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Development of an electrochemical sensor for the determination of the flavonoid luteolin in peanut hull samples

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ABSTRACT

We describe the development of an electrochemical sensor based on glassy carbon electrodes modified with multiwalled carbon nanotubes dispersed in low molecular weight polyethylenimine for the determination of luteolin in peanut hulls. A well defined quasi-reversible surface redox couple was found using cyclic and square wave voltammetries for luteolin in 1.0 mol L^{-1} $HClO_4$ aqueous solutions. The best accumulation potential and the accumulation time were 0.55 V and 20 min, respectively. An optimal ratio of 1:5 for multiwalled carbon nanotubes/polyethylenimine was used to prepare dispersions.
The linear range was from 2.4×10^{-3} to 1.75 μmol L^{-1}. The luteolin contents determined in two peanut hull samples were (1.18 ± 0.08) and (1.47 ± 0.09) g per 100 g of sample, being in very good agreement with those values obtained from the same samples using HPLC. The limits of detection and quantification were 5.0×10^{-10} and 1.5×10^{-9} mol L^{-1}, respectively. The reproducibility and the repeatability were 8.0 and 7.3%, respectively. The modified glassy carbon electrode was stable even after 23 days.

1. Introduction

Luteolin (3′,4′,5,7-tetrahydroxy-flavone, LUT) is one of the most bioactive flavonoids. Its chemical structure is shown in Fig. 1. LUT is found in high amounts in thyme, peppermint, parsley, celery, green pepper, perilla leaves, and chamomile tea [1,2].

It is well known that LUT has a broad range of biochemical and pharmacological properties. It has antioxidant, anti-inflammatory, anticancer, anti-allergic, anti-platelet and anti-ulcer properties. In addition, LUT can be used for antiviral activities, cataract prevention, and antithrombotic action [3–6]. Recent studies have also shown that LUT could suppress the oxidative damage of DNA [7].

Different analytical methods have been reported for the determination of LUT. They include thin-layer chromatography [8], gas chromatography (GC) [9], high-performance liquid chromatography (HPLC) [10–17], and capillary electrophoresis (CE) [18–20], coupled with various detection techniques, such as UV spectrophotometry, diode-array, electrochemical array, etc. Even though these techniques have made possible the highly selective and sensitive quantification of LUT, they present some disadvantages such as high cost, high consumption of time and reagents, and high complexity of operation.

Since the discovery of carbon nanotubes (CNTs) in 1991 [21], they have attracted the attention of scientists all over the world. CNTs are often used for the development of electrochemical sensors due to their multiple advantages such as low overvoltages for the oxidation/reduction of different analytes, fast response times, high sensitivity (mainly connected with the increase of the electroactive surface area), and highly stable responses [22,23]. Therefore, CNT-based electrochemical sensors represent an interesting alternative for the determination of different analytes [24,25].

Non-functionalized multiwalled carbon nanotubes (MWCNTs) are insoluble in most solvents. To overcome this problem, different strategies have been used such as dispersion of CNTs in concentrated acid solutions [26,27], organic solvents as N,N′-dimethylformamide [28,29], and polymers such as Nafion [30,31], chitosan [32–34], DNA [35], polylysine [36] and polyhistidine [37]. Highly branched polyethylenimine (PEI), a polymeric amine with a high positive charge density [38,39] has been successfully used to increase the level of MWCNT solubility, demonstrating an excellent performance either in batch or in flow systems [22,40–42].

In this work, we describe an electrochemical sensor for the direct quantification of LUT based on the use of glassy carbon electrodes modified with MWCNTs dispersed in low molecular weight PEI in 1.0 mol L^{-1} $HClO_4$ aqueous solutions using cyclic (CV) and square

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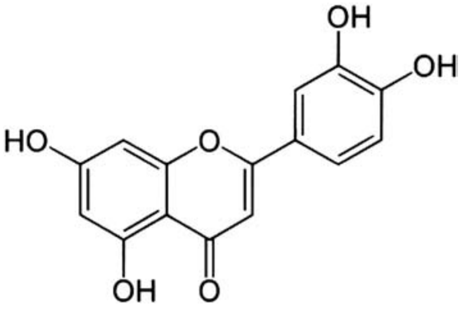

Fig. 1. Chemical structure of luteolin.

wave (SWV) voltammetries. We also discuss the analytical applications of the sensor for the quantification of LUT in samples of peanut hulls.

2. Materials and methods

2.1. Reagents and materials

LUT, ascorbic acid (AA), and PEI (MW ~600 g mol^{-1}) were purchased from SIGMA. MWCNTs (diameter (30 ± 15) nm, length 1–5 μM) were obtained from Nano-Lab (USA). Phosphate buffer solutions (PBS) of pH 7.40 were prepared using Na_2PO_4H (Merck p.a.) and KPO_4H_2 (Merck p.a.). Ultrapure water ($\rho = 18$ MΩ cm) from a Millipore-MilliQ system was used for preparing all solutions. Samples of peanut hulls were obtained from a local farmer. $HClO_4$, methanol and ethanol were from Merck p.a. Acetic acid and acetonitrile were from Sintorgan (HPLC degree).

