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Electroanalysis of phenylalanine in food supplement by differential pulse voltammetry technique: Application of economical and handmade Sonogel-Carbon electrodes improved with a fast polarization step

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ABSTRACT

Sonogel-Carbon electrodes have been applied successfully in the determination of phenylalanine (Phe). These handmade economical sensor devices exhibit better electroanalytical performance than commercial electrodes, and by a fast surface polarization step the electrochemical signal was significantly increased. Improvements in the electrochemically active surface area and electrical conductivity were demonstrated. Excellent figures of merits were assessed, such as a sensitivity value of 68.19 ± 3.11 μA mM^{-1} cm^{-2} comparable to other previously reported devices and limit of detection of 31.92 ± 3.75 μM. Sonogel-Carbon electrodes exhibit worthy selectivity in the presence of other amino acids and electroactive species. Finally, satisfactory recovery values between 96 and 101 % were obtained when this material was applied in the determination of Phe in a food supplement sample. Sonogel-Carbon electrodes stand out compared to other reported sensor devices due to their simple, low-cost and eco-friendly manufacturing; their reusability; and their robust and selective electrochemical performance.

1. Introduction

Sonogel-Carbon (SNG-C) electrodes were developed more than twenty years ago as an economical and simple handmade alternative to expensive commercial transducers. The manufacturing of this conductive material is ultra-fast and eco-friendly. Briefly, the use of high-energy ultrasound for only ten seconds led to the gelation of a silicon oxide network without the requirement of any environmentally harmful organic solvent or supplementary energy source. This methodology is consistent with the 12 principles of green chemistry: less hazardous synthesis; safe products due to the absence of toxicity in the ceramic material; reduction of solvents, auxiliaries and derivatization; energy efficiency; or degradation design, as the glass and carbon component are easily recyclable [1]. Moreover, the manufacturing conditions let reduce costs to approximately only 0.36 € per electrode. These devices can be reused multiple times as well, as a simple surface mechanical renewal led to a new bare electrochemical platform [2,3]. The feasibility of modifying the matrix of the sonogel material with other components, such as conducting polymers [4] or carbon nanoallotropes [5], enhances their electroanalytical performance and expands the potential applications. Several previous papers have demonstrated the applicability of these sensors in the determination of both organic and inorganic analytes and the analysis of several types of real samples: beverages [6], drugs [7], biological fluid [3], environmental waters and wastes [4], etc. Among them, some applications in the analysis of diverse metabolites can be found, such as the successful determination of the amino acid

Abbreviations: SNG-C, Sonogel-Carbon; Phe, Phenylalanine; Tyr, Tyrosine; GC, Glassy carbon; MTMOS, Methyltrimethoxysilane; DPV, Differential pulse voltammetry; CV, Cyclic voltammetry; EIS, Electrochemical Impedance Spectroscopy; BBS, Borate buffer solution; CA, Chronoamperometry; R_{ct}, Charge transfer resistance; LOD, Limit of detection; PANI, Polyaniline; CB, Carbon black.

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tryptophan in fruit juices [8].

In this sense, the analysis of metabolites, such as amino acids, is very useful in diverse applications. Some of the most interesting ones are the diagnosis and monitoring of diseases and mental disorders [9] or the geographical discrimination and identification of food samples [10,11]. Particularly, phenylalanine (Phe) is an essential amino acid that can be found in several protein-rich sources such as dairy products, meat, fish, eggs or nuts [12]. Its main biological role is to serve as carbon skeleton source for the pathway synthesis of tyrosine (Tyr) and, subsequently, the catecholamines neurotransmitter group (dopamine, norepinephrine and epinephrine) [13]. Analysis and monitoring of this metabolite are necessary, however, due to the existence of the phenylketonuria disease [14]. This metabolic disorder is characterized by recessive mutations in the phenylalanine hydroxylase gene, an enzyme that converts Phe into Tyr. Phe accumulates in blood and some tissues, particularly the ones of the brain, causing severe and irreversible neurological damage. Moreover, the deficiency of Tyr leads to insufficient production of the neurotransmitter previously mentioned [15]. Untreated phenylketonuria leads to intellectual disability, developmental delay and many other diseases [16]. For this reason, several electrochemical, fluorometric and colourimetric biosensors for the detection of Phe have been developed in the last years [17]. Alternatively, Phe has been used as chemomarker in food industries for the discrimination of cheeses [18] or the identification of geographical denomination-protected Parmigiano Reggiano cheese [10].

The devices developed so far, however, are usually non-economical and complex sensing systems which require demanding manufacturing processes [19]. The sensor device developed by Sakar et al. was composed of a capped metallic nanoparticles membrane that was bound to a sensing platform by using a hydrothermal reactor in extreme conditions (180 °C for 18 h). Moreover, the manufacturing requires at least three days [20]. Alternatively, a molecular imprinted polymer-based sensor device reported by Ermiş and coworkers was prepared by synthesizing a novel thiophen-3-carbonyl tryptophan monomer. However, the synthesis procedure of this compound includes the use of organic solvents such as tetrahydrofuran and ethyl acetate, hazardous reagents such as triethylamine or thionyl chloride, and the emerging contaminant 1-H-benzotriazole [21]. Other sensors are composed of high-cost materials such as carbon nanotubes [22], reduced graphene oxide [23], specific aptamer [24] or enzymes [25].

