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Electrochemical Sensor for the Quantification of Dopamine Using Glassy Carbon Electrodes Modified with Single-Wall Carbon Nanotubes Covalently Functionalized with Polylysine

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Abstract: We report a dopamine electrochemical sensor based on the modification of glassy carbon electrodes (GCE) with polylysine-functionalized single-wall carbon nanotubes (SWCNT-PLys). The resulting electrodes (GCE/SWCNT-PLys) showed a significant improvement in the electrooxidation of dopamine with drastic decrease in the peak potentials separation and important enhancement in the associated currents. Dopamine was detected by differential pulse voltammetry-adsorptive stripping with medium exchange at nanomolar levels even in the presence of high excess of ascorbic and uric acids. The sensor was successfully used for the quantification of dopamine in urine samples enriched with the neurotransmitter.

Keywords: Covalently-functionalized carbon nanotubes · Glassy carbon · Dopamine · Polylysine

1 Introduction

Carbon nanomaterials have received great attention for technological applications and fundamental research due to their unique properties [1–4]. In particular, the feasibility to modify the carbon nanostructures with different molecules and their inherent electrocatalytic activity has made them extremely useful in the area of electrochemical (bio)sensors [5–7].

Regarding carbon nanotubes (CNTs), important efforts have been done in the last years to solve the inconvenience of their poor solubility in most solvents and make possible, in this way, the preparation of homogeneous and stable dispersions, which are required for a robust and reproducible coverage of the electrodes surfaces [8–12]. Particularly, polymers facilitate the dispersion of CNTs either by non-covalent or covalent functionalization allowing the building of supramolecular architectures more easily dissolved in aqueous solvents [13–15]. Most of the protocols for the covalent functionalization of CNTs rely on their harsh initial oxidation to generate different oxygenated groups, mainly carboxylic residues which can be used for further incorporation of different (bio)molecules [16,17]. Even when the covalent functionalization produces a disruption in the electronic properties of CNTs, the significant increment of the defects produced as a consequence of the oxidation process, makes possible a positive balance between the changes in conductivity and reactivity [18–22].

Inspired by the work of Jalit et al. [23,24] who demonstrated the efficient non-covalent functionalization of multi-walled carbon nanotubes (MWCNTs) with polylysine (Plys), we recently reported the behavior of single-walled carbon nanotubes (SWCNT) covalently functionalized with Plys (SWCNT-Plys) and the analytical usefulness of glassy carbon electrodes (GCE) modified with SWCNT-Plys as platform for the development of glucose electrochemical biosensors [25].

The goal of this work was the development of an electrochemical sensor for dopamine (Do) obtained by modification of GCE with SWCNTs covalently modified with Plys. Do is an ubiquitous biomarker involved in many mammalian functions and pathological disorders like Parkinson's, Huntington's and schizophrenia [26]. It is present in numerous biological fluids, from brain organelles to blood or urine [27]. The levels of Do fluctuate depending on the biological location, physiological state or age.

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For example, normal blood values go between 120 and 450 μM depending on the diet [28,29]. The synaptic concentration in "substance nigra" in the Parkinson's disease drops to values lower than 10 nM while in the case of stress, the urine concentrations rise up several times higher than the physiological value of 1.3 μM [30,31]. Do concentration also changes drastically with drug consumption and dependence (up to 50 μM in the caudate nucleus) [32,33]. Therefore, it is crucial to obtain sensitive and selective methodologies for the quantification of Do.

Electrochemical sensing has demonstrated to be an excellent strategy for the quantification of different species due to their widely known advantages [34]. Do electrochemical quantification at most of the solid electrodes presents two inconveniences, one is the electrode fouling upon oxidation [35], and the other is the interference of metabolically related compounds like ascorbic acid (AA) and uric acid (UA) due to their close oxidation overvoltages [36].

In the following sections we discuss the optimization of the experimental conditions to obtain an efficient SWCNT-Plys dispersion, its robust deposition at GCE (GCE/SWCNT-Plys), the sensitive and selective quantification of Do in the presence of high excess of UA and AA and the application for the quantification of Do in urine samples.