2.2. Apparatus

CV and SWV experiments were carried out with an Epsilon (BAS, USA) potentiostat. Electrochemical measurements were performed in a 5 mL Pyrex cell. Working electrodes were either a bare glassy carbon electrode (GCE, CH Instruments, 3 mm diameter) or a GCE modified with MWCNT dispersions in PEI. A platinum wire and an Ag/AgCl, 3 mol L^{-1} NaCl (BAS, RE-5B) were used as counter and reference electrodes, respectively.

Scanning Electron Microscopy (SEM) images were obtained with a Field Emission Gun Scanning Electron Microscopy (FE-SEM, Zeiss, ΣIGMA model).

LUT was also determined using a HPLC Waters 2998 system equipped with a quaternary pump model Waters e2695, diode array detector, and a C18 (Luna) column (100 × 4.6 mm). The injection volume was 15 μL. The mobile phase flow rate was kept constant at 0.5 mL min^{-1}. A gradient elution was used, starting with 60% of solvent A (H_2O/acetic acid (99:1)) and 40% solvent B (methanol/acetonitrile/acetic acid (89:10:1)), followed by a linear variation up to 100% of solvent B in 30 min.

2.3. Preparation of GCE and GCE/MWCNT–PEI

GCE electrodes were pre-treated by polishing with alumina slurries of 1.0, 0.3, and 0.05 μm for 1 min each, and were sonicated in water bath for 15 s.

MWCNT–PEI dispersions were obtained by mixing 1.0 mg of MWCNTs with 1.0 mL of PEI solutions of different concentrations prepared in 50:50 v/v ethanol/water, followed by sonication for 45 min.

Polished GCE electrodes were modified with MWCNT–PEI dispersions by dropping an aliquot of 20 μL on the top of the electrode and allowing the solvent to evaporate at room temperature.

2.4. Sample preparation

LUT extraction was carried out by milling 2.5 g of peanut hulls in an analytical mill. The extract was then placed in an Erlenmeyer flask containing 50 mL of methanol, with constant stirring for 24 h at room temperature. Finally, the solution was filtered using a Whatman filter paper [43].

2.5. Procedure

Before starting the experiments, the CGE/MWCNTs–PEIs were cycled 10 times between −0.200 and 0.900 V in a 1.0 mol L^{-1} $HClO_4$ aqueous solution at a scan rate of 0.050 V s^{-1}. LUT accumulation on the electrode surface was performed at 0.55 V for 20 min under stirring conditions (see below), and the voltammetric stripping was performed in the same solution.

Square wave voltammograms were recorded in the potential range from 0 to 0.8 V. Other experimental parameters were amplitude of the square wave, $\Delta E_{sw} = 0.025$ V, staircase potential, $\Delta E_s = 0.005$ V and frequency, f = 40 Hz.

3. Results and discussion

3.1. Optimization and characterization of MWCNT–PEI dispersions

The electrochemical behavior of AA in PBS (pH 7.40) was used to optimize and characterize MWCNT–PEI dispersions. Fig. 2 shows cyclic voltammograms for 1.0×10^{-3} mol L^{-1} AA obtained at bare GCE (dotted line), GCE/PEI (dashed line) and GCE/MWCNTs–PEI (solid line). At bare GCE, AA is oxidized at about 0.3 V, with a peak current (I_p) of 20.5 μA. When GCE was modified with PEI, the peak potential (E_p) for AA oxidation decreased by 0.092 V, while the peak current remained almost the same (18.6 μA). The decrease in the overvoltage for AA oxidation is attributed to the facilitated charge transfer due to the favorable electrostatic interactions with the electrode surface. A remarkable decrease in the oxidation overvoltage (~0.3 V) compared to GCE was found at GCE modified with the MWCNT–PEI dispersion, demonstrating that the catalytic activity of MWCNTs associated with PEI largely facilitates the charge transfer. The I_p obtained for the electrooxidation of AA at GCE/MWCNTs–PEI shows an important enhancement (almost 3 times) compared to GCE and GCE/PEI, demonstrating the benefits of the huge increase in the electroactive area due to the presence of MWCNTs.

We also studied the effect of the MWCNTs/PEI ratio on the efficiency of the dispersion and the performance of GCE modified with MWCNTs/PEI using AA as a redox probe. The evaluated MWCNT/PEI ratios were

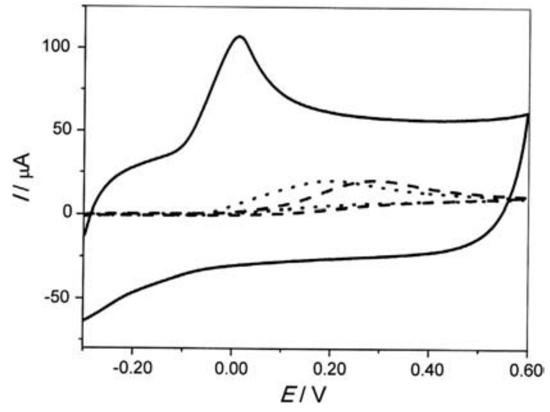

Fig. 2. Cyclic voltammograms of 1.0×10^{-3} mol L^{-1} AA in 0.1 mol L^{-1} PBS of pH 7.40 at bare GCE (…), GCE modified with 5.0 mg mL^{-1} PEI (– – –), and GCE modified with 1.0 mg mL^{-1} MWCNTs/5.0 mg mL^{-1} of PEI (—). v = 0.100 V s^{-1}.

