

AgNP-Decorated SBA-15 for MWCNT Paste Modified Electrode: a Sensor for Simultaneous Voltammetric Determination of Paracetamol and Sulfamethoxazole

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Abstract: A new electrochemical sensor based on a carbon nanotube paste electrode modified with a Santa Barbara Amorphous material (SBA-15) decorated with silver nanoparticles, namely CNT/SBA/Ag-PE, was developed. It was successfully applied for individual and simultaneous determination of both paracetamol (PC) and sulfamethoxazole (SMZ) medicines. The electrode exhibited a linear dynamic range of 0.12–110 $\mu\text{mol L}^{-1}$ for

paracetamol and 0.06–70 $\mu\text{mol L}^{-1}$ for sulfamethoxazole, and detection limits of 38 and 19 nmol L^{-1} , respectively. The proposed sensor offered high sensitivity, fast response time and the potential for detecting both drugs simultaneously. The CNT/SBA/Ag-PE enabled the simultaneous determination of PC and SMZ in urine samples with high recovery rates.

Keywords: silver nanoparticles · ordered silica materials · carbon nanotube paste electrode · medicines determination · ionic silsesquioxane

1 Introduction

Sulfamethoxazole (4-Amino-N-(5-methyl-3-isoxazolyl) benzenesulfonamide, SMZ), a sulfonamide antibiotic representative, is one of the most frequently administered antimicrobial agents, especially in association with trimethoprim (TMP), for the treatment of several human infections [1]. Regarding its pharmacokinetic properties, SMZ undergoes acetylation by N-acetyltransferase enzyme in the liver and approximately 20% of a dose is eliminated unchanged in urine [2,3]. Drug-induced liver injury is a potential complication of many drugs and SMZ/TMP therapies have been associated with hepatic failure [4]. On the other hand, in the course of an infection, fever is a common outcome of immune response and paracetamol (N-acetyl-p-aminophenol, acetaminophen, PC) is an effective analgesic and antipyretic agent [5]. Although not harmful at therapeutic doses, PC is commonly taken in overdose, being also a common cause of acute liver injury [4,6]. Therefore, as both SMZ and PC are associated with liver toxicity, it is of considerable importance to detect and determine these drugs, even at trace level, in biological systems. Chromatographic, spectroscopic and electrochemical methods can be used for the quantification of drug compounds for analytical purposes. The latter presents some advantages such as low-cost instrumentation, possibility of miniaturization and fast response, which allow in-field applications [7].

Structured forms of carbon have attracted increasing attention in electrochemical sensing devices due to their excellent conductivity, biocompatibility and mechanical resistance [7,8] and they have been applied in the determination of PC or SMZ medicines in different

systems. Carbon modified electrodes with ionic liquid [9], chitosan [10], clays [11], metal nanoparticles [12,13], valine [14], and carboxyl group [15] were reported. Also screen-printed carbon electrodes have been employed [16–18]. In a recent work, even unmodified multi-walled carbon electrode was used for these purposes [19]. However, as far as we know, there are no reports on simultaneous determination of PC and SMZ.

Ordered mesoporous silica-based materials were discovered in the 1990s. The most investigated one, SBA-15 [20], has a highly ordered hexagonal pore structure, with cylindrical mesopores and a narrow distribution of pore diameter and due to its excellent properties (high surface area, high pore volume, high hydrothermal stability) it is widely used in different areas such as catalysis, adsorption and separation processes, [21–22]. More recently, the use of SBA-15 in sensor devices is drawing more attention, since it presents interconnected nanopores on its mesopore wall allowing fast diffusion and low resistance to

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Supporting information for this article is available on the WWW under <https://doi.org/10.1002/elan.202060090>

electron transfer [23–25]. The high surface area of SBA-15 is also appropriate for the immobilization of functional species including ionic silsesquioxanes, reportedly known as metal nanoparticles stabilizing and adhesion agents [26,27]. The metal nanoparticles enhance the analytical performance of the electrodes, improving electron transfer, sensitivity and signal/noise ratio [28,29].

In this work, SBA-15 was decorated with silver nanoparticles stabilized by ionic silsesquioxane in order to develop a novel electrochemical sensor based on modified carbon nanotube paste electrode. The sensor was successfully applied in the selective and simultaneous determination of PC and SMZ.

2 Experimental

2.1 Reagents

All chemicals were analytical grade and used without previous purification. The silsesquioxane containing the ionic 1,4-diazoniabicyclo-[2.2.2]octane group was synthesized according to recently described procedure [30]. Multi-walled carbon nanotubes (MWCNT, Nanocyl NC 7000), glucose (Vetec) and ascorbic acid (AA, Quimex). Sulfamethoxazole (SMZ), paracetamol (PC), uric acid (UA), dopamine (DA) and trimethoprim (TMP) were acquired from Sigma-Aldrich.

2.2 Material Synthesis (SBA/AgNP)

Firstly, the silver nanoparticles (AgNP) were synthesized according to a slightly modified reported procedure [31]: 100 mg of ionic silsesquioxane was dissolved in 10.0 mL of distilled water, followed by successive additions of 1.0 mL of AgNO_3 (0.05 mol L^{-1}) and 5 mL of NaBH_4 (0.02 mol L^{-1}). The so-obtained colloidal dispersion of silver nanoparticles underwent a two-fold dilution with water and was designated as AgNP. Subsequently, the SBA-15 support was synthesized based in previous reports [20,21]. The SBA-15 (100 mg) was suspended in 10 mL of AgNP and the system was mechanically stirred for 72 h in closed flask at 20°C . The composite material obtained (assigned as SBA/AgNP) was subjected to water washing, centrifugation, and drying under vacuum for 4 h at 50°C .

2.3 Material Characterization

X-ray diffraction was performed in a Siemens diffractometer D500, with CuK α as source (0.154056 nm). The field emission scanning electron microscopy (FESEM) images of SBA/AgNP were obtained in a Zeiss Auriga microscope. The UV-Visible spectrum of AgNP was registered with an UV-160 1P Shimadzu and the diffuse reflectance UV-Visible spectrum of SBA/AgNP material was recorded in an Agilent CARY 5000 spectrophotometer applying Kubelka-Munk approach [31]. N_2 adsorption-desorption isotherms were recorded using Tristar II 3020 Kr Micromeritics instrument. The materials were vacuum-

treated for 15 h at 110°C . The BET (Brunauer, Emmett and Teller) and BJH (Barret, Joyner and Halenda) methods were applied to estimate the specific surface area and pore size distribution [32].

2.4 Electrochemical Measurements

The SBA-15 and SBA/AgNP materials were used to modify a carbon nanotube paste electrode by mixing 6 mg of each material, 6 mg of MWCNT and $50 \mu\text{L}$ of mineral oil. This was the optimal proportion for the preparation of stable and easy-to-handle electrodes, hereafter assigned as CNT/SBA-PE and CNT/SBA/Ag-PE, respectively. A part of the paste was placed in a cavity having 2 mm of diameter, in contact with a platinum disk glued to a glass tube. The copper wire fused to another side of the disk served as an external electric contact. Unmodified CNT-PE electrode, prepared in the same way, was also used for the sake of comparison analyses. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were carried out with an IviumStat potentiostat/galvanostat with the use of a conventional three-electrode cell. A silver chloride electrode (3 mol L^{-1} KCl) and platinum wire were used as the reference and auxiliary electrodes, respectively. All electrochemical experiments were performed at room temperature (20°C) in 0.04 mol L^{-1} Britton-Robinson buffer (BRBs, $\text{pH} = 5.7$), in the presence of NaNO_3 (0.5 mol L^{-1}) as supporting electrolyte.

2.5 Optimization of Conditions for Determining PC and SMZ

The effects of differential voltammetric pulse amplitude and time, step potential and scan rate have been investigated within a wide range. The well-shaped peak with both the highest peak current and the best signal to background ratio was found at the following values: amplitude of 50 mV, time of 50 ms, step potential of 1 mV and scan rate of 10 mV s^{-1} . DPVs using different concentrations of NaNO_3 supporting electrolyte were employed to determine 0.5 mol L^{-1} NaNO_3 as the optimal concentration (Figure S1). The pretreatment potentials were studied in the range from -1.8 to 0.4 V with fixed pretreatment time of 20 s, 40 s and 100 s (BRBs at $\text{pH} 5.7$) containing PC ($46 \mu\text{mol L}^{-1}$) and SMZ ($25 \mu\text{mol L}^{-1}$).