2 Experimental

2.1 Materials and Reagents

Single walled carbon nanotubes (SWCNT) synthesized by the electric arc reactor method using Ni/Y catalyst (AP-SWNT grade) were purchased from Carbon Solutions Inc. (Riverside, California). They were purified by air oxidation at 350°C for 2h and then refluxed in 3 M HCl for 2 h. Sodium dodecylbenzenesulfonate (SDBS), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), *N*,*N*-diisopropylethylamine (EDIPA) were purchased from Sigma-Aldrich in reagent grade. Polylysine hydrochloride (Plys) (Reference P9404, molecular weight >30000) and dopamine (Do) were received from Sigma. AA and sodium phosphates were received from Baker. UA was from Merck. Ultrapure water (ρ= 18 MΩ cm^{-1}) from a Millipore-MilliQ system was used for preparing all the solutions. A 0.050 M phosphate buffer solution (PBS) pH 7.40 was employed as supporting electrolyte. Other chemicals were reagent grade and used without further purification. All the experiments were conducted at room temperature.

2.2 Apparatus

The electrochemical experiments were performed with a Epsilon (BAS) potentiostat. The electrodes were inserted into the cell (BAS, Model MF-1084) through holes in its Teflon cover. A platinum wire and Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) were used as counter and reference electrodes, respectively. All potentials are referred to the latter. A magnetic stirrer (BASi Cell stand) set at 800 rpm and a stirring bar provided the convective transport during the amperometric measurements. The characterization of the SWCNT covalently modified with Plys was performed by FTIR and thermogravimetric measurements [25].

2.3 Synthesis of Functionalized SWCNT

The synthesis of SWCNT covalently modified with Plys was published elsewhere [25]. Shortly, SWCNT were oxidized in a H_2SO_4/HNO_3 mixture, afterwards, the oxidized SWCNT were dispersed in water with SDBS by ultrasonication and the mixture was purged with Ar. Carboxylic groups of the oxidized SWCNTs were activated with HBTU for further amidation with the amine groups of Plys. The reaction product was purified by dialysis and dried by lyophilization. Modified SWCNTs were characterized by FTIR, TGA and Kaiser test as it was previously reported [25].

2.4 Preparation of the Working Electrodes

2.4.1 Preparation of SWCNT-Plys Dispersion

The SWCNT-Plys dispersion (0.30 mg/mL) was prepared by adding H_2O:EtOH (2:1 v/v) to the SWCNT-Plys powder, followed by bath sonication for 15 minutes.

2.4.2 Preparation of GCE with SWCNT-Plys (GCE/SWCNT-Plys)

The GCE was polished with alumina slurries of 1.0, 0.30, and 0.05 μm for 2 min each. Before functionalization, the electrode was cycled in a 0.050 M phosphate buffer solution pH 7.40 for ten times from −0.300 V to 0.800 V at 0.050 Vs^{-1}. Then, it was modified with the SWCNT-Plys dispersion in the following way: an aliquot of 10 μL was dropped on top of a polished GCE and then the solvent was allowed to evaporate at room temperature for 1 h. The modified electrodes were cycled for ten times between −0.300 V and 0.800 V at 0.050 V s^{-1} before starting the electrochemical experiments.

2.5 Procedure

Do quantification involved the following steps:

- *Preconcentration:* at open circuit potential for a given time in a solution of Do prepared in 0.050 M phosphate buffer solution pH 7.40 under stirring.
- *Washing:* with phosphate buffer solution for 10 seconds.
- *Stripping*: in phosphate buffer solution (0.050 M; pH 7.40) by differential pulse voltammetry (DPV). The DPV parameters are the following: scan rate of

