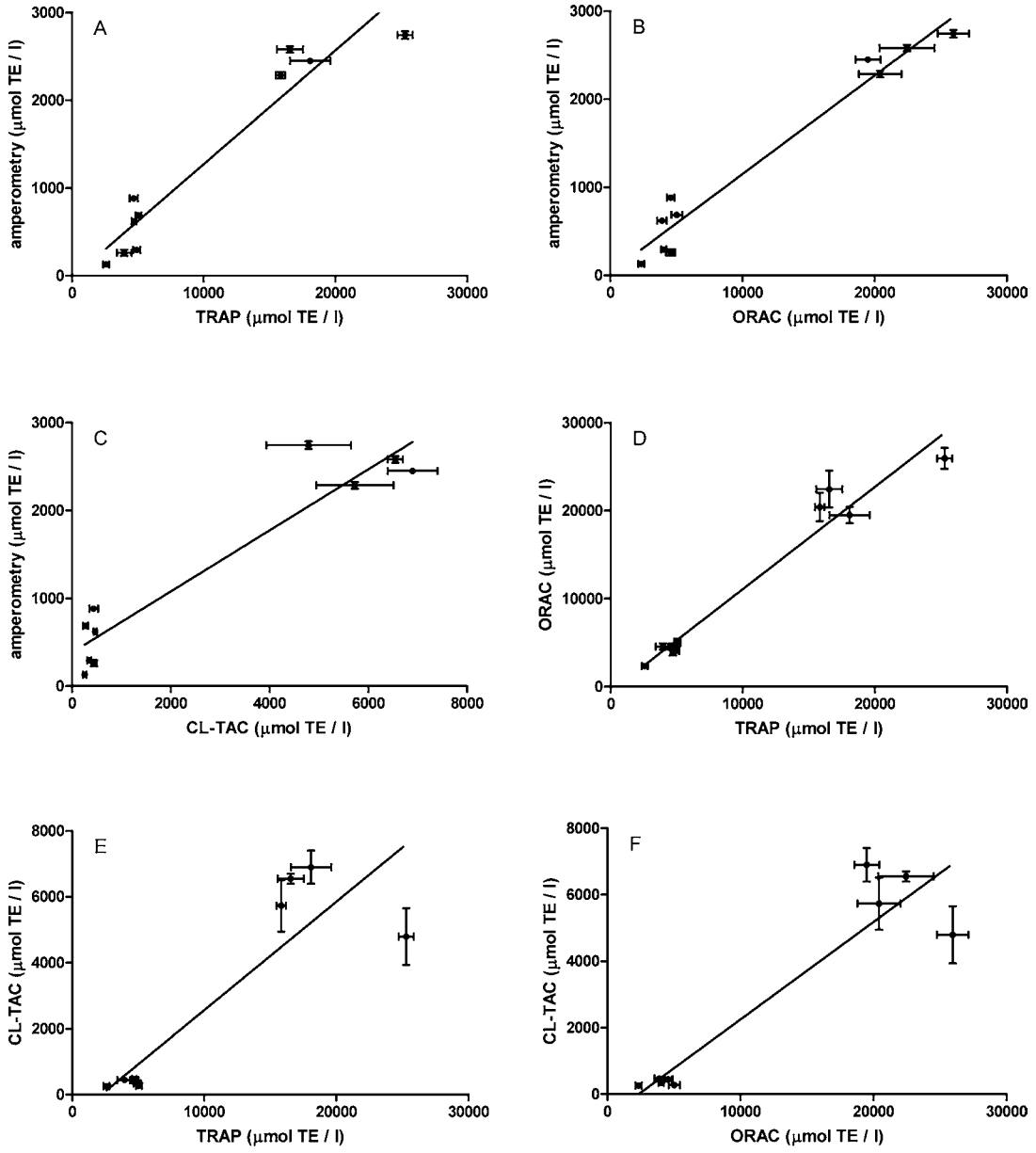

Figure 7. Correlations among various measurements (amperometric, ORAC, TRAP, and CL-TAC) of antioxidant activity of wine samples determined at pH 7.4, expressed in μmol of Trolox equivalents per liter of wine: (A) amperometry vs TRAP ($y = 0.1298x - 42.95$; $r^2 = 0.920$); (B) amperometry vs ORAC ($y = 0.112x - 30.97$; $r^2 = 0.963$); (C) amperometry vs CL-TAC ($y = 0.3476x + 382.5$; $r^2 = 0.904$); (D) ORAC vs TRAP ($y = 1.162x - 540.7$; $r^2 = 0.967$); (E) CL-TAC vs TRAP ($y = 0.3291x - 721.2$; $r^2 = 0.8184$); (F) CL-TAC vs ORAC ($y = 0.2932x - 687.0$; $r^2 = 0.884$).

Thus, higher potentials were not evaluated. The aforementioned facts were the basis for the application of an 800 mV working potential throughout the experimental work.

Although in common flow systems the amperometric current rises with the elevation of the analyzed solution's flow rate, the situation is different for the microdialysis-based sampling. The increasing flow rate elicits a drop in the collection efficiency (i.e., relative recovery) of a microdialysis probe. As a result, (growing) dilution of the analyte in the dialysate counteracts the effect of increased mass transport (i.e., absolute recovery) of the analyte to the electrode surface and so accounts for the decline in the amperometric current (Figure 4).