In this work, we propose the application of SNG-C electrodes in the electrochemical determination of Phe in dietetic supplements. These handmade electrodes are fabricated using an easy and fast procedure with an eco-friendly character due to the absence of organic solvents and the use of high-energy ultrasound. Moreover, they are affordable (0.36 € per unit) and can be mechanically renewed to be applied several times [2]. The use of this sensor device on the electroanalysis of Phe is suggested as an economical and meaningful alternative to commercial electrodes or more expensive, complex and disposable sensor devices reported in the literature.

The performances of the SNG-C sensor device and a commercial glassy carbon (GC) electrode are compared. A polarization step of the electrode surface was tested to enhance the electrochemical performance, and the change of active surface area and resistance were calculated. Calibration curves of SNG-C electrodes were carried out to assess the figure of merits (sensitivity, limit of detection, repeatability, reproducibility, renewability), comparing the results with those of other sensors reported in the literature. The selectivity of the resulting electrodes was tested as well using other amino acids and electroactive species. Finally, it was applied successfully in the analysis of Phe in a food supplement sample.

2. Material and methods

2.1. Reagents and materials

All reagents employed were of analytical grade and used as received without further purification. Methyltrimethoxysilane (MTMOS) was purchased from Merck (Darmstadt, Germany). L-Phenylalanine, L-tyrosine, L-tryptophan, L-glycine, L-valine, L-alanine, ascorbic acid, uric acid, dopamine hydrochloride, potassium hexacyanoferrate (II) and sulfuric acid were from Sigma Aldrich (Sigma, Steinheim, Germany). L-methionine and glucose were purchased from Fluka Chemical (Charlotte, NC, USA). Boric acid, sodium borate and hydrochloric acid were from Carlo Erba (Cornaredo, Italy). Graphite powder was from Alfa Aesar (Johnson Matthey GmbH, Germany). All solutions were prepared with double distilled water. Glass capillary tubes, i.d. 1.15 ± 0.05 mm, were used as the bodies of the electrodes. L-phenylalanine food supplement (750 mg Phe/tablet) from Warnke Vitalstoffe (Germany) was used as real sample. The composition of this sample is described in Section S1 of the Supplementary Material.

2.2. Instrumentation

The synthesis of Sonogel-Carbon material was carried out using a high-energy ultrasound generator, Sonicator 4000 MISONIX (MISONIX, Inc. Farmingdale, NY, USA) equipped with a 13-mm-diameter titanium tip, which provides 600 W of maximum output power. Electrochemical studies were made using an Autolab PGSTAT 12 potentiostat/galvanostat (Ecohemie, Utrecht, the Netherlands) connected with a personal computer. Processing data was made using GPES (General Purpose Electrochemical System) and FRA (Frequency Response Analyzer) software. All the voltammograms obtained by differential pulse voltammetry (DPV) technique were processed using baseline correction tool from GPES. The measurements were carried out in a three-electrode electrochemical cell at room temperature, with the following composition: Ag/AgCl/KCl 3 M as reference electrode, platinum wire as counter electrode, and the Sonogel-Carbon (geometric area: 1.04×10^{-2} cm^2) as the working electrodes. Glassy-Carbon electrode (geometric area: 3.14 $\times$ 10^{-2} cm^2 from Metrohm) was used as working electrode to perform some electrochemical measurements for comparison purposes.

SEM images were registered using a Nova NANOSEM 450 instrument (FEI Company, Hillsboro, OR, USA). AFM images were recorded using a Dimension Icon microscope (Bruker, Billerica, MA, USA) operating in Peak Force Tapping mode using ScanAsyst-Air probes.

2.3. Preparation of Sonogel–Carbon electrodes

The SNG-C electrodes were prepared as reported elsewhere [2]. Briefly, a reaction mixture was prepared from 500 μL of the MTMOS precursor and 100 μL of a 0.2 M hydrochloric acid solution. The mixture was sonicated for 10 s using a high-power ultrasound probe at 40 % of amplitude. Next, 500 mg of spectroscopic-grade graphite was added and homogeneously mixed into a paste. Then, the fabrication of the electrodes was carried out by filling capillary tubes with the prepared material. The electrodes were ready to be used after a gentle polishing of the surface with P1200 emery paper (Struers, Germany) and establishing electrical contact by inserting a copper wire.

2.4. Electrochemical measurements

Before being used, SNG-C electrodes were electrochemically polarized in 0.1 M H_2SO_4 aqueous solution by two polarization steps at −0.7 V for 10 s, and at +1.8 V for 10 s, respectively. This electrochemical cycle was repeated eight times.

Electroactive surface area of SNG-C electrodes was determined via cyclic voltammetry (CV) in presence of 5 mM $K_4[Fe(CN)_6]$ and 0.5 M KCl. As instrumental parameters, potential range from –0.2 to + 0.6 V

and diverse scan rates were set up. Charge transfer resistance of the SNG-C electrodes was calculated using electrochemical impedance spectroscopy (EIS) by performing the resistance measure at different frequencies in a solution containing 2 M KNO_3, 10 mM $K_3[Fe(CN)_6]$ and 10 mM $K_4[Fe(CN)_6]$. The instrumental parameters were as follows: frequencies range from 0.1 Hz to 10 kHz, 50 as number of frequencies, 5 mV as amplitude and a potential value of 0.25 V.

