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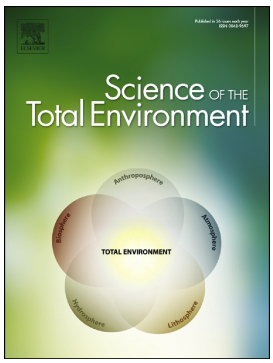

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Electrochemical strategies for gallic acid detection: Potential for application in clinical, food or environmental analyses

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Abstract

Polyphenols are important to human health thus making it interesting and necessary to identify and assess methods for their detection. Gallic acid (GA) is a well-known antioxidant compound, found in tea leaves, various fruits, fruit seeds and in fruit-derived foods and beverages. In this study, to electrochemically detect this compound and assess the potential for GA detection, different analytical conditions at pH values of 5.8, 7 and 8 were tried. Two types of device were used for GA detection: (1) Lazar ORP-146C reduction-oxidation microsensors, coupled with a Jenco device, for estimation of antioxidant capacities of different electroactive media, and (2) screen-printed carbon sensors coupled with a mobile PalmSens device using differential pulse voltammetry (qualitative and quantitative GA determination). These proposed methods were validated by analysing some real samples: wine, green tea, apple juice and serum fortified with GA. Detection was evaluated in terms of specific calibration curves, with low limit of detection (LOD) and limit of quantification (LOQ), low response time, and high sensitivities. The analytical characteristics obtained recommend these methods to be tested on more other types of real samples. Our proposed methods, used in the established conditions of pH, may have further application in other clinical, food or environmental samples analyses in which the results of total antioxidants contents are usually expressed in GA equivalents.

Keywords: electrochemical detection; gallic acid; differential pulse voltammetry; screen-printed carbon electrode; redox microsensor

1. Introduction

Polyphenols are universally present in plant-derived foods and their effects on human health have been extensively studied over the past twenty years. Their metabolites have been associated with the health benefits of plant-derived foods (Abdel-Daim et al., 2018; Bispo et al., 2017). The use of natural products in discovering and developing anticancer drugs is frequent, with over 50% of medicinal products used in antitumor clinical trials being isolated from natural sources (Tietbohol et al., 2017).

Among these natural products, plant-derived compounds are important sources of multiple anticancer clinical agents, plants being well known as sources of molecules with pharmacological activity (Pellegrina et al., 2005). Some studied compounds, such as gallic acid (3,4,5-trihydroxybenzoic acid, GA) and its derivatives have been extensively evaluated for their antitumor activity against various cell lines (Petrea, et al., 2018; Sourani et al., 2016).

GA has been identified as having various biological activities. Antibacterial (Dos Santos et al., 2018), antiviral (You et al., 2018), anti-inflammatory, antihistaminic and antitumor activities, scavenging of free radicals and protection against cardiovascular diseases (Mudnic et al., 2010; Seob et al., 2018), antioxidant (Asnaashari et al., 2014; Suwalsky et al., 2016; Hsu et al., 2016), anticancer (Wang et al., 2014; Locatelli et al., 2013; Verma et al., 2013), antidiabetic (Kong et al., 2018; Huang et al., 2016), etc., have all been reported. Studies have shown that GA can inhibit the mitochondrial dysfunction with a significant neuroprotective effect on cerebral ischemia/reperfusion injury (Sun et al., 2017). The beneficial effects of GA derivatives (methyl, propyl, octyl and dodecyl gallate) are also known for their widespread use as antioxidants in the pharmaceutical and cosmetic industries (Nayeem et al., 2016; Otrisal et al., 2017).

GA is one of the main natural phenolic acids existing in tea leaves (both green and black tea), grapes (grape seeds), fruits such as strawberries, berries and other fruits, as well as in many other processed herbs and drinks – red tea and red wine (Suwalsky et al., 2016; Hsu et al., 2016). Detection of GA is often considered as an indicator of the authenticity of fruit juices and different alcoholic beverages, and in recent years it has been illegally added to

liquors (Ng et al., 2000). GA is also found in several hardwood species such as oak (oak bark) and chestnut; also, it can be obtained under acid hydrolysis of hydrolysable tannins (Liu et al., 2016; Prikryl et al., 2018; Zhang et al., 2014). Consequently, quantitative determination of GA concentration can be made for food, medicinal plants and, last but not least, in body fluids (Shahrzad and Bitsch, 1998). GA usually indicates the total level of antioxidants (total antioxidant capacity) of the real samples and the results are expressed as Gallic Acid Equivalents (GAE) (Xiong et al., 2017).

Thus, we can underline the importance of identifying different viable alternatives to detect GA from different matrices. In recent years, different detection methods have been developed and optimized to detect GA in different samples using thin-layer (Dhalwal et al., 2008) or liquid chromatography (Shahrzad and Bitsch, 1998; Narumi et al., 2014; Denderz and Lehotay, 2014; Keckes et asl., 2013). The antioxidant activity (AOA) of wine samples has been evaluated spectrophotometrically, as a measure of radical scavenging activity, using a 1,1-diphenyl-2-picryl-hydrazyl free radical molecule (DPPH) (Brand-Williams et al., 1995; Leong and Shui, 2002; Di Lorenzo et al., 2017). A 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate (ABTS) assay with some modifications was performed (Re et al., 1999).

Electrochemical and kinetic methods are highly useful for detection and monitoring of electroactive chemical/biochemical species (Bungau et al., 2017; Bungau et al., 2012; Badea and Badea, 2006; Supalkova et al., 2006; Tukimin et al., 2017; Radovici et al., 2003). Some of the biotechniques used are based on receptor-receipt interaction (Eremia et al., 2013; Garcia et al., 2015).

We performed an intensive and systematic review of the existing research about GA detection using electrochemical methods with carbon-based electrodes. Moreover, it was possible to identify the gaps in this area and to underline the additional value of the present study. The trend of the identified articles showed an increased interest in GA only after 2000. Some papers referred to biosensors using laccase (Vlamidist al., 2017) and tyrosinase (Datta et al., 2017) as analytical tools for GA detection and other plant-derived antioxidants. GA was also mentioned as a reagent used to modify different carbon electrodes (multi-walled

carbon nanotubes and electropolymerized GA (poly(gallic acid)/MWNT/GCE) (Ziyatdinova et al., 2018) to detect other bioactive compounds.

With the advantages of electroanalytical methods (Petkovic et al., 2015; Sangeetha and Narayanan, 2014; Jara-Ulloa et al., 2013; Tashkhourian et al., 2013; Liang et al., 2016; Tashkhourian and Nami-Ana, 2015; Raymundo-Pereira et al., 2015; Ščorc et al., 2018), different studies have been conducted to determine GA, using different designs of transducer systems.

Analysing the scientific literature, we found articles dealing with detection of GA or with determination of its antioxidant action by means of electrochemical methods using carbon bare electrodes or carbon modified electrodes. It is noteworthy that some articles referred to the screen-printed electrode commercially available from Dropsens (inexpensive and reliable), but no one used the experimental conditions tested in this study (pH, connection with the mobile PalmSens device, useable for lab work – on turn connected either to a laptop or to a remote system in the field). As far as we know, the second experiment tested in this research is the first that uses a data reporting method analysed with this type of redox microelectrode for the analysis of GA antioxidant action.

In this study we attempt to fill in the gaps left by previously proposed methods found in the literature, recommending stable, fast, inexpensive and reproducible electrochemical detection methods to determine GA and its antioxidant power, in both standard and real samples. Our team, therefore, used two different electrochemical methods:

1) a new Lazar ORP-146C reduction-oxidation microsensor, coupled with a Jenco device to read potential GA applications, and for potential redox detection of GA, in order to estimate the antioxidant capacities of different electroactive media;
2) a commercially available screen-printed sensor linked to a PalmSens device, that uses carbon as a working electrode.

These two proposed methods were not tested before and are expected to have cost effective and excellent performances as to GA detection when compared with the previously reported chemically modified electrodes. The aim of this paper is, above all, to make use of the best conditions and to discuss those in which the pH of carrier solutions differed.

2. Materials and Methods

2.1. Reagents

A stock solution with a concentration of 0.1M was prepared using GA (Sigma Aldrich Corporation, Saint Louis, Missouri, USA) in distilled water. Buffer solutions with pH 5.8, 7.0, and 8.0 were obtained by mixing, in different ratios, 0.1M solutions of sodium phosphate monobasic (Poch S.A, 44-101 Gilwice, Poland) and sodium phosphate dibasic (Poch S.A, 44-101 Gilwice, Poland) (Ruffien-Ciszak et al., 2006).

2.2. Equipment

Differential pulse voltammetry experiments were carried out at room temperature, under dark conditions, using the electrochemical sensor interface PalmSens 3 (Palm Instruments BV, The Netherlands), controlled by a PC running PSTrace software version 4.7. Single-compartment three-electrode transducers DRP C110 (DropSens, Llanera, Spain) were used. These are screen-printed carbon electrodes with a ceramic substrate: L33 x W10 x H0.5 mm with electric contacts made of silver, carbon (4 mm diameter) as the working electrode, a counter electrode also of carbon and a reference electrode made of silver (Gruene et al., 2018).

Redox measurements were taken with a Jenco device (Jenco Instruments, San Diego, California, USA), Model: 6230N coupled with a redox microsensor - ORP-146C (Shelf scientific, Lazar Research Laboratories, Inc., Los Angeles, CA, USA). An extremely small size sensor, with an electrode tip diameter of only 1 mm, it is used for large or small volumes (5 μL) (http://www.shelfscientific.com/cgi-bin/tame/newlaz/redoxn.tam).

The Lazar Model ORP-146C micro oxidation-reduction electrode utilizes a specially formulated platinum redox sensing element, in conjunction with a silver/silver chloride reference electrode, to measure the oxidation-reduction potential in solution volumes down to 10 microliters. The microelectrode has both the redox sensor and the reference electrode built into a single electrode body. The reference junction is a fluoropolymer film, and the electrode flexible capillary stem is also composed of a fluoropolymer formulation. The reference solution storage compartment uses an epoxy body. The micro redox electrode has a

range of measurement of oxidation-reduction potential from -1500 mV to +1500 mV. Accuracy of the micro redox electrode is +/- 0.5 mV.

