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Grafted-double walled carbon nanotubes as electrochemical platforms for immobilization of antibodies using a metallic-complex chelating polymer: Application to the determination of adiponectin cytokine in serum

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ABSTRACT

An electrochemical immunosensor for adiponectin (APN) using screen printed carbon electrodes (SPCEs) modified with functionalized double-walled carbon nanotubes (DWCNTs) as platforms for immobilization of the specific antibodies is reported. DWCNTs were functionalized by treatment with 4-aminobenzoic acid (HOOC-Phe) in the presence of isoamylnitrite resulting in the formation of 4-carboxyphenyl-DWCNTs. The oriented binding of specific antibodies toward adiponectin was accomplished by using the metallic-complex chelating polymer Mix&Go™. The HOOC-Phe-DWCNTs-modified SPCEs were characterized by cyclic voltammetry and compared with HOOC-Phe-SWCNTs/SPCE. The different variables affecting the performance of the developed immunosensor were optimized. Under the selected conditions, a calibration plot for APN was constructed showing a range of linearity extending between 0.05 and 10.0 μg/mL which is adequate for the determination of the cytokine in real samples. A detection limit of 14.5 ng/mL was achieved. The so prepared immunosensor exhibited a good reproducibility for the APN measurements, excellent storage stability and selectivity, and a much shorter assay time than the available ELISA kits. The usefulness of the immunosensor for the analysis of real samples was demonstrated by analyzing human serum from female or male healthy patients.

1. Introduction

The particular structural and electronic properties of double-walled carbon nanotubes (DWCNTs), consisting of two concentric cylindrical graphene layers, have attracted the interest for their use in fabricating molecular electronic devices (Wu et al., 2012). As it is well known, DWCNTs are the simplest configuration of multiwalled carbon nanotubes (MWCNTs), and combine the outstanding properties of this material with those from SWCNTs. It has been found that DWCNTs-modified electrodes possess a better electrochemical behavior than those using the single-walled counterpart, providing fast electron transfer with significant overpotential reduction for various species (Pumera, 2007). A higher reactivity has also been claimed for DWCNTs, which is probably due to the larger number of lattice defects with respect to SWCNTs. With respect to MWCNTs, several beneficial properties have been claimed for DWCNTs such as the improved lifetimes and high stability under aggressive chemical, mechanical and thermal treatments (Green and Hersam, 2011). Furthermore, it has been seen that, upon chemical modification of DWCNTs, the outer cylinder acts as a protective sheath that preserves the electronic properties of the inner tube (Moore et al., 2011). Therefore, covalent sidewall chemistry can be performed onto DWCNTs without loss of the intrinsecal properties (Brozena et al., 2010).

Despite the excellent electrochemical properties and the special performance after functionalization, a very few examples of DWCNTs-based sensing platforms can be found in the literature. An enzyme biosensor for xanthine using a carbon paste electrode modified with DWCNTs was proposed (Anik and Çevik, 2009). Glucose oxidase adsorbed onto oxidized DWCNTs followed by casting onto a glassy carbon electrode was also used for the preparation of a glucose biosensor (Pumera and Smid, 2007). Regarding electrochemical immunosensors, only one configuration

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using DWCNTs has been reported (Punbusayakul et al., 2013). This work described a label-free approach with as-grown DWCNTs for the detection of *Salmonella typhimurium*.

Immobilization of antibodies constitutes a crucial step in the construction of electrochemical immunosensors. Among the various alternatives, the use of suitable functional groups for covalent binding with proteins has proven to be convenient to ensure bioconjugates stability (Ronkainen et al., 2010). Different functionalized materials and processes have been proposed to implement this strategy. Grafting methods applied to carbon surfaces have demonstrated to be a powerful route to incorporate linking groups in a simple and rapid way.In this context, screen-printed carbon electrodes grafted with 4-carboxyphenyl radical (HOOC-Phe-SPCEs) were used by our group to fabricate an amperometric immunosensor for estradiol, by covalent binding of streptavidin and immobilization of the biotinylated antibody (Ojeda et al., 2012). HOOC-Phe-SPCEs further modified with aminophenylboronic acid were also used to prepare an immunosensor for adrenocorticotropin hormone (ACTH) (Moreno-Guzmán et al., 2012b) as well as a multiplex configuration for the simultaneous determination of ACTH and cortisol (Moreno-Guzmán et al., 2012a). Grafted nanotubes have also been used for the development of electrochemical immunosensors. For example, aryldiazonium salt chemistry was employed for patterning of SWCNTs forest onto glassy carbon electrodes and the subsequent preparation of an electrochemical immunosensor for the simultaneous determination of endosulfan and paraoxon through the immobilization of the respective haptens (Liu et al., 2014). Functionalization of graphene by electrografting of aryldiazonium salt followed by activation with glutaraldehyde and covalent immobilization of β-lactoglobulin antibodies was also used for the preparation of a β-lactoglobulin immunosensor which was applied to food analysis (Eissa et al., 2012).