2.6 The Urine Samples

In the preparation of the artificial urine, a procedure described by Sgobbi [16] was used. The precise weights of NaCl (0.292 g), KCl (0.16 g), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.112 g), Na_2SO_4 (0.224 g), KH_2PO_4 (0.14 g), NH_4Cl (0.1 g) and urea (2.5 g) were dissolved in a small portion of distilled water, transferred to a 100 mL volumetric flask and the volume was completed with a BRBs of $\text{pH} 7$. The human urine of a healthy person was used without purification and additional dilution. The PC and SMZ amounts were

added to prepare model solutions of both kinds of urine. The obtained systems were used in the recovery tests.

3 Results and Discussion

3.1 Material Characterization

The XRD analysis for small angles (0.2° – 5.0°) is presented in Figure 1. Typical diffraction patterns of hexagonal ordered pore structure are clearly seen, evidencing the peaks assigned to 100, 110 and 200 planes (Inset Figure 1) [20]. The peak at 0.86° is due to the (100) distance, estimated as 10.3 nm. The other two peaks are attributed to the reflections of (110) and (200) planes, associated with the ordered hexagonal pore structure. As SBA/AgNP material presents the same diffraction peaks of SBA-15, it is possible to state that the ordered pore structure was preserved even after the addition of the silver nanoparticles. The ordered pore structure of SBA-15 material was confirmed by the FESEM image shown in the inset Figure 1, in which the cylindrical pores can be clearly seen.

The UV-Vis spectra of the AgNP dispersion and SBA/AgNP material are presented in Figure 2, where the plasmon resonance band due to silver nanoparticles is observed. The presence of a single peak in both spectra indicates spherical nanoparticle shape [33]. In the AgNP dispersion this band appears with a maximum at 402 nm, while for solid SBA/AgNP there is a shift in the band position towards lower wavelength, which was interpreted as a consequence of the changes in the refraction index caused by the solid matrix [34]. The absorption in the violet region of the spectrum results in intense yellow color for AgNP water dispersion, as well as a yellowish color for the solid SBA/AgNP material, as can be seen in the inset of Figure 2. The maintenance of the plasmon band in the SBA/AgNP spectrum is clear evidence that silver nanoparticles were incorporated to the SBA-15 silica.

Figure 3 shows the N_2 adsorption-desorption isotherms of SBA-15 and SBA/AgNP materials along with the BJH pore size distribution curves. The isotherms present well-defined parallel adsorption and desorption inflexions around relative pressures of 0.7, which are typical of ordered mesoporous structure, in agreement with the X-

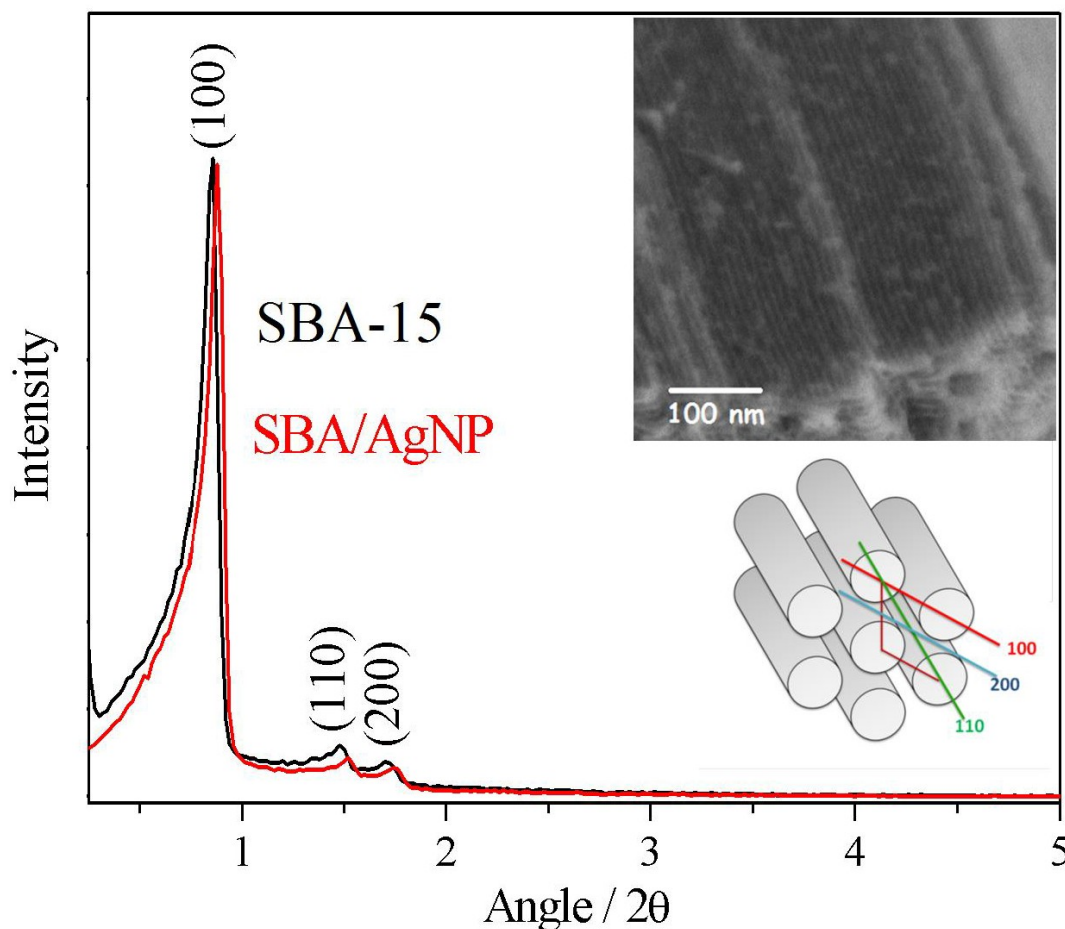


Fig. 1. XRD of SBA-15 and SBA/AgNP materials. Inset Figure the representation of hexagonal packing of cylindrical mesopores and FESEM image of SBA/AgNP material acquired with 80000x of magnification.

