Federico J. V. Gomez*
Adrian Spisso
María Fernanda Silva

Instituto de Biología Agrícola de Mendoza (IBAM-CONICET), Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina

Received April 25, 2017
Revised July 12, 2017
Accepted August 4, 2017

Research Article

Pencil graphite electrodes for improved electrochemical detection of oleuropein by the combination of Natural Deep Eutectic Solvents and graphene oxide

A novel methodology is presented for the enhanced electrochemical detection of oleuropein in complex plant matrices by Graphene Oxide Pencil Grahite Electrode (GOPGE) in combination with a buffer modified with a Natural Deep Eutectic Solvent, containing 10% (v/v) of Lactic acid, Glucose and H_2O (LGH). The electrochemical behavior of oleuropein in the modified-working buffer was examined using differential pulse voltammetry. The combination of both modifications, NADES modified buffer and nanomaterial modified electrode, LGH-GOPGE, resulted on a signal enhancement of 5.3 times higher than the bare electrode with unmodified buffer. A calibration curve of oleuropein was performed between 0.10 to 37 μM and a good linearity was obtained with a correlation coefficient of 0.989. Detection and quantification limits of the method were obtained as 30 and 102 nM, respectively. In addition, precision studies indicated that the voltammetric method was sufficiently repeatable, %RSD 0.01 and 3.16 ($n = 5$) for potential and intensity, respectively. Finally, the proposed electrochemical sensor was successfully applied to the determination of oleuropein in an olive leaf extract prepared by ultrasound-assisted extraction. The results obtained with the proposed electrochemical sensor were compared with Capillary Zone Electrophoresis analysis with satisfactory results.

Keywords:
Enhanced electrochemical detection / Graphene oxide / Oleuropein / Olive / NADES / Pencil graphite electrodes
DOI 10.1002/elps.201700173

1 Introduction

Oleuropein (OLE) is a secoiridoid polyphenol found only in plants belonging to Oleaceae family including olive tree (*Oleaeuropea L*). Its content is influenced by varietal and environmental factors. Several health benefits are attributed to OLE, including antioxidant, antiinflammatory, anticancer, antiviral, antimicrobial and antiatherogenic effects. Being oleuropein the most abundant biophenol in olive leaves [1–3], several relevant applications for the revalorization of olive oil industry by-products can be proposed. Olive leaves have been associated with human health since ancient times for its usage in folk medicine to treat several diseases [4]. Interestingly, it has been reported that the properties of olive leaf extracts include radio-protective [5] and anti-proliferative effects on leukaemia cells by inducing apoptosis [6], in addition to cytotoxic activity on human breast cancer cells [7, 8], and improvement of gentamicin nephrotoxicity [4, 9]. Indeed, the consumption of wine and olive products is related with the French Paradox (the observation that French people have a relatively low incidence of coronary heart disease while having a diet relatively rich in saturated fats). Thus, very interesting possibilities arise for the use of olive cake and olive mill wastewater extracts as food additives or pharmaceuticals.

Reliable, sensitive and robust methodologies for the rapid determination of OLE in olive leaves, olive oil industry by-products, functional foods and pharmaceuticals are needed. OLE has been determined by classical approaches, including liquid chromatography and electrochemical techniques [1, 3, 10–12].

Electrochemical methods provide low-cost, fast and simple alternatives in the trace analysis of bioactive compounds [1]. Electrode modifications can provide extraordinary advantages over conventional electrodes in terms of sensitivity and electrochemical performance including enhanced selectivity and catalytic activity. In this sense, nanomaterials offer specific electroanalysis properties that are exclusive for the nanoscale. Graphene, a single layer of carbon atoms with a

Correspondence: Dr. María Fernanda Silva Instituto de Biología Agrícola de Mendoza (IBAM-CONICET), Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Alte. Brown 500, Chacras de Coria, Mendoza 5505, Argentina
E-mail: msilva@fca.uncu.edu.ar

Abbreviations: **BRB**, Britton-Robinson buffer; **GOPGE**, Graphene Oxide Pencil Grahite Electrode; **GPL**, Graphite pencil leads; **NADES**, Natural Deep Eutectics Solvents; **OLE**, Oleuropein; **PGE**, pencil graphite electrode

*Additional corresponding author: Dr. Federico J. V. GomezE-mail: fedeg33@gmail.com

Colour Online: See the article online to view Figs. 1, 4 and 5 in colour.

honeycomb 2D lattice, has attracted considerable attention in recent years as electrode modifier due to large surface to volume ratio and high conductivity and electron mobility at room temperature [13–15]. Nevertheless, there are no reports concerning the use of graphene as electrode modifier for electrochemical determination of OLE.

Besides the common electrochemical characteristics of carbonaceous electrode materials, the pencil graphite electrode (PGE) offers outstanding further analytical benefits including good mechanical stability, high signal to noise ratio, disposability, commonly commercially availability and extremely low cost [16].