1:1, 1:2, 1:3, 1:4, 1:5 and 1:6 (results not shown). In all cases, a marked decrease in the overvoltage for the oxidation of AA was found. However, this decrease was almost independent of the MWCNT/PEI ratio (the E_p varied from 0.004 to 0.032 V for the different dispersions). Regarding the oxidation current, as light increase was observed with increasing amounts of PEI, i.e., 49 μA and 62 μA were obtained from electrodes modified with 1:1 and 1:6 MWCNT/PEI dispersions, respectively. However, the best compromise between the efficiency of the dispersion and the sensitivity, robustness and reproducibility of the resulting GCE/MWCNTs/PEI was reached with the 1:5 MWCNT/PEI dispersions. Moreover, the 1:5 MWCNT–PEI dispersions were stable even after 23 days. Therefore, a 1:5 MWCNT/PEI ratio was selected for further studies. Under these conditions, the E_p for AA oxidation was 0.004 V, and I_p was 56 μA.

Fig. 3 displays typical SEM pictures for GCE modified with MWCNTs–PEI prepared with different ratios 1:1 (A) and 1:5 (B) at different magnifications (1000× for A1 and B1; and 30,000× for A2 and B2). At low magnifications, both MWCNT–PEI dispersions cover the whole GCE surface, although there are conglomerates of CNTs. Pictures at higher magnifications show more clearly the presence of CNTs dispersed in the polymeric matrix. The images obtained for 1:5 MWCNTs–PEI display a more efficient dispersion of the bundles.

3.2. Application of GCE/MWCNT–PEI electrodes for the determination of luteolin

Fig. 4A displays cyclic voltammograms obtained in 1.0 mol L^{-1} $HClO_4$ aqueous solutions after 20 min accumulation in 1.0 μmol L^{-1} LUT at bare GCE (dotted line) and GCE/PEI (solid line). On these surfaces, the electrochemical signals are poorly defined. At GCE/MWCNTs–PEI, LUT is oxidized under the same experimental conditions, showing a quasi-reversible surface redox couple with a well defined oxidation peak at 0.680 V ($I_p = 28.2$ μA) (Fig. 4B, solid line). No signal was observed for 1.75 μmol L^{-1} LUT without the previous adsorption step (dotted line in Fig. 4B). This behavior clearly indicates a strong adsorption of LUT on GCE/MWCNTs–PEI (1:5), promoted not only by the electrostatic interactions between LUT and PEI, but also by π–π interactions between the structure of LUT and MWCNTs.

It is well known that SWV is a fast and sensitive technique to quantify the amount of initially adsorbed substrate, considering its ability to discriminate against capacitive currents [44]. Thus, SWV was chosen to carry out further studies.

Fig. 5 shows the forward (I_f), reverse (I_r) and net (I_n) currents from SW voltammograms obtained in 1.0 mol L^{-1} $HClO_4$ aqueous solutions, after accumulation of 1.0 μmol L^{-1} of LUT at the GCE/MWCNTs–PEIs. These results clearly suggest both the adsorption of LUT and the quasi-reversible nature of the surface redox couple, considering the shape of I_f, I_r and I_n voltammetric responses obtained [44], as shown in Fig. 5. The selective interaction of LUT with the GCE/MWCNT–PEI electrode surface could be explained by considering the different functional groups containing oxygen, such as carboxylic acid, lactone, o-quinone, p-quinone, carbonyl, phenol, π–π interactions, etc., all of which may be present on the electrode surface [45]. The interaction between these groups and the −OH groups present in the chemical structure of LUT might be responsible for the adsorption of LUT on GCE/MWCNTs–PEIs.

The effect of the accumulation time, t_{acc}, at 0.55 V on the LUT adsorptive oxidation was evaluated using 1.75 μmol L^{-1} LUT. The oxidation current increased linearly with the adsorption time of up to 20 min, and then it leveled off for times longer than 20 min. The effect of the accumulation potential, E_{acc}, on the LUT adsorptive oxidation was evaluated between −0.3 and 0.8 V using a $t_{acc} = 20$ min. The LUT adsorptive