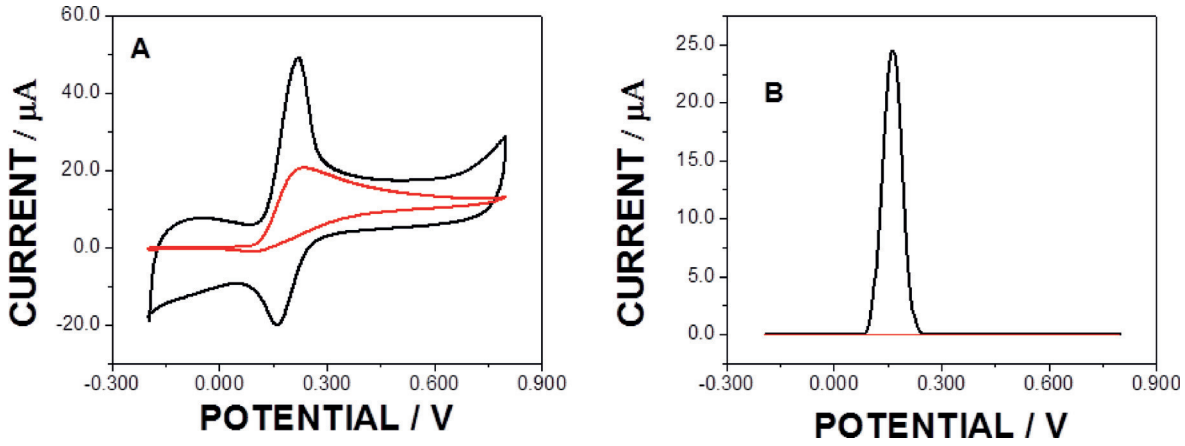

Fig. 1. A) Cyclic voltammograms for 1.0×10^{-3} M Do at bare GCE (red line) and GCE/SWCNT-Plys (black line). Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. Scan rate: 0.050 Vs^{-1}. B) Differential pulse voltammograms obtained at bare GCE (red line) and GCE/SWCNT-Plys (black line) in a 0.050 M phosphate buffer solution pH 7.40 after 5.0 min accumulation at open circuit potential in a 2.0×10^{-6} M Do solution.

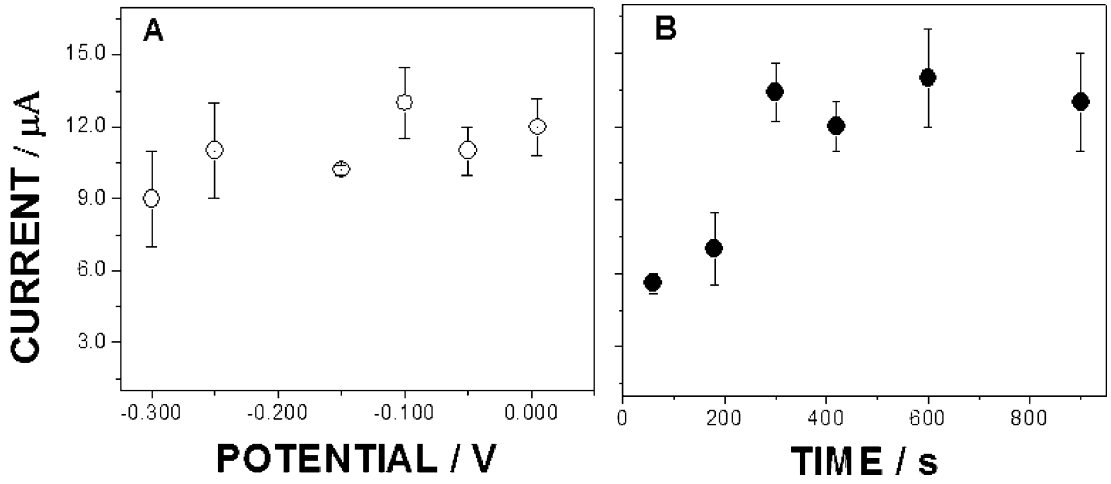

Fig. 2. A) Oxidation currents obtained at GCE/SWCNT-Plys by DPV-adsorptive stripping with medium exchange after accumulation in a 1.0×10^{-6} M Do solution for 5.0 min at different potentials (A) or for different times at 0.005 V (B). Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

20 mVs^{-1}, potential increment 0.004 V, pulse amplitude 0.050 V, pulse with 0.050 and pulse period 0.2 s.

3 Results and Discussion

Figure 1A shows cyclic voltammograms obtained at GCE (red line) and GCE/SWCNT-Plys (black line) in a 1.0×10^{-3} M Do solution at 0.050 Vs^{-1}. At bare GCE the oxidation of Do occurs at (0.266 ± 0.009) V, the oxidation current is (20 ± 1) µA and the current for the reduction of dopaminequinone, the product of Do oxidation, is almost negligible. At GCE/SWCNT-Plys, Do is oxidized at (0.220 ± 0.002) V and the peak current is (43 ± 5) µA. At variance with GCE, at GCE/SWCNT-Plys, the dopaminequinone reduction peak is clearly defined with an associated current of (23 ± 3) µA and a peak potential separation of (0.058 ± 0.003) V.

Figure 1B displays differential pulse voltammograms obtained at GCE (red line) and at GCE/SWCNT-Plys (black line) in a 0.050 M phosphate buffer solution pH 7.40 after accumulation of Do for 5.0 min from a 2.0×10^{-6} M Do solution. No signal is obtained at GCE under these experimental conditions, evidencing a poor adsorption of the neurotransmitter. On the contrary, at GCE/SWCNT-Plys a clearly defined oxidation signal is obtained at 0.163 V after accumulation of Do. Therefore, the presence of CNTs at the electrode surface not only improved the electrochemical behavior of Do but also facilitated the adsorption of the catecholamine at SWCNT-Plys modified GCE.