In a previous work [17], we demonstrated a new application for Natural Deep Eutectics Solvents (NADES); as enhancer agents for electrochemical detection of quercetin and another polyphenols. However, the use of NADES as potential enhancers of PGE approaches has not yet been evaluated. NADES are constituted of metabolites that are naturally present in all types of cells and organisms [18]. The applications of NADES as a green alternative to conventional solvents have dramatically expanded since they were coined in 2011 [19] because of their excellent properties such as non-volatility, low costs, biodegradability, non-toxicity, sustainability, and simple preparation methods. When certain cell primary metabolites such as sugars, organic acids, urea and choline chloride are mixed together there is a considerable reduction of the melting point and consequently the formation of liquids even at very low temperatures [19, 20]. NADES are composed of a mixture consisting of a hydrogen bond acceptor (HBA), with a hydrogen bond donor (HBD). Thus, their physicochemical properties are "tunable" by optimizing the chemical nature of the mixture, synthesis procedure and water content [21].

To our knowledge there is no literature evidence concerning the combination of NADES with nanomaterials modified electrodes. Thus, the main objective of the present work was to develop a simple, low cost and reliable methodology for the enhanced electrochemical detection of OLE in complex matrices by Graphene Oxide Pencil Grahite Electrode (GOPGE) in combination with a buffer modified with Natural Deep Eutectic Solvents. Our results indicated substantial improvement at sensitivity and selectivity compared to bare PGE. The proposed methodology was successfully applied for the determination of OLE in olive leaf extracts obtained by ultrasound assisted extraction. Indeed, the results obtained with the proposed electrochemical sensor were compared with a capillary zone electrophoresis (CZE) method for OLE.

2 Materials and methods

2.1 Reagents

OLE was purchased from Sigma Chemical (St. Louis, MO, USA). D (-) Fructose, D (-) Glucose, Lactic acid and Citric acid were obtained from Biopack (Buenos Aires, Argentina). Sodium hydrogen phosphate and orthophosphoric acid were purchased from Carlos Erba Reagents (Milano, Italy). Boric and acetic acids from J. T. Baker (Xalostoc, Mexico), potassium chloride and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Ultrapure water (18 MΩ cm) was obtained from Milli-Q system (Millipore, Bedford, MA, USA).

2.2 Equipment

All electrochemical measurements were performed, at room temperature, on an USB-based portable electrochemical station μStat 200 Bipotentiostat (Dropsens, Oviedo, Spain) controlled by DropView 200 software. A conventional three-electrode system was consisted of disposable pencil graphite electrode (PGE) as the working electrode, with an Ag/AgCl/3 M KCl as a reference electrode and a platinum wire as the auxiliary electrode. A Plantec mechanical pencil, Model 9512 (Argentina), was used as a holder for the pencil lead. Measurements were performed in a glass cell containing 5 ml of solution. During DPV measurements, stirring was achieved with a magnetic stirring bar.

CE measurements were carried out using a Capel 105 M (Lumex, St Petersburg, Russia) equipped with an UV detector and a 0–25 kV high-voltage power supply. The data were collected on a PC configured with Elforun software (version 3.2.2). The capillary columns used for separation were bare fused-silica capillaries 57 cm full length, 50 cm effective length, 75 μm ID and 375 μm OD from MTC MicroSolv Technology Corporation (Eatontow, USA). The capillary tube was conditioned daily prior to its use by flushing with water (2 min), 0.10 M NaOH for 3 min, followed by water for another 2 min and, finally, with the running buffer (Boric acid pH = 9) for 4 min. The separation voltage was 20 kV and the capillary temperature was 25°C. Samples were injected by hydrodynamic injection at 30 mbar for 3 s. Electropherograms were recorded at 254 nm. Between runs, the capillary was flushed with water (2 min), 0.10 M NaOH (2 min), water (2 min) and fresh buffer (2 min). The capillary tube was rinsed with 0.10 M NaOH for 10 min, then with water for 10 min, every day after use.

Scanning electron microscope (SEM) characterization was made with a JEOL JSM-6610 Series Scanning Electron Microscope (JEOL, Tokyo, Japan) with the acceleration voltage 5.0 kV with 1 μm resolution.

2.3 NADES synthesis

The optimized NADES synthesis was carried out easily following the heating and stirring method described by Dai et al. [20]. Three different NADES were prepared using inexpensive and natural components, in the following combinations and ratios: Citric acid, Glucose and H_2O (CGH, 1:1:2); Lactic acid, Glucose and H_2O (LGH, 5:1:3); Citric acid, Fructose and H_2O (CFH, 1:1:2). The two-component mixture with calculated amounts of water were placed in a bottle with a stirring bar and cap and heated in a plate at 80°C with agitation

till a clear liquid is formed (60 min). The stability of synthesized NADES was tested and they were stable for at least 2 months after its preparation [17].

