Batch-injection analysis with amperometric detection of the DPPH radical for evaluation of antioxidant capacity

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ABSTRACT

This work proposes the application of batch-injection analysis with amperometric detection to determine the antioxidant capacity of real samples based on the measurement of DPPH radical consumption. The efficient concentration or EC50 value corresponds to the concentration of sample or standard required to scavenge 50% DPPH radicals. For the accurate determination of EC50, samples were incubated with DPPH radical for 1 h because many polyphenolic compounds typically found in plants and responsible for the antioxidant activity exhibit slow kinetics. The BIA system with amperometric detection using a glassy-carbon electrode presented high precision (RSD = 0.7%, n = 12), low detection limit (1 μmol L−1) and selective detection of DPPH (free of interferences from antioxidants). These contributed to low detection limits for the antioxidant (0.015 and 0.19 μmol L−1 for gallic acid and butylated hydroxytoluene, respectively). Moreover, BIA methods show great promise for portable analysis because battery-powered instrumentation (electronic micropipette and potentiostats) is commercially available.

1. Introduction

The antioxidant capacity is typically provide by the reaction of a solution containing a mixture of antioxidant compounds with target free radicals. Several antioxidant capacity methods are based on free-radical-scavenging ability including 2,2′-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) (ABTS⁺), peroxyl superoxide (O₂⁻) and 2,2-diphenyl-1-picrylhydrazyl (DPPH⁰). The DPPH scavenging radical method is one of the most widely employed methods to determine the antioxidant capacity. This method is based on a measurement of the consumption of the DPPH radical by an antioxidant compound (generally phenolic compounds). The most commonly used method to evaluate the ability to consume the DPPH radical is the spectrophotometric measurement of the decrease in the absorbance of the DPPH radical after the reaction (Alam, Bristi, & Rafiquzzaman, 2013; Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002; Gulcin, 2012; Huang, Boxin, & Prior, 2005; Liu, 2010; MacDonald-Wicks, Wood, & Garg, 2006; Magalhães, Segundo, Reis, & Lima, 2008).

Other detection techniques have been also used to measure the consumption of the DPPH radical such as electrochemical ones. The electrochemical behavior of the DPPH radical was investigated by cyclic voltammetry, which involves a mono-electron transfer, electrochemically reversible and controlled by diffusion (Ahmed, Tabassum, Shakeel, & Khan, 2012). Cyclic voltammetry of DPPH was reported at a bare glassy-carbon electrode (GCE) and GCE modified with multi-walled carbon nanotubes (CNT-GCE) (Amatatongchai, Laosing, Chailapakul, & Nacapricha, 2012). The CNT-GCE exhibited slight improvement based on the decrease in peak separation (ΔE, from 52 to 22 mV), which indicates a fast electron transfer provided by the carbon nanotubes. The same authors proposed the use of flow-injection analysis (FIA) coupled with amperometric determination of DPPH consumption for the evaluation of antioxidant capacity of plants, in which a continuous flow of a DPPH solution was carried to the working electrode (CNT-GCE) (Amatatongchai et al., 2012). Similarly, the use FIA with amperometric detection at a gold screen-printed electrode was proposed to determine the antioxidant capacity of different wines also based on the consumption of DPPH; while the samples were first pre-incubated with DPPH and then injected in a continuous buffer flow (Andreí, Bunea, Tudorache, Gaspar, & Vasilescu, 2014). The time required for the complete reaction (pre-incubation step) of the samples (or antioxidants) with DPPH depends on the structure of the antioxidant compounds. Some polyphenols can react more rapidly than others can, such as quercetin and catechin that are more reactive than vanillic and p-coumaric acids (Gizdavic-Nikolaïdis et al., 2004; Kilmartin, 2001).
The kinetics of the antioxidant reaction with DPPH can be classified as rapid (<5 min), intermediate (5–30 min) and slow (>30 min) (Jiménez-Escrig, Jiménez-Jiménez, Sánchez-Moreno, & Saura-Calixto, 2000). Many polyphenolic compounds found in plants and responsible for the antioxidant activity, present low kinetic behavior classified as slow and thus require longer times of reaction with DPPH (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998). For this reason, the accurate determination of antioxidant capacity of plant samples requires longer pre-incubation times between DPPH and samples.