DPV was used for the electrochemical determination of Phe. The instrumental parameters were as follows: 5 mV as step potential, 25 mV of modulation amplitude, 0.5 s as interval time, and 0.05 s of modulation time. The electrochemical measurements for Phe detection were performed in 0.1 M borate buffer solution (BBS) at pH 9.4 with 0.1 M KCl, as supporting electrolyte. The current peaks obtained at +0.87 V (vs. Ag/AgCl/KCl 3 M) of working potential were evaluated considering a linear tangent baseline.

Calibration plots were built in presence of different Phe concentrations ranging from 0.1 to 1.3 mM. The measurements for each concentration were done in triplicate to estimate the repeatability of the sensor, whereas the calibration was carried out with several electrodes to assess their reproducibility. Interference studies were performed considering the influence of different amino acids (valine, alanine, methionine, glycine and tyrosine) and electroactive species (ascorbic acid and glucose) on the electrochemical signal of 0.2 mM Phe solution at the same concentration level.

2.5. Food supplement sample analysis

Phe food supplement was analyzed by crushing and grinding a tablet with an agate mortar into fine powder. Next, the sample was diluted in 1 L of distilled water by using stirring and slight heating. The prepared solution was diluted 30 times in 10 mL of 0.1 M BBS solution adjusted at pH 9.4. Subsequently, electrochemical analysis with DPV was carried out using two different methodologies. On the one hand, direct measurement was carried out in the treated sample and the Phe concentration was determined by using the linear regression equation. On the other hand, a standard addition method was employed using several Phe spiked concentrations (0.058; 0.115; 0.172; 0.229, and 0.287 mM). The analysis of the real sample was carried out in triplicate.

3. Results and Discussion

3.1. Preliminary studies for the sensing of Phe and optimization of polarization step

The performance of the SNG-C sensor device was tested with CV in a 1 mM Phe solution. A notable oxidation peak at +0.92 V can be observed when comparing the voltammogram recorded in the working solution with the background signal, as shown in Fig. S1 of the Supplementary Material. However, the peak corresponding to the Phe oxidation cannot be observed when a GC electrode was applied in the same solution. These results suggest that the electrochemical performance of SNG-C electrodes toward Phe is better than the one of GC sensor device. Therefore, further studies were carried out to assess the promising application of SNG-C electrodes in the electroanalysis of Phe.

The electrochemical responses of GC and SNG-C electrodes were recorded in a 1 mM Phe solution with DPV. A slight oxidation peak was observed around +0.87 V with SNG-C sensor devices, whereas no notable peak can be discerned with the GC electrode. Polarization of electrodes surface in acid media was performed to enhance their electrochemical performance, as described in section 2.4. Fig. 1A shows the electrochemical response of both electrodes before and after the treatment. The current intensity of the oxidation peak increases in the case of SNG-C electrodes after the polarization, from 0.198 to 0.998 μA. A negligible response of 0.043 μA was observed with the polarized GC electrode in contrast. Hence, the pre-treatment of the SNG-C electrode surface led to an obvious improvement in the detection of Phe. This can be attributed to an increase in the surface area of the electrode by an opening of the closed-pore structure of the graphitic ceramic matrix, as well as the formation of oxygen-containing functional groups that enhanced the physicochemical and electrochemical properties of the sensor material, as reported previously. Ilangovan and coworkers detected an increase of the oxygen content on the surface of an electrochemically polarized GC electrode using the XPS technique, whereas Abdel-Aziz team reached the same conclusion applying EDX. Moreover, they characterized the treated surface by means of FTIR and RAMAN and attributed the signals registered to carbonyl, carboxyl and acid-anhydride groups. Scanning tunneling microscopy was applied by Shi and coworkers on this kind of surface to determine a boost of the roughness undergone during the polarization step [26–29]. Hence, similar impact of the electrochemical polarization on the SNG-C electrodes is expected, which will be study in this work.