2.4 Samples

Olive leaves samples were cut into small pieces, lyophilized and then the dried samples were homogenized with liquid nitrogen. The homogenates were extracted with 1 mL of 80% ethanol or 80% methanol. For this purpose, 100 mg of the homogenate were transferred to an extraction tube with the solvent, vortexed and then the extraction was accelerated by ultrasonication for 60 min at 35°C. The leaves extract was stored at −18°C until use.

2.5 PGE preparation

The pencil lead can be extruded to different lengths, to yield different surface areas. As expected, the length of the pencil lead (exposed to the sample) has a profound effect upon the response. Thus, the graphite leads were cut into half and introduced into a mechanical pencil holder so that 1.5 cm of the pencil lead remains outside. The PGE was connected to the instrument through a metal wire soldered at the metallic top of the pencil holder. During measurements 1 cm of the graphite lead was introduced into the solution to be analyzed while the holder was kept in the upright position.

GO and MWCNT were obtained from Sigma Chemical (St. Louis, MO, USA). GO was dispersed to obtain a 0.50 mg/mL dispersion in water by ultrasonication in a bath for 30 min. MWCNTs were dispersed to obtain 0.50 mg/mL dispersion in dimethyl formamide (DMF) by ultrasonication in a bath for 60 min. Pencil graphite electrodes were dipped to these solutions for 10 min. Then electrodes were dipped to deionize water for 10 min and dried.

The electrochemical behavior of OLE in working buffer was examined using differential pulse voltammetry (DPV). DPV was performed with a potential range from −0.5 to +1.0 V, with 5 mV step potential, 25 mV pulse potential, 20 mV/s scan rate, 0.01 s pulse time and 3 s equilibration time.

3 Results and discussion

3.1 Selection of the working electrode

From the literature it is well known that during their electrooxidation, phenolic compounds may foul the electrode surface by covering it with a non-conducting polymeric film [22]. Therefore, in order to ensure the determination reproducibility, a tedious time consuming electrode surface cleaning step is necessary before each measurement. Therefore the use of a disposable and renewable electrode, like the PGE, represents an outstanding alternative to traditional electrodes [23]. In the same way, the nature of the working electrode active surface influences the voltammetric behavior of an analyte and thus the shapes of the voltammograms could substantially differ. Graphite pencil leads (GPL) are composites made of three conducting components, i.e. graphite, lead and a binder. The hardness of the GPL and thus also their names depend on the graphite:lead ratio. GPL denoted as B (from blackness) contain more graphite and are softer whereas the harder H (from hardness) pencils have lead as the major component. HB pencils have an equal ratio of graphite and lead [24, 25]. The type of the GPL may influence the voltammetric behavior of an analyte [16]. H, B, HB and 2B GPLs were evaluated on OLE differential pulse voltametry determination. The highest electrochemical signal was obtained with HB graphite pencil leads. Thus, HB GPL was selected as the working electrode for further studies. The electrochemical signal obtained is associated with the oxidation of the catechol group of OLE [1, 26].

3.2 Selection of the supporting electrolyte and pH

In order to select the optimum supporting electrolyte, DPVs were recorded on the PGE for 18 μM OLE solutions prepared in Boric acid (BA), acetate buffer solution (ABS), phosphate buffer solution (PBS) and Britton-Robinson buffer (BRB) at different pH values within the following range: 1 to 50 mM. Figure 1 shows the comparison of DPV responses for different pH values of 5 mM BRB. As can be seen, the pH of the supporting electrolyte influences the shape and magnitude of OLE voltammetric peaks and the peak potentials were highly pH dependent. The best results were obtained for 5 mM of BRB, pH 9.

3.3 OLE signal improvement

In a previous work [17], we have demonstrated that the addition of Natural Deep Eutectic Solvents to electrolyte, improves the electrochemical detection of phenolic compounds. Also, it is well known that carbon nanomaterials such graphene and multiwall carbon nanotubes have a positive effect in terms of sensitivity on voltammetric response for polyphenols. So, in order to explore the performance of NADES as modifiers of background electrolyte in combination with GO and MWCNT modified PGEs for electrochemical detection of OLE, several experiment were carried out. Figure 2 shows the DPVs comparing the best results obtained for the different modifications evaluated.

First, three different NADES were synthesized following the procedure described in Section 2.3 and added to BRB buffer solution at different concentrations (5 to 20% v/v). The highest electrochemical signal was obtained for BRB containing 10% (v/v) of Lactic acid, Glucose and H_2O (LGH, 5:1:3). When BRB modified with LGH was tested with the bare GPE (LGH-PGE), the oxidation peak current for OLE increased significantly (3.4 times). It is worth mentioning

