Analytical Letters

ISSN: 0003-2719 (Print) 1532-236X (Online) Journal homepage: https://www.tandfonline.com/loi/lanl20

Discrimination of Tea by the Electrochemical Determination of its Antioxidant Properties by a Polyaniline – DNA – Polyphenazine Dye Modified Glassy Carbon Electrode

T. N. Kulikova, A. V. Porfireva, V. V. Vorobev, A. A. Saveliev, G. K. Ziyatdinova & G. A. Evtugyn

To cite this article: T. N. Kulikova, A. V. Porfireva, V. V. Vorobev, A. A. Saveliev, G. K. Ziyatdinova & G. A. Evtugyn (2019): Discrimination of Tea by the Electrochemical Determination of its Antioxidant Properties by a Polyaniline – DNA – Polyphenazine Dye Modified Glassy Carbon Electrode, Analytical Letters, DOI: 10.1080/00032719.2019.1618321

To link to this article: https://doi.org/10.1080/00032719.2019.1618321

Published online: 30 May 2019.

ANALYTICAL LETTERS
https://doi.org/10.1080/00032719.2019.1618321

Taylor & Francis
Taylor & Francis Group

FOOD ANALYSIS

Discrimination of Tea by the Electrochemical Determination of its Antioxidant Properties by a Polyaniline – DNA – Polyphenazine Dye Modified Glassy Carbon Electrode

T. N. Kulikova[a], A. V. Porfireva[a], V. V. Vorobev[b], A. A. Saveliev[c], G. K. Ziyatdinova[a], and G. A. Evtugyn[a]

[a]Chemistry Institute named after A. M. Butlerov of Kazan Federal University, Kazan, Russian Federation; [b]Interdisciplinary Center of Analytical Microscopy of Kazan Federal University, Kazan, Russian Federation; [c]Institute of Environemntal Sciences of Kazan Federal University, Kazan, Russian Federation

ABSTRACT

Voltammetric sensors have been developed on the bases of polyaniline and polymeric forms of methylene blue, methylene green, and neutral red obtained by multiple cycling of the potential. The integrity of the layers and expansion of the pH range of redox activity have been achieved by intermediate adsorption of native DNA that stabilized the oxidized polyaniline form. The formation of the coating layer was confirmed by electrochemical impedance spectroscopy and scanning electron microscopy. Model oxidants (ascorbic acid, hydroquinone, and quercetin) in the concentration range from 1×10^{-6} to 1×10^{-3} mol L^{-1} influenced the measured redox peaks of polymeric dyes with standard deviations from 2.4 to 5.5%. The peak currents were used for classification of tea brands using principal component analysis and linear discrimination analysis. The average discrimination of the tea brands purchased on local market was approximately 100%. The assessment of antioxidant properties of tea infusions was in agreement with results obtained using a coulometric bromine titration.

ARTICLE HISTORY
Received 28 March 2019
Accepted 9 May 2019

KEYWORDS
Antioxidant assessment; electropolymerization; polyaniline; polyphenothiazine dyes; tea brand discrimination; voltammetric sensor

Introduction

Antioxidants are natural or synthetic species that are present or added to foodstuffs to prevent oxidative deterioration of natural components (Gülçin 2012). Antioxidants interact with reactive free radicals by electron transfer to form inactive low-toxic products unable to propagate the reaction. Antioxidants prevent many severe consequences accompanying oxidative stress that include neurodegenerative diseases and oxidative damage of biopolymers. As a result, antioxidants intake suppresses mutagenic and cancerogenic effects related to reactive oxygen and nitrogen species and improve the immune status of organisms, health, and in general the quality of life.

For this reason, the determination of antioxidant activity is considered to be an indispensable part of food quality assessment (Serafini and Peluso 2016). At the present time, the total quantity of antioxidants called also known as the antioxidant capacity

CONTACT: Gennady Evtugyn gennady.evtugyn@kpfu.ru Chemistry Institute named after A. M. Butlerov of Kazan Federal University, 18 Kremlevskaya Street, Kazan, 420008, Russian Federation.

has been determined by spectrophotometry (Aleixandre-Tudo et al. 2017), capillary electrophoresis (Hurtado-Fernández et al. 2010), and high-performance liquid chromatography (Niederländer et al. 2008). These methods are based on the direct determination of colored stable radicals and reducing agents or on the quantification of low-molecular products of oxidative degradation of biomolecules (oxidative peroxidation of lipids).

Electrochemical methods have gained growing interest in the past years due to advantages over conventional instrumentation. These advantages include simple, fast, and inexpensive measurement protocol, possibility to classify antioxidants in accordance with the mechanism of their action, and a variety of tunable parameters to reach maximal sensitivity. In addition, electrochemical sensors intended for the antioxidant detection are compatible with portable instrumentation.

Electrochemical measurements can be performed in the presence of many interferences with rather mild limitations due to extreme pH and dissolved oxygen (Pisoschi, Cimpeanu, and Predoi 2015; Hoyos-Arbeláez, Vázquez, and Contreras-Calderón 2017). In the potentiometric protocol, the redox potential is recorded in the sample after the addition of ferricyanide ions (Ivanova, Gerasimova, and Gazizullina 2019). In voltammetric methods, direct or mediated currents related to the antioxidant content are recorded (Gonzalez, Vidal, and Ugliano 2018; Vilas-Boas et al. 2019). In this case, additional information on the mechanism of oxidation and nature of the by-products formed is commonly available, although the modification of the electrode surface complicates the application of these sensors.

Electropolymerization is widely used for the deposition of redox active insoluble products on electrodes by the oxidation of appropriate monomers (Lange, Roznyatovskaya, and Mirsky 2008; Evtugyn and Hianik 2016). Due to easy formation and a variety of redox properties, electropolymerized products are frequently implemented in electrochemical sensors as mediators of electron transfer or carriers of biomolecules and receptors.

This property is especially true for polyaniline that exerts electroconductivity together with polypyrrole and polythiophene and their derivatives. For this reason, these polymers are often used in electrochemical biosensors for wiring enzymes and nucleic acids and for their immobilization by entrapment in growing polymer film (Peng et al. 2009; Zanardi, Terzi, and Seeber 2013; Inzelt 2018). Among electroconductive polymers, polyaniline is the most commonly used in sensor applications. However, its function is limited to acidic media because only the protonated form of half-oxidized polymer (emeraldine salt) exerts electroconductivity and reversible electron transfer (Prakash 2002). The applications of polyaniline in enzyme sensors have involved the determination of glucose (Arslan, Ustabaş, and Arslan 2011), sulfite (Bahmani et al. 2010), catechol (Chen et al. 2013), hydrogen peroxide (da Silva et al. 2013), urea (Buron et al. 2014; Meibodi and Haghjoo 2014), and acetylcholinesterase substrate and inhibitors (Somerset et al. 2007; Cesarino et al. 2012). Polyaniline may associate with nucleic acids and extend the pH region of redox activity due to the stabilization of emeraldine salt by electrostatic interactions (Shi, Xiao, and Willner 2004; Bardavid et al. 2008).

Other redox active polymers may be formed in the oxidation of functionalized heteroaromatic and aromatic systems which are mostly used as biochemical dyes, pH-, and

redox-indicators (Pauliukaite et al. 2010; Kul, Pauliukaite, and Brett 2011). They have been successfully utilized in the mediated oxidation of pharmaceuticals (Jin, Huang, and Kong 2007; Bergamini, Santos, and Zanoni 2013; Dorraji and Jalali 2016), antioxidants (Manasa et al. 2017), metabolites (Dai et al. 2008; Ouyang et al. 2015; Tekbaşoğlu et al. 2017), neurotransmitters (Moon et al. 2018), and enzyme cofactors (Gao et al. 2010; Topçu, Dağcı, and Alanyalıoğlu 2016). Among them, phenothiazine dyes, such as methylene blue and methylene green, have found broad application as redox probes for labeling nucleic acids and antibodies (Komori et al. 2009; Pauliukaite et al. 2010). Neutral red, a phenazine dye, has found to be most effective in the mediation of the oxidation of nicotinamide adenine dinucleotide (Pauliukaite and Brett 2008; Omar et al. 2016).

The application of electropolymerized phenothiazine and phenazine dyes as electrode modifiers is often complicated by their instability on the electrodes, the high contribution of the dye monomers entrapped in the polymer, and the insufficient reversibility of redox conversion. Their combination with polyaniline is expected to improve the performance of electrochemical sensors and increase the variation of the response toward antioxidants necessary for their characterization.

Recently we proposed the characterization of the oxidative damage of nucleic acids co-immobilized with electropolymerized dyes by monitoring changes in the potentials of the redox peaks of poly(methylene blue), poly(methylene green), and poly(neutral red) (Kuzin et al. 2016). In this work, electropolymerized polyaniline was successfully applied for the first time as the support for the physical adsorption of nucleic acids followed by the deposition of polymeric forms of the dyes. The redox activity of the coating depended on the intrinsic transfer of hydrogen ions caused by antioxidant oxidation. Changes in the appropriate peak currents recorded by cyclic voltammetry were used for classification of the tea brands that varied in their antioxidant content.

Experimental

Reagents

Deoxyribonucleic acid (DNA, low molecular weight) from salmon sperm, aniline, oxalic acid, methylene blue (3,7-bis(dimethylamino)phenazathionium chloride), methylene green ([7-(dimethylamino)-4-nitrophenothiazin-3-ylidene]-dimethylazanium chloride), neutral red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride), and doxorubicin were purchased from Sigma-Aldrich, Germany. All other reagents were of analytical grade. The pH of working solutions was controlled with Britton–Robinson buffer (0.04 mol L^{-1} acetic acid, 0.04 mol L^{-1} phosphoric acid, and 0.04 mol L^{-1} boric acid with 0.05 mol L^{-1} sodium sulfate as the electrolyte) denoted below as the working buffer. Deionized Millipore water was used for preparation of working solutions.