Figure 2A displays the effect of the adsorption potential between −0.300 and 0.000 V obtained from DPV in a phosphate buffer solution after adsorption of Do for 5.0 min from a Do solution (1.0×10^{-6} M) and medium exchange. The oxidation current remains almost constant

Table 1. Effet of the amount of SWCNT-Plys in the dispersion on the DPV signal for Do obtained by adsorptive stripping and medium exchange. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. Accumulation time: 5.0 min. Accumulation potential : 0.005 V.

Amount of SWCNT-Plys ($mg\,mL^{-1}$)	Current (μA)
0.10	7.3±0.3
0.30	12±2
0.50	7±2
0.70	9±1

within this potential range, being the open circuit potential (0.005 V) the selected one for further studies. Figure 2B depicts the effect of the adsorption time on the accumulation of 1.0×10^{-6} M Do at open circuit potential. The profile shows a fast increase at short times and a trend to saturation after 300 s, being this time the selected one for Do quantification.

We also investigated the effect of the amount of SWCNT-Plys dispersed in the mixture ethanol/water from 0.1 to 0.5 $mg\,mL^{-1}$ on the Do oxidation current. As Table 1 displays, Do oxidation peak current increases as the amount of SWCNT-Plys in the dispersion used to modify the electrode increases from 0.10 to 0.300 $mg\,mL^{-1}$. This effect is due to the increment in the electroactive area of the SWCNT-Plys modified GCE. No additional improvement was obtained by modification of GCE with dispersions prepared using higher amounts of SWCNT-Plys due to a more difficult dispersion of SWCNT-Plys. The amount selected for further work was 0.30 $mg\,mL^{-1}$ SWCNT-Plys in ethanol/water (1:2 v/v). The solvent used for preparing SWCNT-Plys dispersions demonstrated to be an important aspect (not shown). The deposition on GCE of SWCNT-Plys dispersed in pure water or pure ethanol was not efficient enough to obtain robust sensors. On the contrary, the incorporation of ethanol as co-solvent improved the quality of the dispersions and further depositions at the electrode surface. The best analytical performance for Do was obtained for GCE modified with SWCNT-Plys prepared in ethanol/water 1:2.

The calibration plot for Do after 5.0 min accumulation at open circuit potential and medium exchange is presented in Figure 3. The linear range goes from 1.0×10^{-7} M to 2.0×10^{-6} M Do, with a sensitivity of $1.26\times10^{7}\,\mu A\,M^{-1}$ ($r^2=0.9997$), a detection limit of 16 nM and a quantification limit of 48 nM (taken as $3\times\sigma/S$ and $10\times\sigma/S$, respectively, where σ is the standard deviation of the blank signal and S the sensitivity). Table 2 displays the most significant analytical parameters of other methodologies for the quantification of Do based on CNTs recently reported. Our sensor presents lower detection limit than most of the proposed strategies [37–42] and a slightly higher compared to GCE modified with PCTA and ionic liquid [43] and a combination of nanomaterials (CNTs and AuNP).

Considering that AA and UA are common interferents in the electrochemical quantification of Do due to their

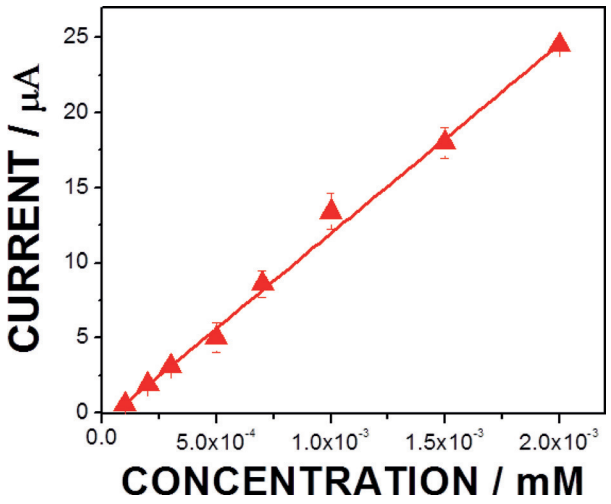

Fig. 3. Calibration plot for Do obtained at GCE/SWCNT-Plys by DPV-adsorptive stripping with medium exchange after accumulation in Do solutions of different concentrations for 5.0 min at 0.005 V. Supporting electrolyte: phosphate buffer solution pH 7.40.

close oxidation overvoltages at GCE, we evaluate the DPV-adsorptive behavior of AA and UA at GCE/SWCNT-Plys. Figure 4A displays the DPV response obtained at GCE/SWCNT-Plys after adsorption of AA at open circuit potential from a 1.0×10^{-3} M AA solution and medium exchange. No response is observed, indicating that under these experimental conditions, AA is poorly adsorbed at the electrode surface. Similar study was performed with 3.0×10^{-4} M UA (Figure 4B) and, at variance with AA, UA can be adsorbed at GCE/SWCNT-Plys and oxidized at 0.280 V with an associated current of (6±1) μA.