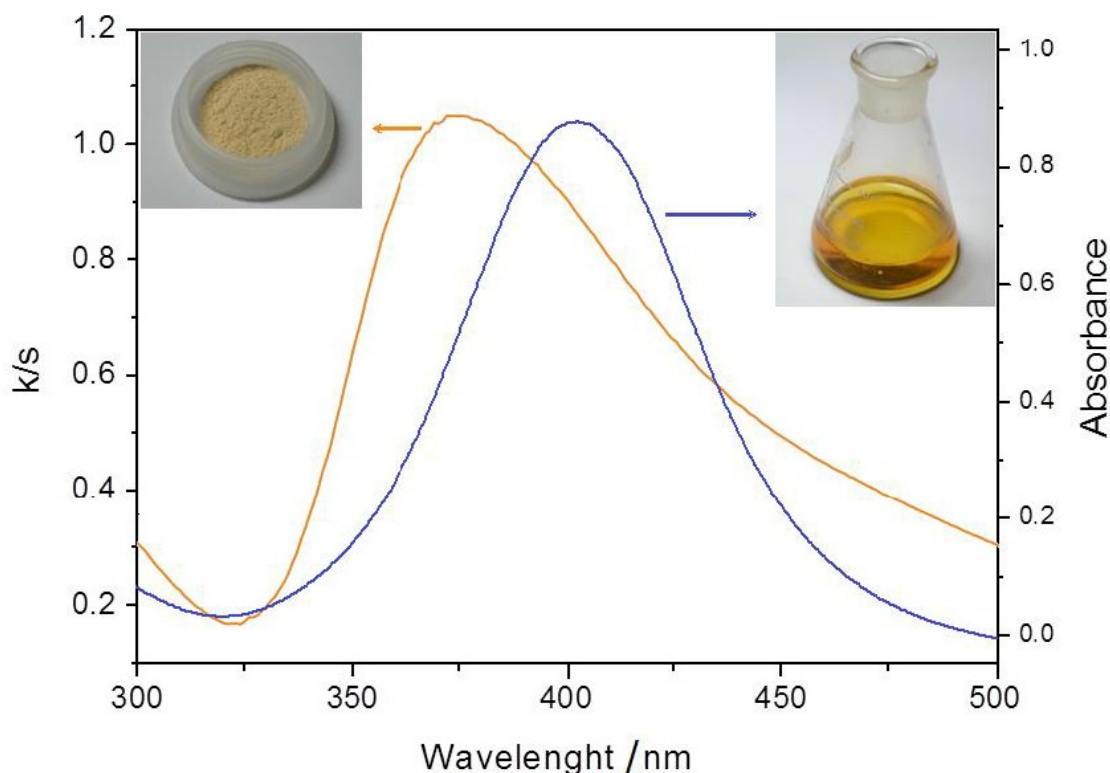


Fig. 2. UV-Vis spectra of AgNP aqueous dispersion and SBA/AgNP material. Inset Figure the pictures of AgNP aqueous dispersion and SBA/AgNP material.

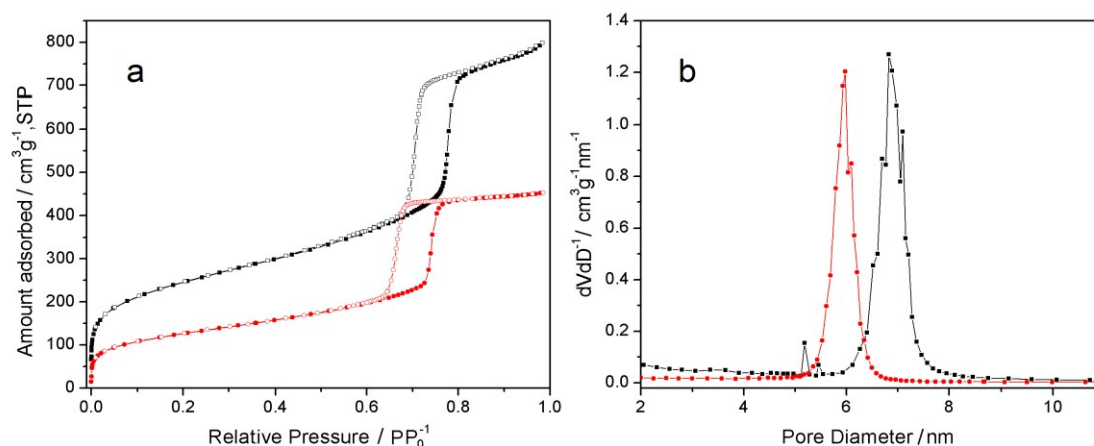


Fig. 3. Textural analysis of SBA-15 and SBA/AgNP materials. a) N_2 adsorption-desorption isotherms; b) BJH pore size distribution.

ray diffraction and FESEM images. Although the isotherms of both materials present a similar profile, they present important differences. After the addition of the AgNP aqueous dispersion there is a shift towards lower adsorbed nitrogen amounts. The surface area and pore volume of SBA-15 were $840 \text{ m}^2 \text{ g}^{-1}$ and $1.12 \text{ cm}^3 \text{ g}^{-1}$, respectively, while the SBA/AgNP material presented lower values, $440 \text{ m}^2 \text{ g}^{-1}$ and $0.658 \text{ cm}^3 \text{ g}^{-1}$, respectively. Also, a slight shift in the position of the hysteresis can be

observed. This shift is responsible for the average pore diameter decrease from 6.9 nm to 5.9 nm, as can be seen in the pore size distribution curves of Figure 3b. These textural changes were attributed to the presence of silver nanoparticles inside of the pores.

The electrochemical behavior of the carbon nanotube paste electrode modified with SBA-15 material, in the absence of AgNP (CNT/SBA-PE) and in the presence of AgNP (CNT/SBA/AgNP-PE) is presented in Figure 4a. As

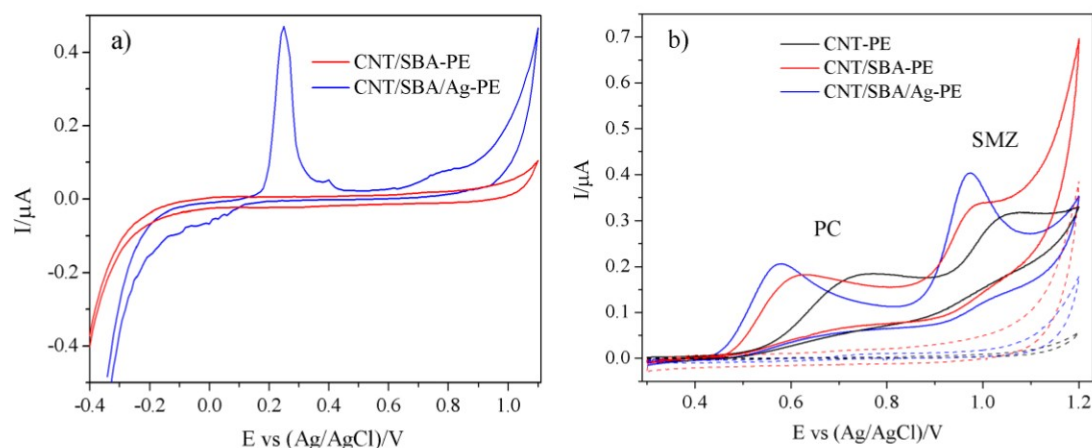


Fig. 4. Cyclic voltammograms of different modified electrodes: a) AgNP presence effect; b) Full lines: in the presence of PC ($46 \mu\text{mol L}^{-1}$) and SMZ ($25 \mu\text{mol L}^{-1}$); dashed lines: in the absence of these drugs. Conditions: 0.04 mol L^{-1} of BRBs with pH 5.7, 0.5 mol L^{-1} of NaNO_3 and scan rate of 20 mV s^{-1} .

can be seen, the cyclic voltammogram (CVs) of CNT/SBA-PE shows no electrochemical process. However, the CV obtained for CNT/SBA/AgNP-PE shows a well-defined peak at $+0.25 \text{ V}$ corresponding to the oxidation process of Ag^0 to Ag^+ [13,35]. A quasi-reversible reduction of Ag^+ process is observed approximately at $+0.03 \text{ V}$. This result confirms that AgNP are immobilized on the SBA surface and the CNT/SBA/AgNP-PE can exhibit electrocatalytic properties due to the AgNP presence.

The electrochemical responses of CNT/SBA-PE and CNT/SBA/AgNP-PE electrodes to PC and SMZ, as well as those of unmodified carbon nanotube electrode (CNT-PE) are shown in Figure 4b. Well-defined oxidation peaks of PC and SMZ for CNT/SBA/Ag-PE are observed at approximately $+0.57 \text{ V}$ and $+0.97 \text{ V}$, respectively. As can be seen, the oxidation of both drugs is an irreversible process in the examined potential range. The oxidation peaks potentials for CNT/SBA-PE and CNT/SBA/AgNP-PE were shifted towards lower values in comparison with the ones for unmodified CNT-PE. Additionally, the improvement in the CNT/SBA/AgNP-PE responses in relation to the CNT/SBA-PE ones, such as high peak resolution and shift to even lower potentials, was interpreted as a consequence of the AgNP presence on the SBA-15 surface that increases the electroactive area of the electrodes. Therefore, for both analytes, the CNT/SBA/Ag-PE was chosen for further determination of PC and SMZ, since it presents a better performance due to the synergistic effects such as the conductivity of the carbon nanotubes, high surface area of the SBA-15 support and the electrocatalytic properties of AgNP.