The oxidation of the nucleic acid was performed by the dissolution of 1 mg of nucleic acid from salmon sperm in 0.9 mL of 25 mmol L^{-1} KCl followed by the addition of 0.1 mL of 4 mmol L^{-1} $CuSO_4$ and 1.3 μL of 30% H_2O_2.

Apparatus

Electrochemical measurements were performed at ambient temperature using an Autolab PGSTAT 302 N potentiostat-galvanostat (Metrohm Autolab b.v., the Netherlands) and CHI 660B voltammograph (CH Instruments, Austin, TX, USA). The three-electrode working cell was equipped with the Pt wire as the auxiliary electrode and a Ag/AgCl/3 mol L^{-1} KCl reference electrode (Metrohm Autolab). The electropolymerization and DNA deposition were performed on the working electrode that was composed of a glassy carbon rod pressed in the polytetrafluoroethylene tube and mechanically polished to a mirror-like surface. The geometric working area was determined to be 1.67 mm^2. On the opposite side, a stainless steel rod was mounted as the current collector.

The electrochemical impedance spectra were recorded with the FRA module of the Autolab PGSTAT 302 N potentiostat-galvanostat in the presence of 0.1 mmol L^{-1} $K_3[Fe(CN)_6]$ and 0.1 mmol L^{-1} $K_4[Fe(CN)_6]$ with the amplitude of the applied sine potential equal to 5 mV. The frequency was varied from 100 kHz to 0.04 Hz using a sampling rate of 12 points per decade. The calculations of the capacitance and the charge transfer resistance from the electrochemical impedance spectra were performed using NOVA software (Metrohm Autolab).

Scanning electron microscopy images were recorded on a Merlin field emission instrument (Zeiss, 30 kV, 300 pA). The samples were preliminary coated with a Au/Pd layer in vacuum using a T150ES splutter coater (Quorum Technologies, UK).

Electrochemical sensor assembly

The working electrode was polished with a silica polishing kit (CH Instruments), rinsed with deionized water, ethanol, 1.0 mol L^{-1} sulfuric acid, 1.0 mol L^{-1} sodium hydroxide and acetone, placed in the working cell, and electrochemically cleaned by multiple cycling of the potential in 0.2 mol L^{-1} sulfuric acid between −0.5 and 1.3 V at a scan rate of 50 mV s^{-1}. Next, the electrode was rinsed with deionized water and transferred into 0.5 mol L^{-1} oxalic acid containing 0.7 mmol L^{-1} aniline that was freshly distilled under vacuum.

The electropolymerization of aniline was performed by running four cycles of the potential between −0.3 and 1.1 V at a scan rate of 50 mV s^{-1}. The electrode was washed again and dried in a flow of air. Next, it was placed upside down, and 2 μL of 1 mg mL^{-1} aqueous DNA solution was spread on the working area. The electrode was capped with an Eppendorf plastic tube to prevent drying. After 15 min of incubation, the electrode was rinsed twice with deionized water and immersed in 0.1 mol L^{-1} phosphate buffer containing either 0.5 mmol L^{-1} methylene blue, methylene green, or neutral red. In case of neutral red, the buffer additionally contained 0.1 mol L^{-1} $NaNO_3$ necessary for effective polymerization.

The pH of reaction media was maintained at pH 6.0 for neutral red, pH 7.0 for methylene blue, and pH 8.0 for methylene green. The conditions for effective electropolymerization of the dyes were reported previously (Evtugyn et al. 2008; Pauliukaite and Brett 2008; Kuzin et al 2016). The electropolymerization was performed by running six cycles of the potential between −0.5 and 1.1 V, and the electrode was transferred to

working buffer without monomers and stabilized by repeated cycling of the potential between −0.5 and 0.5 V for 10 min.

The electrodes were rinsed again and used for antioxidant testing and tea fusion assessment. When not in use, the electrochemical sensors were stored at 4 °C under dry conditions for a period of up to six months.

Tea classification

Five tea brands were purchased from a local market. The tea extracts were prepared by the infusion of the tea bag in 150 mL of boiling water for 5 min. The antioxidants of the tea infusion were determined by a coulometric titration with electrogenerated bromine (Ziyatdinova et al. 2006). The results were expressed by the charge in coulombs sufficient for the full oxidation of antioxidants present in 100 mL of the tea infusion.

For statistical analysis, the R 3.4.4 software was used. Repetitive stratified random resampling at sensor level with replacement of the data set, e.g., bootstrapping, (Fahidy 2008) was used to obtain a close approximation to the true distribution. For principal component analysis, various tea brands were marked with different colors on the score plot and their similarity parameters were assessed.

The linear discriminant analysis was performed using the *lda* function of the MASS software package with 500 random sets obtained by bootstrapping. The antioxidant capacities were generated from the mean values and standard deviations. The statistical analysis of the antioxidant capacity dependence was demonstrated using a significance level of 5% for all sensors.

Results and discussion

Characterization of the surface coating

For polyaniline deposition, the original protocol previously developed for electropolymerization of aniline was applied. Oxalic acid increased the nucleation of the polymer for the first polymerization cycles (Shamagsumova et al. 2015). These results positively affected the adsorption of DNA and repeatability of the sensor characteristics. Aniline polymerization was performed by repeated cycling of the potential in 0.5 mol L^{-1} oxalic acid containing 0.7 mmol L^{-1} of aniline. Full coverage of the electrode surface was reached by the fourth to sixth cycles (Kulikova et al. 2019). The peak currents on the voltammograms attributed to the redox conversion of polyaniline were quite reproducible, with a standard deviation of 2.3% for six individual sensors, and stable following three weeks of electrode storage in dry conditions.

A similar protocol was proposed for the electropolymerization of methylene blue, methylene green, and neutral red. However, in the acidic media necessary for polyaniline deposition, the efficiency of dye polymerization was found to be low. Appropriate peak currents slowly increased with the number of potential cycles and returned to lower values after transfer of the electrodes into the working buffer in the absence of monomers. This phenomenon may be due to the electrostatic repulsion of the positively charged products of the dye polymerization from the polyaniline protonated in acidic media.

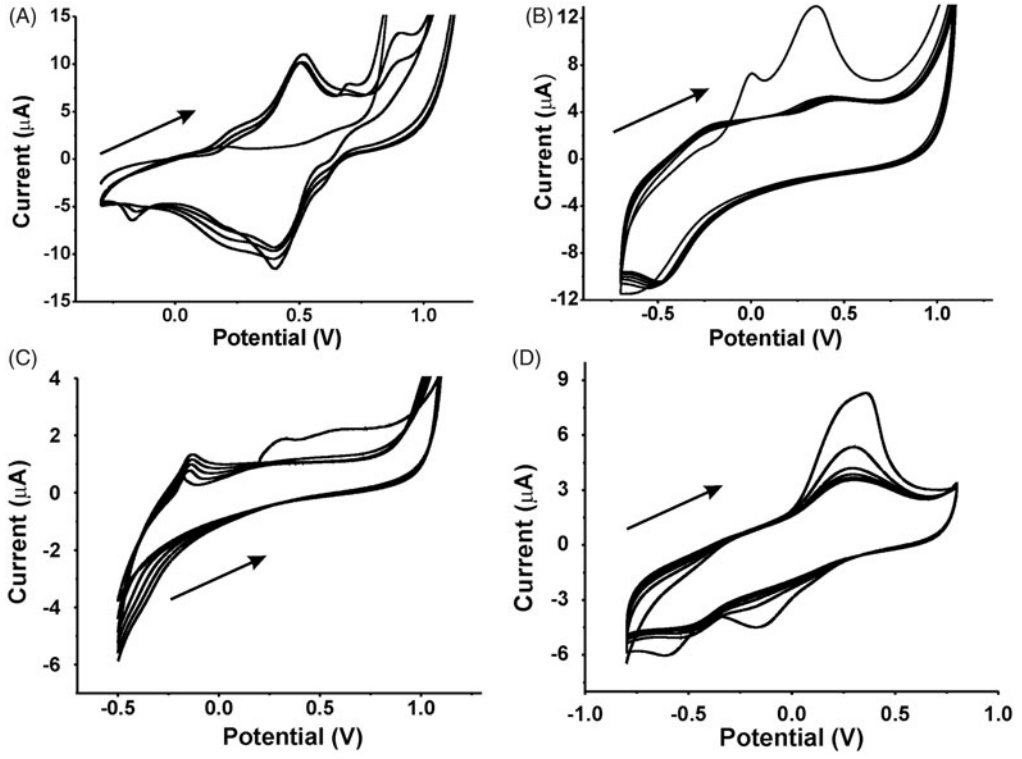

Figure 1. Electrochemical deposition of polyaniline from 0.5 mol L^{-1} oxalic acid containing (a) 0.7 mM aniline, (b) methylene green, (c) methylene blue, and (d) neutral red on DNA adsorbed on polyaniline from Britton–Robinson buffer, pH 7.0. The arrows indicate the direction of the potential scan.

In order to integrate film and improve conditions of electron transduction, the DNA was electrostatically adsorbed on the polyaniline support. After an incubation period, the electrode was washed with deionized water and working buffer to remove excess biopolymer. The variation of DNA concentration from 0.125 to 4.0 mg mL^{-1}, aliquot volume from 1 to 5 μL, and incubation time from 5 to 40 min did not significantly affect the subsequent polymerization of the dyes. Probably similar DNA quantities were adsorbed on the surface of polyaniline using the above conditions.

Figure 1 shows the cyclic voltammograms recorded on the electrode during the polyaniline deposition and following the adsorption of deoxynucleic acid and electropolymerization of the dyes. The dye deposition decreased the polyaniline peak currents from 10 to 12 to 2 to 3 μA. During the first cycle of the potential, the currents were much higher due to the incomplete coverage of the electrode. Subsequently the peak currents related to the polyaniline redox response disappeared by the third cycle so that only peaks attributed to the redox conversion of the dyes remained.