Adiponectin (APN), a 244-amino acid protein expressed in white adipose tissue, is an anti-inflammatory adipocytokine involved in glucose and lipid metabolism (Thanakun et al., 2014). The concentration of APN correlates positively with high-density lipoprotein-cholesterol levels, and negatively with glucose and insulin concentrations as well as with liver fat content, visceral adiposity and degree of obesity or body mass index (BMI). Low levels of APN are observed in diabetic patients, and also are associated with hypertriglyceridemia. All these relationships have led to consider APN as a possible biomarker for metabolic syndrome (Brooks et al., 2007), and monitoring of adiponectin levels is envisaged as a promising target for prevention and treatment of obesity, insulin resistance, hyperlipidemia and atherosclerosis. In this context, it has been reported that the mean APN level found in plasma of healthy females was 11.4 μg/mL (Matsui et al., 2012), with a very similar value found also in men, 11.6 μg/mL (Katsuki et al., 2008). Other authors have reported lower APN concentrations in women serum ranging between 6.0 and 7.8 μg/mL (Koh et al., 2008). The determination of APN in biological samples is usually performed by colorimetric immunoassay. For such purpose, various ELISA kits for serum and plasma are commercially available. The most sensitive configurations use sandwich-type immunoassays with HRP-labeled IgG or HRP-streptavidin bound to anti-APN or biotinylated anti-APN secondary antibodies. These assays provide detection limits of various tenths of pg/mL and dynamic ranges extended up to various tenths of ng/mL. The long time of analysis, which in some cases lasts more than four hours, can be identified as the major drawback for these methods.

In this work, the first immunosensor for APN is reported. Moreover, a novel electrochemical transduction based on screen-printed carbon electrodes modified with functionalized DWCNTs as platforms for immobilization of the specific antibodies, was

used. Functionalization of DWCNTs was carried out by treatment with 4-aminobenzoic acid in the presence of isoamylnitrite, resulting in the formation of 4-carboxyphenyl- DWCNTs (HOOC-Phe-DWCNTs). Then, an original strategy involving the use of metal complexes for the oriented binding of anti-APN was applied. Mix&Go™, a commercial polymeric coating that contains several metallic complexes selected for their efficiency to bind proteins (Muir et al., 2007; Ooi et al., 2014), was used. According to Ooi et al. using slow exchanging metal complexes such as chromium (III) in oligomeric form, there is multicomponent chelation to the surface while retaining potential to similarly bind proteins. The polymer forms strong multi-valent interactions with electron donating groups such as carboxylate (Wu et al., 2008). Particularly, in the case of antibodies, Mix&Go firstly uses small molecule ligands to bind Fc domains as an anchoring point, these ligands mimicking binding domains from proteins A and G, and extending as two adjacent sidechains from a polymer backbone. Secondly, metal complexes that are integrated into the polymer chains enhance binding of Fc domains providing a higher stability. Furthermore, the polymeric metal complexes can also create a multiplicity of interactions with the p-electrons of the phenyl ring, which makes this strategy particularly suitable for the preparation of the immunosensor using HOOC-Phe-DWCNTs (Muir et al., 2007). Accordingly, the combination of 4-carboxyphenyl radical grafted-DWCNTs with the use of Mix&Go™ for antibody immobilization resulted in a successful alternative that could be used as the general route for preparing a variety of electrochemical immunosensors with applications as point-of-care devices.