In addition, the behavior of the CNT/SBA/Ag-PE was evaluated at different scan rates in PC and SMZ solutions. The obtained voltammograms are presented in Figures S2a and S3a (Supporting Information). The anodic peak currents are linearly dependent on the square root of the scan rates for both PC and SMZ (Figures S2b and S3b,

$R^2 \sim 0.999$). The slopes of the $\log I_{\text{pa}}$ vs $\log v$ were 0.434 and 0.56 for PC (Figure S2c) and SMZ (Figure S3c), respectively. These results indicate that oxidation for PC is controlled by diffusion alone, while for SMZ it is controlled by diffusion with a minor contribution of adsorption [36].

The effect of the pH on the oxidation process of PC and SMZ was evaluated by differential pulse voltammetry and the results are presented in the Figures S4a and S4b, respectively. The highest signal was attained at pH of 5.7 for both analytes. An increase in the peak potential was observed as the pH decreases for both PC and SMZ, showing that the oxidation process is pH dependent. The relationship of peak potential as a function of pH showed linearity (insets of Figures S4a and S4b, respectively), presenting a slope of 0.045 V pH^{-1} for PC and 0.055 V pH^{-1} for SMZ. These values are close to the Nernst equation value (0.059 V pH^{-1}), indicating that the oxidation process of the species, on the electrode surface, presents an equal number of electrons and protons. Therefore, according to the literature, the oxidation process of PC and SMZ involves two electrons and two protons [37–39].

3.2 Analytical Characteristics

In order to evaluate the performance of CNT/SBA/Ag-PE as a sensor for PC and SMZ determination, the optimized conditions were set, and they are presented in Supporting Information. Differential Pulse Voltammograms (DPVs) were recorded in solution with different concentrations of PC and SMZ (Figure 5). For PC solely (Figure 5a), a well-defined oxidation peak is observed at *ca.* 0.520 V , and a linear response between PC concentration and peak current is accomplished in the range of 0.1 to $800 \mu\text{mol L}^{-1}$, which is better seen on the calibration plot in the inset of Figure 5a, along with the linear equation. For SMZ, the oxidation process is observed at

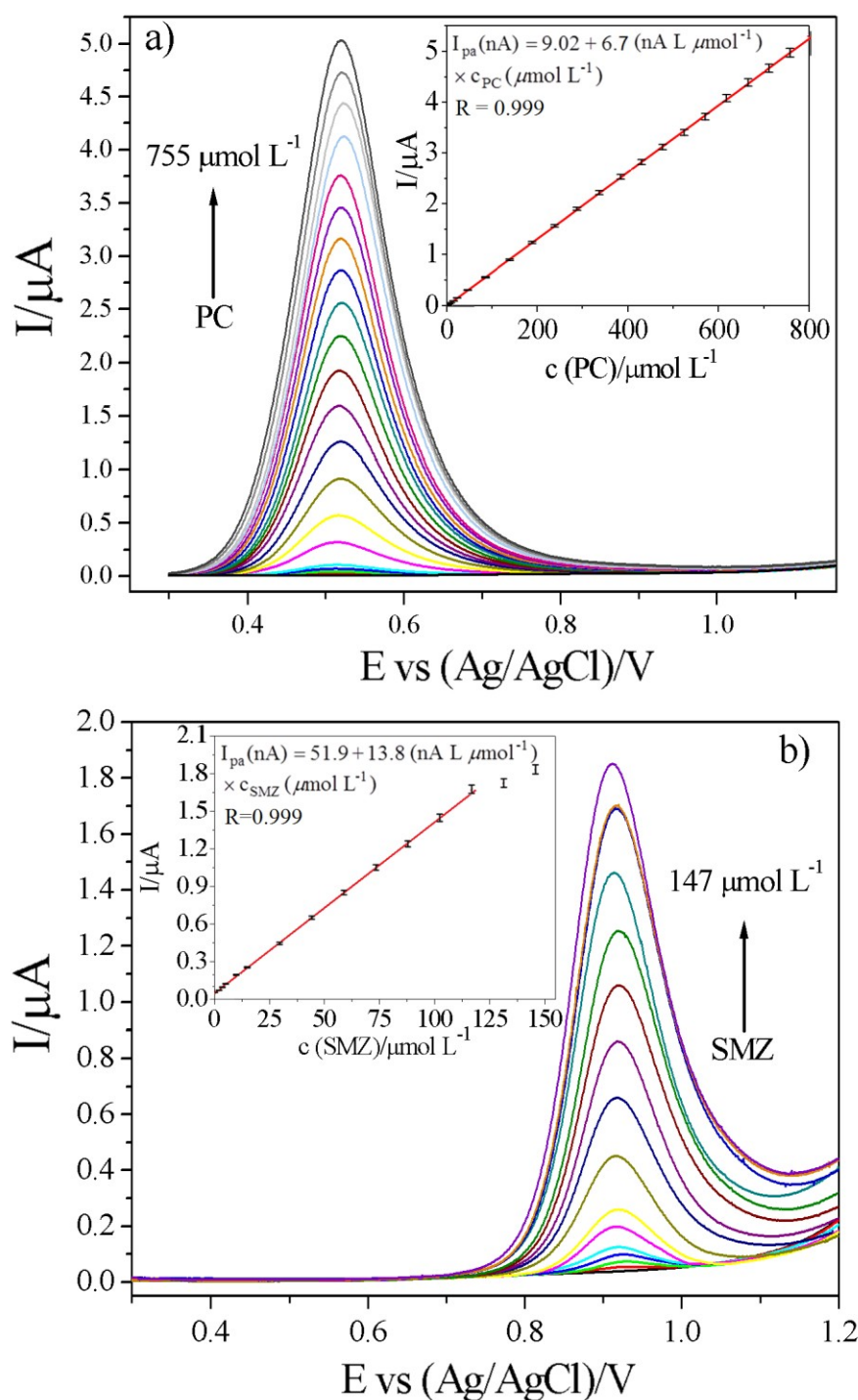


Fig. 5. DPVs of CNT/SBA/Ag-PE in solutions with: a) varied PC concentration; b) varied SMZ concentration; Inset Figures the calibration plots and linear equations. Supporting electrolyte: 0.04 mol L^{-1} of BRBs with pH 5.7, 0.5 mol L^{-1} of NaNO_3 .

ca. 0.920 V , also as a single and well-defined peak (Figure 5b). The peak current increases linearly with the SMZ concentration, in the range of 0.06 to $120 \mu\text{mol L}^{-1}$. Inside Figure 5b is depicted presents the calibration plot and its linear equation. The calculated detection limits are within the nanomolar range, namely, 31 nmol L^{-1} and 19 nmol L^{-1} for PC and SMZ, respectively, and they were

estimated as $3 \times \text{SD}/S$, where SD is the standard deviation obtained from eight blank solution measurements and S is the sensitivity, expressed by the slope of the calibration curve, $6.7 \text{ nA L } \mu\text{mol}^{-1}$ for PC and $13.8 \text{ nA L } \mu\text{mol}^{-1}$ for SMZ (insets of Figures 5a and 5b) [40].

CNT/SBA/Ag-PE responses to PC and SMZ occur far from each other ($\Delta E_{\text{pa}} = 400 \text{ mV}$). Thus, it is feasible to

envisage a simultaneous detection and determination of these analytes. Figure S5a (Supporting Information) presents the DPVs obtained at different concentrations of PC in the presence of a constant amount of SMZ ($0.43 \mu\text{mol}$) corresponding to $25.5 \mu\text{mol L}^{-1}$ of SMZ. Two peaks ascribed to PC and SMZ are observed, with no significant variation in the peak potential when compared with the individual analysis. The PC peak current intensity increases linearly as the PC concentration increases, while the SMZ peak intensity remains almost constant throughout the analysis. The calibration plots (I vs concentration) for PC in the presence of SMZ are presented in Figure S5b. Detection limits and linear range values are summarized on Table 1, along with the same parameters obtained from the individual analysis.

The same behavior is observed for DPVs registered in solution with varied SMZ concentrations, in the presence of a constant amount of PC ($1.06 \mu\text{mol}$) which corresponds to a $53.0 \mu\text{mol L}^{-1}$ PC solution (Figure S6a). The SMZ peak current intensity increases linearly with the SMZ concentration (Figure S6b), while the PC peak remains almost unchangeable. These results suggest no SMZ interference in the PC determination and no PC interference in the SMZ determination. Therefore, the CNT/SBA/Ag-PE can be applied successfully to the simultaneous determination of PC and SMZ.