Similar to the response on the bare glassy carbon electrode, polymeric dyes showed one reversible peak pair in the potential range from −0.4 to 0.3 V. In accordance with the dependence of the peak current on the scan rate, simultaneous transfer of two electrons and one or two hydrogen ions took place depending on the buffer pH. The comparison of voltammograms recorded on the electrode covered with polyaniline, DNA, and polymeric dyes with those obtained on the bare glassy carbon electrode showed

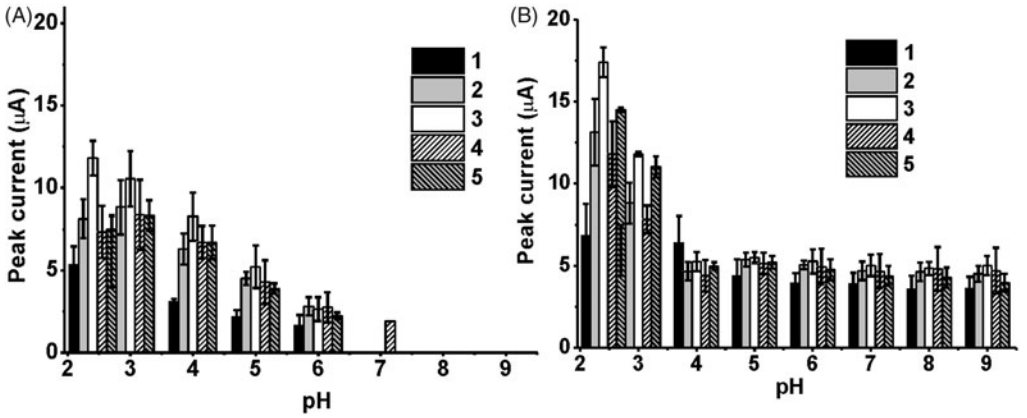

Figure 2. pH dependence of the peak currents of poly(methylene green) (a) oxidation and (b) reduction obtained with the following number of potential cycles in the electropolymerization step: (1) 2 potential cycles for electropolymerization of aniline and 6 potential cycles for electropolymerization of methylene green, (2) 4 potential cycles for electropolymerization of aniline and 6 potential cycles for electropolymerization of methylene green, (3) 6 potential cycles for electropolymerization of aniline and 6 potential cycles for electropolymerization of methylene green, (4) 6 potential cycles for electropolymerization of aniline and 10 potential cycles for electropolymerization of methylene green, and (5) 6 potential cycles for electropolymerization of aniline and 15 potential cycles for electropolymerization of methylene green. Surface layer assembly conditions: 15 min of incubation in 1 mg mL^{-1} DNA solution in Britton–Robinson buffer, pH 7.0, methylene green concentration of 0.5 mmol L^{-1}, scan rate of 50 mV s^{-1}. The results are reported as the average ± standard deviation for three individual sensors.

that the peak shape and height were sensitive to the electroconductivity of underlying polyaniline layer.

As an example, Figure 2 shows the pH dependence of the peak currents of poly(methylene green) on the thickness of polyaniline layer expressed by the number of the potential cycles used on the stage of aniline electropolymerization. Similar dependences were obtained for polymerization of methylene blue and neutral red, although their peak potentials were different from those of poly(methylene green).

The anodic peaks were one-half the size of the cathodic peaks for the same surface coating. At low pH, increasing thickness of the polyaniline layer enhanced the signals of polymeric dyes. At pH values exceeding 6, the anodic peaks were poorly resolved and did not allow quantification of their height. However, the cathodic peaks were stabilized in the same pH region at approximately 4–5 μA.

These phenomena may be explained by the consideration of hydrogen ion exchange within the surface layer. Oxidation reactions on the direct potential cycle produced sufficient hydrogen ions for the stabilization of the conductive (protonated) form of polyaniline. These results show that inner portion of the coating had lower pH values than the bulk solution. Under these conditions, the redox conversion of polymeric dye was limited by electron transduction from the electrode through the polyaniline layer by the protonation of inner polyaniline film.

Preliminary DNA treatment prior to adsorption on polyaniline affected the polymeric dye properties. To demonstrate this phenomenon, the DNA solution was either heated to 95°C and then rapidly cooled in an ice bath or treated with a $CuSO_4/H_2O_2$ mixture

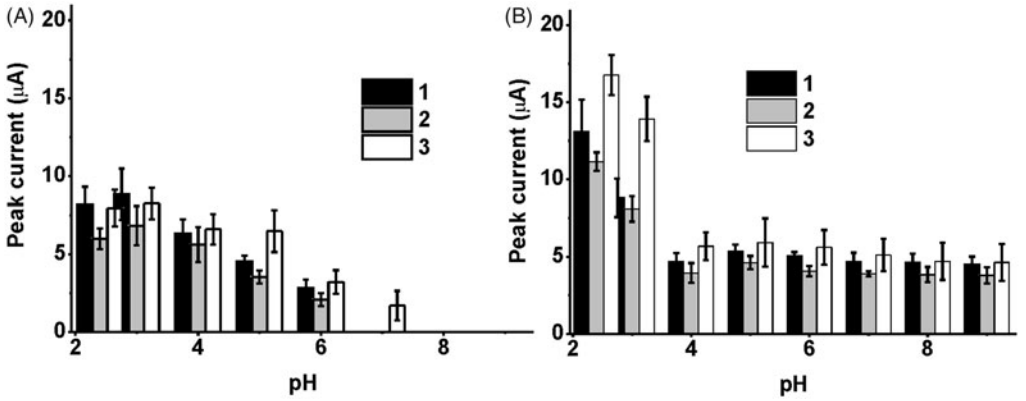

Figure 3. pH dependence of the peak currents of poly(methylene green) (a) oxidation and (b) reduction obtained with the electropolymerized coatings assembled with (1) untreated, (2) thermally inactivated, and (3) oxidized DNA. The number of potential cycles in the electropolymerization step: 4 for aniline polymerization and 6 for methylene green polymerization. Surface layer assembly conditions: 15 min incubation in 1 mg mL^{-1} DNA solution in Britton–Robinson buffer, pH 7.0, methylene green concentration 0.5 mmol L^{-1}, scan rate 50 mV s^{-1}. The results are reported as the average ± standard deviation for three individual sensors.

that generated peroxide radicals (Shen and Anastasio 2012). The pH dependence of the poly(methylene green) peak currents obtained for surface coatings including damaged DNA is presented in Figure 3.

The anodic peak currents obtained with thermally denatured DNA decreased by 12–15% compared to those recorded in the presence of native biopolymer. The peaks of the dyes obtained in the presence of oxidatively damaged DNA were significantly higher, especially in weakly acidic and neutral solutions. Cathodic peaks showed similar behavior but the influence of the oxidation of DNA was more pronounced in acidic media.

The pH variation of the peak currents in repeated cycles and after long incubation in working buffer allowed the exclusion of acidic damage to the DNA structure during the sensor operation. The preliminary treatment of DNA probably affects the conformational flexibility of the biopolymer and hence alters the electrostatic forces participating in the layer assembly.

Electrochemical impedance spectroscopy was employed to confirm the role of electrostatic interactions in the surface layer assembly. At the inner interface of the electrode with polyaniline (Figure 4a), the electron transfer was primarily affected by the pH of solution.

The DNA adsorption slightly decreased the charge transfer resistance due to the stabilization of the charged (electroconductive) form of the polyaniline whose charge was opposite to that of the DNA phosphate residues. The subsequent deposition of the positively charged polymeric dyes did not shift the average value of the resistance but increased its standard deviation by a factor of two. Contrary to that, at the outer interface involving the surface film and solution, Figure 4b shows the presence of a constant charge transfer resistance insensitive to the DNA adsorption but which sharply decayed after electropolymerization of the dyes. This phenomenon may be caused by the

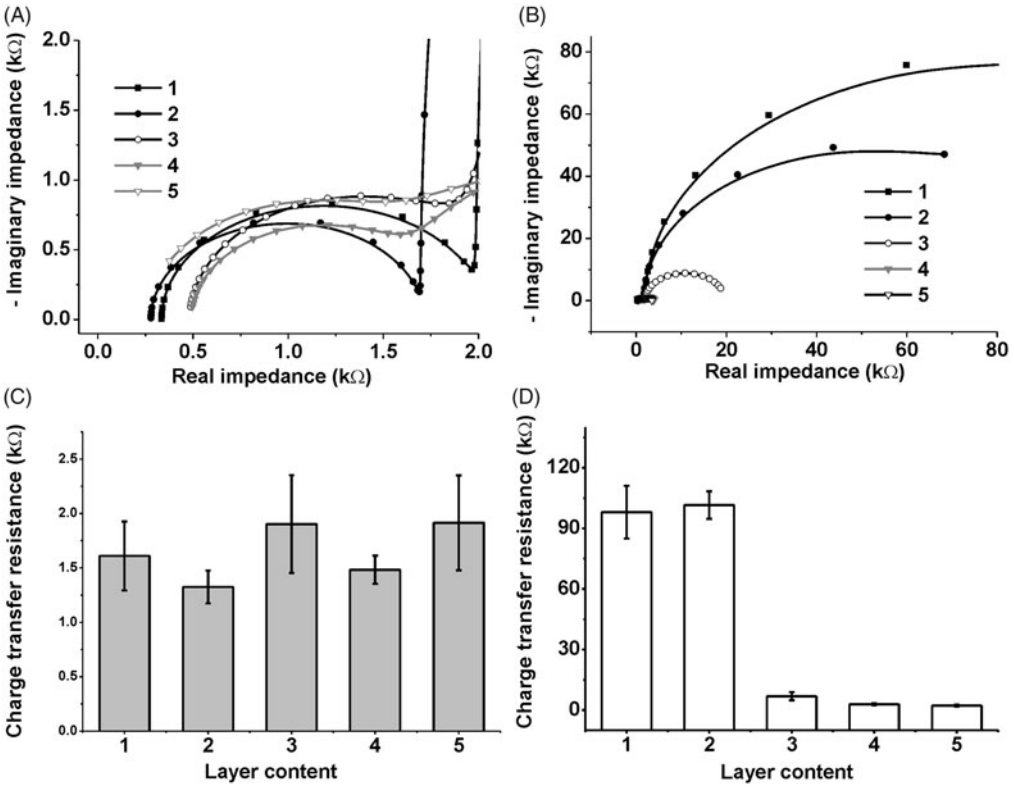

Figure 4. (a, b) Nyquist diagrams and (c, d) charge transfer resistance values corresponded to different modified electrodes calculated from the spectra obtained for different electrode coatings: (1) polyaniline (4 cycles); (2) polyaniline – DNA; (3) polyaniline – DNA – poly(methylene blue); (4) polyaniline – DNA - poly(methylene green); and (5) polyaniline – DNA – poly(neutral red). All dyes were polymerized by 6 cycles of the potential from 0.5 mM solutions at a scan rate of 50 mV s^{-1}. The results are reported as the average ± standard deviation for three individual sensors.

recharging of the layer surface and electrostatic attraction of anionic redox probe (ferrocyanide ions) to the polymeric dyes.