2.3. Methods

The GA stock solution in a 0.1M concentration was obtained fresh before each experiment. Presence of GA was tested in 10 mL buffers with different pH values (pH 5.8; 7; 8), raising the concentration of the active compound to 2 mM. Each solution was tested, using the previously described method (Miccoli et al., 2018) in duplicate and the data were analysed (in order to work with average values and their standard deviations). Differential pulse voltammetry (DPV) was used and all parameters were established using PSTrace software: potential range (-1.0 V to 1.0 V), step potential 0.005 V, pulse potential 0.049 V, pulse time 0.05 s and scan rate 0.01 V/s. A diagram presenting the steps of the procedure is presented in **Figure 1**.

All redox data, collected by the portable microsensor, were obtained simultaneously with the electrochemical measurements (DPV), and under the same experimental conditions (using the same Berzelius glass), for better data correlation. The solution was stirred for 10s before the beginning of each survey, using a magnetic stirrer. Standard GA solutions were analyzed first, in order to obtain the calibration curves. The possible interferences in the presence of ascorbic and/or caffeic acid were analysed under the same conditions. Detection in real samples (homemade white wine, commercial green tea and apple juice) was undertaken using DPV method while homemade white wine and serum were detected using redox microsensor system

2.4. Data registration

Experimental DPV data were plotted as specific voltammograms. Data was obtained using PSTrace v4.7 software concerning the potential of area, specific peaks and peak height. These parameters were considered for data interpretation and qualitative-quantitative analysis. The portable microsensor directly indicated the potential equivalent to the redox reaction from that system.

All experimental data were stored, and all graphics were created with Microsoft Office Excel, using specific experimental values.

3. Results

3.1. Quality assurance for testing different solutions using the differential pulse voltammetry method

The DPV parameters were previously optimized in the lab (data not shown). As mentioned in the description of working procedures, the potential range went from -1.0 V to 1.0 V. However, based on the experimental results the method was optimized to include peaks of interest and to minimize total time of analysis. Data representation reflected a potential range from -0.5 V to 0.6 V.

The GA solutions, in concentrations of 0.1 to 2 mM in the buffer system, and with a pH 5.8, were analysed and specific voltammograms were recorded. Though the total recording was for a potential range of -1.0 V to 1.0 V, the voltammograms were represented within the range of -0.1 V to 0.5 V (**Figure 2**), the range in which characteristic GA peaks were obtained. No peaks were recorded for a potential higher than 0.5V. A single peak corresponding to GA was observed. The average potential for peaks corresponding to GA at pH 5.8 was 0.180 ± 0.021 V. Peak height and area were plotted against GA concentration to obtain calibration lines (**Figure 3**). Good calibrations were obtained with a linearity range of 0.1 to 2.0) mM. The coefficients of determination were calculated with good results. Similar experiments were conducted for pH 7. The corresponding voltammograms and calibration curves are indicated in **Figure 4**.

Two peaks were observed corresponding to the GA in phosphate buffer pH 7 at 0.175 ± 0.024 V (main peak) and at 0.465 ± 0.056 V (smaller), respectively. The characteristic values of the peaks (height and area) were plotted against the GA concentration in **Figure 5**.

Presence of GA at pH 8 and its potential quantification were also tested. The differential pulse voltammograms and calibrations are indicated in **Figure 6** and **Figure 7**. In **Figure 8** two peaks are observed. The average peak potentials corresponding to the GA in phosphate buffer pH 8 are -0.037 ± 0.003 V and 0.137 ± 0.017 V, respectively.

Specific linear relationships of steady-state currents (peak height and peak area) against GA concentrations (0.1 to 0.8 mM) in buffer at pH 8 were observed for both potentials in

which peaks appeared. Above this concentration no linearity was observed. We proposed two ways to quantify the GA concentration, using peak height and peak area for each pH. Based on the standard deviation (SD) of response and the slope of the dependencies, the limit of detection (LOD) and limit of quantification (LOQ) were calculated, using a previously described method (Shrivastava and Gupta, 2011). Calibration lines and correlation coefficients (R^2) are indicated in Figures 3, 5 and 7; LOD, LOQ and sensitivity (slope of calibration line) for each experimental situation are systematized in **Table 1**.

The measurement chain has a specific impact on data reports. The optimization steps that take into consideration diffusion and chemical reaction, as well as the adsorption/desorption inherent in any sensor, all contribute to an applied potential which starts at -0.5 V. Response time ($t_{90,\,max}$) was calculated as the time necessary to obtain 90% of the maximum response value (peak), as similar studies have previously indicated (Ziaian et al., 2015).

GA, as well as caffeic and ascorbic acids, all at the same pH values, were tested to obtain data concerning selectivity of the methods developed for a similar active compound concentration (0.3 mM). The results concerning the potentials corresponding to the DPV peak of these compounds are indicated in **Table 2**.

3.2. Quality assurance for detection using redox microsensors

The linear correlation between concentration and redox potential in GA solutions detected by microsensor are presented in Figure 8 (a-c). The sensitivity values which depend on pH values are depicted in **Table 3**. All the results are in accordance with previous experiments, when the DPV method was used for GA detection (increasing sensitivity from pH 5.8 to 8).

This technique enabled rapid, accurate, and non-invasive determination of the total antioxidant capacity of GA solutions, with results obtained in under 45 seconds, similar to the results reported in other studies using cyclic voltammetry, where the responding time was 1 min (Xiong et al., 2017).

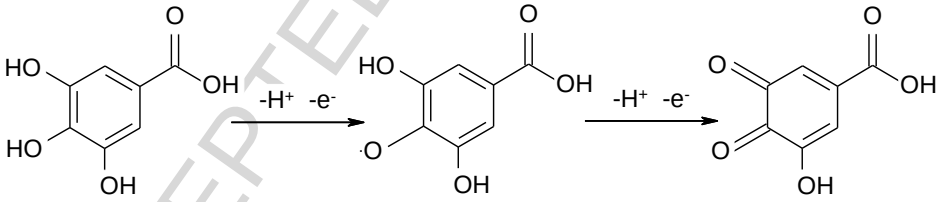
3.3. Analysis of real samples

In order to evaluate the real application of the proposed electrochemical methods, GA

equivalents were assessed in white wine, green tea, apple juice (using DPV method) and in red wine and human serum sample (using redox microsensor). The experiments were performed in triplicate. The results were compared with DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Di Lorenzo et al, 2017; Liu et al., 2013). The standard addition method was used, and the corresponding results are indicated in **Table 4** and **Table 5**. Higly significant correlations were found between DPV and DPPH (R^2=0.9977) and between redox microsensor and DPPH (R^2=0.9998) (**Figure 9** a, b).

4. Discussion

GA is an electroactive compound, characterized by specific redox reactions due to electroactivity resulting from the presence of three hydroxyl groups in the aromatic structure. The two GA hydroxyl groups are deprotonated while two electrons and two protons are generated; the GA is irreversibly oxidized first into a semiquinone radical and then into quinone, according to the equation below (1). The electron exchanges are monitored in the mentioned electrochemical detections because the current response is proportional to the GA concentration.



(1)

Gallic acid Semiquinone radical Quinone (Q)

Several studies have reported on the behaviour of different electrochemical compounds (Bungau et al., 2017; Miccoli et al., 2018; Badea et al., 2017; Rauf et al., 2016) and on the quantity of key phenolic compounds contributing to the total antioxidant capacity of wines (Photinon et al. 2010; Makhotkina and Kilmartin, 2009; Sousa et al., 2004). The main advantage in differential pulse techniques consists in the measurements of $\Delta i/\Delta E$ value, where Δi represents the difference between current intensity values, measured before and after the pulse period (Wang et al., 2012). Another important feature of differential pulse voltammetry is that the double intensity measurement offers peak-shaped results for intensity

dependence versus potential, with a maximum position correlated with the analyte type, and a height depending on the concentration of the active compound (Bungau et al., 2017; Miccoli et al., 2018; Badea et al., 2017; Sochor et al., 2013). An earlier study by Friedman and Jürgens (2000) which demonstrates that caffeic, chlorogenic, and gallic acids are not stable with respect to high pH and that the pH- and time-dependent spectral transformations are not reversible, led us to study, as well, the possible GA detection in different buffers (pH 5.8; 7; 8). Our findings are similar to other previously reported data for GA detection using different methods and/or electrodes (**Table 6**).

The results obtained with screen-printed carbon electrodes and a portable PalmSens system) enabled us to detect GA in all three pH conditions, at room temperature in the dark. We also exploited the area under the anodic current wave as in other studies (Chevion et al., 2000), considering it a suitable parameter similar to peak current intensity, as the total antioxidant capacity of the sample. It appears that for GA, the phosphate buffer pH 7.0 indicated the best analytical detection for the first specific signal, 0.175 ± 0.024 V. Both the linearity established for a wide range of concentrations (0.1 - 2.0 mM) and the stability of the sample were good. Both calibration lines (using peak height and area) indicated low LOD and LOQ values. The GA tested in buffer pH 8.0 indicated two different peaks that can be used for future detection studies, but for a reduced range of linearity (0.1 - 1.0 mM and 0.1 - 0.8 mM for peak height and area, respectively) it showed the same previously tested experimental conditions (pH 5.8; 7). The results obtained in pH 5.8 conditions are good and acceptable (low LOD and LOQ values); the sample is slightly more stable than in the other experiments (only one significant peak was found) due to the medium-acidity of the buffer.

It must be underlined that the appearance of peaks determines improvement in resolution and indicates greater sensitivity versus cyclic voltammetry. Also, to be stressed is that microsensors are important in real complex matrices such as those from fruits and their juices (Blasco et al., 2004), as well as in alcoholic beverages such as wines (Aguirre et al., 2010). The electron transfer from electroactive compounds in aqueous solutions to the electrode surface is important for a high-performance electrochemical sensor and must offer a proper affinity and good electronic conductivity (Kahl et al., 2014).

As most antioxidant molecules have functional groups with acid-based properties (hydroxyl, carboxyl), we also tested the pH influence on the GA analytical signal. Research in the field (Arteaga et al., 2012) has indicated that the peak potential values tended to become less positive as the pH values increased, and electro-oxidation took place more easily. Certain studies have reported GA as using electro-oxidation on a carbon nanotube-modified carbon paste electrode, in a buffer solution with pH 2.50, at 350 mV using Ag/AgCl as the reference electrode (Carneiro et al., 2018).