The use of cyclic voltammetry and differential-pulse voltammetry was proposed to evaluate the antioxidant capacity of different samples and antioxidant standards as a function of the concentration of DPPH (Ahmed et al., 2012; Alvarez-Diduk et al., 2008; Litescu & Radu, 2000). The successful application of these approaches requires that the antioxidants did not present electrochemical responses in the potential range at which the DPPH/DPPH redox pair was detected.

Batch-injection analysis (BIA) was firstly presented by Wang and Taha (1991). The association of BIA with amperometric detection is a powerful tool for the analysis of food, environmental and pharmaceutical samples (Montes, Marra, Rodrigues, Richter, & Muñoz, 2014; Pereira, Marra, Muñoz, & Richter, 2012; Quintino & Angnes, 2004; Silva, Montes, Richter, & Muñoz, 2012; Stefano, De Lima, Montes, Richter, & Muñoz, 2012; Tormin, Cunha, Richter, & Muñoz, 2012). BIA systems employ micropipettes (or simple syringes) to inject microliter aliquots of sample or standards directly onto the working electrode, which is immersed in a large blank solution containing supporting electrolyte. Aliquots of sample or standards are diluted in the large volume of the cell, where the reference and counter electrodes are positioned. The use of an electronic micropipette for injection highly increases precision of analysis in comparison to the use of syringes or common micropipettes. However, the use of internal standard addition was demonstrated in previous works to solve the lack of repeatability associated with variations of injected volume (Gimenes, Santos, Muñoz, & Richter, 2010; Gimenes et al., 2012). Compared with FIA, BIA eliminates the use of valves and pumps typically used in FIA systems and brings the possibility of portable analysis, since there are commercially-available battery-powered potentiosstats and electronic micropipettes (Tormin, Cunha, Silva, Muñoz, & Richter, 2014).

In this work, we propose the use of BIA with amperometric detection to determine the antioxidant capacity of plant and tea samples based on the selective and sensitive amperometric measurement of DPPH consumption.

2. Experimental

2.1. Reagents and solutions

High-purity deionized water (R ≥ 18 MQ cm) obtained from a Milli Direct-Q3 water purification system (Millipore, Bedford, MA, USA) was used for preparing all aqueous solutions. Sodium hydroxide (97% m/m) and ethanol (95% v/v) were purchased from Synth (Diadema, Brazil). Concentrated acetic acid (99.7% v/v), sodium carbonate, and gallic acid were obtained from Vetec (Rio de Janeiro, Brazil). Quercetin (99% m/m) and chlorogenic acid (99% m/m) were purchased from Acros (USA).

A stock solution of 348 μmol L⁻¹ DPPH was prepared by dissolving the appropriate amount in 0.2 mol L⁻¹ acetate buffer (pH 5.5) and ethanol (20:80, v/v). For DPPH solution preparation, a Unique UltraCleaner 1640A ultrasonic bath assisted DPPH dissolution for about 2 h under protection from light to minimize decomposition by light as described in previous work (Amatongchaei et al., 2012).

Leaves of the Moringa oleifera and Eugenia uniflora were collected at Uberlândia, Minas Gerais, Brazil (18° 55' 3.7”S, 48° 15' 38.3”W and 19° 09' 20”S, 48° 23' 20”W). Commercial tea samples were obtained from local stores.

2.2. Instrumentation

All electrochemical measurements were performed using a µ-Autolab Type III (Eco Chemie, Utrecht, Netherlands) controlled by GPES4.9.007 software (General Purpose Electrochemical System). Injections of standard solutions or samples were conducted using an Eppendorf electronic micropipette (multipette® stream), which permits injections from 10 to 1000 μL (using 1 mL combitip®) at a programmable dispensing rate (from 28 to 345 μL s⁻¹). All spectroscopic measurements were performed using a HITACHI model U-2000 spectrophotometer at a wavelength of 517 nm, using 3.0 mL quartz cuvettes.

2.3. Sample preparation

The tea solutions were prepared using 1.7 g of the commercial samples (one whole bag) in 100 mL of deionized-distilled water for infusion (100 °C) for 5 min. One milliliter of the solution was collected and dried. The obtained concentration of sample was 2.3 and 1.6 mg mL⁻¹ for the samples Tea 1 and Tea 2, respectively. The respective yield was 13.5% and 9.4% for Tea 1 and Tea 2.