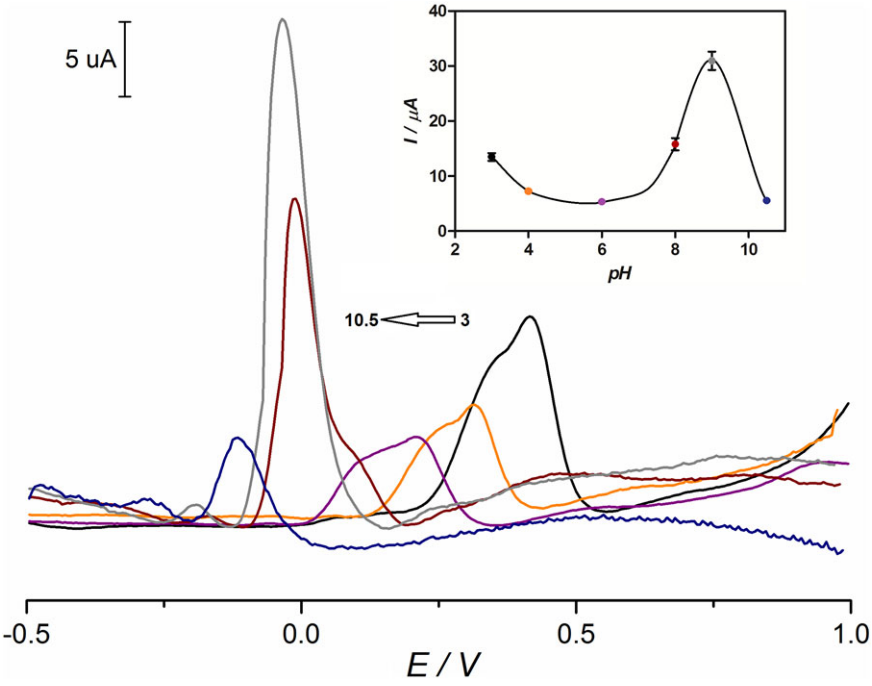

Figure 1. Peak current intensities of 18 μM OLE in 5 mM BRB at different pH values.

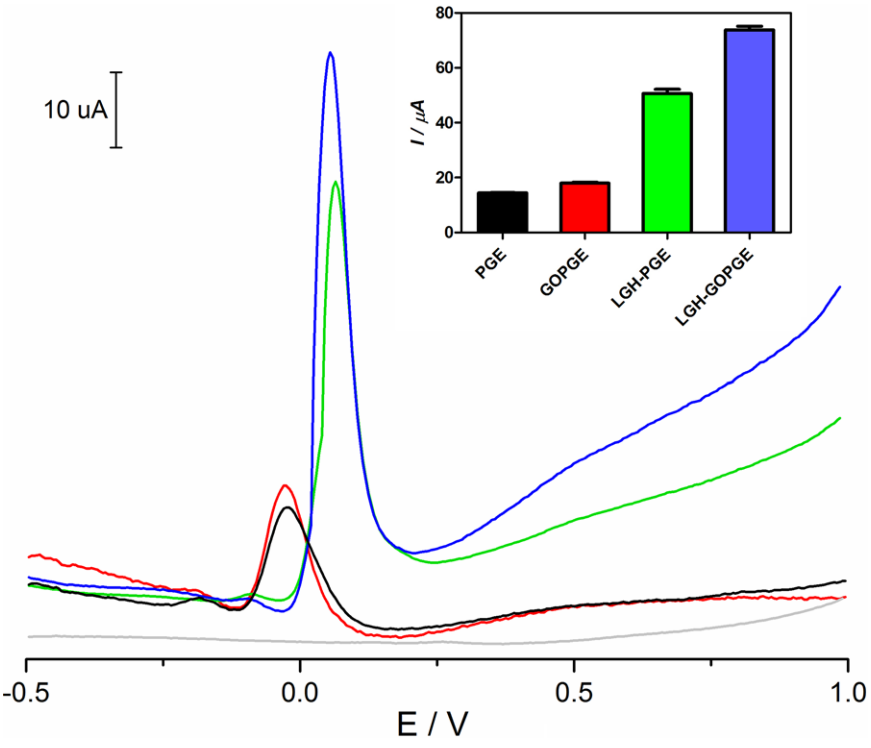

Figure 2. Comparison between peak currents of best results obtained for the different modifications evaluated for 18 μM OLE. PGE (black), GOPGE (red), LGH-PGE (green) and LGH-GOPGE (blue).

while that the analyte peak current intensity increased, the oxidation peak potential mildly shift to a positive oxidation values compared to peak current of OLE in unmodified buffer.

In agreement with previous reports [17], both peak position and shape change; giving evidence that the enhanced response could be explained by kinetic origin. The interaction of OLE with LGH reduces both charge transfer and reaction resistance, and increase the electronic exchange rate, so the peak potential should be shifted negatively. However, as can be seen in Fig. 2, the potential shifted positively. This effect could be explained by the strong interaction of OLE with the structure of the natural eutectic solvent (H-bond).