As in our study, Carneiro et al. (2018) obtained a voltammetric response in GA detection, using microfluidic electro-analytical devices made on a 3D printer (using ABS polymer-acrylonitrile butadiene styrene, combined with cotton yarns as microchannels). Used as an electrochemical detector, these devices demonstrated that the electrochemical oxidation of GA is characterized by two irreversible anode peaks, the first being attributed to the formation of the semiquinone radical, followed by oxidation in its quinone form (Yilmaz et al., 2013). Similarly to ours, these studies also showed that when the carrier buffer pH increased, the peaks (obtained using voltammetry) shifted to less positive potentials, indicating an easier electron donation.

Differential pulse polarography in 0.04 M Britton-Robinson buff er (pH 10.0) indicated high sensitivity for GA determination as a standard phenolic antioxidant. For analysis at -160 mV potential, low interferences were observed with other compounds: ascorbic, oxalic, benzoic, tartaric and ethylenediamine tetra-acetic acids that made this technique useful for fruit juice analysis. The peak potential dependence on pH showed two linear portions broken at pH = 10.5, which corresponds to the GA pKa value that is related to its acid dissociation constant (Vilian et al., 2018). As Table 3 shows, ascorbic and caffeic acids did not interfere with DPV detection of GA in terms of selectivity, a conclusion similar to ones reached in other experiments using salt-templated 3D porous carbon electrode (Vilian et al., 2018).

For analysis of the second sets of experimental data (presented in Figure 8.a-c), detection of GA antioxidant status using the Lazar Model ORP-146C micro oxidation-reduction electrode, indicated good linearity ranges, depending on the pH value of the media (Table 3) due to its different redox reactivity. The fluoropolymer capillary tube of

the microsensor device makes it unbreakable; being more stable than the glass electrodes it thus contributes to a much more stable redox reading. The microsensor can also be connected to a computer, thus the redox potential can be continuously monitored on a PC and a real time curve registered if necessary (http://www.shelfscientific.com/cgi-bin/tame/newlaz/redoxn.tam).

Use of this type of microsensor is rapid for sample analysis and can be applied even to small sample volumes since the immersion depth for the micro-redox electrode is 0.5 mm. It can thus be used in 96 well plates, micro test tubes, serum cups and in capillary, NMR and microcentrifuge tubes. LOD and LOQ values calculated for GA detection were higher than with the previously tested method using DPV. This method made it possible to indicate only the potentials corresponding to electroactive solutions as their total antioxidant status. This will also be important in real samples (extracts, wines), when mixtures of electrochemical compounds are included.

The redox microsensor has also shown good results in estimating the antioxidant capacity index of real samples of white wine and serum. In this situation, similar to other methods used for detection of total antioxidant capacity/status (Di Lorenzo et al, 2017), the real sample data for quantitative estimation of antioxidants is reported as a GA equivalent **(**GAE) (using interpolation by means of a calibration line constructed on a GA concentration base) as recommended in the literature (Sen and Sarkar, 2013). Ideal for measuring the midpoint potential of cytochromes, this redox microsensor may be used successfully in different areas such as biotechnology, biomedical sciences and pharmaceutical or environmental studies.

The novelty of the results obtained in these studies consists in the possible use of the electroanalytic devices in point-of-care applications or in rapid food analysis control, without pre-treatment of surface electrodes (Randviir et al., 2013; Metters et al., 2012; Metters et al. 2011). Since the components (carbon screen-printed sensors and Lazar microsensors) are commercially available, the devices and the proposed methods may be used by scientists in analytical laboratories. However, if the surface electrode undergoes modifications and microsensor characteristics thus improved, costs will naturally rise, and the sensors may

become too expensive for some laboratories. The significance of the electrochemical results was demonstrated in previous studies through correlation studies with the results obtained with conventional spectrophotometric assays for polyphenols using Folin-Ciocalteu procedure (absorbance at 280 nm) (Sanchez et al, 2013), classical spectrophotometric ABTS assay (Chaisuksant et al., 2012) or DPPH method (Di Lorenzo et al., 2017). For our studies, a spectrophotometric method with DPPH (as validation procedure) was used, as previously validated for wine samples (Di Lorenzo et al., 2017). Highly statistic correlations were obtained between DPV and DPPH (R^2=0.9977) and between redox microsensor and DPPH (R^2=0.9998)

The proposed electrochemical protocols for both tested methods are facile, fast and offer the possibility of GA sensing with deviations (RSD%) less than 9% in repeatability between measurements. The reproducibility of GA detection by means of screen printed electrodes was studied using seven electrodes and the relative standard deviation (RSD) was 4.36%. The methods were validated by analysing real samples: wine, green tea, apple juice and serum fortified with GA. The results obtained are similar to other reports (Di Lorenzo et al., 2017; Fernandes and Salgado, 2016). Advantages and drawbacks of these two methods are presented in **Table 7**.

5. Conclusions

We have studied two types of devices that may be used for GA detection: Lazar ORP-146C reduction-oxidation microsensors coupled with a Jenco device to read potential applications for estimation of antioxidant capacities of different electroactive media, and screen-printed carbon sensors coupled with a mobile PalmSens device using differential pulse voltammetry for qualitative and quantitative GA detection. As it is well known, this electrode is cost effective and gives an excellent performance for GA detection when compared with other previously reported chemically modified electrodes.

To the best of our knowledge, it is the first time that the electrochemical methods proposed in this study, under experimental conditions mentioned above, are published in the scientific literature. Compared to optical, mass and thermal sensors, they seem more attractive due to

their remarkable detectability, experimental simplicity and low cost. The suggested methods show many advantages such as fast responses, low detection limits, wide dynamic ranges, and good selectivity in applications demonstrated with a white wine sample. Our results demonstrated a promising feature for diverse life science applications (environmental, clinical, cosmetic, agricultural), thanks to the very good calibration lines obtained for different pH values, with GA being used as the reference standard and results expressed as GA equivalents.

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Contribution: All authors had equal contribution to this paper.

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List of figures

Table 1. Characteristics of DPV experiments for detection of GA (peak heights and areas) measured with screen-printed carbon electrodes, at different pH values

pH	Principal Potential (V)	Linearity range (mM)	Calibration based on	R2	Sensitivity	LOD (mM)	LOQ (mM)	$t_{90,\ max}$ (s)
5.8	0.180±0.021	0.1-2.0	Peak height	0.9935	2.808 μA/mM	0.033	0.099	66.2±1.89
			Peak area	0.9822	0.5307 μA·V/mM	0.055	0.165	
7	0.175±0.024	0.1-2.0	Peak height	0.9915	6.4533 μA/mM	0.032	0.096	65.75±2.16
			Peak area	0.9955	1.3665 μA·V/mM	0.023	0.070	
	0.465±0.056	0.1-2.0	Peak height	0.9607	0.348 μA/mM	0.082	0.248	91.85±5.04
			Peak area	0.9615	0.0379 μA·V/mM	0.102	0.310	
8	-0.037±0.003	0.1-1.0	Peak height	0.9947	0.8258 μA/mM	0.066	0.201	46.67±0.27
			Peak area	0.9689	0.0373 μA·V/mM	0.096	0.354	
	0.137±0.017	0.1-0.8	Peak height	0.9792	9.447 μA/mM	0.058	0.176	62.33±1.53
			Peak area	0.9983	1.5519 μA·V/mM	0.046	0.138	

Table 2. Potentials for the peak of DP voltammogram (V) for gallic, caffeic, and ascorbic acids (0.3 mM) detection

pH	Potentials for peak of DP voltammogram (V)		
	Gallic acid 0.3 mM	**Caffeic acid 0.3 mM**	**Ascorbic acid 0.3 mM**
5.8	0.199±0.012	0.116±0.004	- 0.0136±0.001
7	0.189±0.014 0.453±0.014	0.057±0.001	0.0498±0.004
8	-0.034±0.002 0.133±0.012	0.121± 0.004	0.089±0.003

Table 3. Characteristics of GA detection using redox microsensors, at different pH values

pH	Linearity range (mM)	R^2	Sensitivity (mV/mM)	LOD (mM)	LOQ (mM)
5.8	0.2-2	0.9902	-5.4529	0.049	0.148
7	0.1-2	0.9324	-22.025	0.109	0.331
8	0.1-1.5	0.9576	-43.856	0.074	0.223

Table 4. Detection of gallic acid in real samples (**N=3)** using DPV method with carbon screen printed electrodes, pH 7

Sample	Spiked sample GA (μM)	Content determination					
		DPV method			DPPH method		
		Total found (μM) mean(±SD)	Recovery degree (%)	RSD (%)	Total found (μM) mean(±SD)	Recovery degree (%)	RSD (%)
White wine*	0	6.1(±0.5)	-	8.2	7.0(±0.3)	-	4.3
	100	104.2(±1.1)	98.2	1.1	111.2(±0.4)	103.9	0.4
Green tea juice**	0	28.5(±0.7)	-	2.5	32.4(±0.6)	-	1.9
	100	131.2(±0.8)	102.1	0.6	131.1(±0.5)	99.0	0.4
Apple tea juice**	0	52.8(±1.2)	-	2.2	54.3(±0.8)	-	1.5
	100	151.1(±0.7)	98.9	0.5	158.5(±0.8)	102.7	0.5

* Homemade wine; **sample from local supermarket

Table 5. Detection of gallic acid in real samples (**N=3)** using spiked procedure and corresponding recovery, with redox microelectrode, at pH 5.8

Sample	**Spiked sample GA (μM)**	**Content determination**					
		Redox microelectrode method			**DPPH method**		
		Total found (μM) mean(±SD)	**Recovery degree (%)**	**RSD (%)**	**Total found (μM)** mean(±SD)	**Recovery degree (%)**	**RSD (%)**
White wine	0	6.4(±0.5)	-	7.81	7.0(±0.3)	-	4.28
	200	204.2(±0.5)	98.9	0.24	223.8(±0.4)	108.1	0.18
Serum	0	1912.3(±0.7)	-	0.04	1890.5(±0.6)	-	0.04
	200	2100.8(±0.7)	109.9	0.04	2110.2(±1.2)	100.9	0.06