Extracts of leaves of the M. oleifera and E. uniflora, known popularly as Moringa and Brazilian cherry, respectively, were prepared and analyzed. Vegetal material was dried at 35 °C until 7% humidity was obtained. The extracts were prepared by maceration using 15 g of the powdered leaves, and 200 mL of 98 and 80% ethanol for Moringa; 80% acetone and 70% ethanol for Brazilian cherry. Maceration was soaked in solvent for 7 days. After this, the extract was filtered, the solvent was removed by rotator evaporation at 40 °C. The process was repeated three times to achieve complete extraction. The dried extracts were transferred to amber flasks and stored at 4 °C until analysis. The yields of Moringa extracts were 31.2 % for 70% ethanol and 11.5% for 98% ethanol. The yields of Brazilian cherry extracts were 37.1% for 70% ethanol and 37.3% for 80% acetone.

2.4. Electrochemical cell and electrodes

The reference and auxiliary electrodes were a miniaturized Ag/AgCl (saturated KCl) (Pedrotti, Angnes, & Gutz, 1996) and a platinum wire, respectively. A 3 mm commercial GCE (Metrohm) was used as was the working electrode. Cleaning of the GCE surface was performed mechanically on a felt-polishing pad using an alumina powder suspension (0.3 μm) and copiously rinsing with deionized water.

The initial investigation of the electrochemical process of DPPH and antioxidants in ethanol–acetate buffer solution was carried out by cyclic voltammetry. Amperometric measurements were performed using a homemade electrochemical batch-injection cell previously described in the literature (Pereira et al., 2012; Silva et al., 2012). The BIA cell presented an internal volume of 180 mL and was constructed from a glass cylinder (of 7 cm internal
diameter) and two polyethylene covers, which were firmly fitted on the top and bottom of the cylinder. The top cover contained 3 holes for the counter and reference electrodes and micropipette tip (combitip® syringe shape). The micropipette tip (with a regular external diameter of 6 mm) was firmly introduced into the hole (6.1 mm diameter) in the center of the cover in such a way that the injection procedure was highly reproducible. The bottom cover contained a single hole (which was also precisely located at the center of the cover) in which the working glassy-carbon electrode (GCE) was inserted (positioned oppositely to the micropipette tip). Teflon tape was used to fix the electrode in the hole and to prevent leakage. The distance between the electrode and micropipette tip was adjusted to around 2 mm (wall-jet configuration).

A mixture of 0.2 mol L⁻¹ acetate buffer (pH 5.5) and ethanol (40:60 v/v) was used as supporting electrolyte solution in all electrochemical measurements.

2.5. Electrochemical analysis

A 0.5 mL of the 348 μM DPPH solution was added into 2.0 mL ambar microtubes followed by the addition of 1.5 mL of each sample at different concentrations. The concentration ranges were as follows: 0.9–5.2 μg mL⁻¹ of Tea 1 sample; 4.0–22.0 μg mL⁻¹ of Tea 2 sample; 3.0–9.1 μg mL⁻¹ of Brazilian cherry (70% ethanol and 80% acetone extracts); 11.2–131.2 μg mL⁻¹ of 98% ethanol Moringa and 26.2–86.2% of 70% ethanol Moringa extracts. The antioxidant standards GA and BHT were also analyzed in a similar manner in the following concentration ranges: 0.5–9.5 μg mL⁻¹ and 10–40 μg mL⁻¹, respectively. Both plant extracts, antioxidant standards (GA and BHT) and DPPH were dissolved in a solvent mixture containing 0.2 mol L⁻¹ acetate buffer and ethanol (40:60 v/v). After 1 h at room temperature and protected from light, the solutions contained in the microtubes were injected by the BIA system for the amperometric determination of the remaining DPPH. The scavenging capacity based on the amperometric measurements of DPPH that corresponds to the percentage of DPPH radical scavenging was determined by:

\[
\text{DPPH radical scavenging} = \left[ 1 - \frac{(I_{\text{sample}} - I_{\text{blank}})}{I_{\text{control}}} \right] \times 100 \tag{1}
\]

where \(I_{\text{sample}}\) is the current for the residual DPPH from the reaction between DPPH and sample (or standard) after 1 h, \(I_{\text{blank}}\) is the current of the blank (1.5 mL of the electrolyte and 0.5 mL of sample or standard solution), and \(I_{\text{control}}\) is the current for the initial amount of DPPH established as the control (1.5 mL of electrolyte and 0.5 mL of the DPPH solution).