Secondly, PGEs were modified as mentioned in Section 2.5 with MWCNT and GO. The following experimental con-

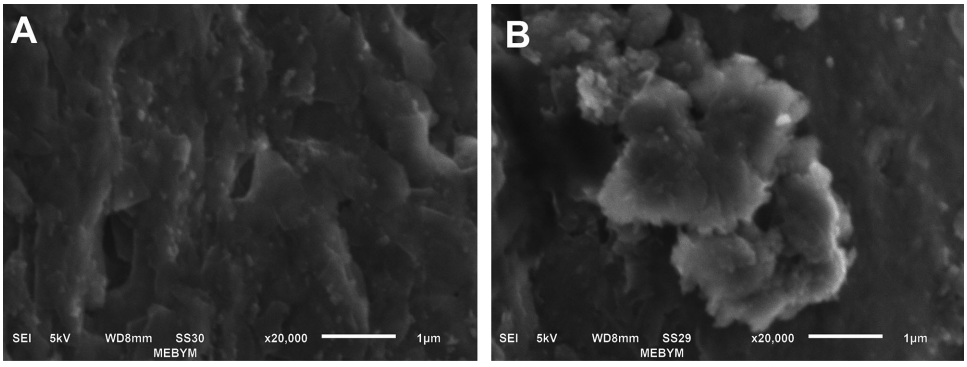

Figure 3. SEM surface characterization of PGE (A) and GOPGE (B).

ditions were tested: modification method (drop casting or dipping), modification time (5 to 60 min) and nanomaterial concentration (0.05 to 0.50 mg/mL). The best results were obtained for graphene oxide 0.5mg/mL and 10 min dipping modification. When GOPGE with unmodified buffer was evaluated, the oxidation peak currents for OLE increased (1.3 times) while the oxidation peak potentials did not shift to a less positive oxidation values compared to bare PGE.

Thus, in the third stage a combination of both modifications (LGH-GOPGE) was evaluated. In this case, an impressive signal enhancement (5.3 times) for OLE was obtained and, as previously observed, peak potentials mildly shift to a positive oxidation values compared to peak current of OLE in unmodified buffer. So, the optimal conditions were as follows: 10% LGH 0.5 mg/mL GO dipped modified PGE.

3.4 Surface characterization

SEM characterization was made for bare and GO modified PGEs. Figure 3 clearly shows that the graphite layers of PGE (Fig. 3A) were covered by GO nanosheets (Fig. 3B).

Additionally, the effective surface areas of PGE and GOPGE were obtained by cyclic voltammetry with 5 mmol/L $K_3[Fe(CN)_6]$ as a probe at different scan rates. For a reversible process, the equation $I_P = (2.69 \times 10^5)n^{3/2}AD^{1/2}C\upsilon^{1/2}$ is applied [27], where I_P refers to the peak current and A is the electrode area (cm^2), C is the concentration of $K_3[Fe(CN)_6]$ and υ is the scan rate. Herein, for 5 mM $K_3[Fe(CN)_6]$, $n = 1$, $D = 7.6\ 9\ 10^{-6}$ $m^2\ s^{-1}$ (0.1 mM KCl). The effective surface areas of the GPE and GOGPE were computed to be 0.120 and 0.211 cm^2, respectively. The GOPGE effective surface area is much larger than that of the bare GPE, showing that the bare electrode was modified efficiently by GO.

3.5 Effect of scan rate on the oxidation of OLE

The effect of scan rate on the electrooxidation of OLE at the LGH-GOPGE was investigated by cyclic voltammetry to acquire information about electrochemical mechanism from the relationship between peak current and scan rate of potential. The cyclic voltammograms of 18 μM OLE in 5 mM BRB

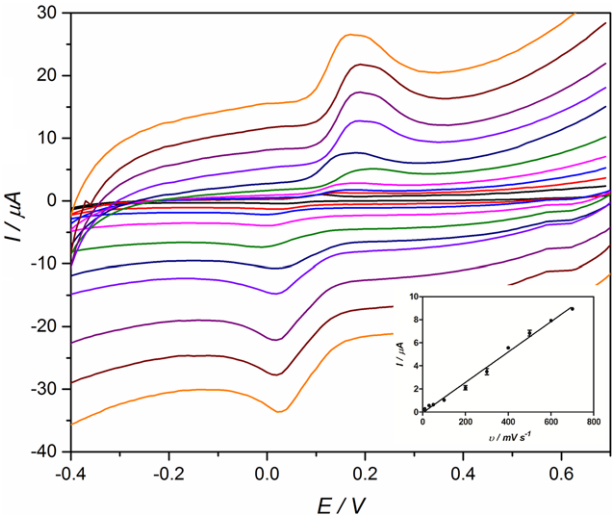

Figure 4. Cyclic voltammograms of 18 μM OLE in 5 mM in LGH-GOPGE at different scan rates. Inset: Relationship between peak current and scan rate for 18 μM OLE in LGH-GOPGE.

with 10% of LGH were recorded at different scan rates from 10 to 700 mV/s (Fig. 4). As shown in Fig. 4 inset, a linear correlation ($R^2 = 0.990$) was obtained between the peak current and the scan rate, indicating that the oxidation process is controlled by adsorption. The regression equation was $I_{pa}\ (\mu A) = 13.5\upsilon\ (mV/s) - 59.2$.