Table 6. Reported data for the electrochemical detection of GA

Electrode	Method	Sample	Characteristics	Reference
A functionalized graphene oxide/poly(p-amino-hippuric acid)-sodium dodecyl sulfate nanocomposite modified glassy carbon electrode (APTS@GO/PPAH-SDS/GCE)	CV (cyclic voltammetry), DPV (differential pulse voltammetry), amperometry	Black tea Tab water	Linear range of 0.006-2000 μM; LOD 1.7 nM for GA (S/N=3) using amperometric method; Determination in the as real samples	(Baghayeri et al., 2018)
Carbon paste electrode (CPE) modified by nanocomposite containing zirconia nanoparticles (ZrO(2)NPs), choline chloride (ChCD) and gold nanoparticles (AuNPs) to construct ZrO2-ChCI-AuNPs/CPE	DPV	Green tea Fruit juice Human urine samples	Linear response 0.22- 55 μM; LOD 25 nM; Applied for the independent simultaneous determination of GA and UA (uric acid)	(Shahamirifard et al., 2018)
Modified GC electrodes (titanium nitride) or wolfram carbide-doped reduced graphene oxide, labeled as TNrGO and WCrGO, respectively	CV	Sweet wines	LOD 1.1 μM and 3.1 μM for TNrGO and WCrGO, respectively; Linear ranges: for TNrGO 4.5-76 μM and for WCrGO 10-100 μM	(Stanković et al., 2017)
Glassy carbon electrode modified with multi-walled carbon nanotubes and poly-quercetin (polyquercetin/MWNT/GCE)	DPV	Tea	The linear dynamic range 10-750 μM; LOD 0.10 μM; The tea antioxidant capacity evaluation (AOC).	(Ziyatdinova et al., 2017)
Organic electrochemical transistors (OECTs) based on poly(3,4-ethylenedioxythiophene): poly (styrene sulfonic acid) (PEDOT: PSS) Functionalizing the gate electrodes of the OECTs with nanocomposites of poly (diallyl dimethylammonium chloride) (PDDA) and multiple carbon nanomaterials	Doping/de-doping of the channel upon gate polarization	Tea	LOD 10 nM of GA; Rapid and accurate assessment of total phenol content of practical tea samples	(Xiong et al., 2017)

Activated screen printed carbon electrode (ASPCE)	Amperometry	Green tea Apple juice	Linear range 0.01-1799.6 μM LOD 0.031 μM	(Raja et al., 2017)
Multiwalled carbon nanotube-modified carbon paste electrode (MWCNT-CPE), and MIP and MWCNT-modified CPE (MIP-MWCNT-CPE)	Electrochemical detection	Apple, pineapple, orange juices A commercial green tea drink	Nano-sensors (MIP-MWCNT-CPE) have higher sensitivity compared with bare CPE, MWCNT-CPE, and MIP-CPE; Linear range 0.12-380.0 μM LOD 47.0 nM	(Shojaei et al., 2016)
Glassy carbon electrode modified with cerium dioxide nanoparticles in a 0.02 M BrijA(R)35	DPV	Spices	pH 7.4; Analytical range 50-2490 μM; LOD 11.9 μM	(Ziyatdinova et al., 2016)
A modified electrode prepared by modification of the CPE with graphene nano-sheets	DPV	plant samples	Analytical range 0.3-150 μM; LOD 0.11 μM	(Sheikh-Mohseni, 2016)
Gold micro-clusters (AuMCs) electrodeposited on sulfonate functionalized graphene (SF-GR) electrode	DPV	Black tea Cortex mouton Urine samples	pH 4; Linear range 0.05-8.0 μM; LOD 10.7 nM	(Liang et al., 2016)
Single-walled carbon nanotubes (SWCNT) covalently functionalized with polytyrosine (Polytyr)	Amperometry	Tea extracts	Linear range 0.5- 170 μM; LOD 8.8 nM Determination of total polyphenolic	(Eguílaz et al., 2016)
Thin electroactive poly(melamine) film immobilized on a pre-anodized screen-printed carbon electrode (SPCE*/PME)	FIA (flow-injection amperometry)	Green tea	Linear response to GA in pH 3 and pH 7.0; LOD 0.076 μM; RSD 3.9% is obtained for 57 successive measurements of 50 mM GA in pH 7	(Su and Cheng, 2015)
Gel-RGONS modified glassy carbon electrode (Gel-RGONS/GCE)	LSV (linear sweep voltammetry)	Black tea	Linear response 1.0 – 100 μM; LOD 0.47 μM	(Chekin et al., 2015)
The hybrid material of chitosan (CS), fishbone-shaped Fe2O3 (fFe(2)O(3)), and	DPV	Red / white wines	Linear relationship with the logarithmic values of GA concentration over the range from 1-100	(Gao et al, 2015)

electrochemically reduced graphene oxide (ERGO) modified glassy carbon electrode			μM; LOD 0.15 μM; Antioxidant capacity index of real samples	
SiO2 nanoparticle modified carbon paste electrode using multiwalled carbon nanotubes (MWCNTs) and graphite	Electrochemical	Tea Orange juice samples	Concentration range 0.8 – 100 μM; LOD 0.25 μM; Sensitivity 1790.7 μA/mM	(Tashkhourian and Nami-Ana, 2015)
Electrochemical sensor based on [Cu(2)tpmc](ClO4)(4) immobilized in PVC matrix and coated on graphite (CGE) or classy carbon rod (CGCE)	DPV	White, rose and red wine samples	Voltammograms for both sensors, recorded in HNO_3 (supporting electrolyte), at pH 2 and analytical range 0.25-100 μM; 2 linear calibration curves (for higher and lower GA concentration range); LOD for CGE 0.148 μM; LOD for CGCE 4.6 μM; Determination of the antioxidant capacity based on GA equivalents for	(Petković et al., 2015)
Electro-generated o-dianisidine derivative (o-DD)-stabilized multi-walled carbon nanotube (MWCNT)-modified glassy carbon electrode (GCE/o-DD@MWCNT)	Chronoamperometry	Real grape juice Water samples	Detection at 0.16 V vs. Ag/AgCl in pH 7 PBS; Sensitivity 0.4580 μA /μM; Detection range of 100-1300 μM; LOD 144 nM	(Sundaram et al., 2015)
Electrochemical sensor based on poly-epinephrine /glassy carbon electrode, PEP/GCE	AdSV (adsorptive stripping voltammetry)	Black tea sample	Linear range 1.0 - 20.0 μM LOD 0663 μM	(Abdel-Hamid et al., 2013)
Glassy carbon (GC) or platinum (Pt) working electrode	CV amperometry	Tea	Optimum response at pH 5.8; Linear range 1-250 μg/mL; LOD 2.34 μg /mL Quantitative estimation of antioxidants in samples were expressed as gallic acid	(Sen and Sarkar, 2013)

			equivalent (GAE)	
Modified carbon paste electrode was prepared by incorporating the TiO2 nanoparticles in the carbon paste matrix	CV DPV EIS	Black/green teas	Buffered solutions with pH 1.7 Linear dynamic range 2.5 -150 μM; LOD 0.94 μM	(Tashkhourian et al., 2013)
Glassy carbon working electrode	CV and flow injection with amperometric detection (FI-AD)	Ginger infusion	pH 7.0; Antioxidant capacity of real sample was expressed as gallic acid equivalent (GAE) unit	(Chaisuksant et al, 2012)
Glassy carbon electrode	Flow-based coupled electrochemical technique - sequential injection (SI) with AD	Commercial instant ginger infusion beverages	pH 7.0; GA used as standard antioxidant and the capacity reported as gallic acid equivalent (GAE) unit	(Chan-Eam et al., 2010)
Sensor based on a modified carbon paste electrode with multi-walled carbon nanotubes	CV chronocoulometry, LSV, DPV disk electrode voltammetry	*Punica granatum, Myrtus communis, Itriphal extracts*	LOD 0.2 μM	(Ghoreishi et al., 2011)
Working electrodes - carbon paste electrodes (CPEs) prepared from 3 types of carbonaceous materials: graphite (G-CPE), carbon microspheres (μS-CPE) and carbon nanotubes (CNT-CPE); the reference electrode - Ag|AgCl/KClsat. and the counter electrode - platinum plate	CV	No real samples tested	An array of electrodes has been constructed using the three types of electrodes. LOD: 11.32 μM - G-CPE; LOD: 13.24 μM – μS-CPE; LOD: 13.26 μM - CNT-CPE.	(Apetrei et al., 2011)
Screen printed carbon electrode DRP C110	DPV	White wine	LOD: 23-103 μM (depending on pH – Table 1)	Our work

		Green tea Apple juice		
Redox microsensor - ORP-146C	Redox measurements	White wine Serum	LOD: 49-109 μM (depending on pH –Table 3)	Our work

Table 7. Advantages/drawbacks of the methods

	DPV method	**Redox microsensor**
Advantages	Working at different pH values: 5.8; 7; 8 Low LOD and LOQ Commercially available electrode Possibility to be used in telemonitoring (remote system) – PalmSens device with Bluetooth Fast Inexpensive Reproducible Applicable: case of biological samples Selective versus caffeic and ascorbic acid	Working at different pH values: 5.8; 7; 8 Low LOD and LOQ Commercially available electrode Portability – redox potential can be continuously monitored on a PC and a real time curve can be obtained Unbreakable because of fluoropolymer capillary tube of the microsensor device Fast Inexpensive Reproducible Applicable: case of biological samples Reusable electrode
Drawbacks	Single use electrode	Not selective – it could indicate the total antioxidant power/capacity, in GAE

Highlights

. Gallic acid is a well-known antioxidant found in tea leaves, fruits, fruit seeds and beverages

. Microsensors for estimation of antioxidant capacities of different electroactive media

. Screen-printed carbon sensors for qualitative and quantitative GA detection.

. The procedures may be further applied in other clinical, food or environmental analyses.