To minimize the time delay of the electrochemical detector response owing to the tubing connecting the active part of the microdialysis probe with the CFE, further experiments were performed at a higher flow rate (30 μL/min producing ca. 1 min lag time).

Correlation of Amperometric Technique of Wine Analysis with Standards. Routinely, voltammetric analyses of wines are performed at pH 3.6 and are standardized to the responses of gallic acid and/or catechin dissolved in "artificial wine solution". However, antioxidant capacities are evaluated in nearly neutral (pH 7.4) solutions using ORAC, CL-TAC, and TRAP, and raw data are standardized to Trolox. Thus, the electrochemical behaviors of Trolox and catechin (Figure 5) were studied by CV at pH 3.6 and 7.4 (the pH value of artificial wine solution was adjusted using 1 M NaOH).

Cyclic voltammograms of Trolox reveal one anodic peak exhibiting a greater degree of reversibility at pH 3.6 compared to pH 7.4. The heights of Trolox anodic peaks at both pH values are comparable. This finding is in agreement with the

previous reports on Trolox electrochemistry, in which nearly identical CV anodic peak currents were found in acidic and neutral solutions.[22] The electrochemical behavior of Trolox (TrOH) is characterized by the mechanism[22] involving one electron oxidation ($TrOH \rightarrow TrOH^{\bullet} + e^{-}$), followed by rapid deprotonation $TrOH^{\bullet} \rightarrow TrO^{\bullet} + H^{+}$ and the second electron transfer $TrO^{\bullet} \rightarrow TrO^{+} + e^{-}$. TrO^{+} is subsequently transformed by ensuing nucleophilic reaction into nonelectroactive species. The cyclic voltammogram of catechin contains two anodic peaks. The electrochemistry of catechin involves two two-electron−two-proton oxidation steps. The first peak is unequivocally ascribed to oxidation of catechol −OH groups on the B-ring. The second peak is irreversible and is thought to correspond to the oxidation of the *m*-diphenol groups on the A-ring. The degree of irreversibility and hence the appearance of the second peak are dependent on the state of the electrode surface.[23,24] The results of the experiments using microdialysis-coupled amperometry with Trolox and catechin dissolved in artificial wine solutions adjusted to pH 3.6 and 7.4 are shown in Figure 6.

At the set potential of 800 mV, nearly pH-independent current responses are found for both catechin and Trolox. Due to the different numbers of transferred electrons (two for Trolox vs four in the case of catechin), the amperometric response at 800 mV is expected to be higher for catechin than for Trolox, assuming similar reactivity of Trolox and catechin toward free radicals used in the antioxidant capacity tests. The experimental value of $TEAC_{catechin}$ = 2.7 determined amperometrically as the ratio of slopes of calibration lines for catechin and Trolox (Figure 6) at pH 7.4 (2.9 at pH 3.6) is in good agreement with the literature data from $ABTS^{\bullet+}$ decolorization assays. A value of 2.95[25,26] was found for 0.1 mM catechin at pH 7.4 using $ABTS^{\bullet+}$ generated by the reaction of ABTS with peroxydisulfate,[25] or electrogenerated[26] and 2.85[27] was determined by scavenging the cation radical $ABTS^{\bullet+}$ induced by the reaction of metmyoglobin with H_2O_2. Some methods (e.g., peroxynitrite scavenging activity, FRAP) give TEAC values of catechin close to 1, indicating that only two-electron oxidation of catechin takes place in these tests.[27] The coincidences between the amperometric responses determined at both pH 3.6 and 7.4 for Trolox and catechin indicate that the microdialysis probe recoveries are similar for Trolox and catechin and do not depend on the pH of the solution.

Comparison of Amperometric Method to TRAP, ORAC, and CL-TAC. The antioxidant capacities of 10 different wines from various countries (four Cabernet Sauvignon red wines, four white wines (Chardonnay), and two rosé wines (Zinfandel rosé and Cinsault-Grenache)) were evaluated using the amperometric method proposed in this work as well as standard methods of antioxidant capacity evaluation, that is, TRAP, ORAC, and CL-TAC. The values of antioxidant capacities are given in Table 2. The results are expressed as the mean ± standard deviation (SD). The experiments were carried out in triplicates.

Correlations between antioxidant capacities of wines determined by the aforementioned methods are shown in Figure 7. To express correlations, the Pearson correlation coefficient (r^2) was used, and linear regressions were calculated. A p value of <0.05 was considered to be significant.

A significant correlation was found between amperometric measurement and TRAP as well as between amperometric measurement and ORAC (r^2 values are comparable to r^2 of the dependence between TRAP and ORAC). Less significant correlation was found between amperometry and the CL-TAC method. Whereas the average ratios between the antioxidant capacities of red and white wines were similar for the amperometric, TRAP, and ORAC methods (5.81, 4.36, and 5.76, respectively), the figure determined by CL-TAC was much different (17.8). The apparent inconsistency may emerge from (a combination of) interfering red wine constituents affecting the activity of horseradish peroxidase enzyme, on the one hand, and/or the lower values of white wines' antioxidant capacity, on the other.

For wine analysis, amperometry using carbon fiber microelectrode coupled online with microdialysis offers good reproducibility and allows convenient automation using a 96-microtitration plate format. The application of the method to the analysis of other beverages or types of foods depends on finding suitable protocols of electrode regeneration.

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Notes

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