Several electrochemical polarization methods were tested on SNG-C

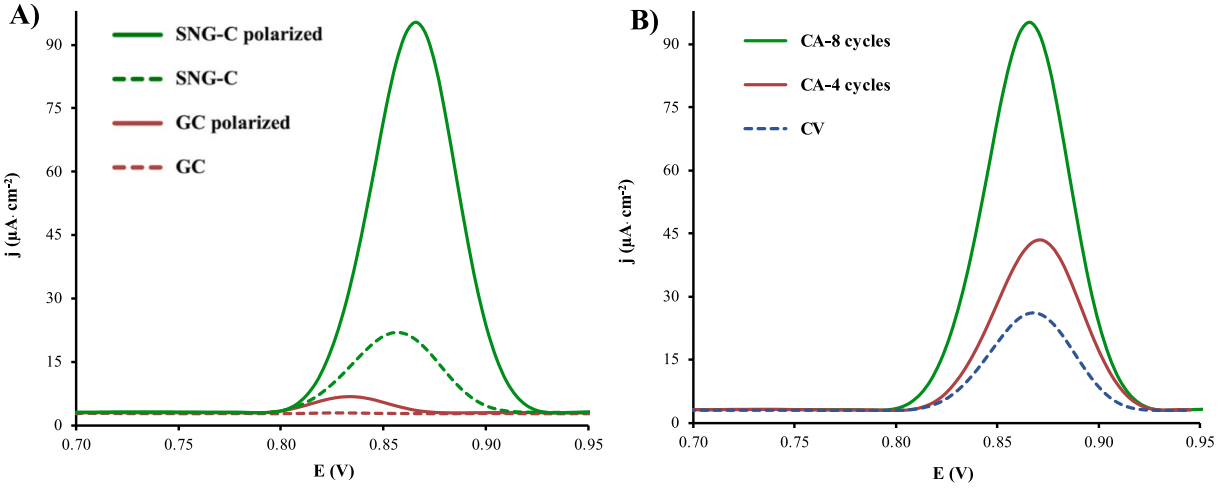

Fig. 1. (A) DPV scans recorded in 1 mM Phe, 0.1 M BBS (pH 9.4) with 0.1 M KCl, with a GC (red line) and a SNG-C (green line), before (dashed line) and after polarization (solid line) in 0.1 M H_2SO_4 solution. **(B)** Scans recorded with SNG-C electrodes polarized with CV (blue dash line), 4 cycles of CA (red solid line) and 8 cycles of CA (green solid line). CA cycles comprised two polarization steps of −0.7 V and + 1.8 V for 10 s each one. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

electrodes to improve their performance, as described in section 2.4. Firstly, cyclic voltammetry (CV) and chronoamperometry (CA) techniques were employed to perform the polarization in acid media. A higher current intensity peak was found in 1 mM Phe solution with the electrode polarized with CA in 0.1 M H_2SO_4 solution, as shown in Fig. 1B. Secondly, chronoamperometry cycles from 4 to 12 were tested as well. Each cycle comprises two polarization steps with different potential applied in each case: −0.7 V s and +1.8 V for 10 s each one. A meaningful improvement was observed when the number of polarization cycles was increased from 4 to 8. However, the performance of the polarization with 12 cycles did not suppose a significant improvement. Finally, a treatment performed in basic media (0.1 M NaOH) led to similar results. Therefore, polarization with 8 CA cycles in 0.1 M H_2SO_4 media was selected as the optimal procedure.

Next, a surface area evaluation was carried out with SNG-C electrodes in order to assess the observed improvement. Voltammograms of 5 mM $[Fe(CN)_6]^{-4}$ were recorded at different scan rates with SNG-C electrodes before and after the electrochemical polarization. Peak currents were plotted against the square root of the scan rate with excellent relationships in both cases ($R^2 = 0.999$), as it can be observed in Fig. 2A. The electrochemically active surface area was calculated using the slope of this plot and the Randles-Sevcĭk equation (Eq. (1) and (2)) [30]:

$$I_p = \left(2.686 \times 10^5\right) n^{3/2} A\, C D^{1/2} \nu^{1/2} \quad (1)$$

$$k = \left(2.686 \times 10^5\right) n^{3/2} A\, C D^{1/2} \quad (2)$$

where I_p is the peak current, n is the number of electrons involved in the reaction (1), A is the surface area of the electrode, D is the diffusion coefficient (6.67×10^6 cm^2/s) [31], C is the concentration of the electroactive specie (5×10^{-6} mol/cm^3), ν is the scan rate and k is the slope of peak current versus the square root of scan rate ($I_p/\nu^{1/2}$). Higher peak current and slope were obtained with the polarized SNG-C electrode (Fig. 2B). The surface of SNG-C electrodes increases a 27 % after the electrochemical pre-treatment. Moreover, the roughness factor for the polarized electrode was 1.19. This change in the electrochemically active area could partially explain the enchantment in the electroanalytical performance of polarized SNG-C electrodes. Additional information about the effect of the treatment on the electrode surface is discussed in Section S2 of the Supplementary Material.

The polarization step was further studied using Electrochemical Impedance Spectroscopy (EIS). As expected, the Nyquist plots (Fig. 2C) for both configurations are very similar: a semicircular part corresponding to the electron transfer process can be observed at high frequencies, whereas a linear part related to the Warburg impedance and the analyte diffusion is observed at low frequencies. The charge transfer resistance (R_{ct}) of the electrode interface was calculated using the diameter of the semicircular part. The R_{ct} value for the untreated SNG-C electrodes was 1469 ± 126 Ω (n = 3), whereas for the polarized electrodes this value decreased by almost half to 791 ± 62 Ω (n = 3). These results suggest that an increase in the electrical conductivity and the electron transfer capability of the sensor device is achieved due to the surface polarization step. Hence, a fast electrochemical treatment of the electrodes led to a notable improvement in their physicochemical properties.

Moreover, SNG-C electrodes modified with conducting polymers and nanostructured carbon materials, which showed antifouling and electrocatalytic properties in the detection of other molecules in previous studies, were tested. Configurations containing polyaniline (SNG-C-PANI) [4], poly(3,4-ethylenedioxythiophene) (SNG-C-PEDOT) [32], and carbon black (SNG-C/CB) [5] were polarized and applied in a solution of Phe displaying lower DPV response than the unmodified SNG-C material, as shown in Fig. S2 (Supplementary Material). These results suggest that the modification of the SNG-C matrix led to poorer electrochemical performance for the specific determination of Phe. The hypothetical formation of active oxygen-containing groups on the carbon composite proposed previously could been hindered by the presence of other materials on the electrode surface. The configuration SNG-C-PEDOT possesses a greater amount of modifier than the rest of the materials tested and displayed the worst electrochemical response in this application by far. The presence of large graphitic surfaces on the electrode would be mandatory for the growth of these oxygen-containing groups, with a crucial role in the electroanalytical performance of the sensor device in the determination of Phe. Moreover, the effective electrochemical polarization of CB surface is a slow process, as reported previously [33], and the formation of active surface groups might be partially prevented in the configuration containing that nanomaterial. Therefore, subsequent studies of this work were carried out using non-modified SNG-C electrodes.