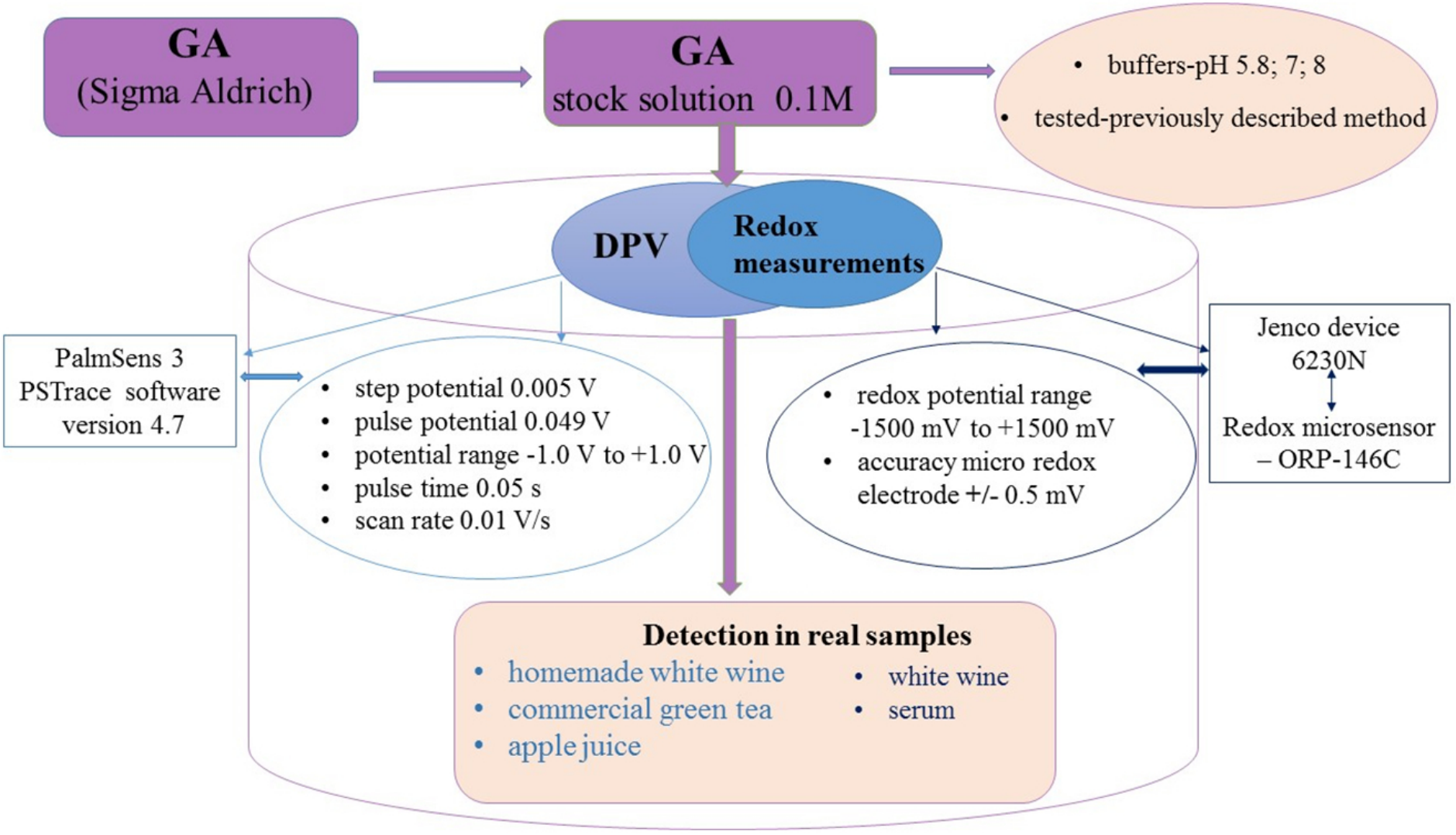

GA
(Sigma Aldrich)
GA
stock solution 0.1M
• buffers-pH 5.8; 7; 8
• tested-previously described method
DPV
Redox measurements
PalmSens 3
PSTrace software
version 4.7
• step potential 0.005 V
• pulse potential 0.049 V
• potential range -1.0 V to +1.0 V
• pulse time 0.05 s
• scan rate 0.01 V/s
• redox potential range -1500 mV to +1500 mV
• accuracy micro redox electrode +/- 0.5 mV
Jenco device
6230N
Redox microsensor
– ORP-146C
Detection in real samples
• homemade white wine
• commercial green tea
• apple juice
• white wine
• serum

Figure 1

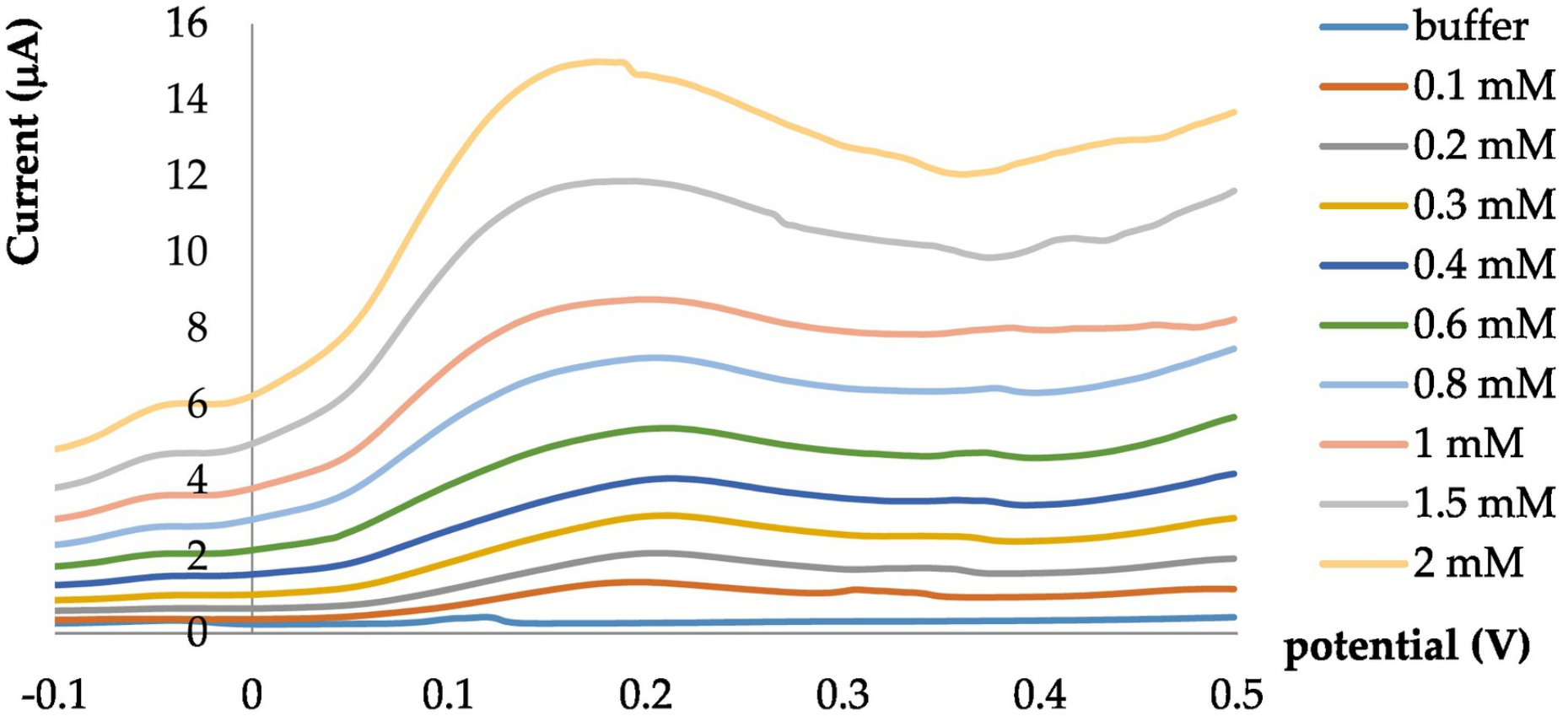
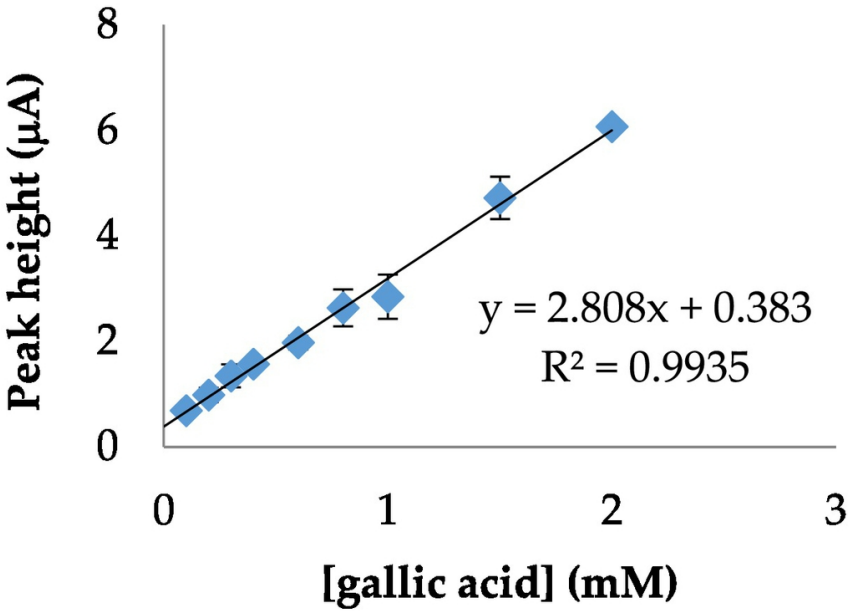
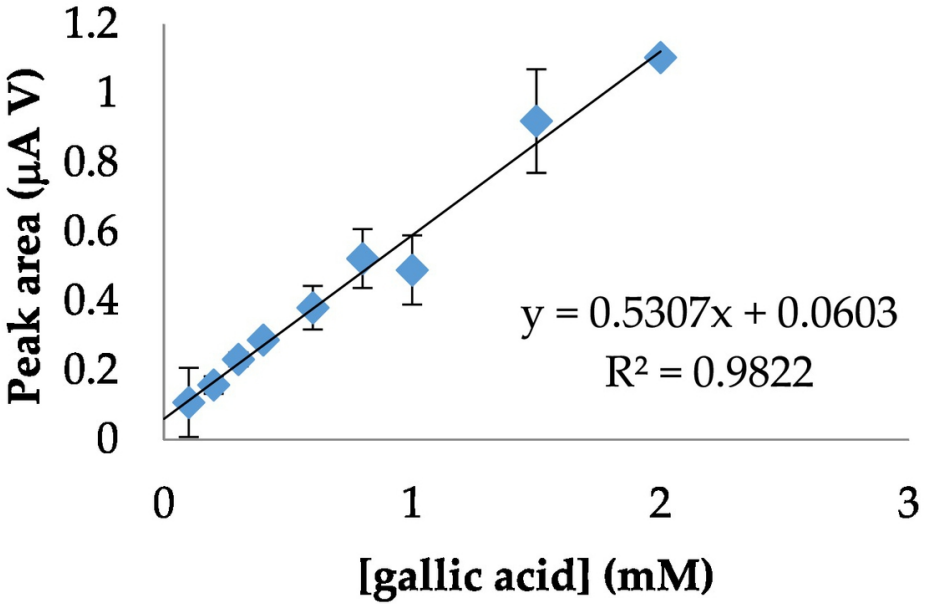