2. Experimental

2.1. Reagents and solutions

Mouse polyclonal human anti-adiponectin (anti-APN) was from Abnova. Human adiponectin peptide (APN), rabbit polyclonal biotinylated anti-APN antibody (Biotin-anti-APN), and streptavidin labeled with HRP (HRP-Strept) were from Abcam. High-Pressure CO (HiPCO) SWCNTs were purchased from NanoIntegris (purified grade). DWCNTs (purity $>98\%$) were purchased from XinNano Materials, Inc., (Barrejón et al., 2015). Hydroquinone (HQ) and hydrogen peroxide (30%, w/v) were from Sigma. Mix&Go™ polymer was from Anteo Diagnostics. Sodium di-hydrogen phosphate and di-sodium hydrogen phosphate were from Scharlau. Bovine serum albumin (BSA) was from GERBU Biotechnik, GmbH. Casein from bovine milk (Sigma) and semi-skimmed milkpurchased in a local supermarket were also used. Buffer solutions used were 0.1 M phosphate buffer solution of pH 7.4 (PBS), and 25 mM 2-(N-morpholino)ethanesulfonic acid solution of pH 5.0 (MES). Human ceruloplasmin (Cp, Abcam), protein C reactive (CRP, Fitzgerald), tumor necrosis factor alpha (TNFα, BD Pharmigen), and ghrelin (GHRL, Anaspec) were tested as potentially interfering compounds. Deionized water was obtained from a Millipore Milli-Q purification system (18.2 MΩ cm).

2.2. Apparatus and electrodes

All electrochemical measurements were made with a PGSTAT 12 potentiostat from Autolab. The electrochemical software was the general-purpose electrochemical system (GPES) (EcoChemie B. V.). Screen printed carbon electrodes (SPCEs, 110 DRP, 4 mm ⌀) were purchased from DropSens (Oviedo, Spain). These electrodes include a silver pseudoreference electrode and a carbon counter electrode. For homogenization of the solutions, an Optic Ivymen System constant temperature incubator shaker (Comecta S.A.) was used. All experiments were performed at room temperature.

2.3. Samples

Human serum samples from both female and male healthy patients (Abyntek, Refs. SG609–2 and SG610–2, respectively) were analyzed. Adiponectin concentrations in these samples were also determined by using a colorimetric ELISA kit from Abcam (Ref. ab 108786), were 7.5 ± 0.5 μg/mL (female) and 10.0 ± 0.6 μg/mL (male).

2.4. Procedures

2.4.1. Functionalization of SWCNTs and DWCNTs

SWCNT and DWCNT were covalently functionalized with carboxyphenyl moieties by the widely applied methodology of, *in situ* generated, aryl diazonium salts addition (Bahr and Tour, 2001). Functionalization of SWCNT was performed under classical conditions according to the procedure described by Palacin et al. (2009). Furthermore, functionalization procedure and characterization for DWCNTs were described in (Barrejón et al., 2015). Measurement of the degree of functionalization in both hybrids was performed by the I_D/I_G ratio in Raman spectra as well as from TGA curves. Fig. S1 (Supplementary material) shows the results obtained for SWCNTs.

2.4.2. Preparation of the immunosensors

The immunosensors for APN were prepared following the steps illustrated in Fig. 1. After functionalization of DWCNTs (step 1), 3 μL of a 0.5 mg HOOC-Phe-DWCNTs per mL aqueous dispersion which also contained 15 mg/mL Tween 20™ were casted on the SPCE surface and dried under IR radiation. Tween 20 acted as a suspension stabilizer and its role in reducing nonspecific protein adsorption via primarily hydrophobic interactions has been also reported (Brogan et al., 2004). Then, 3 μL of Mix&Go polymer solution were dropped onto the HOOC-Phe-DWCNTs/SPCE and left standing for 1 h at room temperature. After washing with distilled water, 5 μL of a 1/75 diluted anti-APN solution in 25 mM MES buffer of pH 5.0 were incubated for 1 h on the electrode surface (step 2 in Fig. 1). This pH value was that of the Mix&Go solution and the buffer employed was that recommended by the supplier to be used with Mix&Go. After washing with distilled water, 5 μL of a 2% BSA solution were added allowing incubation for 30 min. Step 3 in Fig. 1 illustrates 5 μL dropping of a standard APN solution or the sample in 0.1 M PBS of pH 7.4 (this value was selected because it is the physiological value) onto the electrode surface and left standing for 1 h. Thereafter, 5 μL of 2.0 μg/mL Biotin-anti-APN solution was added to the APN-anti-APN-Phe-DWCNTs/SPCE and incubated for 15 min. Finally, 5 μL of a 1/1000 diluted HRP-Strept solution was drop casted and, after incubation for 15 min, the determination of APN was accomplished by dropping 45 μL of a 1 mM HQ solution onto the surface of HRP-Strept-Biotin-anti-APN-APN-anti-APN-Phe-DWCNTs/SPCE immunosensor horizontally positioned and applying a potential of −0.20 V. Once the background current was stabilized (approx. 100 s), 5 μL of a 50 mM H_2O_2 solution was added and allowed standing for 200 s. The steady state current corresponding to the electrochemical reduction of benzoquinone was used as the analytical readout.