3.2. Evaluation of the electrochemical reaction mechanism

The oxidation of Phe is a pH-dependent process due to the involvement of protons, as described in a previously proposed reaction mechanism [21]. Therefore, the effect of the solution pH on the electrochemical oxidation of Phe at the SNG-C electrode was investigated using DPV at different pH values. A very low and amorphous peak was found in phosphate buffer solution at pH 7, whereas no signal was observed in acid media of acetic-acetate buffer solution at pH 4. However, a well-defined peak is found in basic media. Several boric-borate

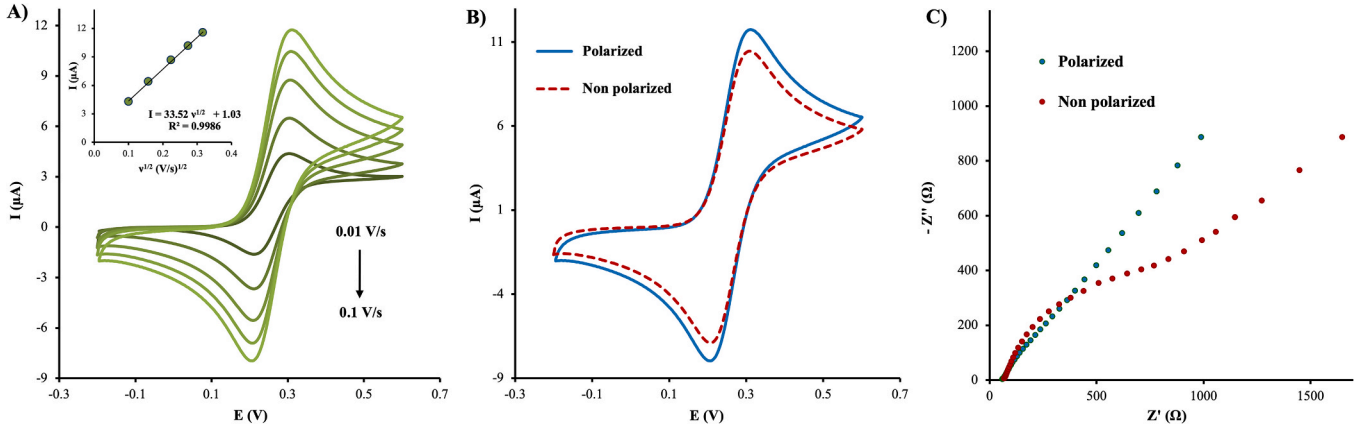

Fig. 2. A) Cyclic voltammograms recorded with a non-polarized SNG-C electrode in 5 mM $K_4[Fe(CN)_6]$ and 0.5 M KCl solution at different scan rates. The inset displays linear regression of the peak current versus the square root of the scan rate. **B)** Cyclic voltammograms recorded with a non-polarized SNG-C (dashed line) and a polarized SNG-C (solid line) electrodes in 5 mM $K_4[Fe(CN)_6]$ and 0.5 M KCl solution at a scan rate of 0.1 V/s. **C)** Nyquist plots recorded in 10 mM $K_3Fe(CN)_6$/$K_4Fe(CN)_6$, and 2 M KNO_3 solution.

buffer solutions with ranging pH from 8.6 to 10.6 were tested, as it can be observed in Fig. 3. The increase of pH led to a shift of peak potential to more negative values, as shown in the plot of the figure inset. A significant linear relationship ($R^2 = 0.987$) was established between these parameters, which suggests that the oxidation reaction is proton dependent. The higher current intensity peak was found at pH 9.4 and at a potential of +0.87 V. In consequence, this solution was selected as the optimal working media for the calibration of SNG-C electrodes carried out with Phe and the application of this sensor device in the analysis of a real food supplement sample.

Finally, the electrochemical reaction mechanism of Phe was studied by means of the effect of scan rate in the peak current intensity. Several CV voltammograms recorded in a 1 mM Phe solution at diverse scan rates in the range from 25 to 175 mV/s can be observed in Fig. S3. As expected, the peak current intensity increases with the scan rate and the oxidation potential shifts to more positive potentials. A good linear relationship ($R^2 = 0.986$) can be established between the current intensity peak and the scan rate, as well as between current intensity and the square root of the scan rate ($R^2 = 0.996$) (Fig. S4). These results indicate that the electrochemical oxidation of Phe at the SNG-C electrode surface is a mixed diffusion-adsorption controlled process. Moreover, the slope value of 0.52 observed in the plot of the logarithm of current intensity versus the logarithm of scan rate (Fig. S5) reinforces this hypothesis, as a value between 0.5 and 1 evidences a mechanism controlled by a mixed diffusion-adsorption process [34]. This fact emphasizes the role of active oxygen-containing groups formed during the surface polarization step proposed previously. These electroactive points can enhance the adsorption of Phe molecule and, hence increase the peak current intensity obtained with the sensor device, as it has been observed in the optimization studies. A proposal scheme of the reaction mechanism and the role of surface-active groups is displayed in Fig. S6 of Supporting Material. The oxidation reaction proposed by Ermiş and coworkers was considered [21]. Briefly, Phe molecules diffuse on the working solution and get adsorbed on the SNG-C surface by active surface groups. These oxygen-contained groups created on the graphite would attract and adsorb the Phe molecules by hydrogen bond weak interactions. There, the analyte would be oxidized during the voltammetry scan and the corresponding signal current would be detected by the transductor material. Consequently, the polarization of the SNG-C electrodes is an essential step in the methodology proposed to determine Phe in food supplement samples.