Considering that the adsorption of AA at GCE/SWCNT-Plys is very poor and that the adsorbed UA is oxidized at a potential 120 mV more positive than that for Do, we performed the quantification of Do in the presence of 1.0×10^{-3} M AA and 3.0×10^{-4} M UA (Figure 5A). The sensitivities obtained from the DPV-adsorptive stripping with medium exchange are $1.37\times10^{7}\,\mu A\,M^{-1}$ ($r^2=0.997$) and $1.28\times10^{7}\,\mu A\,M^{-1}$ ($r^2=0.998$) in the presence of AA (empty circles) and UA (full circles), respectively. Taking into account that the sensitivity obtained for Do alone was $1.26\times10^{7}\,\mu A\,M^{-1}$, there are not significant difference from the statistical point of view between the sensitivities for Do in the presence and absence of the interferents, clearly demonstrating that it is possible to quantify Do even in the presence of a large excess of AA and UA. Figure 5B shows typical DPV responses for 1.5×10^{-6} M Do alone and in the presence of 1.0×10^{-3} M AA and 3.0×10^{-4} M UA, where is evident that neither AA nor UA interferes in the determination of Do. The oxidation signal for a mixture of 1.5×10^{-6} M Do + 1.0×10^{-3} M AA + 1.0×10^{-3} M UA matched in a 99.96% the signal obtained for 1.5×10^{-6} M Do alone.

The reproducibility obtained for 1.0×10^{-6} M Do using the same dispersion was 5.5% (average signal for 8 elec-

Table 2. Analytical characteristics of different Do electrochemical sensors based on the use of CNTs. poly(Tyr)/MWCNTs-COOH: poly (tyrosine)/carboxyl functionalized multi-walled carbon nanotubes composite film modified electrode; HCNTs: helical carbon nanotubes; PDDA: poly(diallyl dimethyl ammonium chloride); GCE/MWCNT@PDOP@PtNPs: composite, MWCNT, PDOP: polydopamine; PtNPs:Pt nanoparticles; Fe(III)P/MWCNT/GCE: chloro [3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropanoate (2−)]iron(III)/multiwalled carbon nanotubes (Fe(III)P/MWCNTs) composites and a modified glassy carbon electrode (GCE); IL: ionic liquid: [BMIM] [BF_6]; PTCA: 3,4,9,10-perylene tetracarboxylic acid; GS: graphene sheets. DPV-Ads: differential pulse voltammetry-adsorptive stripping.

Platform	Detection	Sensitivity ($\mu A\,\mu M^{-1}$)	DL (μM)	Linear Range (μM)	Analytical performance	Ref.
Nitrogen doped graphene	DPV	0.03195	0.25	0.5–170	Simultaneous AA, DA and UA	[37]
GCE/poly(Tyr)/ MWCNTs-COOH	DPV	–	0.02	0.1–30	Simultaneous AA, DA and UA	[38]
GCE/ MWCNT@PDOP@PtNPs	DPV	1.03	0.08	0.25–20	Mixture: DA,UA,AA	[39]
GCE/PDDA@HCNTs		2.058 [a]	0.08	2.5–10	Real sample: fetal bovine serum	[40]
GE/Fe(III)P/MWCNT	Amperometry	–	0.09	0.7–3600	Real sample: urine	[41]
GCE/GS-MWCNT-PTCA-IL [a]	DPV	0.0150	0.0012	0.03–3820	Detection of Do in the presence of AA and UA. Real sample: pharmaceutical preparation.	[42]
GCE/MWCNT-PEI	DPV-Adsorptive stripping	0.72	0.1	0.5–50	Detection of Do in the presence of UA. Real sample: urine	[43]
GCE/SWCNT-Plys	DPV-Adsorptive stripping	12.6	0.016	0.1–2.0	Detection of Do in the presence of AA and UA. Real sample: urine	This work

[a] Density current/j ($mA\,cm^{-2}$).