2.4.3. Determination of APN in human serum

APN determination in human serum was performed by applying the procedure described above to samples fifty times diluted with PBS. The measured steady-state currents at −0.20 V were interpolated into the linear portion of the calibration plot obtained with APN standard solutions.

3. Results and discussion

3.1. Electrochemical characterization

The electrochemical characterization of SPCE surfaces modified with HOOC-Phe-DWCNTs was performed by cyclic voltammetry. Fig. 2 compares cyclic voltammograms recorded using 1 mM

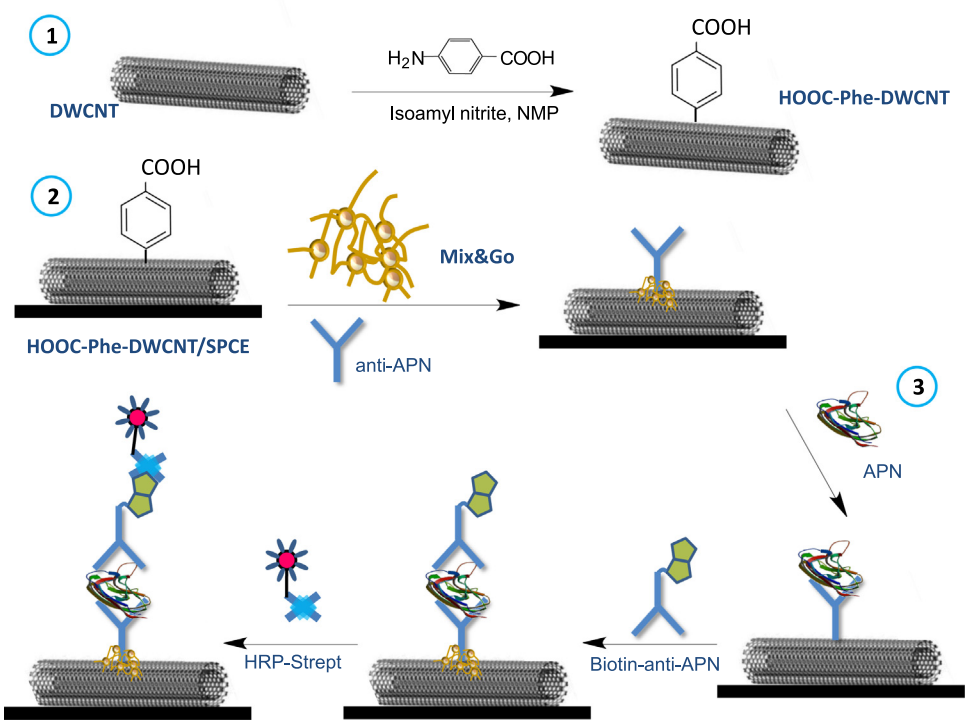

Fig. 1. Schematic display of the different steps involved in the construction of an amperometric immunosensor for APN involving functionalized DWCNTs and oriented immobilization of anti-APN by using the metallic-complex chelating polymer Mix&Go™.