The DPVs for the simultaneous determination of PC and SMZ using CNT/SBA/Ag-PE are presented in Figure 6. A linear increase in the intensity of the peak current is observed for both analytes with the simultaneous increase in their concentrations. The respective calibration curves for PC and SMZ are shown alongside

Figure 6, and the analytical parameters are presented on Table 1. The detection limits for both PC and SMZ, in the simultaneous determination, remain the same as their individual ones. Their sensitivities, which are expressed by the slope of the linear equation, are also well preserved, namely, $6.3 \text{ nA } \mu\text{mol}^{-1}$ for PC and $14.3 \text{ nA } \mu\text{mol}^{-1}$ for SMZ. These results indicate the feasibility of quantifying both analytes, PC and SMZ, at the same time, using the CNT/SBA/Ag-PE, with good sensitivity and low detection limits.

Table 1 presents comparative analytical characteristics of recently reported sensors for PC and SMZ, such as limit of detection and linear concentration range. As it can be seen, the performance of the developed electrode CNT/SBA/Ag-PE is comparable with the best reported sensors.

As a way of estimating the repeatability (the intra-assay precision) of the CNT/SBA/Ag-PE, five experiments were performed on the same day, in the same electrochemical cell with the same samples, using BRBs with pH 5.7 and 0.5 mol L^{-1} of NaNO_3 containing PC ($96 \mu\text{mol L}^{-1}$) and SMZ ($50 \mu\text{mol L}^{-1}$). The relative standard deviations (RSD) for five successive analyses were 1.2 % for PC and 1.7 % for SMZ. The reproducibility was estimated in a series of experiments with five equally prepared CNT/SBA/Ag-PE electrodes. The obtained RSD values were equal to 2.1 % for PC and 2.8 % for SMZ. Moreover, measurements were also carried out with five electrodes prepared over five different days. In this case, the RSD values were 3.8 % for PC and 4.1 % for SMZ, considering the same concentration level.

Regarding the possibility of determining PC and SMZ in urine samples, and considering that urine can contain

Table 1. Comparative performance of sensors for PC and SMZ.

Electrode	Method	Analyte	Linear range, $\mu\text{mol L}^{-1}$	LOD, nmol L^{-1}	Ref.
fMWCNT-[BMIM]PF ₆ /GCE ¹	SWV	PC	0.3–3.0	67	[9]
fMWCNT/CTS–Co/GCE ²	DPV	PC	0.1–400	10	[10]
EP/MWCNTs/pPGE ³	DPV	PC	0.06–60	18	[11]
PEDOT/Au/GCE ⁴	amperometry	PC	0.15–5881	41	[12]
GCE/rGO–AgNP ⁵	DPV	SMZ	1.0–10	0.6	[13]
GO/poly(Val)/CPE ⁶	DPV	PC	5–60	290	[14]
carboxyl/MWCNTs/GCE ⁷	amperometry	SMZ	0.5–110	94	[15]
SPCE/CB-ERGO ⁸	–	PC	9.9–95	1500	[16]
MWCNT/PBnc/SPE ⁹	DPV	SMZ	0.1–10	38	[17]
AArGO/SPE ¹⁰	DPV	SMZ	0.5–50	40	[18]
MWCNT/PE ¹¹	DPV	SMZ	0.35–30	100	[19]
CNT/SBA/Ag-PE	DPV	PC	0.1–800	31	This work
CNT/SBA/Ag-PE	DPV	SMZ	0.06–120	19	This work

¹glassy carbon electrode covered with composite of functionalized multi-walled carbon nanotubes and 1-butyl-3-methylimidazolium hexafluorophosphate; ²glassy carbon electrode modified with nanocomposite consisting of functionalized multi-walled carbon nanotubes and complex of chitosan with Co (II) ions; ³clay graphite MWCNT electrode; ⁴glassy carbon electrode modified with poly (3,4-ethylenedioxythiophene) and Au@graphene core-shell nanoparticles; ⁵glassy carbon electrode modified with reduced graphene oxide and silver nanoparticle composite; ⁶carbon paste electrode modified with polyValine and graphene oxide; ⁷glassy carbon electrode modified with carboxyl multiwalled carbon nanotubes;

⁸screen printed carbon electrode modified with carbon black and electrochemically reduced graphene oxide composite; ⁹screen-printed electrode modified with multiwalled carbon nanotubes decorated with Prussian blue nanocubes; ¹⁰screen-printed electrode modified with ascorbic acid reduced graphene oxide; ¹¹multi-walled carbon nanotube paste electrode.

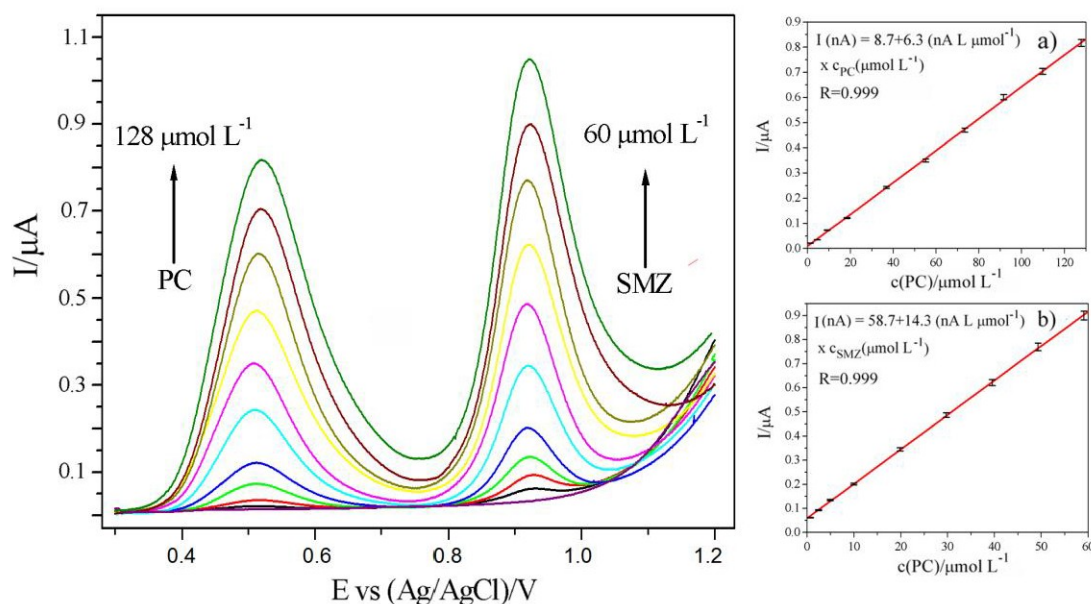


Fig. 6. DPVs of CNT/SBA/Ag-PE in solutions with varied concentrations of both PC and SMZ. Supporting electrolyte: 0.04 mol L⁻¹ of BRBs with pH 5.7, 0.5 mol L⁻¹ of NaNO₃. Aside Figure 6 the calibration curves of simultaneous analysis a) PC and b) SMZ.

different redox active interferences, the analytical performance of the CNT/SBA/Ag-PE electrode was investigated using DPV technique in the presence of potential interferences, such as ascorbic acid, uric acid, dopamine, glucose and trimethoprim. The interferences (except UA) did not show an oxidation process in the studied potential range. The oxidation peak of UA was observed at 0.380 mV and high concentrations of UA (> 100 μmol L⁻¹) influence the shape of PC peak oxidation. The effect of the interferences on the background current was evaluated by using varied concentrations of these substances. As shown in Figures S7a and S8a, background current of PC and SMZ oxidation peaks increase dramatically in the presence of AA, DA and UA, while the addition of glucose or trimethoprim results in minor changes. However, it is possible to observe in Figures S7b and S8b that the signals of both PC and SMZ anodic peaks are enhanced by the same amplitude in each studied system. The recoveries vary in the range of 98–102 %. The obtained results indicate that the CNT/SBA/Ag-PE is a promising electrode to be applied as a sensor for the determination of PC and SMZ in urine samples by using the standard addition method.