The \(EC_{50}\) value (efficient concentration), denoting the concentration of sample (or standard) required to scavenge 50% DPPH radicals, was calculated by graphical regression analysis of the scavenging capacity versus sample (or standard) concentrations.

The time of 1 h reaction between DPPH and samples (or antioxidant standards) was based on the low kinetic of reaction for polyphenolic compounds typically found in the samples analyzed in this work as described in the literature (Sánchez-Moreno et al., 1998).

2.6. Spectrophotometric analysis

The procedure was based on the method described in the literature (Sousa et al., 2014). An aliquot of 0.5 mL of the 348 μM DPPH solution was added in a quartz cuvette followed by the addition of 1.5 mL of each sample at different concentrations. The concentration ranges of the samples were prepared at the same concentrations prepared for the BIA electrochemical determinations also dissolved in a solvent mixture containing 0.2 mol L⁻¹ acetate buffer and ethanol (40:60 v/v). After 1 h at room temperature and protected from light, the absorbance was determined at 517 nm. The scavenging capacity that corresponds to the percentage of DPPH radical scavenging was determined by:

\[
\text{DPPH radical scavenging} = \left[ 1 - \frac{(Abs_{\text{sample}} - Abs_{\text{blank}})}{Abs_{\text{control}}} \right] \times 100 \tag{2}
\]

where \(Abs_{\text{sample}}\) is the absorbance of the reaction between DPPH and the sample (or standard) after 1 h, \(Abs_{\text{blank}}\) is the absorbance of the blank (1.5 mL of the solvent and 0.5 mL of sample or standard solution) and \(Abs_{\text{control}}\) is the absorbance of the control (1.5 mL of the solvent and 0.5 mL of the DPPH solution).

The \(EC_{50}\) values obtained by spectrophotometry were calculated using the same procedure of the electrochemical analysis.

The kinetic of the reaction between DPPH and the different samples was monitored by spectrophotometry for 1 h in intervals of 5 min.

2.7. Determination of total phenolic content in the extracts

This analysis was performed according to the procedure described in the literature (Fernandes et al., 2015). A portion of 2 mL of 7.5% (m/v) sodium carbonate freshly prepared was added into a mixture containing 0.5 mL of sample extract in methanol at 250 μg mL⁻¹ (except for the tea samples that were used 130 μg mL⁻¹) and 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent aqueous solution. The mixture was kept at 50 °C for 5 min and then the absorbance was determined at 760 nm. The same procedure was performed using 0.5 mL of methanol for obtaining the blank. The total phenolic content of samples was determined using a graphical regression analysis of gallic acid standard solutions in methanol (10, 20, 30, 40, 50, 60, 70, and 80 μg mL⁻¹) versus absorbance. The results were expressed as mg of gallic acid equivalent (GAE) per gram of extract.

3. Results and discussion

The proposed BIA method for the determination of the total antioxidant capacity was based on the amperometric detection of DPPH that remains from its reaction with samples or antioxidants standards. Nevertheless, the first step of this work was to evaluate the BIA method with amperometric detection for the accurate and sensitive determination of DPPH in hydroethanolic medium.

The electrochemical behavior of DPPH by cyclic voltammetry (voltammograms not shown) using a GCE was investigated in phosphate buffer (pH 7), based on previous work (Amatatongchai et al., 2012), and acetate buffer (pH 5.5), both mixed with 60% (v/v) ethanol. The electrolyte solution that provided the highest voltammogram which presents the mean (n = 3) of each current as a function of applied potentials (87 μmol L⁻¹ DPPH).

The electrochemical reduction of DPPH to the respective anion radical (Ahmed et al., 2012) started at 0.3 V reaching a plateau of current between 0.2 and −0.2 V. Based on this hydrodynamic
voltammogram, a potential of 0.05 V was selected for the electrochemical reduction of DPPH during amperometric measurements.

After selecting the electrolyte and applied potential to obtain the highest response for DPPH reduction, BIA parameters such as injection speed of the programmable micropipette and injected volume were evaluated (Fig. 2A and B).