3.3. Analytical calibration of Phe and figures of merits assessment

Analytical calibration of Phe was carried out with the SNG-C sensor device by assaying different concentrations of the analyte ranging from 100 to 1300 μM. DPV was used as the electrochemical technique, employing the instrumental parameters mentioned in section 2.4. The oxidation peak of Phe, located at a working potential of +0.87 V (vs. Ag/AgCl/KCl 3 M), increased with each addition, as observed in Fig. 4. The calibration plot was represented by the density current anodic peak versus different concentrations of Phe. The corresponding linear regression equation (Eq. (3)) of this calibration was as follows:

$$j(\mu A cm^{-2}) = (68.19 \pm 3.11)[Phe](mM) - (0.64 \pm 0.03) \quad (3)$$

with a good correlation coefficient (R^2) of 0.999 ($S_{y/x} = 0.98$, n = 9, 5 points overall, $P = 0.05$). The sensitivity was calculated by means of the slope of the regression curve. The value obtained was 68.19 ± 3.11 μA mM^{-1} cm^{-2} (n = 9). The limit of detection (LOD) was also calculated as three times the standard deviation of the intercept divided by the slope (LOD = 3 S_q/m). In this case, the SNG-C exhibits a LOD of 31.92 ± 3.75 μM (n = 9). The repeatability of the sensor in the determination of Phe was calculated as well by measuring each concentration by triplicate, whereas reproducibility was assessed by comparing the results obtained with different electrodes (n = 9). Relative standard deviation values of 1.51 and 4.57 % were obtained for repeatability and reproducibility, respectively. Finally, the surface renewability of these electrodes was tested by mechanically polishing an electrode after being calibrated. This electrode was polarized again and the calibration with Phe was carried out. A negligible variation coefficient of 3.61 % was calculated between the measures before and after the surface renewal.

Alternatively, SNG-C electrodes modified with the Carbon Black (CB) nanomaterial and the conducting polymer polyaniline (PANI) were tested as well in the calibration with Phe (see Fig. S7). Anti-fouling and electrocatalytic features have been reported elsewhere in the application of these sensors in the analysis of other analytes [4,6]. The sensor device with only CB exhibited a sensitivity of 12.66 ± 1.16 μA mM^{-1} cm^{-2} and a LOD of 138 μM, whereas the inclusion of PANI in this configuration led to a sensitivity value of 29.25 ± 2.00 μA mM^{-1} cm^{-2} and a LOD of 115

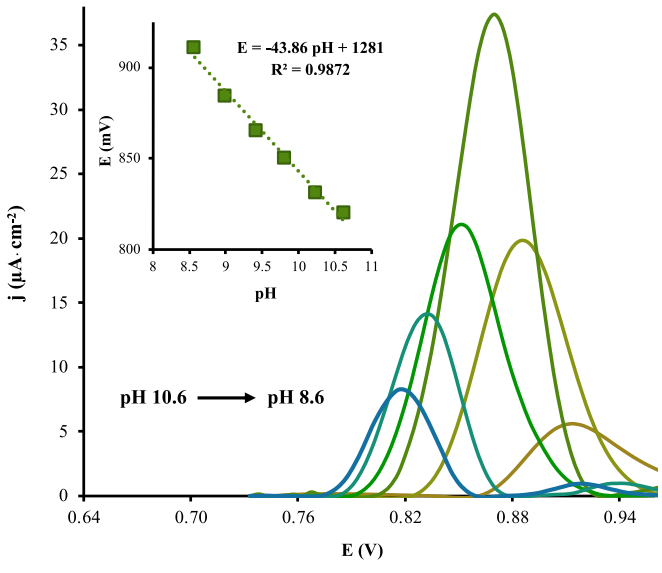

Fig. 3. Differential pulse voltammograms recorded with a SNG-C electrode in presence of 0.5 mM Phe in BBS solution at different pH values (8.6; 9.0; 9.4; 9.8; 10.2 and 10.6). The inset displays linear regression of peak potential versus solution pH.