In order to evaluate the practical applicability of CNT/SBA/Ag-PE, the analyses of synthetic and human urine samples were performed by using the DPV technique and standard addition method. The results (mean ± SD) for three determinations and the corresponding recovery values are presented in Table 2. It can be clearly seen that there were no significant differences between added and detected concentrations of drugs in artificial urine sample, while recovery values are slightly higher (within 3–4 %) in humane urine sample. The obtained results indicate the

Table 2. Determination of PC and SMZ by CNT/SBA/Ag-PE in urine.

System	Analyte	[Added] μmol L ⁻¹	[Found] μmol L ⁻¹ ^a	Recovery %
Synthetic Urine + SMZ	SMZ	4.1	4.2 ± 0.1	102 ± 2
Synthetic Urine + PC	PC	6.7	6.8 ± 0.1	101 ± 1
Synthetic Urine + PC	SMZ	4.1	4.2 ± 0.2	102 ± 3
+ SMZ	PC	6.7	6.6 ± 0.2	99 ± 1
Human Urine + SMZ	SMZ	4.1	4.2 ± 0.2	102 ± 3
Human Urine + PC	PC	6.7	6.9 ± 0.3	103 ± 4
Human Urine + PC	SMZ	4.1	4.3 ± 0.2	104 ± 3
+ SMZ	PC	6.7	6.9 ± 0.3	103 ± 4

^a analysis performed in triplicate.

CNT/SBA/Ag-PE can be applied to the determination of PC and SMZ in urine samples using the standard addition method.

4 Conclusions

In this work, a novel electrochemical sensor based on an electrode comprised of carbon nanotubes modified with SBA-15 and decorated with silver nanoparticles was tested. This sensor (CNT/SBA/Ag-PE) exhibited sensitive and selective detection for PC and SMZ individually and collectively for PC and SMZ determination in urine samples. Using DPV, the detection limits were 31 nmol L⁻¹ and 19 nmol L⁻¹ for PC and SMZ, respectively. The electrode exhibited stability, good reproducibility and repeatability for drug detection with a low relative standard deviation (less 5 %). The above excellent analytic sensing properties are attributed to synergistic characteristics such as low electron transfer resistance of

the MWCNT, high surface area of SBA-15 and electrocatalytic activity of the AgNP. The methodology was applied to the determination of PC and SMZ in both artificial and real urine and it showed high recovery rates (99–105 %). The obtained results demonstrate a potential applicability of CNT/SBA/Ag-PE electrode as a sensor for practical biomedical and environmental uses.

Acknowledgements

The authors thank FAPERGS, CNPq, CAPES and the Ministry of Education and Science of Ukraine for their financial support and grants. The authors also thank NANO-UFRGS and CMM-UFRGS for the access to their facilities.

Conflict of Interest

The authors declare no conflict of interest.

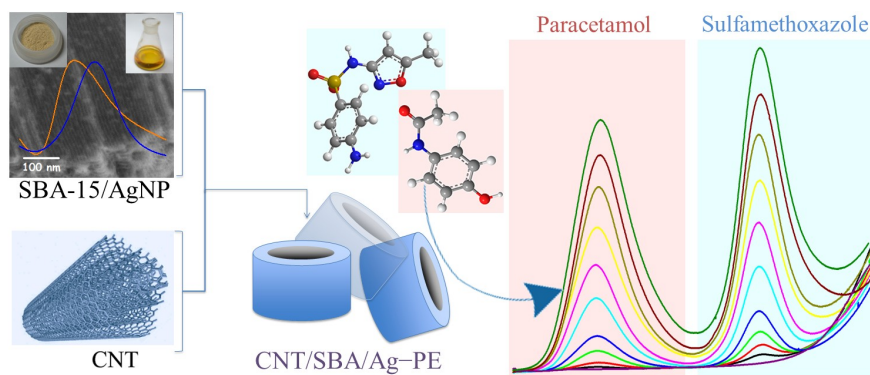
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Received: March 28, 2020

Accepted: July 14, 2020

Published online on ■■■, ■■■



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1 – 10

AgNP-Decorated SBA-15 for MWCNT Paste Modified Electrode: a Sensor for Simultaneous Voltammetric Determination of Paracetamol and Sulfamethoxazole





Mesoporous structured silica modified with niobium oxide and cobalt hematoporphyrin applied to the simultaneous electrochemical evaluation of oxalic and uric acids

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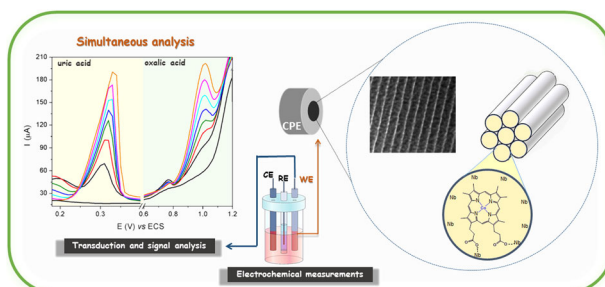
Received: 29 April 2021 / Accepted: 1 September 2021

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Abstract

Mesoporous SBA-15-type silica, with cylindrical pores hexagonally ordered, was obtained and modified with niobium oxide in a highly dispersed way. Subsequently, a further modification with cobalt hematoporphyrin was performed. The ordered pore structure of the SBA-15 was preserved after successive modifications, maintaining its textural properties. The material was used to modify a carbon paste electrode that was successfully applied to the individual and simultaneous detection of both oxalic acid and uric acid, by using cyclic voltammetry and differential pulse voltammetry. For individual evaluation, the obtained detection limits for oxalic acid and uric acid were 9.94 and $0.17 \mu\text{mol}\cdot\text{L}^{-1}$, respectively. However, for the simultaneous evaluation of both analytes, the detection limits were 2.83 and $0.14 \mu\text{mol}\cdot\text{L}^{-1}$, for oxalic acid and uric acid, respectively.

Graphical Abstract



Keywords Santa Barbara Amorphous materials · Niobia · Sol-gel · Grafting · Electrochemical sensor · CPE

Highlights

- Well-ordered silica grafted with niobia and cobalt hematoporphyrin.
- SBA-15 texture preserved after successive modifications with niobium oxide and hematoporphyrin macromolecule.
- Simultaneous electrochemical evaluation of oxalic acid and uric acid.
- Modified carbon paste electrode for sensitive evaluation of uric and oxalic acids.

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1 Introduction

Mesoporous SBA-15-type silica materials exhibit high surface area and cylindrical hexagonally ordered mesopores [1]. The preparation of this type of material is based on the hydrolysis and polycondensation reactions of silicon alkoxide precursors and a template is employed to build the well-organized pore structure. This synthesis strategy allows specific textural characteristics to be obtained that lead to materials with high pore volumes, unimodal pore size distributions and interconnected micropores and mesopores [2] which allow their use as sorbents [3], catalysts [4, 5], drug delivery systems [6], as well as matrices for the development of electrochemical sensors [7]. Such silica moieties with high surface areas and high pore volumes led to a high electroactive area, which is an important parameter to obtain efficient electrodes. It was demonstrated that the best silica materials for manufacturing CPEs were ordered systems, such as SBA-15, which presents high porosity, interconnected micropores and cylindrical mesopores, enabling electron transfer and allowing the easy diffusion of species to specific sites [8].

SBA-15 silica materials can be chemically modified by grafting reactions to produce metal oxide coatings covalently bonded to the silica surface [9]. By using planned and well-controlled experimental conditions, the grafting reactions allow highly dispersed and amorphous metal oxides to be obtained, in this way preventing pore blocking and maintaining the textural characteristics of the SBA-15 silica matrix [10, 11]. In the same way, the modification of silica surfaces with thin coatings of metal oxides, such as niobium oxide, provides reactive Lewis and Brønsted acid sites [11–13]. These sites facilitate enabling interactions with other species with specific organic functional groups, such as carboxylate [14] and allow the immobilization of electroactive macromolecules [9, 15], which is useful for the development of electrochemical sensors.