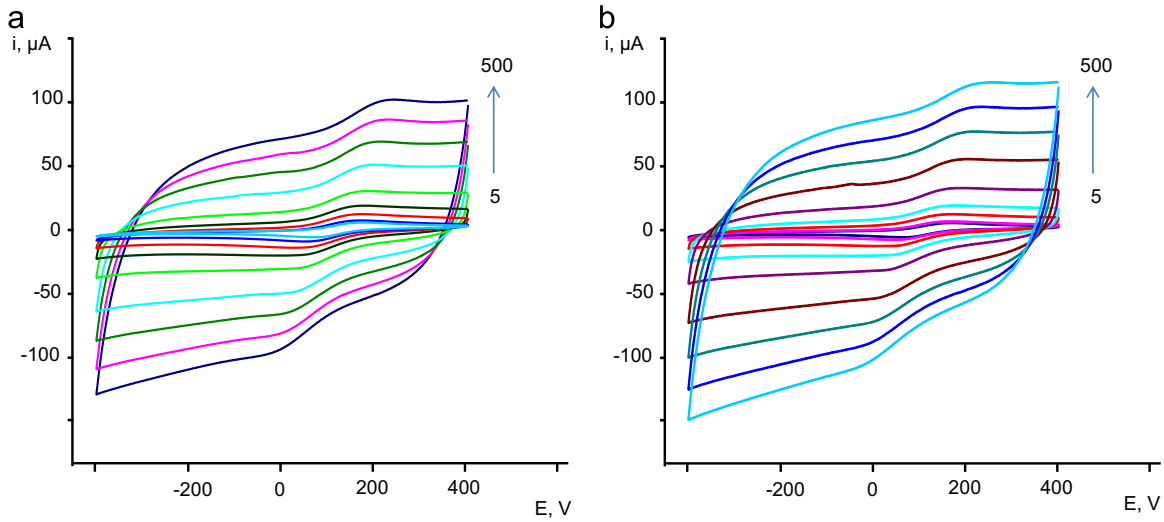

Fig. 2. Cyclic voltammograms of 1 mM $Fe(CN)_6^{4-}$ solution in 0.1 M KCl of pH 3 at HOOC-Phe-DWCNTs/SPCE (a) and HOOC-Phe-SWCNTs/SPCE (b), at different potential scan rates.

$Fe(CN)_6^{4-}$ as redox probe in 0.1 M KCl of pH 3 at (a) HOOC-Phe-DWCNTs/SPCE and (b) HOOC-Phe-SWCNTs/SPCE modified electrodes, at potential scan rates over the 5–500 mV/s range. As it can be seen, a similar voltammetric behavior was apparent at both modified electrodes, although a somewhat larger background current was observed at the SWCNTs-modified surface. Using Nicholson's method (Nicholson, 1965; Bard and Faulkner, 2001), the standard heterogeneous rate constant, k^0, of the $Fe(CN)_6^{4-}$ electrochemical reaction at the modified electrodes is calculated according to the following equation:

$$k^0 = \Psi\left[D_O \pi \nu \frac{nF}{RT}\right]^{1/2}\left(\frac{D_R}{D_O}\right)^{\alpha/2}$$

where the terms have the usual meaning, and the values used for the diffusion coefficients are 6.3×10^{-6} cm^2/s for D_R (ferrocyanide), and 7.6×10^{-6} cm^2/s for D_O (ferricyanide). The dimensionless kinetic parameter Ψ values were obtained by interpolation of the measured E_p values from the cyclic voltammograms recorded at different potential scan rates into the ψ vs. ΔE_p data set provided by Nicholson, and assuming $\alpha=0.5$. The calculated rate constant values were $(2.0\pm0.3)\times10^{-3}$ cm/s and $(1.5\pm0.3)\times10^{-3}$ cm/s, at HOOC-Phe-DWCNTs/SPCE and HOOC-Phe-SWCNTs/SPCE, respectively. These k^0 values suggested faster electrode kinetics at the double-walled CNTs modified interface which is in good agreement with that reported by Moore et al. (2011) using Si-aminophenyl-DWCNTs or -SWCNTs electrodes with $Ru(NH_3)_6^{3+/2+}$ as the electrochemical probe. Furthermore, the k^0 values obtained were also consistent with the only datum found in the literature (Salinas-Torres et al., 2011) at a GCE/SWCNTs and using $Fe(CN)_6^{3-/4-}$ as redox probe, 1.31×10^{-3} cm/s.