The introduction of DNA and subsequent electropolymerization of the dyes were also monitored by scanning electron microscopy (Figure 5). The results show that the underlying polyaniline coating had a flat, poorly defined structure that was probably due to mechanical scratches during the mechanical polishing of the glassy carbon electrode. Biopolymer molecules formed round aggregates, which were combined in branched chains. The electrodeposition of the dyes increased the roughness of the support and formed netted filaments around the DNA aggregates. As expected, the electropolymerization on the adsorbed DNA did not disaggregate the biopolymer particles but increased the average roughness of underlying surface.

Influence of antioxidants

All of the modified electrodes were characterized in model antioxidant solutions frequently found in food and beverages. For this purpose, standard solutions of ascorbic

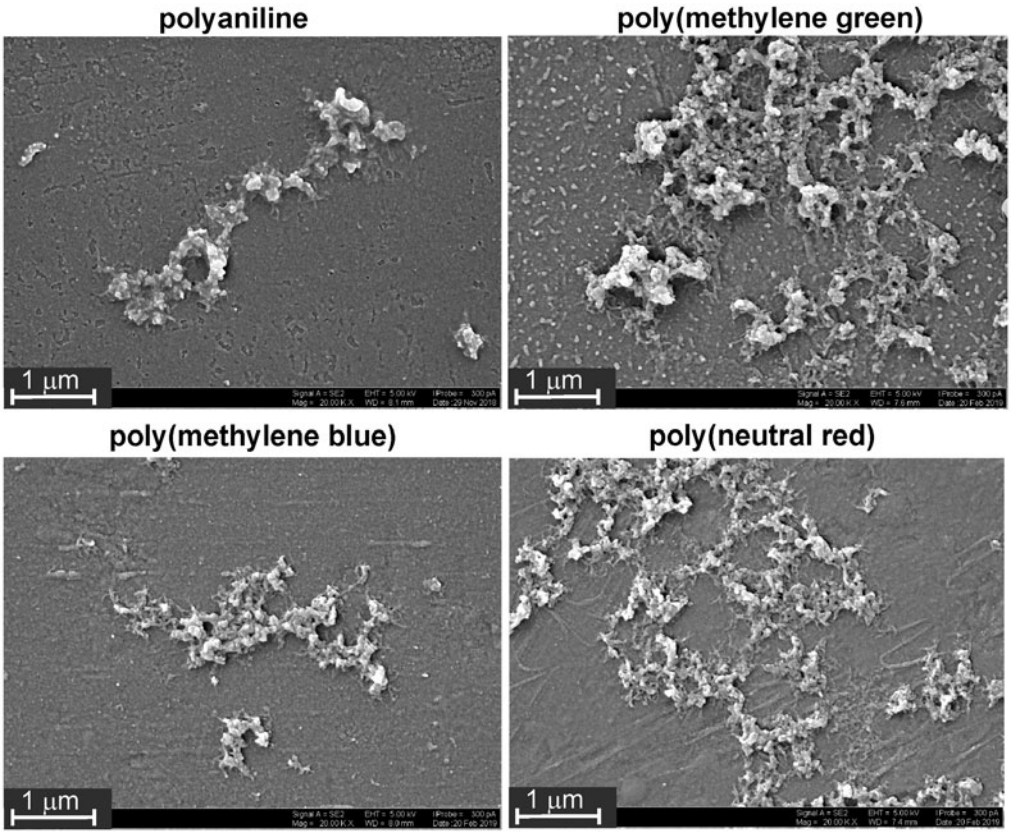

Figure 5. Scanning electron microscopy images for a glassy carbon electrode covered with polyaniline and physically adsorbed DNA and also covered with polymerized dyes. In all cases, 4 cycles were applied for the aniline polymerization and 6 for the dyes.

acid, hydroquinone, and quercetin were prepared and added to the working buffer in 1:1 (v/v) ratios to final concentrations of 1×10^{-6}, 1×10^{-5}, 1×10^{-4}, and 1×10^{-3} mol L^{-1}. Next, cyclic voltammograms were recorded on the modified electrodes with various combinations of polyaniline and polymeric dyes. In most cases, the addition of antioxidants did not affect the shape of the peaks on voltammograms attributed to the redox conversion of the dyes but increased their values due to competitive redox reactions.

Figure 6 shows voltammograms recorded on the glassy carbon electrode modified with polyaniline, DNA, and poly(methylene blue) in the presence of 1.0×10^{-5} mol L^{-1} additives as an example. The peaks on the voltammograms are similar independent of antioxidant. However, their responses on the unmodified electrode were different. Thus, quercetin and hydroquinone formed one or two peaks on the anodic branch depending on the pH, while ascorbic acid did not form its own peaks in the selected potential window.

The peaks on the modified electrodes were symmetrical indicating reversible electron transfer. Their absolute height regularly decreased with pH due to the lower conductivity of polyaniline underlying layer. The anodic currents were higher in basic media for hydroquinone and quercetin oxidized on bare glassy carbon. The cathodic peaks

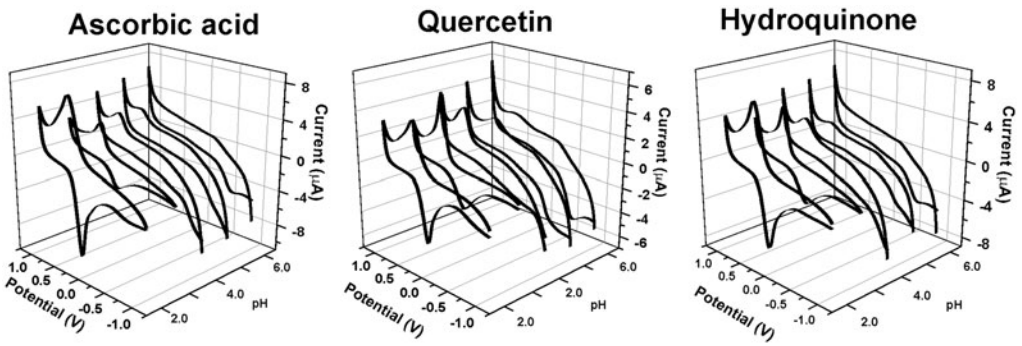

Figure 6. Cyclic voltammograms recorded on a glassy carbon electrode modified with polyaniline using 4 polymerization cycles, DNA and poly(methylene blue) with 6 polymerization cycles in the presence of 1.0×10^{-5} mol L^{-1} ascorbic acid, hydroquinone, and quercetin at various pH values in buffer solutions. Surface layer assembly conditions: 15 min incubation in 1 mg mL^{-1} DNA solution in Britton–Robinson buffer, pH 7.0, methylene blue concentration 0.5 mmol L^{-1}, and scan rate 50 mV s^{-1}.

decreased at pH values exceeding 4, while anodic peaks did not change in weakly acidic and neutral media and remained constant up to pH 7.0.

Although the peak currents varied with the antioxidant concentration, the determination was complicated by the contradictory effects of the underlying polyaniline layer. Thus, increasing the concentration of ascorbic acid at pH 2.0 enhanced the oxidation dye peak due to the regeneration of reduced form of the dye by its reaction with an oxidant. In addition, the reduction peaks recorded in the presence of different concentrations of ascorbic acid changed in the opposite direction (Figure 7).

The absolute height of the peaks decreased in the range poly(neutral red) $>$ poly(methylene blue) $\geq$ poly(methylene green). These results coincide with the changes of the formal redox potentials of the surface coatings determined as the half-sum of the appropriate peak potentials (0.192 V $<$ 0.225 V $<$ 0.238 V). Contrary to that, the influence of ascorbic acid on the dye redox activity at pH 6.0 disappeared, and the peak currents varied insignificantly in comparison with the deviation established for a set of individual sensors in the absence of antioxidants.

Hydroquinone more significantly influenced the poly(methylene green) peaks compared to ascorbic acid in acidic media and the other antioxidants at weakly acidic and neutral pH values. These results may be due to the higher number of hydrogen ions released during the oxidation of this compound compared to ascorbic acid. The decay of the anodic peak current in the presence of hydroquinone with pH from 2.0 to 6.0 is more pronounced for poly(methylene blue) than for poly(neutral red).

Coatings of poly(methylene green) were intermediate, although their redox potential was approximately the same as for poly(methylene blue). With respect to quercetin, its influence was a maximum for the coatings of poly(methylene green), whereas the response of poly(methylene blue) and poly(neutral red) are closer to the value for ascorbic acid. This phenomenon may be due to the higher lipophilicity of quercetin compared to the other antioxidants and milder effect of internal pH changes on its weak hydrophobic interactions with the polymeric dye chains.