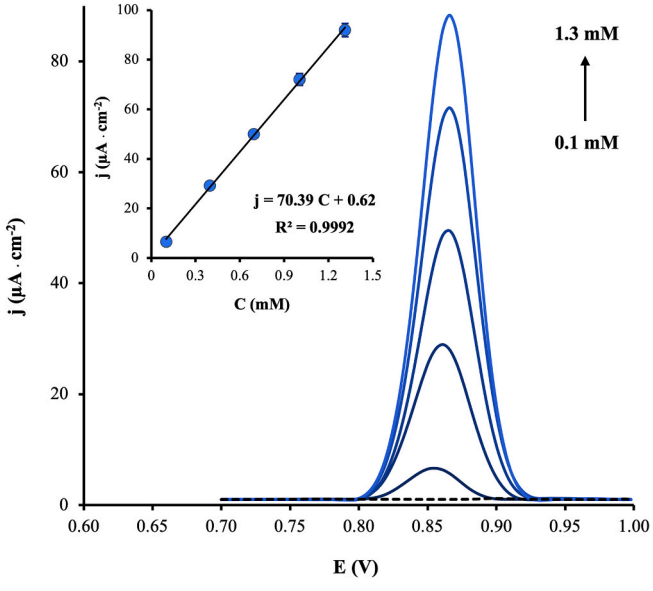

Fig. 4. Differential pulse voltammograms recorded with an SNG-C electrode in presence of different concentrations of Phe: 0.10; 0.40; 0.69; 1.00, and 1.31 mM. The inset displays the calibration plot obtained. The buffer solution was 0.1 M BBS at pH 9.4 with 0.1 M KCl.

μM (n = 6). Therefore, the synergetic effect of both modifiers improves the electroanalytical performance of the device with only CB. However, these figures of merits are worse than the ones obtained with the original SNG-C sensor and the modified electrodes were discarded for this application.

One of the most interesting features of this application is the absence of a significant decline in the electrochemical performance of the SNG-C electrodes after several measurements with Phe. No electrochemical or mechanical surface renewal were required for the continuous use of these sensor devices at concentration levels of Phe lower than 1.5 mM. These results suggest that fouling phenomena problems in the SNG-C electrode surface are not significant in this concentration working range. The absence of significant fouling effects on the electrode surface boosts the sensor device stability, being the only factor that would have an impact on this crucial parameter. Hence, the analysis of Phe using SNG-C electrode is fast, easy and repeatable.

Furthermore, a brief comparison of the analytical parameters of the SNG-C electrodes toward Phe and the performance of other sensors reported in the literature are shown in Table 1. These sensor devices resulted in working linear ranges with lower concentrations than the SNG-C electrodes. Usually, this is very convenient to diminish the influence of the matrix samples (e.g.: interferents, fouling effects) in the measurements via dilution. However, in our case the proposed device is able to detect Phe at high concentrations without suffering usually found fouling phenomena, and, as it will be demonstrated in the next section, with an adequate selectivity even at high interferent concentrations. The sensitivity displayed by the SNG-C electrodes is similar to the values reported for other sensors. Concerning LOD, lower values can be found in the literature. However, the LOD of the SNG-C electrodes is adequate for the application in food sample analysis such as milk or dairy products. Standard Phe total content in whole milk powder is 79 mM according to the U.S. Dairy Export Council, several times higher than the LOD of the SNG-C electrodes [35].

Nevertheless, the main feature of the SNG-C electrodes in contrast to other reported sensor devices is their simplicity. The synthesis of the sonogel material is ultra-fast, eco-friendly and very easy, as the formation of the matrix is promoted by applying high-energy ultrasound for only 10 s and in absence of any organic solvent or supplementary source of energy. Moreover, the manufacturing of the electrodes is cheap (0.36 € per unit, as detailed in Section S3 of Supplementary Material), as all the reagents and materials employed are economical and an insignificant amount of energy is used. Finally, the good repeatability, reproducibility and renewability of these electrodes allow to reuse them multiple times in the analytical application proposed. On the other hand, sensor devices found in the literature are composed of high-cost materials with complicated, time-consuming and environmentally harmful manufacturing procedures.

Table 1
Comparison of the analytical performance of different sensors devices in the determination of Phe.

Sensor device	Linear range (mM)	Sensitivity (μA mM^{-1} cm^{-2})	LOD (mM)	Reference
MIP/ß-CD–MWNTs/PAN/CE	0.0005–0.1	140.7	0.000001	[22]
β-CD/RGO/GCE	0.0004–0.04	–	0.00001	[23]
PDA-β-CD-GCE	0.0002–0.04	–	0.00002	[36]
LNT/CPE	0.025–0.5	54.08	0.003	[37]
L-cys@Fe_3O_4/CCY	0.001–0.1	0.002	0.0058	[20]
SNG-C	0.1–1.3	68.19	0.031	This work

MIP molecular imprinted polymer, *ß-CD* ß-cyclodextrin, *MWNTs* multi-walled carbon nanotubes, *PAN* polyaniline, *CE* carbon electrode; *RGO* reduced graphene oxide, *GCE* glassy carbon electrode, *PDA* polydopamine, *LNT* perovskite $LaNi_{0.5}T_{i0.5}O_3$, *CPE* carbon paste electrode, *L-cys@Fe_3O_4* L- cysteine-capped Fe_3O_4 nanoparticles, *CCY* conductive carbon yarn.