3.6 Analytical performance

The analytical performance was also evaluated using the LGH-GOPGE. The DPV responses of OLE at different concentrations were recorded as shown in Fig. 5. Table 1 shows the analytical figures of merit of the proposed approach. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the formula LOD = 3 SD/b and LOQ = 10SD/b, respectively, where SD is the standard deviation of ten reagent blank determinations and b is the slope of the calibration curve.

In order to evaluate the reproducibility of the method, several GO modified GPEs were tested for the electrochemical measurement of OLE. The oxidation peak currents of this

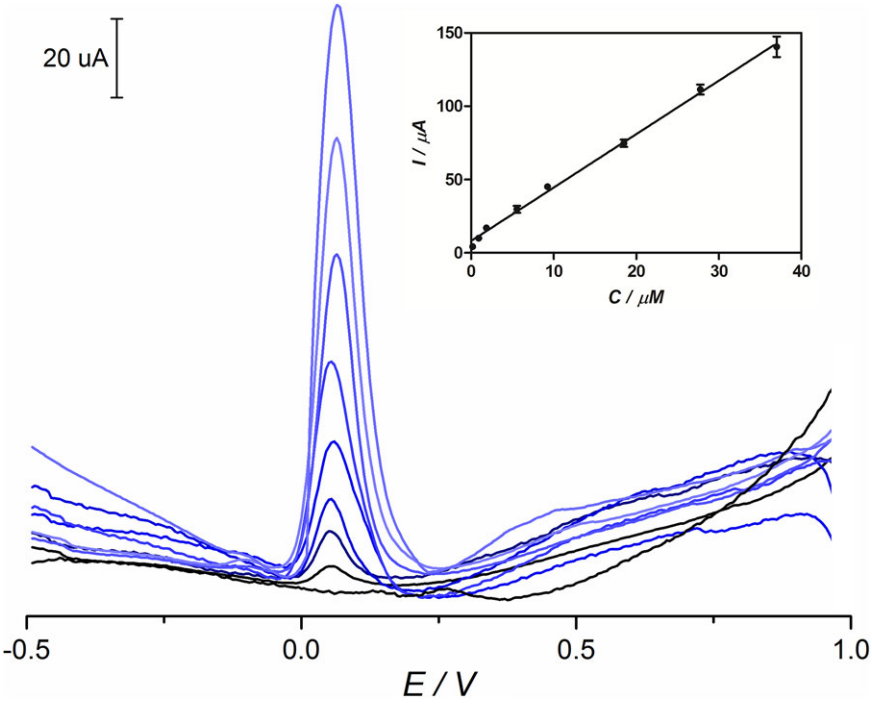

Figure 5. DPV responses for different concentrations of OLE within the range 0.018-37 μM in LGH-GOPGE. Inset: Calibration plot.

Table 1. Analytical parameters of the proposed methodology

Parameter	
Regression equation[a)]	y = 8.14 + 3.64x
R^2	0.989
Linear range (μM)	0.10 to 37
LOD (μM)	0.030
LOQ (μM)	0.102
%RSD potential[b)]	0.01
%RSD intensity[b)]	3.16

a) Regression equation is y = a + bx where y is the voltammetric current (μA) and x is the analyte concentration (μM).
b) $n = 5$.

analyte decreased randomly slightly with several measurements, perhaps due to surface sorption and fouling observed. Therefore, the GOPGEs were only used for single measurements and the reproducibility between multiple electrodes was evaluated by the parallel determination of the oxidation peak current of 18 μM of OLE. The relative standard deviation (%RSD) for peak current was 3.16 ($n = 5$). This RSD suggested that the reproducibility and the precision of detection were satisfactory.

3.7 Interference effect

The influences of some species on the determination of oleuropein at LGH-GOPGE were examined. Representative phenolic compounds and some cations commonly present in plant matrices were selected at practical concentrations. The oxidation peak currents of OLE were measured individually in pH 9 LGH modified BRB containing the interferents and the peak change in current was then checked. The results indicated that the following species did not interfere in the determination of 18 mM OLE as the peak current change was <10%: 100 mM Tyrosol, 100 mM p-Coumaric acid, 100 mM p-Amino benzoic acid, 100 mM quercetin, 100 mM Syringic acid and 100 mM luteolin. Also, the 100-fold concentrations of K^+, Na^+, Cu^{2+}, Ca^{2+}, Pb^{2+}, Zn^{2+}, Br^-, PO_4^{3-} did not interfere.