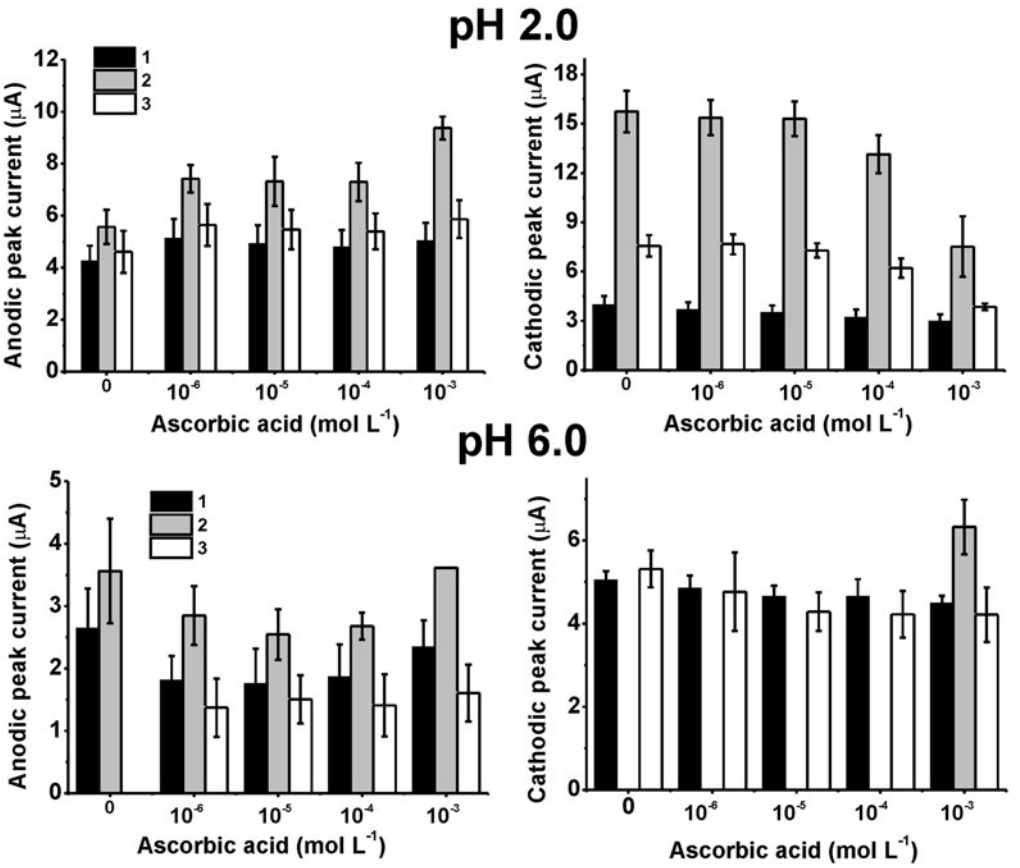

Figure 7. Anodic and cathodic peak currents of poly(methylene blue), poly(methylene green), and poly(neutral red) in the presence of ascorbic acid at pH 2.0 and 6.0. In all cases, 4 cycles were applied for the aniline polymerization and 6 cycles for the dyes: (1) poly(methylene green), (2) poly(neutral red), and (3) poly(methylene blue). Surface layer assembly conditions: 15 min incubation in 1 mg mL^{-1} DNA solution in Britton–Robinson buffer, pH 7.0, dye concentration 0.5 mmol L^{-1}, and scan rate 50 mV s^{-1}. The results are reported as the average ± standard deviation for three individual sensors.

Table 1 summarizes the anodic peak currents recorded on various coatings in the presence of 1×10^{-6} to 1×10^{-3} mol L^{-1} antioxidants. The voltammetric response to the antioxidants in weakly acidic and neutral media is probably more dependent on the polyaniline status (redox equilibrium involving emeraldine salt) than for the redox reactions of the dyes. For this reason, the subsequent tea analysis was performed using acidic conditions at pH 3.0.

All of the electrochemical signals were stable and reproducible for repeated potential cycling using the same solution and a set of electrochemical sensors. The measurement-to-measurement and sensor-to-sensor repeatability of the peak currents were 2.4 and 5.5%, respectively. The characteristics of the sensors remained reproducible for at least six weeks of storage in dry conditions at room temperature. The electrochemical activity of polymeric dyes was monitored within six months of storage at 4°C.

Table 1. Anodic peak currents of electrochemical sensors based on polymeric dyes: poly(neutral red), poly(methylene blue), poly(methylene green) in the presence of various antioxidants from pH 2.0 to 6.0. Dashes (–) indicate the formation of the wave instead of peak and the inability to calculate the peak current.

Antioxidant	pH	Antioxidant concentration (M)	Anodic peak currents (μA)		
			poly(methylene green)	poly(neutral red)	poly(methylene blue)
Ascorbic acid	2.0	0	3.97 ± 0.34	15.7 ± 1.3	7.56 ± 0.66
		1×10^{-6}	3.67 ± 0.36	15.4 ± 2.1	7.66 ± 0.61
		1×10^{-5}	3.48 ± 0.35	15.3 ± 1.1	7.29 ± 0.43
		1×10^{-4}	3.21 ± 0.42	13.1 ± 1.2	6.21 ± 0.26
		1×10^{-3}	2.96 ± 0.24	7.52 ± 0.84	3.85 ± 0.20
Hydroquinone		0	3.62 ± 0.28	3.02 ± 0.23	4.66 ± 0.12
		1×10^{-6}	4.60 ± 0.45	3.38 ± 0.31	5.98 ± 0.28
		1×10^{-5}	4.50 ± 0.28	3.24 ± 0.22	5.88 ± 0.13
		1×10^{-4}	4.33 ± 0.25	3.23 ± 0.38	6.00 ± 0.29
		1×10^{-3}	4.20 ± 0.21	5.80 ± 0.21	6.19 ± 0.37
Quercetin		0	6.90 ± 0.47	4.67 ± 0.20	3.64 ± 0.23
		1×10^{-6}	9.69 ± 0.66	5.65 ± 0.40	4.63 ± 0.40
		1×10^{-5}	9.54 ± 0.69	5.58 ± 0.25	4.46 ± 0.29
		1×10^{-4}	9.21 ± 0.72	5.56 ± 0.39	4.46 ± 0.27
		1×10^{-3}	7.91 ± 0.67	5.15 ± 0.39	3.98 ± 0.11
Ascorbic acid	3.0	0	5.59 ± 0.50	4.37 ± 0.22	3.20 ± 0.21
		1×10^{-6}	9.34 ± 1.20	8.07 ± 0.27	3.69 ± 0.30
		1×10^{-5}	9.71 ± 1.07	8.00 ± 0.20	3.62 ± 0.22
		1×10^{-4}	12.7 ± 1.3	8.07 ± 0.17	3.67 ± 0.20
		1×10^{-3}	15.3 ± 0.7	11.8 ± 1.7	4.25 ± 0.27
Hydroquinone		0	4.91 ± 0.51	4.00 ± 0.16	2.27 ± 0.24
		1×10^{-6}	6.47 ± 0.21	4.10 ± 0.20	6.17 ± 0.26
		1×10^{-5}	6.13 ± 0.21	3.84 ± 0.10	6.32 ± 0.70
		1×10^{-4}	5.95 ± 0.15	3.76 ± 0.12	6.20 ± 0.36
		1×10^{-3}	5.78 ± 0.34	4.28 ± 0.13	6.23 ± 0.44
Quercetin		0	5.62 ± 0.75	2.93 ± 0.14	2.26 ± 0.38
		1×10^{-6}	8.32 ± 0.63	3.21 ± 0.12	5.13 ± 0.21
		1×10^{-5}	8.09 ± 0.61	3.12 ± 0.12	5.17 ± 0.23
		1×10^{-4}	8.31 ± 0.65	3.02 ± 0.19	5.15 ± 0.26
		1×10^{-3}	7.14 ± 0.47	2.68 ± 0.16	3.92 ± 0.15
Ascorbic acid	4.0	0	1.79 ± 0.52	3.92 ± 0.70	1.07 ± 0.25
		1×10^{-6}	3.08 ± 0.28	4.45 ± 0.39	2.27 ± 0.15
		1×10^{-5}	3.14 ± 0.21	4.44 ± 0.27	2.45 ± 0.13
		1×10^{-4}	3.01 ± 0.22	4.41 ± 0.15	2.76 ± 0.20
		1×10^{-3}	4.38 ± 0.69	6.02 ± 0.39	4.16 ± 0.11
Hydroquinone		0	1.88 ± 0.48	2.96 ± 0.07	4.85 ± 0.13
		1×10^{-6}	2.91 ± 0.54	3.42 ± 0.12	5.60 ± 0.17
		1×10^{-5}	3.02 ± 0.36	3.51 ± 0.16	5.39 ± 0.16
		1×10^{-4}	3.00 ± 0.42	3.46 ± 0.17	5.40 ± 0.23
		1×10^{-3}	2.99 ± 0.51	3.58 ± 0.19	5.89 ± 0.12
Quercetin	4.0	0	1.75 ± 0.24	3.17 ± 0.75	1.14 ± 0.25
		1×10^{-6}	3.24 ± 0.49	3.68 ± 0.91	2.28 ± 0.19
		1×10^{-5}	3.21 ± 0.23	3.50 ± 0.79	2.54 ± 0.15
		1×10^{-4}	3.80 ± 0.22	3.55 ± 0.92	2.86 ± 0.16
		1×10^{-3}	3.52 ± 0.28	3.39 ± 0.23	2.54 ± 0.11
Ascorbic acid	5.0	0	1.81 ± 0.30	4.12 ± 0.24	1.47 ± 0.16
		1×10^{-6}	2.16 ± 0.19	4.70 ± 0.37	2.51 ± 0.25
		1×10^{-5}	2.37 ± 0.17	4.06 ± 0.25	2.48 ± 0.14
		1×10^{-4}	2.53 ± 0.22	3.89 ± 0.27	2.60 ± 0.29
		1×10^{-3}	3.18 ± 0.35	5.88 ± 0.28	3.66 ± 0.31
Hydroquinone		0	1.09 ± 0.15	4.36 ± 0.28	0.81 ± 0.25
		1×10^{-6}	2.37 ± 0.21	3.15 ± 0.14	1.52 ± 0.16
		1×10^{-5}	2.21 ± 0.15	2.92 ± 0.15	1.68 ± 0.15
		1×10^{-4}	1.97 ± 0.23	2.70 ± 0.27	1.81 ± 0.19
		1×10^{-3}	1.83 ± 0.15	3.08 ± 0.28	2.80 ± 0.10