Fig. 3 shows cyclic voltammograms of a 1.0 mM $Fe(CN)_6^{4-}$ solution prepared in PBS, at HOOC-Phe-DWCNTs/SPCE and anti-APN-Phe-DWCNTs/SPCE. As expected, due to the immobilization of the insulating protein, a remarkable decrease in the redox probe peaks occurred, which confirmed the successful attachment of APN antibodies. All the steps involved in the immunosensor preparation were also monitored by electrochemical impedance spectroscopy using 1.0 mM $Fe(CN)_6^{3-/4-}$ as redox probe in 0.1 M PBS of pH 7.4. Fig. S2 (Supplementary material) shows the Nyquist plots obtained.

3.1. Optimization of the experimental variables involved in the preparation of the immunosensors

The different variables affecting the performance of the developed immunosensor were optimized. These studies involved the evaluation of: a) the loading of functionalized DWCNTs onto SPCE; b) the loading of immobilized anti-APN onto DWCNTs/SPCE coated with Mix&Go™ polymer; c) the time for incubation of APN onto anti-APN-Phe-DWCNTs/SPCE; d) the concentration of Biotin-anti-APN onto APN-anti-APN-Phe-DWCNTs/SPCE, and the time for incubation; e) the concentration of HRP-Strept onto Biotin-anti-APN-APN-anti-APN-Phe-DWCNTs/SPCE, and the time for incubation; f) the blocking step. Details on these optimization studies can be found in Supplementary material and in Figs. S3–S7, and summarized in Table S3.

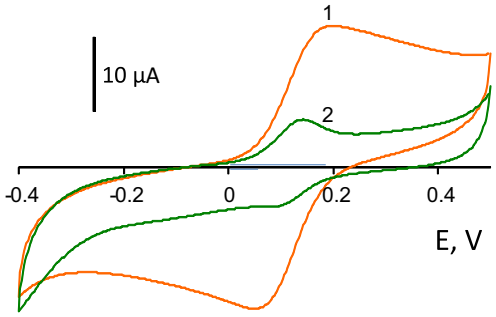

Fig. 3. Cyclic voltammograms of a 1.0 mM $Fe(CN)_6^{3-/4-}$ solution prepared in 0.1 M PBS of pH 7.4, at HOOC-Phe-DWCNTs/SPCE (1) and at anti-APN-Phe-DWCNTs/SPCE (2); $\nu=50$ mV/s.

3.2. Analytical characteristics of the immunosensor

The calibration plot for APN constructed with the HRP-Strept-Biotin-anti-APN-APN-anti-APN-Phe-DWCNTs/SPCE immunosensor under the optimized working conditions is shown in Fig. 4. Error bars were calculated from measurements carried out with three different immunosensors in each case. The steady state current vs. APN concentration followed the adjusted equation $I(\mu A)=0.467\log C(\mu g/mL)+0.071$ ($r^2=0.997$), with a range of linearity extending between 0.05 and 10.0 μg/mL APN. This range covers more than two orders of magnitude and it is adequate for the determination of the cytokine in real samples taking into account the expected concentrations, at level of tens of μg/mL (Matsui et al., 2012). The achieved limit of detection, 14.5 ng/mL, was calculated by applying the $3s_b$ criterion, where s_b was estimated as the standard deviation in concentration units 4.83 ng/mL ($n=10$) of measured blank currents (0 ng/mL APN). Reproducibility studies

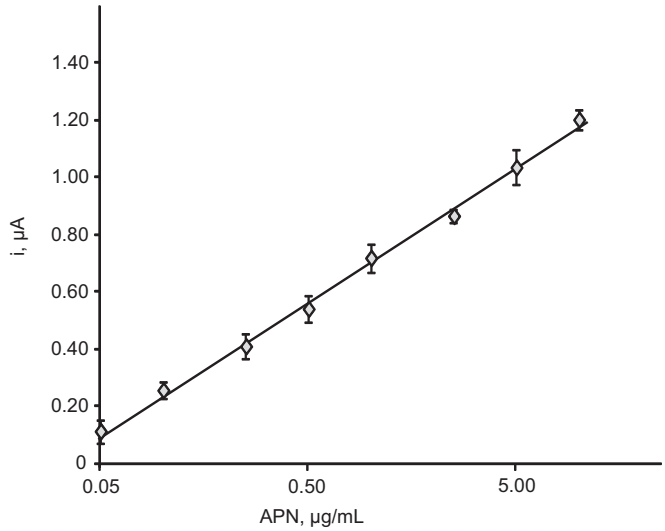

Fig. 4. Calibration plot constructed for APN by amperometry at the HRP-Strept-Biotin-anti-APN-APN-anti-APN-Phe-DWCNTs/SPCE immunosensor. See text and Table 1 for the experimental conditions.