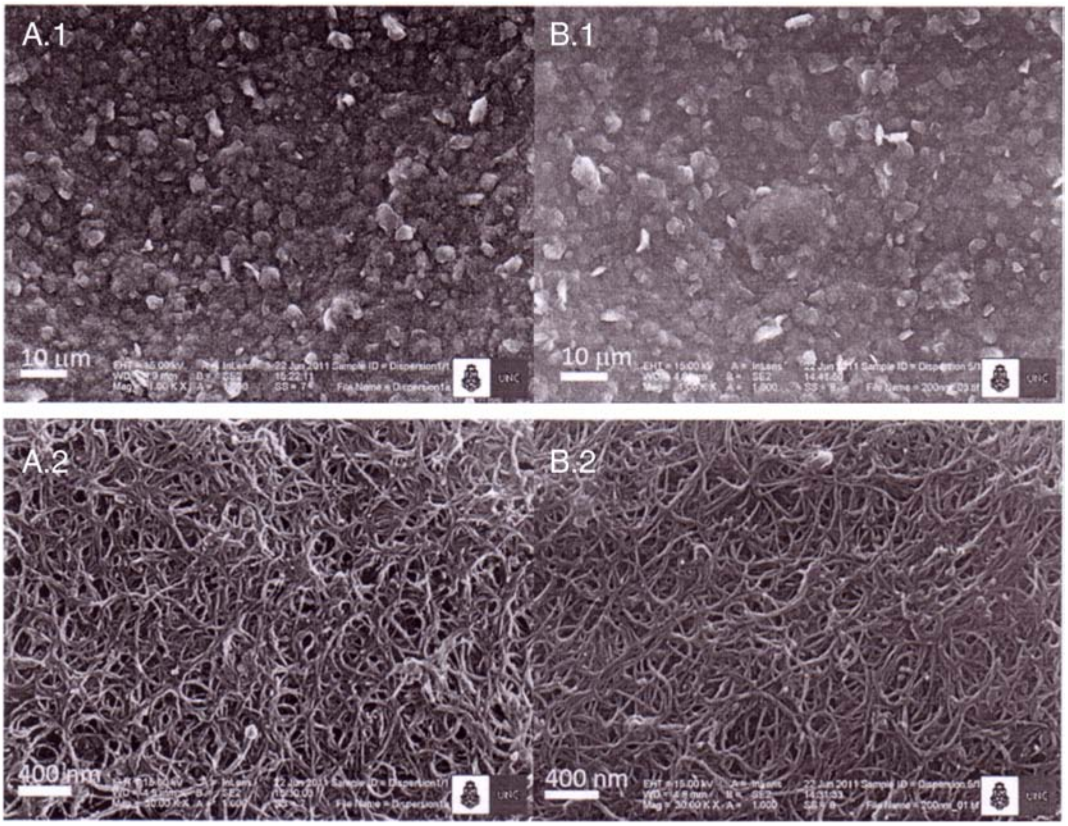

Fig. 3. SEM images of the GCE/MWCNT–PEI electrode prepared with MWCNT/PEI ratios 1:1 (A) and 1:5 (B). Detector In-Lens at 15.00 kV acceleration voltages, magnification 1000× for A.1 and B.1, and 30,000.00× for A.2 and B.2.

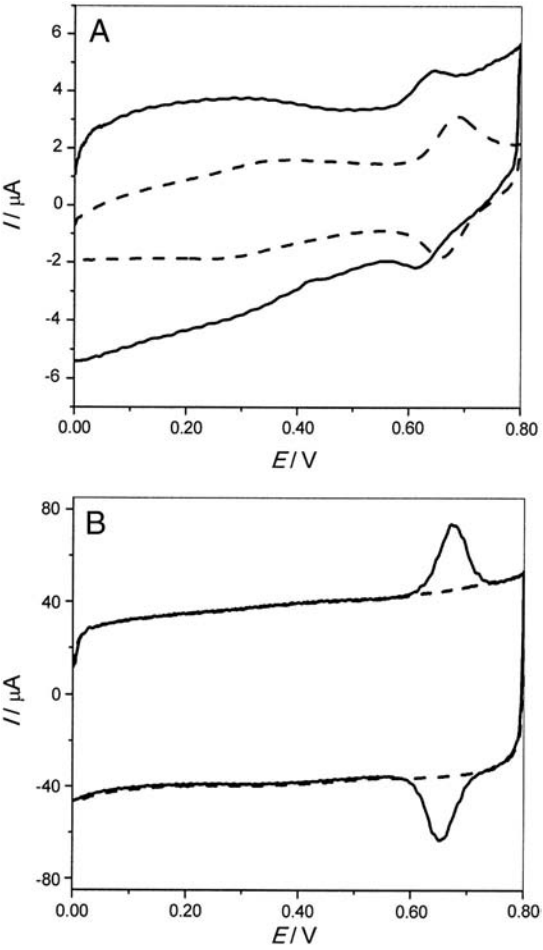

Fig. 4. Cyclic voltammograms obtained in 1.0 mol $L^{-1}$$HClO_4$ after accumulation of 1.0 μmol L^{-1} of LUT at A) bare GCE(– – –) and GCE/PEI(—), and B) GCE/MWCNTs–PEI. Experimental conditions: adsorption of LUT for 20 min at 0.55 V. v = 0.100 V s^{-1}. Other conditions are the same as that in Fig. 2.

oxidation current was independent of E_{acc} between −0.30 and 0.40 V, with a maximum response at 0.55 V (results not shown). Thus, selected conditions for the accumulation of LUT at the modified electrode surface were t_{acc} = 20 min, and E_{acc} = 0.55 V.