3.8 Sample analysis

In order to investigate the possibility of applying the proposed sensor to the quantification of OLE in natural samples, extracts of olive leaves were analyzed by the developed methodology. In this sense, two different extractions from lyophilized leaves were made as been indicated in Section 2.4. Fifty microliters of the extract were diluted in working buffer and analyzed by DPV. Indeed, the results obtained with

Table 2. Determination of OLE in olive leaves using LGH-GOPGE and CE method

Sample	Solvent/ ratio	LGH-GOPGE (mg/g)[a)]	CE (mg/g)[a)]	Error %
Olive leaf	MeOH:H_2O / 4:1	19.75 ± 0.3	17.76 ± 0.5	11.2
Olive leaf	EtOH:H_2O / 4:1	20.02 ± 0.3	18.11 ± 0.4	10.5

a) Values are expressed as mean value ± SD (n = 3).

Table 3. Comparison of electrochemical sensors for OLE

Electrode	Method	LR (μM)	LOD (μM)	Sample	Ref.
MWCNT/GCE	SWV	0.01–0.70	0.003	Olive leaf	[3]
poly[Ni-(PPIX)]/GCE	Amperometry	-	1.21	Olive oil	[29]
DNA-coated CHIT/CPE	DPV	0.30–12	0.090	Olive leaf and Human serum	[1]
SPE	DPV	-	0.463	Olive oil	[30]
Gold wire	ME with AD	-	13	Olive oil	[31]
CNSF	ME with AD	-	<10	Olive oil	[32]
LGH-GOPGE	DPV	0.10 – 37	0.030	Olive leaf	This work

DPV, differential pulse voltammetry; SWV, square wave voltammetry; ME, microchip electrophoresis; AD, amperometric detection; GCE, glassy carbon electrode; CPE, carbon paste electrode; SPE, screen-printed electrode; MWCNTs, multiwalled carbon nanotubes; poly[Ni-(PPIX)], poly[Ni-(proto- porphyrinIX)dimethylester]; CHIT, chitosan; LOD, limit of detection and LR, linear range.

the proposed electrochemical sensor were compared with a capillary zone electrophoresis (CZE) method for OLE previously reported [28] with some modifications. Calibration curve for determining Oleuropein in the sample with CE-UV method was obtained using a series of standard solutions over the concentration range from 2 to 50 mg/L. Thus, the accuracy of the proposed method was compared with the with CE methodology. As can be seen in Table 2, the results obtained by CE and by the electrochemical approach were in good agreement and the relative error was <11.20% indicating that the proposed method is accurate and reliable. In addition, the obtained results demonstrate that the proposed method using GO modified PGE and LGH modified BRB is able for the determination of OLE in complex food samples.

The developed methodology has demonstrated to be better in terms of cost, simplicity and sample throughput compared with traditional methodologies for determination of OLE like HPLC [10–12] and comparable in terms of sensitivity and robustness. Finally, Table 3 lists the main analytical features obtained in the selected related works found in the literature involving the electrochemical sensing of OLE [1,3,29–32]. As can be seen the analytical performance of the proposed method is highly satisfactory in terms of sensitivity, simplicity, dynamic range and cost.

4 Concluding remarks

In this work, a novel electrochemical sensor based on the combination of natural deep eutectic solvent modified buffer and nanomaterial pencil graphite electrode, is presented and evaluated for the first time. The electrochemical response of the sensor was greatly enhanced and displayed outstanding advantages such as single use, disposability, high analytical performance and extremely low cost. The LODs obtained were also competitive with the previous reported. Additionally, the portability of the system makes it very valuable for wide spread use without the need of skilled personnel. All these features make these electrochemical approaches very valuable for polyphenol screening tools before the use of more sophisticated analytical techniques.

The authors gratefully acknowledge financial support provided by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional de Cuyo (UNCuyo).

The authors have declared no conflict of interest.

5 References

[1] Mohamadi, M., Mostafavi, A., Torkzadeh-Mahani, M., *Bioelectrochemistry* 2015, *101*, 52–57.

[2] Segura-Carretero, A., Menéndez-Menéndez, J., Fernández-Gutiérrez, A., *Olives Olive Oil Health Disease Prevention*, Academic Press, New York, Chapter 19, 2010, 167–175.

[3] Cittan, M., Koçak, S., Çelik, A., Dost, K., *Talanta* 2016, *159*, 148–154.

[4] Difonzo, G., Russo, A., Trani, A., Paradiso, V. M., Ranieri, M., Pasqualone, A., Summo, C., Tamma, G., Silletti, R., Caponio, F., *J. Func. Foods* 2017, *31*, 63–70.

[5] Castillo, J., Alcaraz, M., Benavente-García, O., *Olives Olive Oil Health Disease Prevention*, Academic Press, New York, Chapter 102, 2010, 951–958.

[6] Abaza, L., Talorete, T. P. N., Yamada, P., Kurita, Y., Zarrouk, M., Isoda, H., *Biosci. Biotech. Biochem.* 2007, *71*, 1306–1312.

[7] Fu, S., Arráez-Roman, D., Segura-Carretero, A., Menéndez, J. A., Menéndez-Gutiérrez, M. P., Micol, V., Fernández-Gutiérrez, A., *Anal. Bioanal. Chem.* 2010, *397*, 643–654.