(continued)

Table 1. Continued

Antioxidant	pH	Antioxidant concentration (M)	Anodic peak currents (μA)		
			poly(methylene green)	poly(neutral red)	poly(methylene blue)
Quercetin		0	1.26 ± 0.28	1.94 ± 0.24	0.72 ± 0.20
		1×10^{-6}	2.23 ± 0.30	1.95 ± 0.19	1.32 ± 0.13
		1×10^{-5}	2.42 ± 0.35	1.75 ± 0.20	1.34 ± 0.11
		1×10^{-4}	3.13 ± 0.48	2.08 ± 0.22	1.55 ± 0.19
		1×10^{-3}	3.21 ± 0.62	2.02 ± 0.11	1.52 ± 0.10
Ascorbic acid	6.0	0	2.65 ± 0.63	3.56 ± 0.84	–
		1×10^{-6}	1.81 ± 0.39	2.85 ± 0.47	1.37 ± 0.17
		1×10^{-5}	1.76 ± 0.56	2.54 ± 0.41	1.50 ± 0.19
		1×10^{-4}	1.87 ± 0.52	2.68 ± 0.22	1.41 ± 0.10
		1×10^{-3}	2.35 ± 0.43	3.61 ± 0.11	1.60 ± 0.16
Hydroquinone		0	2.87 ± 0.93	3.47 ± 0.59	–
		1×10^{-6}	2.93 ± 0.54	2.99 ± 0.23	–
		1×10^{-5}	2.22 ± 0.62	2.43 ± 0.28	–
		1×10^{-4}	1.96 ± 0.57	2.37 ± 0.12	–
		1×10^{-3}	2.19 ± 0.53	3.10 ± 0.33	–
Quercetin		0	2.95 ± 0.53	3.56 ± 0.24	–
		1×10^{-6}	3.36 ± 0.50	2.56 ± 0.13	1.33 ± 0.13
		1×10^{-5}	2.86 ± 0.43	2.38 ± 0.70	1.20 ± 0.18
		1×10^{-4}	2.56 ± 0.53	2.52 ± 0.89	1.19 ± 0.08
		1×10^{-3}	2.83 ± 0.76	2.33 ± 0.75	1.41 ± 0.12

Tea brand classification

The various responses of the electrochemical sensors toward model antioxidants allow the discrimination of the samples that vary in the identity and concentrations of the antioxidants. The analysis is based on the electronic tongue concept (Vlasov, Legin, and Rudnitskaya 2002) that employs an array of sensors with low selectivity to discriminate samples in accordance with the classes defined *a priori* by means of chemometric approaches. Potentiometric sensor arrays based on solid-contact electrodes covered with polyaniline and macrocyclic ionophores have been successfully used for the discrimination of fruit juices and beverages (Evtugyn et al. 2010) and green tea varieties (Lvova et al. 2003; Evtugyn et al. 2011). In impedimetric sensor arrays, tea classification was performed using large datasets of frequency depending responses (Bhondekar et al. 2010; Kumar et al. 2012). The voltammetric electronic tongues are primarily based on complicated potential modulations [large amplitude pulse voltammetric waveform (Ghosh et al. 2015)] or decomposition of overlapping voltammetric peaks related to individual antioxidants (Ziyatdinova et al. 2014).

In this work, the tea classification by brand was solved using lower numbers of sensors and experimental points. The initial dataset was compiled from responses toward diluted tea that were obtained by the sensors based on polymeric dyes. In order to consider the individual varieties of sensor transducers, four glassy carbon electrodes were selected for modification with polyaniline, DNA, and polymerized forms of dyes. They were utilized for the determination of cathodic and anodic peak currents for each tea brand. A total of 64 signals were processed assuming a normal distribution of the variables.

Figure 8 shows example cyclic voltammograms recorded on an electrode covered with polyaniline – DNA – poly(neutral red) layer in five tea infusions diluted 1:1 (v/v) with working buffer with the average anodic peak currents using the described sensors. Other experimental measurements were obtained in a similar manner using the other electrode coatings.

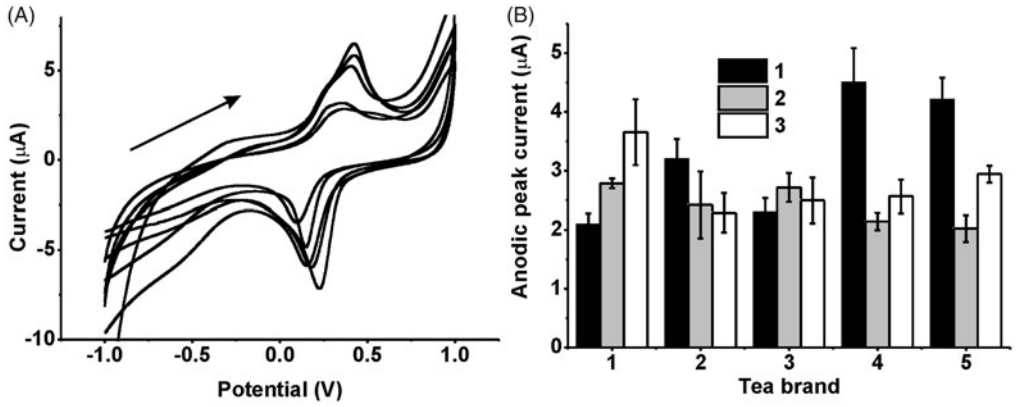

Figure 8. (a) Cyclic voltammograms recorded with the electrode covered with polyaniline, DNA, and poly(neutral red). The arrows indicate direction of the potential scan. (b) Anodic peak currents recorded on electrodes covered with polyaniline, DNA, and polymeric dyes: (1) poly(neutral red), (2) poly(methylene blue), and (3) (poly(methylene green) in the presence of tea fusions diluted by 1:1 (v/v) with pH 3.0 working buffer. Surface layer assembly conditions: 15 min incubation in 1 mg mL^{-1} DNA solution in Britton–Robinson buffer, pH 7.0, dye concentration 0.5 mmol L^{-1}, scan rate 50 mV s^{-1}. The results are reported as the average ± standard deviation for three individual sensors.

The classification of the tea samples by brand was first performed using principal component analysis (Jolliffe 2002). This method is commonly used for the visualization of multidimensional data and for searching internal links between randomized elements of large datasets. Orthogonal transformation in the datasets is performed to convert observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components.

The first coordinate in the new coordinate system (the first principal component) corresponds to the greatest variance by any data projection, the second coordinate to the second greatest projection variance, etc. The efficiency of correlation findings is quantified by the percentage of explained variance. Sensor signals characterizing a certain class of samples (in this case, tea brands) form a group separated from others on the plot of first two principal components (the score plot). The closer the points within a group and the farther the distance between the groups on the core plot, the stronger the internal links and probability of correlation between the experimental data. The score plot of the two first principal components obtained from the dataset for all of the anodic and cathodic peak currents recorded in all five tea infusions is presented in Figure 9. The legend shows the points belonging to specific tea brands.

The appropriate areas on the score plot are well separated, indicating that the signals used for score plotting are sufficient to distinguish the tea brands. The total explained variance was 91.1%, from which 75.7% related to the first principal component and 15.4% to the second principal component.

The classification of the tea brands based on the peak currents recorded on the electrodes with polymer coatings was performed using linear discriminant analysis (Pravdova, Pravda, and Guilbault 2002). In this method, a linear combination of features (peak currents) that separate points belonging to specific tea brands is established

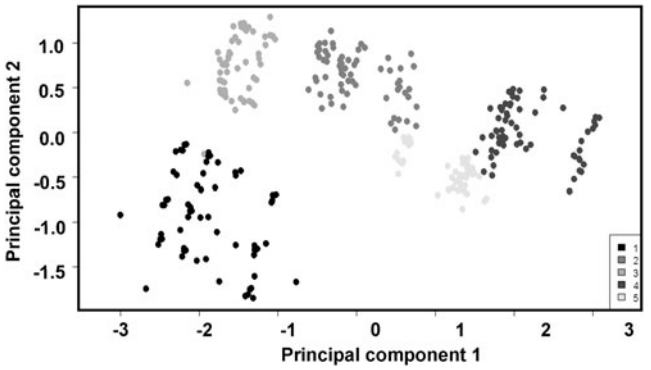

Figure 9. Score plot for the first two principal components obtained from the results of tea brand discrimination based on anodic and cathodic peak currents of the polymeric dyes. The initial data set involved all of the coatings including the polyaniline and polymeric forms of the dyes. The legend identifies the tea samples used for classification.

Table 2. Classification accuracy for tea brands performed by linear discriminant analysis.

	Predicted tea brand				
Actual tea brand	1	2	3	4	5
1	99.8	0.0	0.2	0.0	0.0
2	0.0	94.6	1.7	0.4	3.3
3	0.0	0.3	99.7	0.0	0.0
4	0.0	0.1	0.0	99.0	0.9
5	0.0	0.0	0.0	0.0	100.0

by linear regression. The resulting combination was used as a linear classifier to predict the tea brand of unknown samples. In Table 2, the averaged classification results are summarized for 100 datasets randomly generated by bootstrapping.

The antioxidant capacity of the tea fusion was determined by a coulometric titration with electrogenerated bromine for samples diluted twice with working buffer. The appropriate value varied from to 358 ± 6 to 509 ± 11 coulomb $(100\,\text{mL})^{-1}$ with the standard deviation varying from 0.008 to 0.02. In order to establish the relationship between this value and sensor responses, a linear mixed model was fit using cathodic and anodic peak currents of individual sensors based on three various coatings. The random effects characterizing the nonequivalent behavior of individual transducers were used for electropolymerization. The results show that the model explains 69.9% of the total variance. The largest effect was caused by the sensor coated with poly(neutral red), which alone provided 50.6% of the explained variance. Other parameters introduce contributions that did not exceed 5% each.