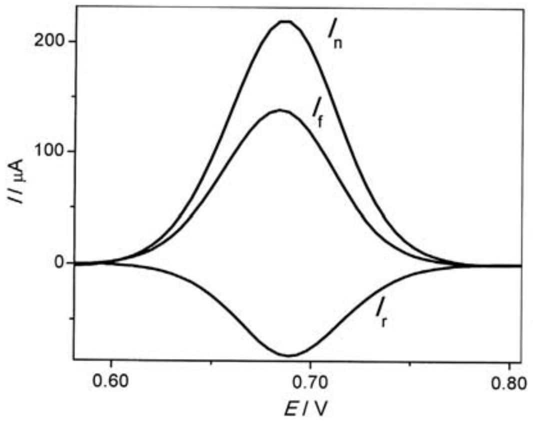

Fig. 5. Anodic stripping square wave voltammograms obtained in 1.0 mol $L^{-1}$$HClO_4$ after accumulation of 1.0 μmol L^{-1} of LUT at GCE/MWCNTs–PEI. t_{acc} = 20 min, E_{acc} = 0.55 V. ΔE_{sw} = 0.025 V, ΔE_s = 0.005 V and f = 40 Hz.

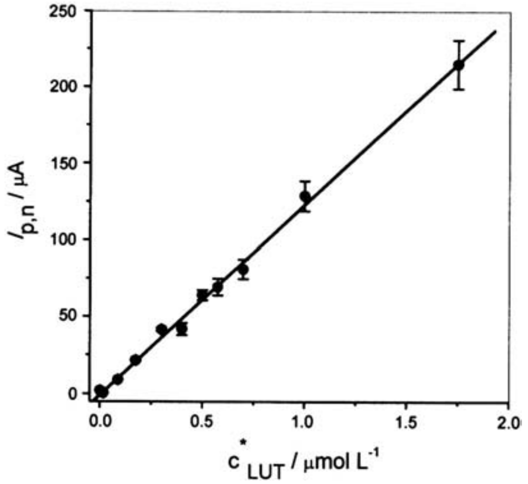

Fig. 6. Calibration plot obtained by anodic stripping square wave voltammetry after accumulation in LUT in 1 M $HClO_4$ aqueous solutions. Other experimental conditions are the same as that in Fig. 5.

The calibration plot obtained in 1.0 mol L^{-1} $HClO_4$ after performing the LUT accumulation at the electrode surface using conditions previously described is shown in Fig. 6. A linear relationship between net peak current, $I_{p,n}$, and LUT bulk concentration, c_{LUT}^* was found in the range from 2.4×10^{-3} to 1.75 μmol L^{-1}. The sensitivity was 124 μA nmol L^{-1} and the linear correlation coefficient, r^2, was 0.9964. The limit of detection, LOD, was 5.0×10^{-10} mol L^{-1} (calculated as 3σ/S, where σ is the blank standard deviation and S is the sensitivity of the calibration curve). The quantification limit, LOQ, calculated as 10σ/S, was 1.5×10^{-9} mol L^{-1} [46].

The reproducibility of MWCNT–PEI dispersions at GCE, evaluated from the sensitivity of LUT calibration curves using five modified electrodes, with six different dispersions, produced a relative standard deviation, RSD, of 8.0%. Repeatability assays were performed by measuring the sensitivity of LUT calibration curves using five electrodes modified with the same dispersion, giving a RSD of 7.3%. These results indicate that the overall process, from the dispersion preparation to the GCE modification with MWCNTs–PEI, is highly reproducible.

The GCE/MWCNTs–PEIs were used to determine LUT in two different peanut hull samples. LUT values obtained in both samples were 1.18 and 1.47 g/100 g. These values are in good agreement with those found by Yen et al. [47], who reported LUT contents in peanut hulls between 0.19 and 2.86 g/100 g, which depend on grain ripeness. On the other hand, our results show values higher than those reported for LUT in peanut hulls by Zhao et al. [48] and Sheng et al. [49]. Zhao et al. found a LUT content of 0.326 g/100 g using differential pulse voltammetry (DPV) at a GCE modified with MWCNTs, while Sheng et al. found LUT contents in the range from 0.64 to 0.88 g/100 g using a MWCNT/poly(ethylene terephthalate) (PET) composite electrode in combination with CE. Moreover, the linear range, and the LOD found by us are better than those values reported by Pang et al. [50], who used a GCE modified with graphene nanosheets (GNs) and hydroxyapatite (HA). Moreover, the electrochemical behavior of LUT has also been studied at macroporous carbon modified GCE in phosphate buffer solutions of pH 7.0 by CV and DPV [51]. This electrochemical sensor showed good statistical parameters, and was then used to determine LUT in capsules of a Chinese medicine. For comparison purposes, different statistical parameters obtained in this study are shown in Table 1 together with those previously reported in the literature.

LUT contents in peanut hull samples were also determined using HPLC. LUT showed a well defined chromatographic peak, with a retention time of 28.5 min. The calibration curve, obtained using HPLC

Table 1
Statistical parameters of different methods.