Despite exhibiting high solubility, oxalic acid chelation with calcium or magnesium leads to the formation of oxalate salts, which have rather low solubility. This process, in addition to removing calcium ions from the blood, can produce renal stones [16, 17]. In this way, the urinary level of oxalic acid has been recognized as an indicator in the diagnosis of renal stones. Another important compound that is present in the human organism is uric acid, which is the product of purine metabolism. The amount of uric acid in the blood or urine also helps to diagnose several diseases, such as hyperuricemia, Lesch–Nyhan syndrome, obesity, gout, diabetes, high blood pressure, and high cholesterol [18, 19]. Several methods have been described to quantify oxalic and uric acids in body fluids, including spectrometry [20, 21] and chemiluminescence [22, 23].

However, these methods are expensive and they involve several sampling steps. Thus, simple and direct methods for monitoring the amount of oxalic and uric acids are desirable. Therefore, electrochemical techniques stand out as being easy and low-cost possibilities. In this context, this work presents a CPE modified with mesoporous SBA-15 material, previously grafted with niobium oxide and cobalt hematoporphyrin (CoHp), which allows the evaluation of these analytes individually or simultaneously. As far as we know, this is the first report of simultaneous evaluation of oxalic acid and uric acid, making the developed electrode very promising for application in diagnosis for both analytes that are simultaneously present in urine.

2 Experimental

2.1 Synthesis of SBA-15

SBA-15 type silica was synthesized from an adapted procedure reported elsewhere [1]. In this procedure, Pluronic® P-123 surfactant was used as a template and tetraethylorthosilicate (TEOS, Sigma, 98%) as a source of silica. For the purpose of this synthesis, 2 g of surfactant were first dissolved in 75 mL of 1.6 mol·L⁻¹ HCl (Merck). The system was maintained at 40 °C under magnetic stirring. Afterward, 4.6 mL of TEOS were added slowly and the system remained under stirring and heating for 24 h. Then the system was transferred to an autoclave and heated at 100 °C for an additional 24 h. The white solid obtained was filtered, washed with distilled water, and dried in an oven at 80 °C for 4 h. Finally, the material was calcined in a muffle furnace for 6 h at 550 °C to remove the template. This procedure was repeated several times to obtain a sufficient amount of SBA-15 material.

2.2 Modification of SBA-15 surface with niobium oxide (SBA/Nb)

SBA-15 material (2.5 g) was previously activated at 120 °C, under vacuum, for 8 h. Subsequently, it was dispersed in toluene (Merck, 99.5%, 30 mL), under N₂ atmosphere, at 80 °C. At the same time, 405 mg of niobium pentachloride, provided by Companhia Brasileira de Mineração e Metalurgia (CBMM) were dissolved in 10 mL of absolute ethanol (Merck, 99.9%), in a glove chamber, under N₂ atmosphere. This solution was added to the flask containing SBA-15 in toluene. The mixture remained under mechanical stirring at 80 °C for 18 h. Afterwards, the supernatant was removed, and the material was washed with the following order of solvents: toluene, ethanol, water, and ethanol. The solid was then

dried at 120 °C, under vacuum. This material is hereafter called SBA/Nb.

2.3 Modification of SBA/Nb with cobalt hematoporphyrin (SBA/Nb/HpCo)

Firstly, the metalation of hematoporphyrin was performed by adapting the method described by Zahavi [24]. Hematoporphyrin (50 mg) was dissolved in 5 mL of chloroform and 7 mL of tetrahydrofuran (Merck). A cobalt acetate (Synth, 50 mg)/acetic acid solution (Dynamics, 4 mL) was added, under N₂ atmosphere and the system maintained under stirring, for 4 h. A mixture of acetic acid:ethyl acetate (1:8 v/v) was then added to precipitate the excess of cobalt, which was separated by centrifugation. Hexane (Merck, 16 mL) was added to the supernatant and the system was left in the refrigerator at 5 °C for 24 h. A CoHp precipitate thus formed was separated by centrifugation, dried under vacuum, and redissolved in 10 mL of ethanol. The CoHp ethanolic solution was added to 2.0 g of SBA/Nb material. The system was maintained under mechanical stirring for 24 h. Finally, the solid was filtered, washed with ethanol and dried at 100 °C under vacuum. The solid obtained was designated as SBA/Nb/HpCo.

2.4 Materials characterization

X-ray diffraction analysis (XRD) was performed using a Siemens model D500 diffractometer, with Cu-K α as source ($\lambda = 0.154056$ nm). The N₂ isotherms were obtained at 77 K, using a Tristar II Kr Micromeritics instrument, after the samples were degassing at 120 °C, for 10 h. BET surface area and BJH pore size distribution methods were applied [25]. Transmission electron microscopy (TEM) images were obtained using a JEOL JEM model 2010, operating at 120 keV. The samples were previously dispersed in isopropyl alcohol, in an ultrasound bath, and dispersed onto a carbon-coated copper grid, before the analyses. Scanning electron microscopy (SEM) images were acquired in a Zeiss Auriga microscope. The samples were previously dispersed on a conductive tape on aluminum support and coated with gold film. EDS analysis was acquired with an electron beam of 15 keV and 5 nA current in a JEOL SEM with a LaB₆ filament and equipped with a Thermo Fisher Scientific SDD spectrometer. The samples were compacted as disks (5.0 ton cm⁻²). UV-Vis spectra were obtained in an Agilent CARY 5000 spectrophotometer, using transmission technique for liquid samples and diffuse reflectance with integrating sphere for solid materials. The Kubelka-Munk method was applied in the latter case: $k/s = [(1-R)^2]/2R$, considering (R) as the diffuse reflectance, absorbed light as (k) and (s) as scattering coefficient.

2.5 Electrochemical measurements

Electrochemical measurements were performed on an IviumStat galvanostat/potentiostat by using a three-electrode cell constituted by: a saturated calomel electrode (SCE) as reference; a platinum wire as counter electrode and a CPE as a working electrode. The modified CPE was prepared by using SBA/Nb/HpCo material (8 mg), analytical grade graphite powder (Sigma, 99%, 12 mg) and mineral oil (5 mg). The components were mixed and a fraction of the paste was deposited in a Teflon cavity with 1 mm depth, connected to a platinum disk (6 mm of diameter) glued to a glass tube with a copper wire. The modified CPE is hereafter referred to as SBA/Nb/HpCo-CPE and was used to evaluate oxalic and uric acid contents (Sigma, 98%) using cyclic voltammetry and differential pulse voltammetry techniques. All measurements were carried out at room temperature in the presence of 1 mol L⁻¹ KCl (Merck, 99.5%) as a support electrolyte. The potential range used for cyclic voltammograms for oxalic acid was between 0.3 and 1.0 V, with 20 mV·s⁻¹ scan rate; for uric acid, the corresponding parameters were 0.1–0.6 V, and 20 mV·s⁻¹, respectively. For differential pulse voltammetry, the potential range used was between 0.1 and 0.4 V, with 5 mV·s⁻¹ scan rate. Phosphate buffer solution (PBS, 0.1 mol·L⁻¹, pH 6.0) was prepared from NaH₂PO₄ (Vetec) and Na₂HPO₄ (Vetec).

3 Results and discussion

Niobium (V) oxide was grafted onto a mesoporous SBA-15 surface to generate the SBA/Nb material by using an anhydrous ethanolic NbCl₅ solution as niobium precursor. The grafting reaction was planned to yield 0.5 mmol of niobium per gram of SBA/Nb. Subsequently, this material was further modified with CoHp to obtain the SBA/Nb/HpCo. Figure 1A shows the XRD patterns of materials at small angles, from 0.5 to 2.8°. All diffractograms presented a typical pattern of mesoporous SBA-15 material with hexagonally ordered cylindrical pores. The peaks at 0.9°, 1.5°, and 1.7° angles are due to the 100, 110 and 200 planes, respectively [1, 5, 10]. The ordered cylindrical pore structure is illustrated in the inset Fig. 1A. The XRD results demonstrate that the hexagonal ordered structure of SBA-15 is preserved, even after the subsequent surface modifications with niobium oxide and CoHp. Figure 1B presents a SEM image of the SBA-15, showing silica particles with similar morphology with dimensions around 500 nm. The TEM image of SBA-15, which is depicted in Fig. 1C, shows a lateral view of the ordered structure of the cylindrical pores.