Current (µA)
16
14
12
10
8
6
4
2
0
-0.1
0
0.1
0.2
0.3
0.4
0.5
potential (V)
buffer
0.1 mM
0.2 mM
0.3 mM
0.4 mM
0.6 mM
0.8 mM
1 mM
1.5 mM
2 mM

Figure 2


Peak height (µA)
8
6
4
2
0
0
1
2
3
[gallic acid] (mM)
y = 2.808x + 0.383
R² = 0.9935

(a)


Peak area (µA V)
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
[gallic acid] (mM)
y = 0.5307x + 0.0603
R² = 0.9822

(b)

Figure 3

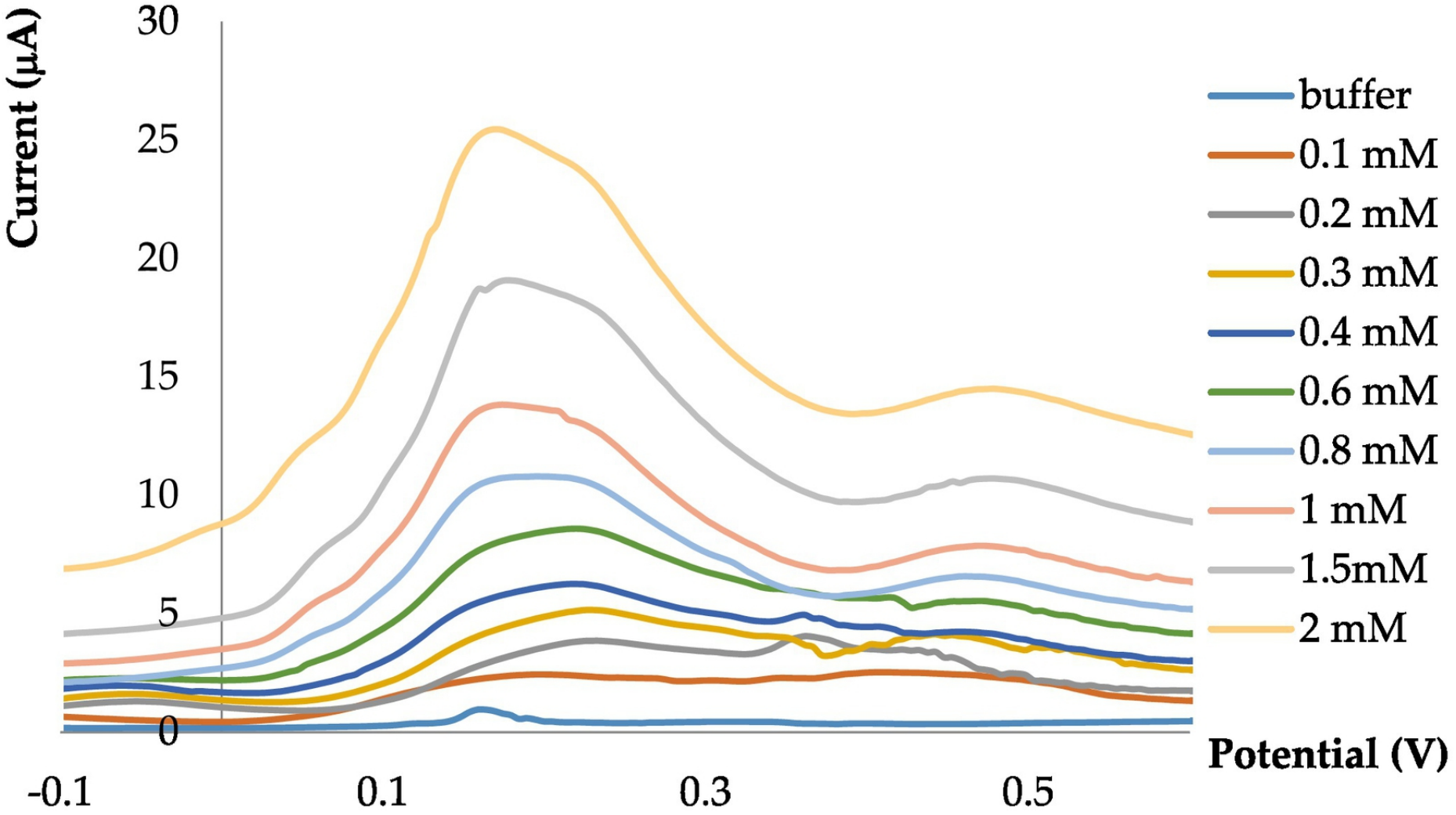
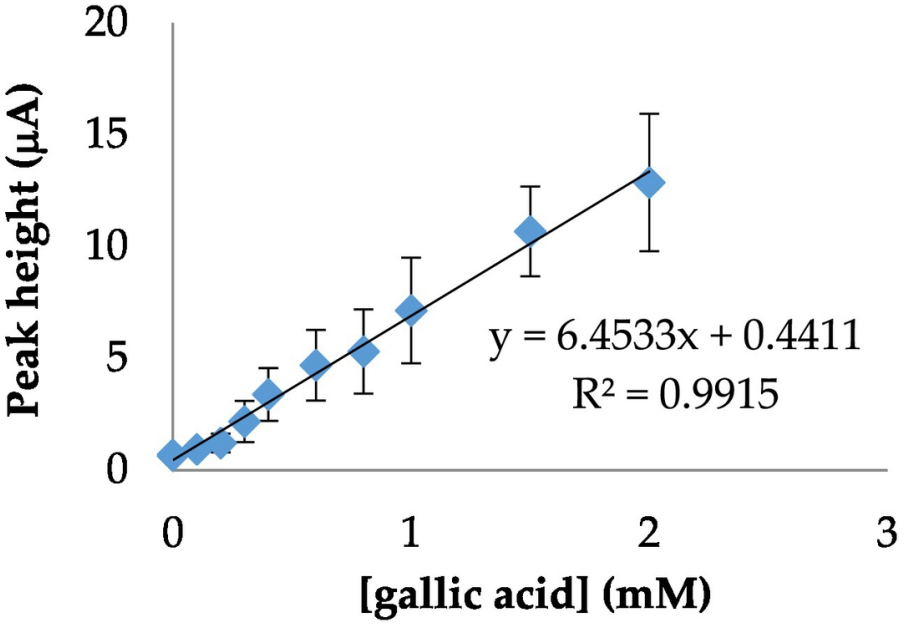
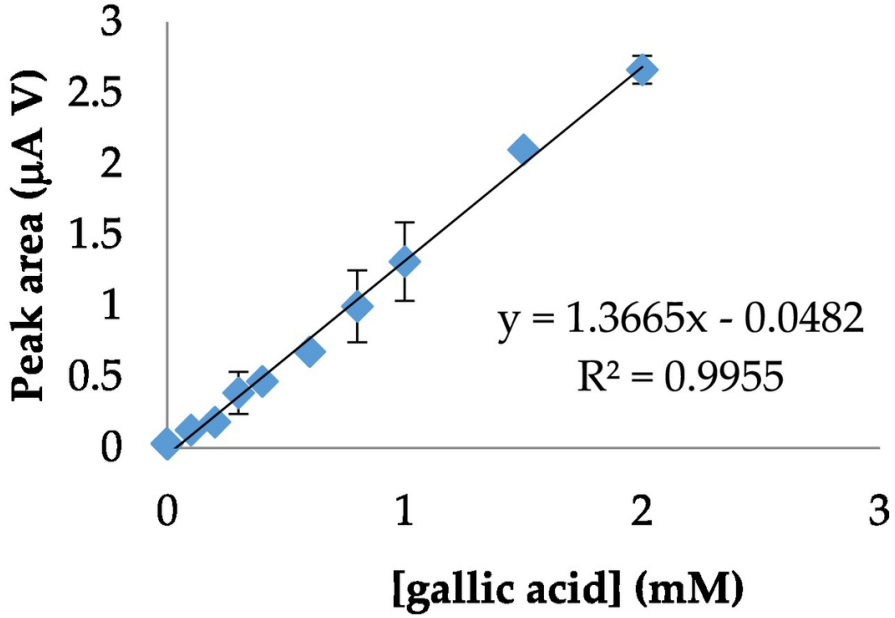

Current (µA)
30
25
20
15
10
5
0
-0.1
0.1
0.3
0.5
Potential (V)
buffer
0.1 mM
0.2 mM
0.3 mM
0.4 mM
0.6 mM
0.8 mM
1 mM
1.5mM
2 mM