Technique	Working electrode	Linear range/mol L^{-1}	LOD/mol L^{-1}	LOQ/mol L^{-1}	Stability of the sensor	Ref.
DPV	GCE/MWNCTs	2.0×10^{-10}–3.0×10^{-9}	6.0×10^{-11}	–	~21 days	[48]
CE	MWCNTs/PET	1×10^{-6}–1×10^{-3}	4.4×10^{-7}	–	15[a]	[49]
DPV	GCE/GNs-HA	2×10^{-8}–1×10^{-5}	1×10^{-8}		30 days	[50]
SWV	GCE/MWCNTs–PEI	2.4×10^{-9}–1.75×10^{-6}	5.0×10^{-10}	1.5×10^{-9}	23 days	This work

[a] Repetitive measurements.

measurements, is expressed as (eight points were taken into account, which were determined by triplicate):

$$\mathrm{Area}(\mathrm{A} \times \mathrm{t}) = -(2 \pm 1) \times 10^{5} + (6.3 \pm 0.3) \times 10^{10} c^{*}_{\mathrm{LUT}}\left(\mathrm{mol\ L^{-1}}\right) \quad (1)$$

where the areas under chromatographic peaks in Eq. (1) are expressed in absorbance per time units.

Table 2 shows LUT contents obtained in peanut hulls using both methods. While these values are in good agreement, the percentage relative error of the chromatographic method (18 and 12.5% for samples 1 and 2, respectively) is greater than that of the electrochemical method (6.8 and 6% for samples 1 and 2, respectively). Thus, the sensor proposed in this study is very simple and fast. In addition, the relative cost compared to chromatography and electrophoresis is considerably smaller. The sensor could be applied for portable designs, and it presents very good analytical parameters, some of which are better than most of the existing methodologies. The sensor was useful for the quantification of LUT in two peanut hull samples, being in excellent agreement with the values determined using HPLC. Based on these results, we can conclude that the GCEs modified with MWCNTs/PEI could provide a satisfactory methodology for LUT determination in peanut hull samples.

4. Conclusions

Most of the electrochemical sensors modified with CNTs dispersed in PEI were based on the use of a highly-branched high molecular weight PEI (>750,000). In this study, we propose the use of a low molecular weight PEI as a dispersing agent of MWCNTs used to build a new platform for the development of an electrochemical sensor. The low molecular weight PEI demonstrated to be an effective dispersing agent for MWCNTs, being the proportion of 1.0 mg of MWCNTs to 5.0 mg mL^{-1} of PEI, the best to obtain a good compromise between sensitivity, stability and reproducibility.

Glassy carbon electrodes modified with MWCNTs–PEI exhibited good performance, reproducibility, repeatability, limits of detection and quantification, and a wide linear range for LUT quantification. This good analytical performance allowed us to determine LUT content in peanut hull samples, providing an advantageous alternative to existing methods for the determination of LUT in real samples.

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Table 2
LUT determination in peanut hull samples using electrochemical and chromatographic methods. Concentrations are expressed as g LUT per 100 g peanut hulls.

Method	Sample 1	Sample 2
Electrochemical	1.18 ± 0.08	1.47 ± 0.09
HPLC	1.1 ± 0.2	1.6 ± 0.2

References

[1] L.E. Craker, J.E. Simon (Eds.), Herbs, Spices and Medicinal Plants: Recent Advances in Botany, Horticulture and Pharmacology, vol. 3, The Howorth Press Inc., New York, 1996.

[2] K. Shimoi, H. Okada, M. Furugori, T. Goda, S. Takase, M. Suzuki, Y. Hara, H. Yamamoto, N. Kinae, Intestinal absorption of luteolin and luteolin 7-O-β-glucoside in rats and humans, FEBS Lett. 438 (1998) 220–224.

[3] E. Middleton, C. Kandaswami, Effects of flavonoids on immune and inflammatory cell functions, Biochem. Pharmacol. 43 (1992) 1167–1179.

[4] N.C. Cooks, S. Samman, Flavonoids: chemistry, metabolism, cardioprotective effects, and dietary sources, J. Nutr. Biochem. 7 (1996) 66–76.

[5] M.T. Fernandez, M.L. Mira, M.H. Florêncio, K.R. Jennings, Iron and copper chelation by flavonoids: an electrospray mass spectrometry study, J. Inorg. Biochem. 92 (2002) 105–111.

[6] Y.J. Park, H.J. Kim, S.J. Lee, H.Y. Choi, C. Jin, Y.S. Lee, A new chromone, 11-hydroxy-sec-O-glucosylhamaudol from *Ostericum koreanum*, Chem. Pharm. Bull. 55 (2007) 1065–1066.

[7] K. Kazuki, U. Mari, Y. Hiroaki, H. Takashi, Bioavailable flavonoids to suppress the formation of 8-OHdG in HepG2 cells, Arch. Biochem. Biophys. 455 (2006) 197–203.

[8] I. Fecka, W. Cisowski, TLC determination of tannins and flavonoids in extracts from some Erodium species using chemically modified stationary phases, J. Planar. Chromatogr. 15 (2002) 429–432.

[9] C.S. Liu, Y.S. Song, K.J. Zhang, J.C. Ryu, M. Kim, T.U. Zhou, Gas chromatographic/mass spectrometric profiling of luteolin and its metabolites in rat urine and bile, J. Pharm. Biomed. Anal. 13 (1995) 1409–1414.