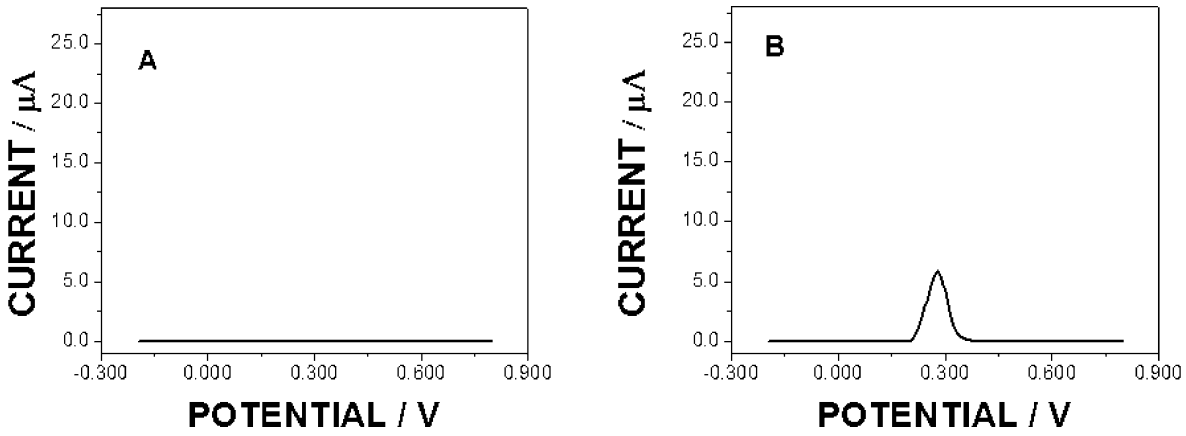

Fig. 4. Differential pulse voltammograms obtained after accumulation at 0.005 V in a 1.0×10^{-3} M AA (A) or 3.0×10^{-4} M UA (B) solutions at GCE/SWCNT-Plys. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

trodes: (13 ± 1) µA). The reproducibility inter-dispersions was 5.9 % (average signal for 8 electrodes: (11 ± 1) µA).

The sensor was challenged with a urine sample diluted with 0.050 M phosphate buffer solution pH 7.40 (1:75). Since Do was not found in the sample, it was enriched with 1.5×10^{-4} M Do and the recovery was (96 ± 9) %.

4. Conclusions

In summary, the combination of the electrocatalytic activity of SWCNTs and the efficient dispersion mediated by the Plys covalently attached to them have allowed the highly sensitive (detection at nM levels) and selective quantification of Do even in the presence of large excess of AA and UA in a simple and fast way without needing of permselective membranes or complicated modification steps, just using a small amount of SWCNTs covalently modified with Plys. The electrode was successfully used for the determination of Do in urine samples. These characteristics make GCE/SWCNT-Plys an interesting analytical tool and open the doors to new challenges in the electroanalytical determination of other bioanalytes and further practical applications.

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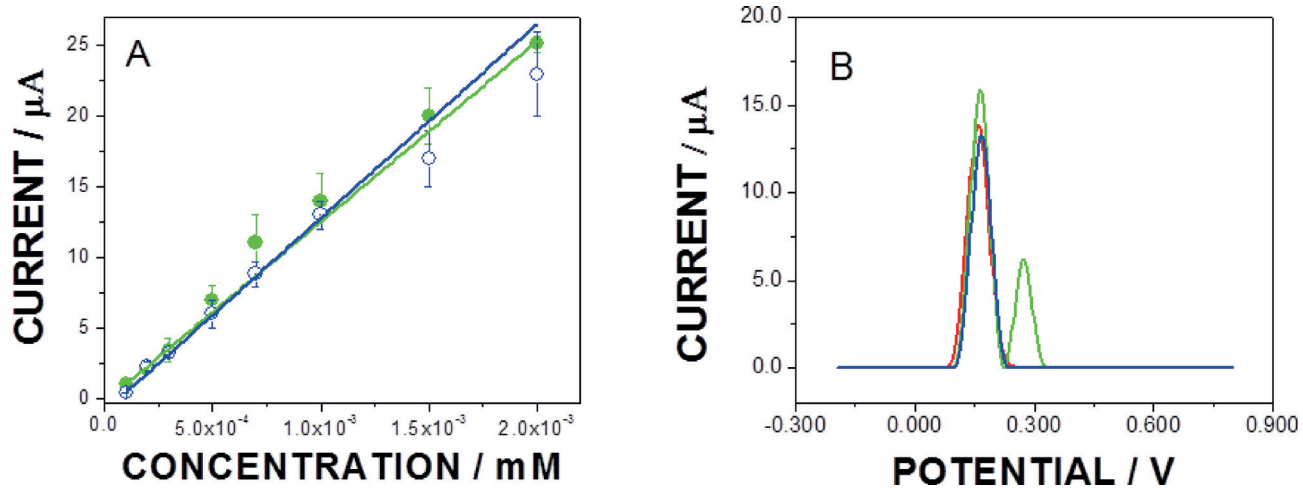