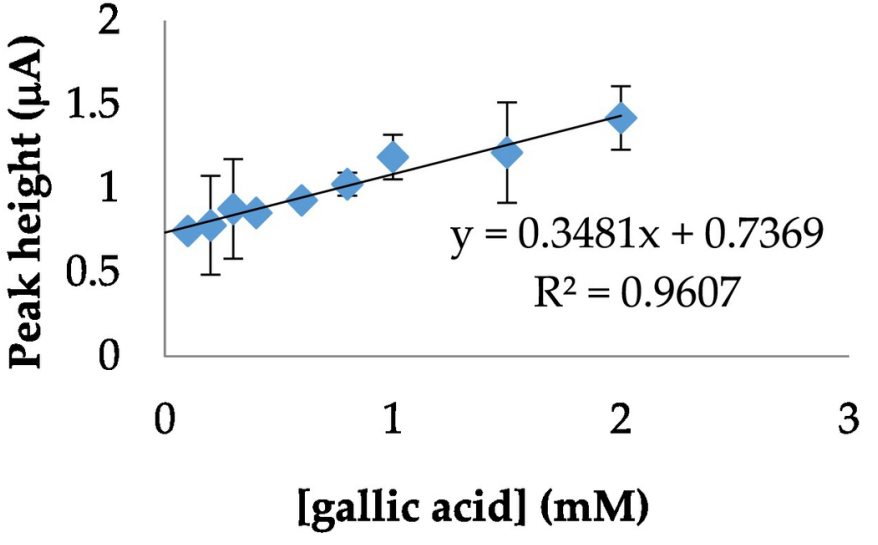
Figure 4



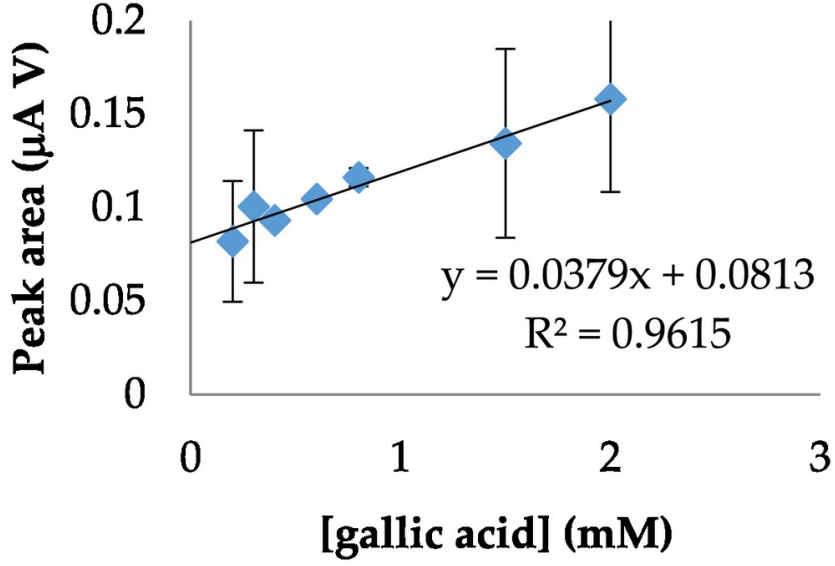
(a1)



(b1)



(a2)



(b2)

Figure 5

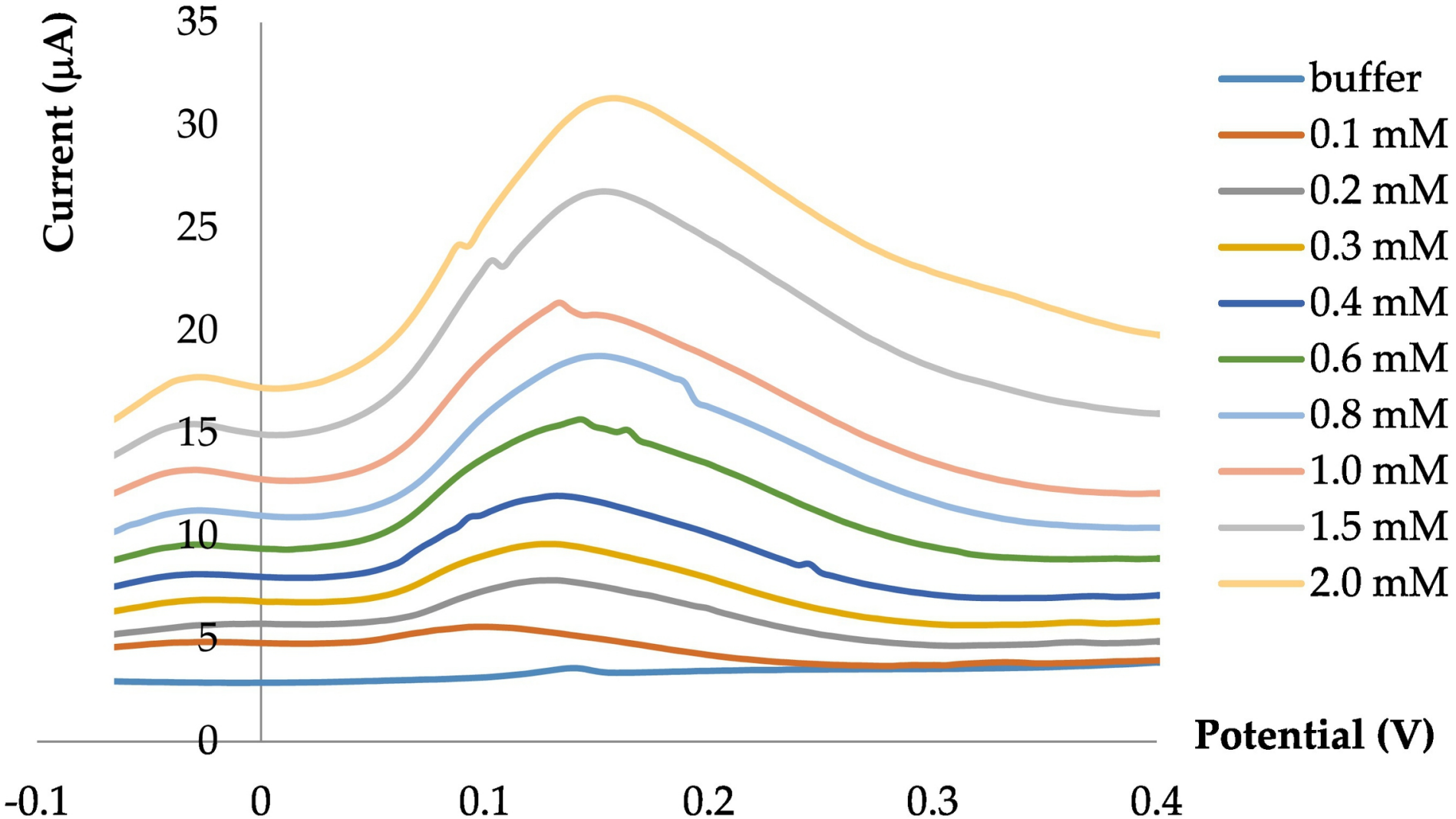
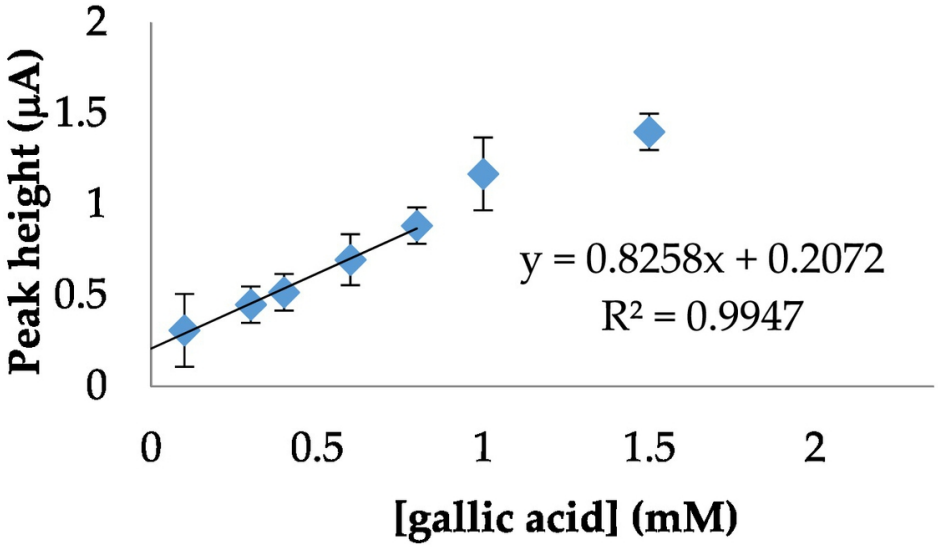
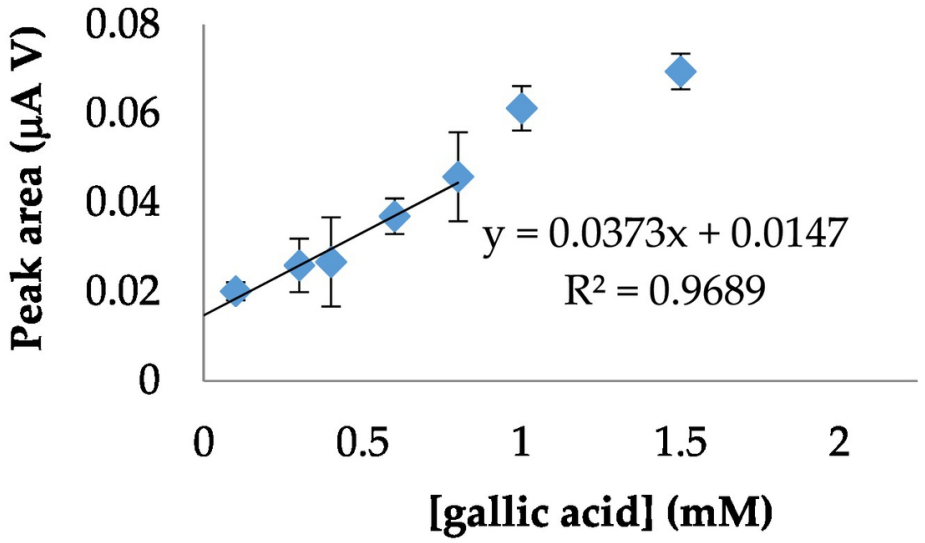

Current (µA)
35
30
25
20
15
10
5
0
-0.1
0
0.1
0.2
0.3
0.4
Potential (V)
buffer
0.1 mM
0.2 mM
0.3 mM
0.4 mM
0.6 mM
0.8 mM
1.0 mM
1.5 mM
2.0 mM