[10] F.F. Liu, C.Y.W. Ang, T.M. Heinze, J.D. Rankin, R.D. Beger, J.P. Freeman, I.O. Lay, Evaluation of major active components in St. John's wort dietary supplements by high pressure liquid chromatography with photodiode array detection and electrospray mass spectrometric confirmation, J. Chromatogr. A 888 (1–2) (2000) 85–92.

[11] F.M. Areias, P. Valentao, P.B. Andrade, F. Ferreres, R.M. Seabra, Phenolic fingerprint of peppermint leaves, Food Chem. 73 (2001) 307–311.

[12] Y.R. Ku, C.Y. Chen, L.K. Ho, J.H. Lini, Y.S. Chang, Analysis of flavonoids in *Vernonia paltula* by high-performance liquid chromatography, J. Food Drug Anal. 10 (2002) 139–142.

[13] S.M. Wittemer, M. Veit, Validated method for the determination of six metabolites derived from artichoke leaf extract in human plasma by high performance liquid chromatography-colorimetric array detection, J. Chromatogr. B 793 (2003) 367–375.

[14] H. Srinivasa, M.S. Bagul, H. Padh, M. Rajani, A rapid densitometric method for the quantification of luteolin in medicinal plants using HPTLC, Chromatographia 60 (2004) 131–134.

[15] D.R. Jin, H. Hakamata, K. Takahashi, A. Kotani, F. Kusu, Separation of flavonoids by semi-micro high-performance liquid chromatography with electrochemical detection, Bull. Chem. Soc. Jpn. 77 (2004) 1147–1152.

[16] Z.M. Qian, H.J. Li, P. Li, M.T. Ren, D. Tang, Simultaneous qualitation and quantification of thirteen bioactive compounds in Flos lonicerae by high-performance liquid chromatography with diode array detector and mass spectrometry, Chem. Pharm. Bull. 55 (2007) 1073–1076.

[17] Y.Z. Zhou, X.X. Liu, X.H. Zheng, J.B. Zheng, Simultaneous determination of quercetin and luteolin in dried flowers by multivariate HPLC-ECD calibration, Chromatographia 66 (2007) 635–637.

[18] Y.Q. Lü, C.H. Wu, Z.B. Yuan, Determination of apigenin and luteolin in Flos buddlejae by hydroxy propyl-β-cyclodextrin micellar electrokinetic capillary chromatography, Chin. J. Anal. Chem. 6 (2005) 805–807.

[19] Q.C. Chu, T. Wu, L. Fu, J.N. Ye, Simultaneous determination of active ingredients in *Erigeron breviscapus* (Vant.) Hand-Mazz. by capillary electrophoresis with electrochemical detection, J. Pharm. Biomed. Anal. 37 (2005) 535–541.

[20] X. Xu, L. Yu, G. Chen, Determination of flavonoids in *Portulaca oleracea* L. by capillary electrophoresis with electrochemical detection, J. Pharm. Biomed. Anal. 41 (2006) 493–499.

[21] S. Iijima, Helical microtubules of graphitic carbon, Nature 354 (1991) 56–58.

[22] M.D. Rubianes, G.A. Rivas, Dispersion of multi-wall carbon nanotubes in polyethylenimine: a new alternative for preparing electrochemical sensors, Electrochem. Commun. 9 (2007) 480–484.

[23] K. Balasubramanian, T. Kurkina, A. Ahmad, M. Burghard, K. Kern, Tuning the functional interface of carbon nanotubes by electrochemistry: toward nanoscale chemical sensors and biosensors, J. Mater. Res. 27 (2012) 391–402.

[24] G.A. Rivas, M.D. Rubianes, M.C. Rodríguez, N.F. Ferreyra, G.L. Luque, M.L. Pedano, S.A. Miscoria, C. Parrado, Carbon nanotubes for electrochemical biosensing, Talanta 74 (2007) 291–307.

[25] B. Pérez-López, A. Merkoci, Nanomaterials based on biosensors for food analysis applications, Trends Food Sci. Technol. 22 (2011) 625–639.