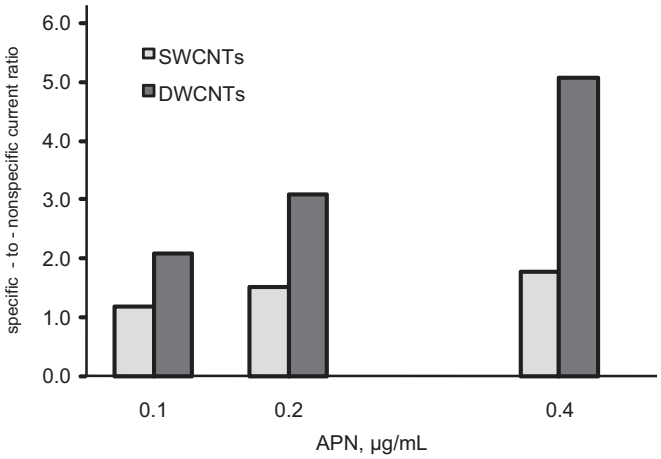

Fig. 5. Specific-to-nonspecific current ratios for 0.1, 0.2 and 0.4 μg/mL APN at HRP-Strept-Biotin-anti-APN-APN-anti-APN-Phe-DWCNTs/SPCE (black) and HRP-Strept-Biotin-anti-APN-APN-anti-APN-Phe-SWCNTs/SPCE (gray). See text and Table 1 for the experimental conditions.

were made by evaluating the amperometric responses obtained with different immunosensors prepared on the same day and on different days using a new HOOC-Phe-DWCNTs/SPCE in each case. The relative standard deviation (RSD) values obtained ($n=8$) were 6.1% and 6.8% for the assays performed on the same day in absence and in presence of 1 μg/mL APN, respectively, whereas RSD ($n=8$) values were 7.8% and 8.5%, respectively, for the measurements made on different days. These results revealed the good level of precision achieved in the fabrication and functioning of the proposed immunosensing platform.

When the analytical characteristics provided by the developed immunosensor are compared with those obtained by ELISA kits, it can be concluded that a much wider APN concentration range is covered by the immunosensor, with the additional advantage of calibration plot linearity. However, the achieved limit of detection was higher than that claimed for ELISA kits from Invitrogen KHP0041 (0.1 ng/mL) (www.lifetechnologies.com/order/catalog/product/KHP0041), or Abcam ab99968 (0.025 ng/mL) (www.abcam.com/adiponectin-human-elisa-kit-ab99968.html). Nevertheless, the criteria used for calculation of these values are not well known. It should be also remarked that the analytical performance of the immunosensor takes associated the advantage of a relatively short time of analysis. In fact, it can be deduced from Table 1 that the whole experimental procedure, once the capture antibody was immobilized, took 2 h. This period of time is much shorter than that required when using commercial ELISA kits for APN (for example, 3 h 20 min (Invitrogen, KHP0041), or 4 h 45 min (Abcam, ab99968).

The responses of the DWCNTs-based immunosensor for APN were also compared with those obtained using a similar approach with immunosensors prepared with functionalized SWCNTs. Fig. 5 displays histograms showing specific-to-nonspecific (i.e. in the absence of APN) current ratios for three different APN concentrations at both anti-APN-HOOC-Phe-DWCNTs/SPCE and anti-APN-HOOC-Phe-SWCNTs/SPCE immunosensors. As it can be observed, while a clear discrimination of low concentrations for the target analyte is feasible at the DWCNTs immunosensor, much lower analytical readouts and a remarkably worse sensitivity were observed with the SWCNTs immunosensor. This is most likely due to the enhanced electrochemical transduction behavior of DWCNTs/SPCEs.

Table 1
Determination of APN in human serum

Serum	Immunosensor, μg/mL	ELISA kit, μg/mL
Female	7.6 ± 0.2[a]	7.5 ± 0.5
Male	10.5 ± 1[a]	10.0 ± 0.6

[a] Mean value $\pm$ ts/$\sqrt{n}$.