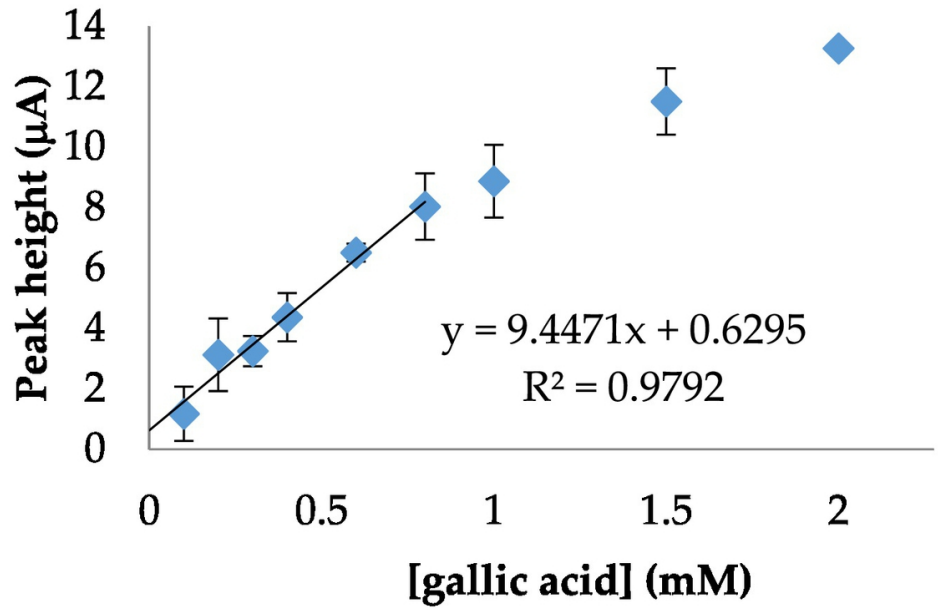
Figure 6



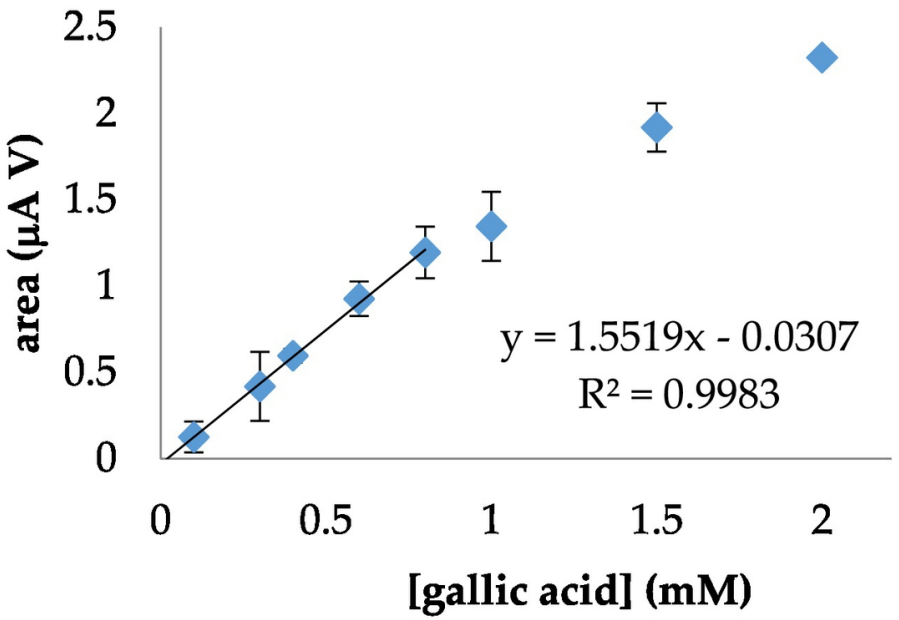
(a1)



(b1)



(a2)



(b2)

Figure 7

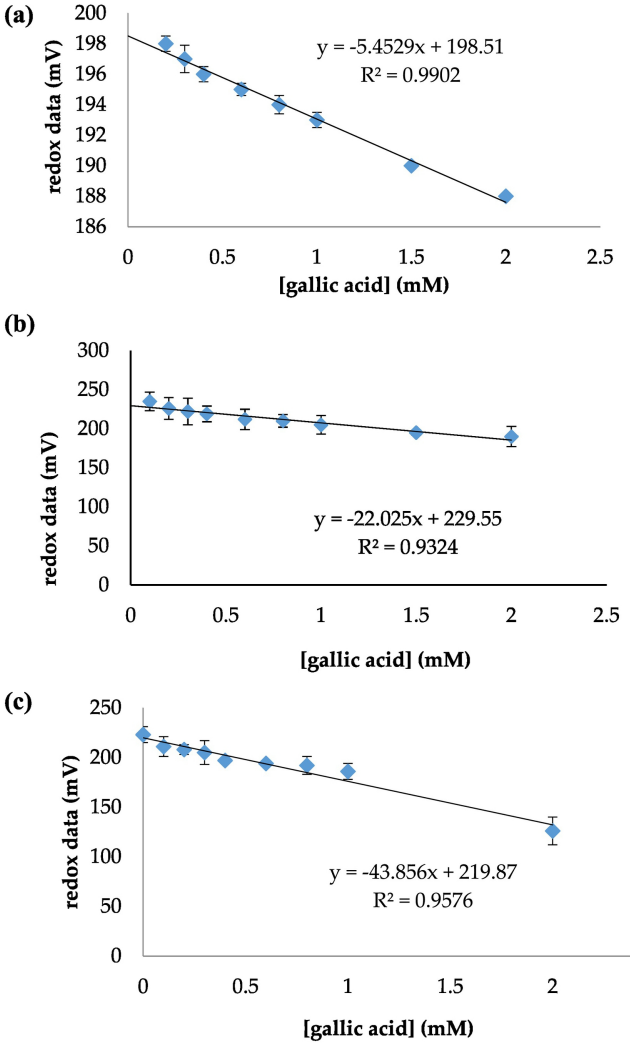
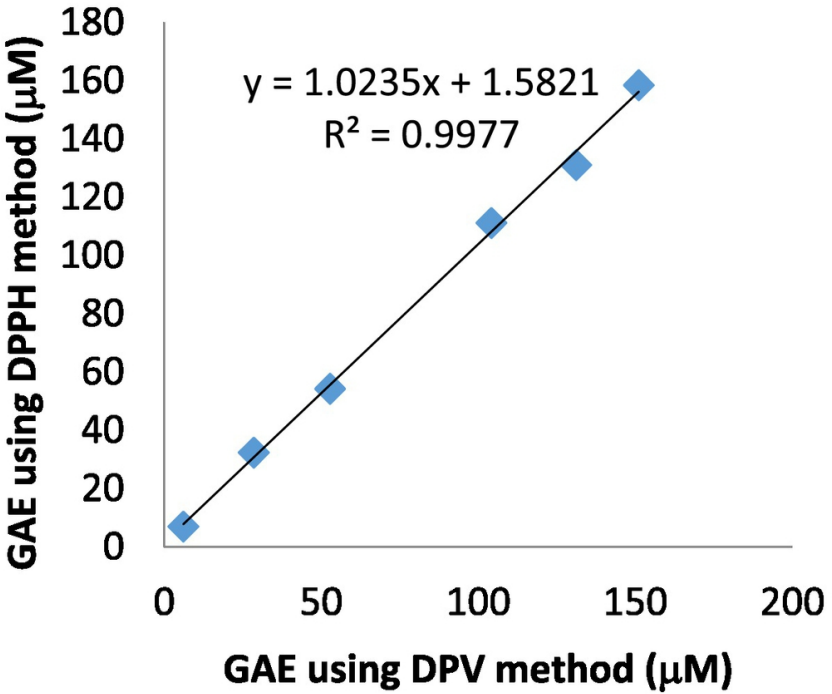
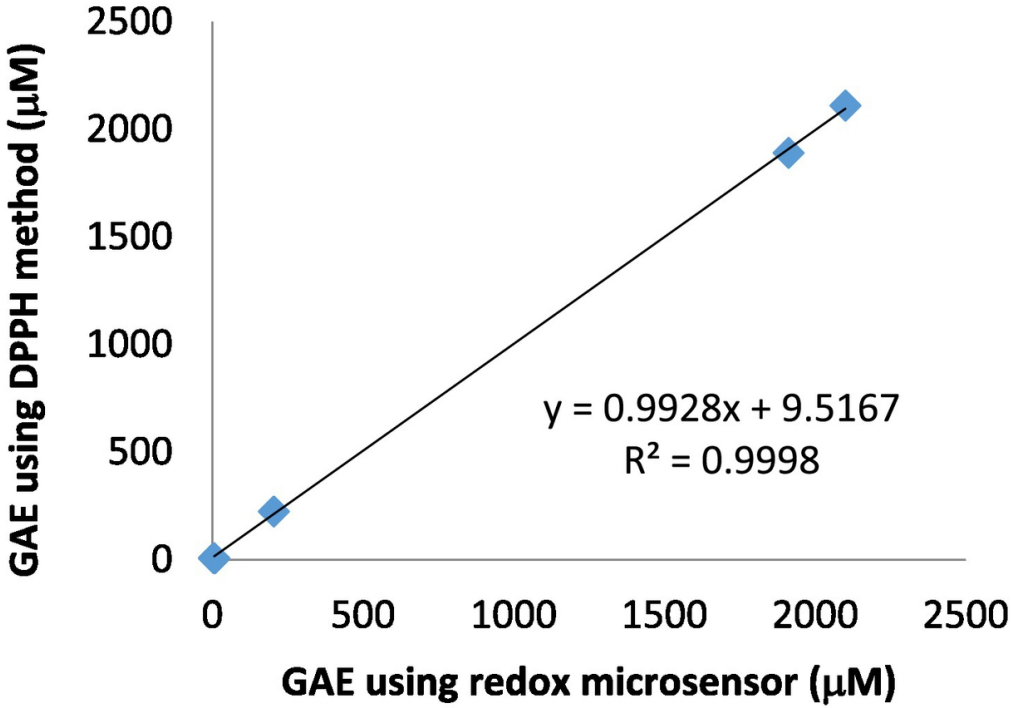

(a)
redox data (mV)
200
198
196
194
192
190
188
186
y = -5.4529x + 198.51
R² = 0.9902
0
0.5
1
1.5
2
2.5
[gallic acid] (mM)
(b)
redox data (mV)
300
250
200
150
100
50
0
y = -22.025x + 229.55
R² = 0.9324
0
0.5
1
1.5
2
2.5
[gallic acid] (mM)
(c)
redox data (mV)
250
200
150
100
50
0
y = -43.856x + 219.87
R² = 0.9576
0
0.5
1
1.5
2
2.5
[gallic acid] (mM)

Figure 8


y = 1.0235x + 1.5821
R² = 0.9977
GAE using DPPH method (µM)
GAE using DPV method (µM)
0
20
40
60
80
100
120
140
160
180
0
50
100
150
200

(a)


y = 0.9928x + 9.5167
R² = 0.9998
GAE using DPPH method (µM)
GAE using redox microsensor (µM)
0
500
1000
1500
2000
2500

(b)

Figure 9