Fig. 5. (A) Calibration plots for Do obtained by DPV-adsorptive stripping with medium exchange after accumulation from Do solutions of different concentrations at GCE/SWCNT-Plys for 5.0 min at 0.005 V in the presence of 1.0×10^{-3} M AA (blue line) or 3.0×10^{-4} M UA (green line). Supporting electrolyte: phosphate buffer solution pH 7.40. (B) Differential pulse voltammograms obtained after accumulation at 0.005 V in a 2.0×10^{-6} M Do alone (red line) or in the presence of 1.0×10^{-3} M AA (blue line) or 3.0×10^{-4} M UA (green line). Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

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References

[1] F. Valentini, M. Carbone, G. Palleschi, *Anal. Bioanal. Chem.* **2013**, *405*, 451.
[2] A. Walcarius, S. D. Minteer, J. Wang, Y. Lin, A. Merkoçi, *J. Mater. Chem.* **2013**, *38*, 4878.
[3] A. T. Lawal, *Talanta* **2015**, *131*,424.
[4] C. I. L. Justino, T. A. P. Rocha-Santos, A. C. Duarte, *Trends Anal. Chem.* **2013**, *45*, 24.
[5] P. Yáñez-Sedeño, J. M. Pingarrón, J. Riu, F. X. Rius, *Trends Anal. Chem.* **2010**, *29*, 939.
[6] E. N. Primo, F. A. Gutierrez, G. L. Luque, P. R. Dalmasso, A. Gasnier, Y. Jalit, M. Moreno, M. V. Bracamonte, M. Eguilaz Rubio, M. L. Pedano, M. C. Rodríguez, N. F. Ferreyra, M. D. Rubianes, S. Bollo, G. A. Rivas, *Anal. Chim. Acta*, **2013**, *805*, 19.
[7] F. Valentini, M. Carbone, G. Palleschi, *Anal. Bioanal. Chem.* **2013**, *405*, 451.
[8] V. Papper, K. Elouarzaki, K. Gorgy, A. Sukharaharja, S. Cosnier, R. S. Marks, *Chem. Eur. J.* **2014**, *42*, 13561.
[9] I. Capek, *Adv. Colloid Interf.* **2009**, *150*, 63.
[10] P. Bilalis, D. Kastsigiannopoulos, A. Avregopoulos, G. Sakellarion, *RSC Adv.* **2014**, *4*, 2911.
[11] G. Dreselhaus, M. A. Pimenta, R. Saito, J.-C. Charlier, S. D. M. Brown, P. Corio, A. Marucci, M. S. Dresselhaus, *Science and Applications of Nanotubes* (Eds: D. Tomanek, R. J. Enbody), Kluwer Academic, New York, **2000**, p. 275.
[12] M. Eguílaz, N. F. Ferreyra, G. A. Rivas, *Electroanalysis*, **2014**, *26*, 2434.
[13] X. Ling, Y. Wei, L. Zou, S. Xu, *Colloid Surf. B* **2014**, *443*, 19.
[14] N. G. Sahoo, S. Rana, J. W. Cho, L. Li, S. H. Chan, *Progr. Polym. Sci.* **2010**, *35*, 837.
[15] S. A. Ntim, O. Sae-Khow, F. A. Witzmann, S. Mitra, *J. Colloid Interf. Sci.* **2011**, *355*, 383.
[16] X. Liu, I. Marangon, G. Melinte, C. Wilhelm, C. Ménard-Moyon, B. P. Pichon, O. Ersen, K. Aubertin, W. Baaziz, C. Pham-Huu, S. Bégin-Colin, A. Bianco, F. Gazeau, D. Bégin, *ACS Nano* **2014**, *11*, 11290.
[17] A. Battigelli, C. Ménard-Moyon, T. Da Ros, M. Prato, A. Bianco, *Adv. Drug Deliv. Rev.* **2013**, *65*, 1899.
[18] I. Dumitrescu, P. R. Unwin, J. V. Macpherson, *Chem. Commun.* **2009**, *45*, 6886.
[19] K. Gong, S. Chakrabarti, L. Dai, *Angew. Chem. Int.* **2008**, *47*, 5446.
[20] P. Cañete-Rosales, M. González, A. Ansón, M. T. Martínez, C. Yáñez, S. Bollo, *Electrochim. Acta* **2014**, *140*, 489.
[21] C. E. Banks, R. R. Moore, J. D. Trevor R. G. Compton, *Chem. Commun.* **2004**, 1804.
[22] C. E. Banks, R.G Compton, *Analyst* **2006**, *131*, 15.
[23] Y. Jalit, M. Moreno, F. A. Gutiérrez, A. Sánchez, M. Chicharro, E. Bermejo, A. Zapardiel, C. Parrado, G. A. Rivas, M. C. Rodríguez, *Electroanalysis* **2013**, *25*, 1116.
[24] Y. Jalit, M. C. Rodríguez, M. D. Rubianes, S. Bollo, G. A. Rivas, *Electroanalysis* **2008**, *20*, 1623.
[25] A. Gasnier, J. M. González-Domínguez, A. Ansón-Casaos, J. Hernández-Ferrer, M. L. Pedano, M. D. Rubianes, M. T. Martínez, G. A. Rivas, *Electroanalysis* **2014**, *26*, 1676.
[26] C. N. Lin, H. C. Liu, S. J. Tsai, T. Y. Liu, C. J. Hong, *Eur. Neurol.* **2002**, *48*, 207.
[27] N. N. Li, E. H. Zheng, X. Chen, S. R. Sun, C. P. You, Y. M. Ruan, X. X. Weng, *Int. J. Electrochem. Sci.* **2013**, *8*, 6524.
[28] S. Lupu, A. Mucci, L. Pigani, R. Seeber, C. Zanardi, *Electroanalysis* **2002**, *14*, 519.
[29] M. Noroozifar, M. Khorasani-Motlagh, A. Taheri, *Talanta* **2010**, *80, 1657.*
[30] *Normal Hormone Reference Ranges. Greenspan's Basic & Clinical Endocrinology*, 9th ed., McGraw-Hill, New York, **2011**.
[31] V. Muchenje, K. Dzama, M. Chimonyo, P. E. Strydom, J. G. Raats, *Meat Sci.* **2009**, *81*, 653.
[32] C. Lüscher, R. C. Malenka, *Neuron* **2011**, *69*, 650.
[33] B. J. Venton, R. M. Wightman, *Anal. Chem.* **2003**, *75*, 414A.