4. Selectivity evaluation via interferences testing

Interference studies were carried out to assess the selectivity of the SNG-C electrodes in the determination of Phe. A 200 μM Phe solution was analyzed by DPV in presence of likely interfering compounds found in food samples as described in section 2.4. The deviation in the electrochemical response was expressed using the following expression (Eq. (4)):

$$Interference(\%) = |(I_{Phe} - I_{(Phe+Int)})/I_{Phe}| \times 100 \quad (4)$$

where I_{Phe} and $I_{(Phe+Int)}$ are the current peaks corresponding to Phe oxidation in the absence and presence of the interferent evaluated, respectively. It is interesting to observe that any signal overlappings were observed in this study due to the high oxidation potential of Phe, as it can be observed in the example of Tyr in Fig. S8A of the Supplementary Material. Results of this study are shown in Fig. S8B, where percentage of the electrochemical response variation are displayed. Briefly, a value of 100 % correspond to the signal of Phe without interferents, whereas percentages higher or lower to this value indicate that the interferent affects the studied signal with an increase or decrease of the current peak intensity, respectively. The first group evaluate was composed by common species such as ascorbic acid or glucose, which had an almost negligible negative interference lower than 5 %. Alternatively, positive interference values of around 10 % were found when using other amino acids such as tyrosine or alanine, among others. These results suggest that the determination of Phe could be carried out in pharmaceutical or food matrices without significant interference from amino acids and other foreign species.

4.1. Real application in a food supplement sample

Analysis of Phe in a food supplement commercial sample was carried out in order to assess the applicability of SNG-C in real and complex matrices. The determination was performed by direct measurement in the diluted sample and calculation of the Phe concentration by using the linear regression equation, as well as applying the standard addition method. These results were compared with the concentration value referenced by the commercial company.

Experimental results are shown in Fig. 5. A well-defined peak with low current intensity can be obtained with the SNG-C sensor device when the sample is added to the cell. Current peak increases when Phe is spiked to the working solution and a good relationship with the concentration is obtained (R^2 = 0.998). Then, the concentration of Phe calculated by using the linear regression equation is 715 ± 20 mg Phe per tablet of the supplement, whereas the concentration calculated by using the standard addition method is 735 ± 19 mg Phe per tablet. Notably, the reference value provided by the supplier is 750 mg Phe per tablet. Recovery values, defined as the ratio between the Phe concentration measured using the electrochemical method and the reference concentration [38], ranging from 93 and 98 % were obtained by direct measurement in the triplicate analysis of Phe food supplements, whereas values ranging from 96 to 101 % were obtained using the standard addition method. Consequently, the SNG-C electrodes can be proposed as a simply-but-effective and economical alternative to the analysis of food samples due to their good performance in real matrices.

5. Conclusions

In this work, SNG-C electrodes have been proposed as electrochemical sensor devices for the determination of Phe in food supplement samples. These simple and economical electrodes exhibit better electroanalytical performance than commercial GC electrodes. Moreover, a simple surface polarization in acid media increases the electrochemical signal obtained, which can be attributed to higher electroactive surface area and the formation of oxygen-containing groups. Figures of merits of

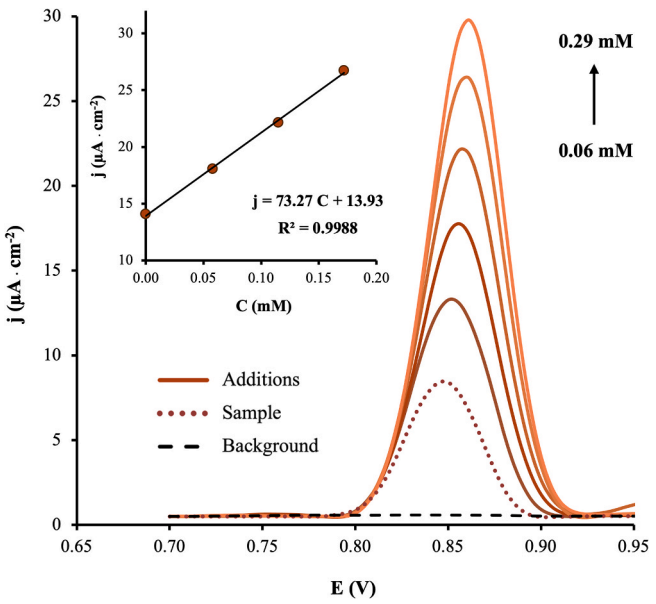

Fig. 5. Differential pulse voltammograms recorded with a SNG-C electrode in presence of a food supplement sample diluted in 0.1 M BBS at pH 9.4. Voltammograms in the background (black dashed line), with sample (red pointed line) and with additions of Phe (red solid line) are represented. Different concentrations of Phe were spiked: 0.058; 0.115; 0.172; 0.229, and 0.287 mM. The inset displays the linear regression of current density versus concentration of Phe. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

these electrodes are comparable to other complex and costly sensors previously reported. The meaningful repeatability, reproducibility and selectivity exhibited denote the robustness of this sensor. Additionally, the surface renewability makes possible to reuse these electrodes multiple times. Finally, these electrodes have been tested successfully in the analysis of Phe in a food supplement sample, with recovery values ranging from 96 to 101 %. Direct measurement in the sample and subsequent application of the linear regression equation has been established as a suitable approach due to similar effectiveness than the standard addition method and easier and faster applicability. Sonogel-Carbon electrodes arise as an efficient and eco-friendly alternative to commercial and hand-made sensor devices in the analysis of pharmaceutical and food samples.

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CRediT authorship contribution statement

Alfonso Sierra-Padilla: Writing – original draft, Investigation, Formal analysis, Data curation. **Alessandro Monari:** Visualization, Validation. **José María Palacios-Santander:** Funding acquisition, Supervision. **Juan José García-Guzmán:** Methodology, Validation. **Laura Pigani:** Supervision, Project administration. **Laura Cubillana-Aguilera:** Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2025.113208.

Data availability

Data will be made available on request.

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