[8] Taamalli, A., Arráez-Román, D., Barrajón-Catalán, E., Ruiz-Torres, V., Pérez-Sánchez, A., Herrero, M., Ibañez, E., Micol, V., Zarrouk, M., Segura-Carretero, A., Fernández-Gutiérrez, A., *Food Chem. Toxicol.* 2012, *50*, 1817–1825.

[9] Tavafi, M., Ahmadvand, H., Toolabi, P., *Iran. J. Kidney Diseases* 2012, *6*, 25–32.

[10] Lemonakis, N., Mougios, V., Halabalaki, M., Skaltsounis, A. L., Gikas, E., *Biomed. Chromatogr.* 2016, *30*, 2016–2023.

[11] Bertolini, T., Vicentini, L., Boschetti, S., Andreatta, P., Gatti, R., *J. Pharm. Biomed. Anal.* 2016, *129*, 198–202.

[12] Peng, L. Q., Li, Q., Chang, Y. X., An, M., Yang, R., Tan, Z., Hao, J., Cao, J., Xu, J. J., Hu, S. S., *J. Chromatogr. A* 2016, *1456*, 68–76.

[13] Ambrosi, A., Chua, C. K., Latiff, N. M., Loo, A. H., Wong, C. H. A., Eng, A. Y. S., Bonanni, A., Pumera, M., *Chem. Soc. Rev.* 2016, *45*, 2458–2493.

[14] Martín, A., Escarpa, A., *TrAC - Trends Analyt. Chem.* 2014, *56*, 13–26.

[15] Ambrosi, A., Chua, C. K., Bonanni, A., Pumera, M., *Chem. Rev.* 2014, *114*, 7150–7188.

[16] David, I. G., Popa, D. E., Buleandra, M., Moldovan, Z., Iorgulescu, E. E., Badea, I. A., *Anal. Meth.* 2016, *8*, 6537–6544.

[17] Gomez, F. J. V., Espino, M., de los Angeles Fernandez, M., Raba, J., Silva, M. F., *Anal. Chim. Acta* 2016, *936*, 91–96.

[18] Espino, M., de los Ángeles Fernández, M., Gomez, F. J. V., Silva, M. F., *Trac. Trend Anal. Chem.* 2016, *76*, 126–136.

[19] Choi, Y. H., van Spronsen, J., Dai, Y., Verberne, M., Hollmann, F., Arends, I. W. C. E., Witkamp, G. J., Verpoorte, R., *Plant Physiol.* 2011, *156*, 1701–1705.

[20] Dai, Y., Witkamp, G. J., Verpoorte, R., Choi, Y. H., *Anal. Chem.* 2013, *85*, 6272–6278.

[21] Bosiljkov, T., Dujmić, F., Cvjetko Bubalo, M., Hribar, J., Vidrih, R., Brnčić, M., Zlatic, E., Radojčić Redovniković, I., Jokić, S., *Food Bioprod. Process.* 2017, *102*, 195–203.

[22] De Carvalho, R. M., Kubota, L. T., Rath, S., *J. Electroanal. Chem.* 2003, *548*, 19–26.

[23] David, I. G., Bizgan, A. M. C., Popa, D. E., Buleandra, M., Moldovan, Z., Badea, I. A., Tekiner, T. A., Basaga, H., Ciucu, A. A., *Food Chem.* 2015, *173*, 1059–1065.

[24] Vishnu, N., Kumar, A. S., *Anal. Meth.* 2015, *7*, 1943–1950.

[25] Dervisevic, M., Çevik, E., Durmuş, Z., Şenel, M., *Mat. Sci. Eng. C* 2016, *58*, 790–798.

[26] Ranalli, A., Contento, S., Lucera, L., Di Febo, M., Marchegiani, D., Di Fonzo, V., *J. Agricul. Food Chem.* 2006, *54*, 434–440.

[27] Arvand, M., Anvari, M., *J. Iranian Chem. Soc.* 2013, *10*, 841–849.

[28] De Fernandez, M. D. L. A., Sotovargas, V. C., Silva, M. F., *J. Am. Chem. Oil Soc.* 2014, *91*, 2021–2033.

[29] Campo Dall'Orto, V., Danilowicz, C., Rezzano, I., Del Carlo, M., Mascini, M., *Anal. Lett.* 1999, *32*, 1981–1990.

[30] Capannesi, C., Palchetti, I., Mascini, M., Parenti, A., *Food Chem.* 2000, *71*, 553–562.

[31] Del Pilar Godoy-Caballero, M., Acedo-Valenzuela, M. I., Galeano-Díaz, T., Costa-García, A., Fernández-Abedul, M. T., *Analyst* 2012, *137*, 5153–5160.

[32] Martín, A., Vázquez, L., Escarpa, A., *J. Mater. Chem. A* 2016, *4*, 13142–13147.