[34] N. Thiyagarajan, J.-L. Chang, K. Senthilkumar, J.-M. Zen, *Electrochem. Commun.* **2014**, *38*, 86.
[35] M. D. Rubianes, A. Sánchez, E. Bermejo, M. Chicharro, A. Zapardiel, G. A. Rivas, *Sens. Actuators B* **2010**, *144*, 274.
[36] P. E. Erden, E. Kilic, *Talanta* **2013**, *107*, 312.
[37] Z. H. Sheng, X. Q. Zheng, J. Y. Xu, W. J. Bao, F. B. Wang, X. H. Xia, *Biosens. Bioelectron.* **2012**, *34*, 125.
[38] S. J. Li, C. Qian, K. Wang, B. Y. Hua, F. B. Wang, Z. H. Sheng, X. H. Xia, *Sens. Actuators B* **2012**, *174*, 441.
[39] M. Lin, H. Huang, Y. Liu, C. Liang, S. Fei, X. Chen, C. Ni, *Nanotechnology* **2013**, *24*, 065501.
[40] B. Zhang, D. Huang, X. Xu, G. Alemu, Y. Zhang, F. Zhan, Y. Shen, M. Wang, *Electrochim. Acta* **2013**, *91*, 261.
[41] C. Wang, R. Yuan, Y. Chai, S. Chen, Y. Zhang, F. Hu, M. Zhang, *Electrochim. Acta* **2012**, *62*,109.
[42] A. Gutiérrez, M. L. Lozano, L. Galicia, N. F. Ferreyra, G. A. Rivas, *Electroanalysis*, **2014**, *26*, 2191.
[43] X. Niu, W. Yang, H. Guo, J. Ren, *J.* Gao, *Biosens. Bioelectron.* **2013**, 41, 225.

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