The storage stability of the anti-APN-Phe-DWCNTs/SPCE conjugates at −20 °C under dry conditions was also evaluated. Various bioconjugates were prepared on the same day, stored and used to construct the corresponding HRP-Strept-Biotin-anti-APN-APN-anti-APN-Phe-DWCNTs/SPCEs immunosensors on different days. Results obtained (not shown) revealed that immunosensor responses remained within the control limits, set at ± 2 times the standard deviation of measurements ($n=3$) carried out on the first day, for at least 15 days. This can be considered as an acceptable storage stability of the conjugates, thus allowing their preparation and storage under the above specified conditions and their use for the fabrication of the immunosensors on request.

3.3. Selectivity studies

Various proteins: BSA, ceruloplasmin (Cp), protein C reactive (CRP), tumor necrosis factor alpha (TNFα), and ghrelin (GHRL) were tested as potential interfering substances for the determination of APN using the developed immunosensor. Selectivity evaluation was accomplished by comparing the immunosensor responses for 0 and 2.5 μg/mL APN in the absence and in the presence of each tested compound at concentrations corresponding to the normal levels that can be expected in human serum. As Fig. 6 clearly shows, no significant differences in the measured response were apparent in any case both with and without the potentially interfering protein, thus demonstrating the high selectivity of the proposed configuration for APN determination.

3.4. Determination of APN in human serum with the developed immunosensor.

The usefulness of the immunosensor for the analysis of real samples was demonstrated by analyzing human serum from female or male healthy patients. The adiponectin concentrations were firstly determined by using a colorimetric ELISA kit from Abcam (Ref. ab 108786). Thereafter, the experimental procedure described in Section 2.4.2 was applied to samples 1:50 diluted

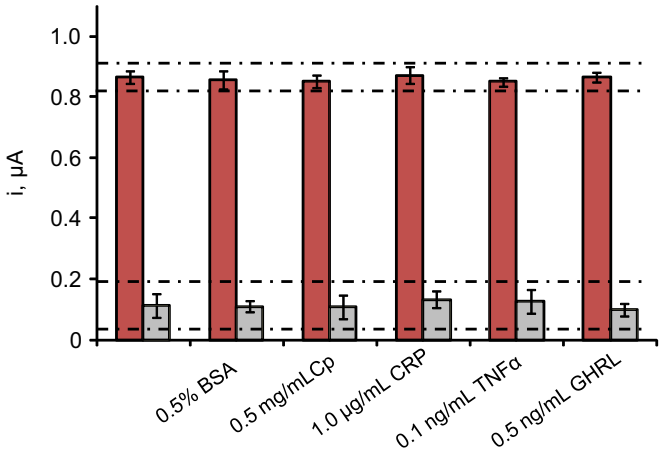

Fig. 6. Effect of the presence of BSA, Cp, CRP, TNF and GHRL on the amperometric responses obtained for 0 (light) and 2.5 (dark) μg/mL APN at the HRP-Strept-Biotin-anti-APN-APN-anti-APN-Phe-DWCNTs/SPCE immunosensor.

with PBS, and the current was measured at −0.20 V. The mean APN concentrations obtained with the immunosensor were compared with those achieved using the ELISA kit (Table 1). A statistical comparison by the Student's *t* test shows that no significant differences between results obtained by both methods existed and, therefore, it could be concluded the usefulness of the developed immunosensor for APN determination at the required concentration levels in human serum.

4. Conclusions

This work clearly reveals that electrochemical platforms consisting of disposable electrodes modified with functionalized double-walled carbon nanotubes can be employed for the successful design of immunosensors suitable to be used as rapid analytical tools with potential application in the preparation of point-of-care devices. This approach combines the potentialities of the so prepared modified electrodes with an oriented immobilization of capture antibodies by means of the metallic-complex chelating polymer Mix&Go™. The proof of concept was demonstrated by preparing an amperometric immunosensor for the obesity biomarker adiponectin that exhibits an excellent analytical performance in terms of selectivity, required sensitivity and reproducibility, and storage stability. Moreover, the usefulness of the developed immunosensor was demonstrated by determining the target analyte at the required concentration levels in human serum and showing important advantages in terms of assay rapidity with respect to commercial ELISA kits.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.06.001.

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