[26] S. Murugesan, K. Myers, V. Subramanian, Amino-functionalized and acid treated multi-walled carbon nanotubes as supports for electrochemical oxidation of formic acid, Appl. Catal. B Environ. 103 (2011) 266–274.
[27] J. Chen, Q. Chen, Q. Ma, Influence of surface functionalization via chemical oxidation on the properties of carbon nanotubes, J. Colloid Interface Sci. 370 (2012) 32–38.
[28] M. Trojanowicz, A. Mulchandani, M. Mascini, Carbon nanotubes-modified screen-printed electrodes for chemical sensors and biosensors, Anal. Lett. 37 (2004) 3185–3204.
[29] M.W. Forney, J.C. Poler, Significantly enhanced single-walled carbon nanotube dispersion stability in mixed solvent systems, J. Phys. Chem. C 115 (2011) 10531–10536.
[30] G.A. Rivas, S.A. Miscoria, J. Desbrieres, G.D. Barrera, New biosensing platforms based on the layer-by-layer self-assembling of polyelectrolytes on Nafion/carbon nanotubes-coated glassy carbon electrodes, Talanta 71 (2007) 270–275.
[31] E.D. Belashova, N.A. Melnik, N.D. Pismenskaya, K.A. Shevtsova, A.V. Nebavsky, K.A. Lebedev, V.V. Nikonenko, Overlimiting mass transfer through cation-exchange membranes modified by Nafion film and carbon nanotubes, Electrochim. Acta 59 (2012) 412–423.
[32] L. Qian, X. Yang, Composite film of carbon nanotubes and chitosan for preparation of amperometric hydrogen peroxide biosensor, Talanta 68 (2006) 721–727.
[33] S. Bollo, N.F. Ferreyra, G.A. Rivas, Electrooxidation of DNA at glassy carbon electrodes modified with multi-wall carbon nanotubes dispersed in chitosan, Electroanalytical 19 (2007) 833–840.
[34] T. Zhao, L. Liu, G. Li, A. Dang, T. Li, Electrochemical determination of melamine with a glassy carbon electrode coated with a multi-wall carbon nanotube/chitosan composite, J. Electrochem. Soc. 159 (2012) K141–K145.
[35] S.S. Jyothirmayee Aravind, S.G. Ramaprabhu, Noble metal dispersed multiwalled carbon nanotubes immobilized ss-DNA for selective detection of dopamine, Sens. Actuators B Chem. 155 (2011) 679–686.
[36] Y. Jalit, M.C. Rodríguez, M.D. Rubianes, S. Bollo, G.A. Rivas, Glassy carbon electrodes modified with multiwall carbon nanotubes dispersed in polylysine, Electroanalytical 20 (2008) 1623–1631.
[37] P. Dalmasso, M.L. Pedano, G.A. Rivas, Dispersion of multi-wall carbon nanotubes in polyhistidine: characterization and analytical applications, Anal. Chim. Acta. 710 (2012) 58–64.
[38] G.L. Luque, N.F. Ferreyra, A. Granero, S. Bollo, G.A. Rivas, Electrooxidation of DNA at glassy carbon electrodes modified with multiwall carbon nanotubes dispersed in polyethylenimine, Electrochim. Acta 56 (2011) 9121–9126.
[39] Y.-T. Shieh, T.-Y. Yu, T.-L. Wang, C.H. Yang, Effects of pH on electrocatalytic activity of carbon nanotubes in polyethylenimine composites, J. Electroanal. Chem. 664 (2012) 139–145.
[40] A. Sánchez Arribas, E. Bermejo, M. Chicharro, A. Zapardiel, G.L. Luque, N.F. Ferreyra, G.A. Rivas, Analytical applications of glassy carbon electrodes modified with multi-wall carbon nanotubes dispersed in polyethylenimine as detectors in flow systems, Anal. Chim. Acta. 596 (2007) 183–194.
[41] F. Gutiérrez, G. Ortega, J.L. Cabrera, M.D. Rubianes, G.A. Rivas, Quantification of quercetin using glassy carbon electrodes modified with multiwalled carbon nanotubes dispersed in polyethylenimine and polyacrylic acid, Electroanalytical 22 (2010) 2650–2657.
[42] G. Gutiérrez, A.S. Gutiérrez, G. García, L. Galicia, G.A. Rivas, Determination of 8-hydroxy-2′-deoxyguanosine using electrodes modified with a dispersion of carbon nanotubes in polyethylenimine, Electroanalytical 23 (2011) 1221–1228.
[43] D. Pin-Der, Y. Dong-Bor, Y. Gow-Chin, Extraction and identification of an antioxidative component from peanut hulls, JAOCS 69 (1992) 814–818.
[44] J.G. Osteryoung, J. O'Dea, in: A.J. Bard (Ed.), Electroanalytical Chemistry, Marcel Dekker, New York, 1987, pp. 209–234.
[45] R.L. McCreery, Carbon electrodes: structural effects on electron transfer kinetics, in: A.J. Bard (Ed.), Electroanalytical Chemistry, Marcel Dekker, New York, 1991, pp. 221–274.
[46] J.C. Miller, J.N. Miller, Estadística para Química Analítica, Addison-Wesley Iberoamericana, S.A., Wilmington, Delaware, 1993.
[47] G.C. Yen, P.D. Duh, C.L. Tsai, Relationship between antioxidant activity and maturity of peanut hulls, J. Agric. Food Chem. 41 (1993) 67–70.
[48] D. Zhao, X. Zhang, L. Feng, Q. Qi, S. Wang, Sensitive electrochemical determination of luteolin in peanut hulls using multi-walled carbon nanotubes modified electrode, Food Chem. 127 (2011) 694–698.
[49] S. Sheng, L. Zhang, G. Chen, Determination of 5,7-dihydrochromone and luteolin in peanut hulls by capillary electrophoresis with a multiwall carbon nanotube/poly(ethylene terephthalate) composite electrode, Food Chem. 145 (2014) 555–561.
[50] P. Pang, Y. Liu, Y. Zhang, Y. Gao, Q. Hu, Electrochemical determination of luteolin in peanut hulls using graphene and hydroxyapatite nanocomposite modified electrode, Sens. Actuators B Chem. 194 (2014) 397–403.
[51] L. Zeng, Y. Zhang, H. Wang, L. Guo, Electrochemical behavior of luteolin and its detection based on macroporous carbon modified glassy carbon electrode, Anal. Methods 5 (2013) 3365–3370.