There are two well-defined linear ranges in the plot of current as a function of dispensing rate. Reduction currents for DPPH increased linearly up to 153 μL s⁻¹, which is in agreement with the theory of wall-jet disk-electrode conditions (Brett, Brett, & Mitoseriu, 1995). After this value of dispensing rate, the current continued to increase but at a lower rate (Fig. 2A). Therefore, the dispensing rate value of 153 μL s⁻¹ was selected for further amperometric measurements.

The peak current increased significantly with increasing injection volume of 20–60 μL, and remained almost constant between 60 and 120 μL (experiment performed at 153 μL s⁻¹). A high relative standard deviation (RSD) was verified for BIA injections of 20 μL, probably due to the low volume injected at relative high dispensing rate (injection time of ~0.13 s; current sampling interval of 0.10 s). The high analytical response and lower RSD value for DPPH was obtained for the injection of 80 μL (Fig. 2B). This volume was maintained for further measurements. It was expected that an increased current would be observed as the injection volume increases due to the large volume of DPPH solution in contact with the working electrode, as reported in previous works using BIA systems (Montes et al., 2014; Pereira et al., 2012; Silva et al., 2012; Stefano et al., 2012; Tormin et al., 2012).

A repeatability study was conducted to evaluate the precision of BIA method for DPPH determination (Fig. 3). This test was obtained from a series of 12 successive injections of solutions containing 87 μmol L⁻¹ DPPH.

The RSD value for this test was 0.7%, which is comparable with data obtained by other BIA methods (Montes et al., 2014; Pereira et al., 2012; Silva et al., 2012; Stefano et al., 2012; Tormin et al., 2012). Such a low RSD attested the highly precise injections provided by the programmable micropipette and by the reproducible positioning of the micropipette tip close to the working electrode surface (~2 mm). The analytical frequency of this BIA method for DPPH detection was estimated (using data from Fig. 3) at 180 injections per hour, which was substantially increased in comparison with conventional amperometric systems applied for DPPH detection (Milardovic, Ivkovic, & Grabaric, 2006).

Fig. 4A shows the responses recorded at +0.05 V for 80 μL triplicate injections of solutions containing increasing and decreasing concentrations of DPPH (a–f: 10–100 μmol L⁻¹). The respective calibration curves are also shown (Fig. 4B). This experiment was performed in acetate buffer in ethanol (40:60, v/v).

A linear behavior with good correlation coefficients (R = 0.999 for increasing and R = 0.997 for decreasing concentrations) was observed between 10 and 100 μmol L⁻¹ DPPH. The slope values (1.88 × 10⁻² and 1.83 × 10⁻² μA L⁻¹ μmol⁻¹, respectively) are in close agreement, which indicates the absence of carryover effects. The limits of detection and quantification for DPPH were estimated to be 1.0 and 3.3 μmol L⁻¹, respectively.

After the selection of the optimal conditions for DPPH detection by BIA with amperometric detection, the determination of the total antioxidant capacity of two standard antioxidants, GA and BHT, was performed. EC₅₀, which is defined as efficient concentration of antioxidant to scavenge 50% DPPH radicals after 1 h reaction, was determined using amperometric measurements for DPPH consumption by different concentrations of both antioxidants. For comparison, the total antioxidant capacity of these two antioxidant standards was also determined by spectrophotometry (Table 1). Table 1 also presents the analytical characteristics of the proposed BIA method based on the DPPH consumption to determine the antioxidant capacity of GA and BHT.

The results in Table 1 shows the agreement between the proposed BIA and spectrophotometric methods based on the
measurement of \( \text{DPPH}^- \) consumption for both antioxidants. Table 1 also reveals the low detection limit values obtained for GA and BHT using the proposed BIA method. The low detection limit for \( \text{DPPH}^- \) using the proposed BIA method is comparable to the value obtained by the FIA method using a modified electrode (CNT-GCE) (Amatatongchai et al., 2012), which is a well-known chemical modifier with electrocatalytic activity toward several electrode reactions (Ji, Kadara, Krussma, Chen, & Banks, 2010). Therefore, the association of BIA amperometric methods with chemically-modified electrodes is very promising for lower detection limits for \( \text{DPPH}^- \).