The results of tea brand classification have shown new applications of electrochemical sensors for food quality assessment and classification based on combined electropolymerized materials. In comparison with other voltammetric systems described in the literature (Bhondekar et al. 2010; Kumar et al. 2012; Ghosh et al. 2015), the classification based on peak currents of redox active coatings relies on a lower number of initial experimental points and sensors and is easier in terms of sensor preparation and measurement protocols.

Conclusions

Electropolymerization allows the production of integrated polymeric films composed of polyaniline, DNA, and polymerized methylene blue, methylene green, and neutral red with intrinsic redox activity. The incorporation of DNA was confirmed by scanning electron microscopy and electrochemical impedance spectroscopy indicating the participation of the biopolymer in composition of the layer and its influence on charge transfer resistance and pH area of the conductivity of polyaniline.

The electrochemical behavior of the coating depended both on the pH and the dye. In acidic media, reversible redox conversion of polymeric dyes take place, while in weakly acidic and neutral media, the remaining redox activity of polyaniline doped with DNA limits the current. In comparison with a single-layer coating, the combination of polyaniline with DNA and polymeric dyes retained electrochemical activity at high pH values.

The electrochemical sensors were characterized for the determination of individual antioxidants and classification of tea by brand. The influence of ascorbic acid, hydroquinone, and quercetin was observed predominantly in acidic conditions with increases in the anodic peaks and decreases in the cathodic peaks due to the dye present. In most cases, the absolute peak current values decreased in the order of poly(neutral red) to (poly(methylene blue) and poly(methylene green).

With increasing pH, the peak currents became more similar because of the enhanced influence of polyaniline into the overall redox activity of the coating. Changes in the peaks also varied with nature of antioxidants, allowing the use of the electrochemical sensor for the discrimination of tea fusions based on the anodic and cathodic peak currents of the sensors. Principal component analysis confirmed discrimination of tea samples in accordance with their brand. Linear discrimination analysis provided approximately 100% accuracy for the tea brands. A linear random model that took into account the individual properties of the sensor transducers demonstrated a relationship between the sensor signals and antioxidant capacity by coulometry with 69.9% explained variance.

The electrochemical sensors based on combination of various electropolymerized materials may find further application in the analysis of foodstuffs and beverages in accordance with their antioxidant capacities. In comparison with other sensor arrays described in the literature for these purposes, the classification by antioxidant properties requires a lower number of individual sensors that were prepared by using a uniform protocol of electropolymerization. The use of conventional constant current voltammetry and tuning electrochemical activity of polymeric dyes by pH variation are other advantages of the developed sensors compared to devices requiring sophisticated instrumentation and additional auxiliary reagents implemented in the surface layer such as ionophores and nanoparticles with electrocatalytic properties.

Funding

The financial support of Russian Science Foundation [grant No. 17-73-20024] is gratefully acknowledged.

ORCID

G. A. Evtugyn http://orcid.org/0000-0002-4694-7836

References

Aleixandre-Tudo, J. L., A. Buica, H. Nieuwoudt, J. L. Aleixandre, and W. Du Toit. 2017. Spectrophotometric analysis of phenolic compounds in grapes and wines. *Journal of Agricultural and Food Chemistry* 65(20):4009–26. doi:10.1021/acs.jafc.7b01724..

Arslan, A., S. Ustabaş, and H. Arslan. 2011. An amperometric biosensor for glucose determination prepared from glucose oxidase immobilized in polyaniline-polyvinylsulfonate film. *Sensors (Basel, Switzerland)* 11(8):8152–63. doi:10.3390/s110808152.

Bahmani, B., F. Moztarzadeh, M. Rabiee, and M. Tahriri. 2010. Development of an electrochemical sulfite biosensor by immobilization of sulfite oxidase on conducting polyaniline film. *Synthetic Metals* 160(23/24):2653–7. doi:10.1016/j.synthmet.2010.10.020.

Bardavid, J., J. Ghabboun, D. Porath, A. B. Kotylar, and S. Yitzchaik. 2008. Formation of polyaniline layer on DNA by electrochemical polymerization. *Polymer* 49(9):2217–22. doi:10.1016/j.polymer.2008.03.014.

Bergamini, M. F., D. P. Santos, and M. V. B. Zanoni. 2013. Electrochemical behavior and voltammetric determination of pyrazinamide using a poly-histidine modified electrode. *Journal of Electroanalytical Chemistry* 690:47–52. doi:10.1016/j.jelechem.2012.11.032.

Bhondekar, A. P., M. Dhiman, A. Sharma, A. Bhakta, A. Ganguli, S. S. Bari, R. Vig, P. Kapur, and M. L. Singl. 2010. A novel iTongue for Indian black tea discrimination. *Sensors and Actuators B: Chemical* 148(2):601–9. doi:10.1016/j.snb.2010.05.053.

Buron, C. C., M. Quinart, T. Vrlinic, S. Yunus, K. Glinel, A. M. Jonas, and B. Lakard. 2014. Application of original assemblies of polyelectrolytes, urease and electrodeposited polyaniline as sensitive films of potentiometric urea biosensors. *Electrochimica Acta* 148:53–61. doi:10.1016/j.electacta.2014.10.026.

Cesarino, I., F. C. Moraes, M. R. V. Lanza, and S. A. S. Machado. 2012. Electrochemical detection of carbamate pesticides in fruit and vegetables with a biosensor based on acetylcholinesterase immobilised on a composite of polyaniline-carbon nanotubes. *Food Chemistry* 135(3):873–9. doi:10.1016/j.foodchem.2012.04.147.

Chen, H., S. Li, S. Wang, Y. Tan, and J. Kan. 2013. A new catechol biosensor immobilized polyphenol oxidase by combining electropolymerization and cross-linking process. *International Journal of Polymeric Materials* 62(12):620–6. doi:10.1080/00914037.2012.761629.

Dai, Z.-H., F.-X. Liu, G.-F. Lu, and J.-C. Bao. 2008. Electrocatalytic detection of NADH and ethanol at glassy carbon electrode modified with electropolymerized films from methylene green. *Journal of Solid State Electrochemistry* 12(2):175–80. doi:10.1007/s10008-007-0378-1.

Dorraji, P. S., and F. Jalali. 2016. Differential pulse voltammetric determination of nanomolar concentrations of antiviral drug acyclovir at polymer film modified glassy carbon electrode. *Materials Science and Engineering: C* 61:858–64. doi:10.1016/j.msec.2016.01.030.

Evtugyn, G. A., S. V. Belyakova, R. V. Shamagsumova, A. A. Saveliev, A. N. Ivanov, E. E. Stoikova, N. N. Dolgova, I. I. Stoikov, I. S. Antipin, and H. C. Budnikov. 2010. Discrimination of apple juice and herbal liqueur brands with solid-state electrodes covered with polyaniline and thiacalixarenes. *Talanta* 82(2):613–9. doi:10.1016/j.talanta.2010.05.016.

Evtugyn, G. A., and T. Hianik. 2016. Electrochemical DNA sensors and aptasensors based on electropolymerized materials and polyelectrolyte complexes. *TrAC - Trends in Analytical Chemistry* 79:168–78. doi:10.1016/j.trac.2015.11.025.

Evtugyn, G. A., A. V. Porfireva, T. Hianik, M. S. Cheburova, and H. C. Budnikov. 2008. Potentiometric DNA sensor based on electropolymerized phenothiazines for protein detection. *Electroanalysis* 20(12):1300–8. doi:10.1002/elan.200704186.

Evtugyn, G. A., R. V. Shamagsumova, E. E. Stoikova, R. R. Sitdikov, I. I. Stoikov, H. C. Budnikov, A. N. Ivanov, and I. S. Antipin. 2011. Potentiometric sensors based on polyaniline

and thiacalixarenes for green tea discrimination. *Electroanalysis* 23(5):1081–8. doi:10.1002/elan.201000586.

Fahidy, T. Z. 2008. On the application of a bootstrapping-resample technique of nonparametric statistics to electroanalytical measurements. *Journal of Electroanalytical Chemistry* 615(2): 159–64. doi:10.1016/j.jelechem.2007.12.006.

Gao, Q., M. Sun, P. Peng, H. L Qi, and C. Zhang. 2010. Electro-oxidative polymerization of phenothiazine dyes into a multilayer-containing carbon nanotube on a glassy carbon electrode for the sensitive and low-potential detection of NADH. *Microchimica Acta* 168(3–4):299–307. doi:10.1007/s00604-009-0283-7.

Ghosh, A., A. K. Bag, P. Sharma, B. Tudu, S. Sabhapondit, B. D. Baruah, P. Tamuly, N. Bhattacharyya, and R. Bandyopadhyay. 2015. Monitoring the fermentation process and detection of optimum fermentation time of black tea using an electronic tongue. *IEEE Sensors Journal* 15(11):6255–62. doi:10.1109/JSEN.2015.2455535.

Gonzalez, A., S. Vidal, and M. Ugliano. 2018. Untargeted voltammetric approaches for characterization of oxidation patterns in white wines. *Food Chemistry* 269:1–8. doi:10.1016/j.foodchem.2018.06.104.

Gülçin, I. 2012. Antioxidant activity of food constituents: An overview. *Archives of Toxicology* 86(3):345–91. doi:10.1007/s00204-011-0774-2.

Hoyos-Arbeláez, J., M. Vázquez, and J. Contreras-Calderón. 2017. Electrochemical methods as a tool for determining the antioxidantcapacity of food and beverages: A review. *Food Chemistry* 221:1371–81. doi:10.1016/j.foodchem.2016.11.017.

Hurtado-Fernández, A., M. Gómez-Romero, A. Carrasco-Pancorbo, and A. Fernández-Gutiérrez. 2010. Application and potential of capillary electroseparation methods to determine antioxidant phenolic compounds from plant food material. *Journal of Pharmaceutical and Biomedical Analysis* 53(5):1130–60. doi:10.1016/j.jpba.2010.07.028.