It is also important to mention that the \( I_{\text{blank}} \) (from Eq. (1)) was negligible for both GA and BHT. This data was obtained after triplicate injections of both antioxidants under optimized conditions of \( \text{DPPH}^- \) detection that resulted in negligible currents. Therefore, the selection of +0.05 V, which was based on the highest amperometric signal for \( \text{DPPH}^- \), is also suitable given the absence of an electrochemical response for several antioxidants, including GA and BHT, that can be found in samples such as the ones analyzed in this work. Both antioxidants (GA and BHT) undergo electrochemical oxidation at more positive potentials than +0.05 V, thus they did not interfere with \( \text{DPPH}^- \) detection.

An interference study was performed. Negligible responses to GA, rutin (RU), caffeine (CAF), quercetin (QT), flavone (FN), flavanone (FNN), tocopherol (TO), catechin (CAT), chlorogenic acid (CA) and BHT (50 \( \mu \text{mol L}^{-1} \) each) were obtained under the optimized conditions for \( \text{DPPH}^- \) determination. It is noteworthy to mention that the antioxidant capacity is determined based on the current for residual \( \text{DPPH}^- \) in solution, so the concentration of antioxidants in samples or standards is probably negligible.

Amperometric measurements in the BIA system optimized for \( \text{DPPH}^- \) detection as well as cyclic voltammetry were applied for all analyzed samples (tea and plant extracts). All samples produced oxidation peaks at potentials more positive than +0.05 V and they...
did not interfere with the DPPH response, which was used to calculate the antioxidant capacity of all samples. The evaluation of the total antioxidant capacity of two plants (Moringa and Brazilian cherry) and two teas was performed in triplicate for each sample. The data obtained by the proposed method were compared with values obtained from DPPH-spectrophotometric method and is shown in Fig. 5.

The results in Fig. 5 show the agreement between the proposed BIA and spectrophotometric methods based on the measurement of DPPH-consumption. At the 95% confidence level, the calculated paired Student t-Test value (2.53) was smaller than the critical value (2.57, n = 6), which indicates that there were no significant differences between the results. It is noteworthy to emphasize that longer reaction times (1 h) are required when a DPPH-based assay is applied to calculate EC50 values because polyphenolic compounds responsible for the antioxidant activity are commonly found in plants, and the reaction between these compounds and DPPH present low kinetics. Lower incubation times may generate inaccurate EC50 values. Fig. 5 presents the kinetic of the reactions between DPPH and the six analyzed samples. These experiments clearly show the requirement of 1 h reaction in order to obtain a constant DPPH concentration, which indicated the end of reaction at 1 h. The sample throughput of the proposed BIA method can be estimated as 90 h⁻¹, considering the possible treatment of 180 samples in 1 h (pre-incubation time) and the analytical frequency of 180 h⁻¹ for DPPH detection.

The lower EC50 (efficient concentration of sample to scavenge 50% DPPH radicals) indicates higher antioxidant capacity. Therefore, the Tea 1 sample presented higher antioxidant capacity than the Tea 2 sample, while the Moringa extracts presented lower antioxidant capacity than Brazilian cherry extracts. Associating this data with the total phenolic content of each extract (Fig. 2), the higher antioxidant capacity of Tea 1 can be correlated with the higher content of phenolic compounds in comparison with Tea 2. This fact is also evident in the Brazilian cherry extracts. Additionally, the Moringa extracts presented the lowest amount of phenolic compounds, which can be correlated with their lower antioxidant capacity in comparison with the other samples.

4. Conclusions

This work has demonstrated the first application of BIA with amperometric detection to determine the antioxidant capacity of real samples based on the measurement of DPPH consumption. The BIA system with amperometric detection provided fast (180 h⁻¹), highly precise (RSD = 0.7%), sensitive and selective detection of DPPH, which contributed to sample throughput of 90 h⁻¹ considering the pre-incubation time of 1 h required for the accurate determination of antioxidant capacity of plant and tea samples. The BIA electrochemical and spectrophotometric methods based on the measurement of DPPH consumption were in agreement for the analyses of real samples (plants and tea). Additionally, one main advantage of amperometric detection over spectrophotometric one is that the second can be affected by the color or turbidity of samples, which will affect the accuracy of the spectrophotometric method. Moreover, all instrumentation required for the application of the BIA proposed method for the determination of antioxidant capacity is portable (commercially-available battery-powered instrumentation), which offers great promise for on-site analyses.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.07.064.

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