Inzelt, G. 2018. *Conducting polymers: Past present, future. Journal of Electrochemical Science and Engineering* 8:3–37. doi:10.5599/jese.448.

Ivanova, A. V., E. L. Gerasimova, and E. R. Gazizullina. 2019. New antiradical capacity assay with the use potentiometric method. *Analytica Chimica Acta* 1046:69–76. doi:10.1016/j.aca.2018.09.025.

Jin, G. Y., F. Huang, and J. L. Kong. 2007. Sensitive determination of clomipramine at poly-ABSA/Pt nano-clusters modified glassy carbon electrode. *Analytical Letters* 40(18):3392–404. doi:10.1080/00032710701689131.

Jolliffe, I. T. 2002. *Principal component analysis (springer series in statistics)*. New York, NY: Springer. 487 pp.

Komori, C., K. Okada, K. Kawamura, S. Chida, and T. Suzuki. 2009. The sonodynamic antitumor effect of methylene blue on sarcoma180 cells in vitro. *Anticancer Research* 29:2411–5.

Kul, D., R. Pauliukaite, and C. M. A. Brett. 2011. Electrosynthesis and characterisation of poly(Nile blue) films. *Journal of Electroanalytical Chemistry* 662(2):328–33. doi:10.1016/j.jelechem.2011.09.007.

Kulikova, T., A. Porfireva, G. Evtugyn, and T. Hianik. 2019. Electrochemical DNA sensors with layered polyaniline – DNA coating for detection of specific DNA interactions. *Sensors* 19(3): 469. doi:10.3390/s19030469

Kumar, R., A. P. Bhondekar, R. Kaur, S. Vig, A. Sharma, and P. Kapur. 2012. A simple electronic tongue. *Sensors and Actuators B: Chemical* 171–172 :1046–53. doi:10.1016/j.snb.2012.06.031.

Kuzin, Y., A. Ivanov, G. Evtugyn, and T. Hianik. 2016. Voltammetric detection of oxidative DNA damage based on interactions between polymeric dyes and DNA. *Electroanalysis* 28(12): 2956–64. doi:10.1002/elan.201600297.

Lange, U., N. V. Roznyatovskaya, and V. M. Mirsky. 2008. Conducting polymers in chemical sensors and arrays. *Analytica Chimica Acta* 614(1):1–26. doi:10.1016/j.aca.2008.02.068.

Lvova, L.,. A. Legin, Y. Vlasov, G. S. Cha, and H. Nam. 2003. Multicomponent analysis of Korean green tea by means of disposable all-solid-state potentiometric electronic tongue microsystem. *Sensors and Actuators B: Chemical* 95(1–3):391–9. doi:10.1016/S0925-4005(03)00445-3.

Manasa, G., R. J. Mascarenhas, A. K. Satpati, O. J. D'Souza, and A. Dhason. 2017. Facile preparation of poly(methylene blue) modified carbon paste electrode for the detection and quantification of catechin. *Materials Science and Engineering: C* 73:552–61. doi:10.1016/j.msec.2016.12.114.

Meibodi, A. S. E., and S. Haghjoo. 2014. Amperometric urea biosensor based on covalently immobilized urease on an electrochemically polymerized film of polyaniline containing MWCNTs. *Synthetic Metals* 194:1–6. doi:10.1016/j.synthmet.2014.04.009.

Moon, J.-M., N. Thapliyal, K. K. Hussain, R. N. Goyal, and Y.-B. Shim. 2018. Conducting polymer-based electrochemical biosensors for neurotransmitters: A review. *Biosensors and Bioelectronics* 102:540–52. doi:10.1016/j.bios.2017.11.069.

Niederländer, H. A. G., T. A. van Beek, A. Bartasiute, and I. I. Koleva. 2008. Antioxidant activity assays on-line with liquid chromatography. *Journal of Chromatography A* 1210(2):121–34. doi:10.1016/j.chroma.2008.09.061.

Omar, F. S., N. Duraisamy, K. Ramesh, and S. Ramesh. 2016. Conducting polymer and its composite materials based electrochemical sensor for Nicotinamide Adenine Dinucleotide (NADH). *Biosensors and Bioelectronics* 79:763–75. doi:10.1016/j.bios.2016.01.013.

Ouyang, X., L. Luo, Y. Ding, B. Liu, D. Xu, and A. Huang. 2015. Simultaneous determination of uric acid, dopamine and ascorbic acid based on poly(bromocresol green) modified glassy carbon electrode. *Journal of Electroanalytical Chemistry* 748:1–7. doi:10.1016/j.jelechem.2015.04.026.

Pauliukaite, R., and C. M. A. Brett. 2008. Poly(neutral red): Electrosynthesis, characterization, and application as a redox mediator. *Electroanalysis* 20(12):1275–85. doi:10.1002/elan.200804217.

Pauliukaite, R., M. E. Ghica, M. M. Barsan, and C. M. A. Brett. 2010. Phenazines and polyphenazines in electrochemical sensors and biosensors. *Analytical Letters* 43(10/11):1588–608. doi:10.1080/00032711003653791.

Peng, H., L. Zhang, C. Soeller, and J. Travas-Sejdic. 2009. Conducting polymers for electrochemical DNA sensing. *Biomaterials* 30(11):2132–48. doi:10.1016/j.biomaterials.2008.12.065.

Pisoschi, A. M., C. Cimpeanu, and G. Predoi. 2015. Electrochemical methods for total antioxidant capacity and its main contributors determination: A review. *Open Chemistry* 13:824–85. doi:10.1515/chem-2015-0099.

Prakash, R. 2002. Electrochemistry of polyaniline: Study of the pH effect and electrochromism. *Journal of Applied Polymer Science* 83(2):378–85. doi:10.1002/app.10025.

Pravdova, V., M. Pravda, and G. G. Guilbault. 2002. Role of chemometrics for electrochemical sensors. *Analytical Letters* 35:2389–419. doi:10.1081/AL-120016533.

Shamagsumova, R., A. Porfireva, V. Stepanova, Y. Osin, G. Evtugyn, and T. Hianik. 2015. Polyaniline-DNA based sensor for the detection of anthracycline drugs. *Sensors and Actuators B: Chemical* 220:573–82. doi:10.1016/j.snb.2015.05.076.

Shen, H., and C. Anastasio. 2012. A comparison of hydroxyl radical and hydrogen peroxide generation in ambient particle extracts and laboratory metal solutions. *Atmospheric Environment* 46:665–8. doi:10.1016/j.atmosenv.2011.10.006.

Shi, L., Y. Xiao, and I. Willner. 2004. Electrical contacting of glucose oxidase by DNA-templated polyaniline wires on surfaces. *Electrochemistry Communications* 6(10):1057–60. doi:10.1016/j.elecom.2004.08.004

Serafini, M., and I. Peluso. 2016. Functional foods for health: The interrelated antioxidant and anti-inflammatory role of fruits, vegetables, herbs, spices and cocoa in humans. *Current Pharmaceutical Design* 22(44):6701–15. doi:10.2174/1381612823666161123094235.

da Silva, J. V., D. M. Pimentel, D. E. P. Souto, R. C. S. Luz, and F. S. Damos. 2013. Application of horseradish peroxidase/polyaniline/bis(2-aminoethyl) polyethylene glycol-functionalized carbon nanotube composite as a platform for hydrogen peroxide detection with high sensitivity at low potential. *Journal of Solid State Electrochemistry* 17(11):2795–804. doi:10.1007/s10008-013-2182-4.

Somerset, V. S., M. J. Klink, P. G. L. Baker, and E. I. Iwuoha. 2007. Acetylcholinesterase-polyaniline biosensor investigation of organophosphate pesticides in selected organic solvents. *Journal of Environmental Science and Health, Part B* 42(3):297–304. doi:10.1080/03601230701229288.

Tekbaşoğlu, T. Y., T. Soganci, M. Ak, A. Koca, and M. K. Şener. 2017. Enhancing biosensor properties of conducting polymers via copolymerization: Synthesis of EDOT-substituted bis(2-pyridylimino)isoindolato-palladium complex and electrochemical sensing of glucose by its copolymerized film. *Biosensors and Bioelectronics* 87:81–8. doi:10.1016/j.bios.2016.08.020.

Topçu, E., K. Dağcı, and M. Alanyalıoğlu. 2016. Free-standing graphene/poly(methylene blue)/AgNPs composite paper for electrochemical sensing of NADH. *Electroanalysis* 28(9):2058–69. doi:10.1002/elan.201600108.

Vilas-Boas, Â., P. Valderrama, N. Fontes, D. Geraldo, and F. Bento. 2019. Evaluation of total polyphenol content of wines by means of voltammetrictechniques: Cyclic voltammetry vs differential pulse voltammetry. *Food Chemistry* 276:719–25. doi:10.1016/j.foodchem.2018.10.078.

Vlasov, Y., A. Legin, and A. Rudnitskaya. 2002. Electronic tongues and their analytical application. *Analytical and Bioanalytical Chemistry* 373(3):136–46. doi:10.1007/s00216-002-1310-2.

Zanardi, C., F. Terzi, and R. Seeber. 2013. Polythiophenes and polythiophene-based composites in amperometric sensing. *Analytical and Bioanalytical Chemistry* 405(2–3):509–31. doi:10.1007/s00216-012-6318-7.

Ziyatdinova, G. K., H. C. Budnikov, V. I. Pogorel'tzev, and T. S. Ganeev. 2006. The application of coulometry for total antioxidant capacity determination of human blood. *Talanta* 68(3):800–5. doi:10.1016/j.talanta.2005.06.010.

Ziyatdinova, G. K., A. A. Saveliev, G. A. Evtugyn, and H. C. Budnikov. 2014. Simultaneous voltammetric determination of phenolic antioxidantswith chemometric approaches. *Electrochimica Acta* 137:114–20. doi:10.1016/j.electacta.2014.06.009.