Figure 2A shows the backscattered electron image of the SBA/Nb/HpCo material and the EDS spectrum is depicted

Fig. 1 **A** XRD analysis of SBA-15, SBA/Nb and SBA/Nb/HpCo materials. The Inset to **(A)** illustrates their cylindrical pore structure; **B** SEM image of SBA-15; **C** TEM image of SBA-15

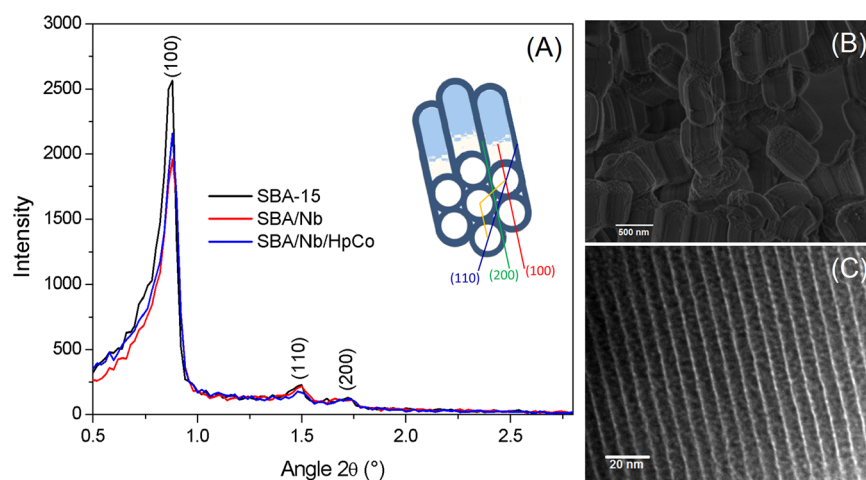
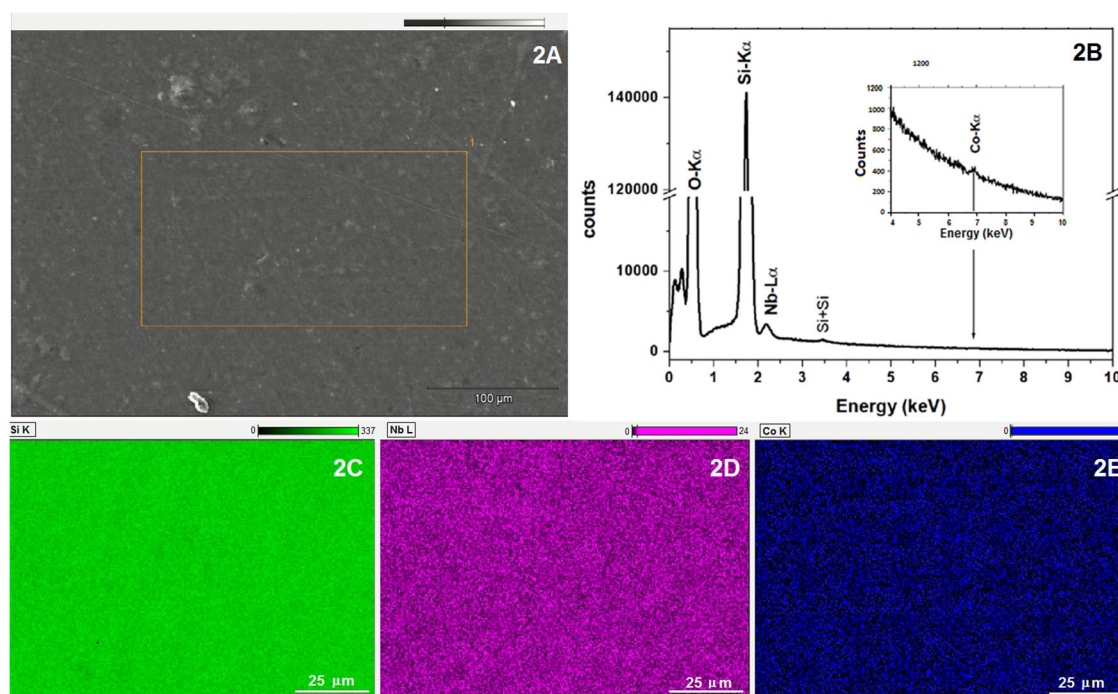
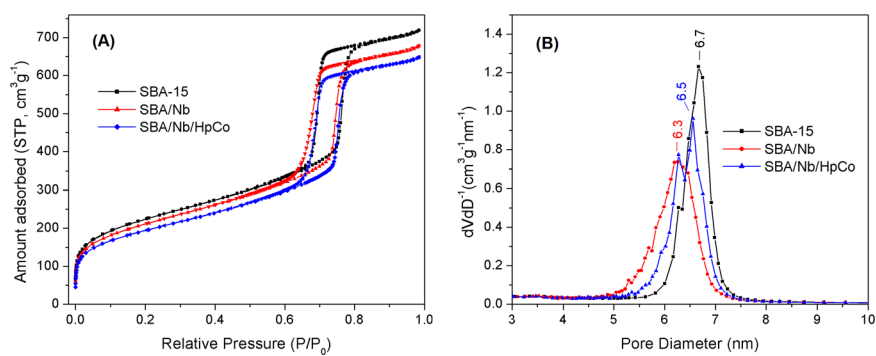


Fig. 2 EDS analysis: **(A)** backscattered electron image of sample pressed at 5.0 ton cm⁻²; **B** EDS spectrum; **C** silicon elemental mapping; **D** niobium elemental mapping and **(E)** cobalt elemental mapping

Fig. 3 Textural analysis of materials: **(A)** N₂ adsorption-desorption isotherms; **B** BJH pore size distribution, obtained from the desorption branch of isotherms



in Fig. 2B. The image is consistent with the pressed powder preparation of the sample (5.0 ton cm^2). The Si-K α and Nb-L α peaks are clearly observed, and they were estimated as 98 wt.% SiO₂ and 2 wt.% Nb₂O₅. In the case of the Co-K α peak, quantification was not possible. Qualitatively, however, the presence of cobalt can be securely established. The elemental mapping is presented in Fig. 2C, 2D and 2E for silicon, niobium and cobalt, respectively. As can be seen, the niobium and the cobalt showed homogeneous distribution at the micrometric level, even though the latter is statistically poor.

Nitrogen adsorption-desorption isotherms are depicted in Fig. 3A. Type IV isotherms with vertical parallel H1 hys-

teresis are clearly seen for all materials. This behavior is typical of materials with cylindrical and highly ordered mesopores [1, 10]. The BJH pore size distribution curves shown in Fig. 3B, which were obtained from the desorption branch of isotherms, confirm the ordered mesopore structure with narrow size distribution, characteristic of SBA-15-type materials. The surface areas and pore diameters are presented in Table 1. After the grafting reaction, from SBA-15 to SBA/Nb, the surface area decreases by 5%, and the pore diameter decreases by 0.4 nm. This result indicates that the niobium oxide is highly dispersed on the surface (SBA/Nb), which is compatible with a coating of about 0.2 nm thickness [11]. Further modification of the surface with CoHp (SBA/Nb/HpCo) leads to an additional decrease in the surface area of about 8%, and also an increase of 0.2 nm in the pore diameter. This behavior, of increasing diameter, was already reported during the immobilization of macromolecules on porous silica materials, and it was interpreted as a possible leaching process which occurs during the CoHp immobilization, resulting in some erosion of the silica pore wall [26]. This interpretation makes sense, since the matrix for immobilizing CoHp (SBA/Nb) has not undergone previous heat treatment, precisely to remain

Table 1 Textural data of materials

Sample	Surface area ($\pm 5 \text{ m}^2 \text{ g}^{-1}$)	Pore size ^a (nm)
SBA-15	768	6.7
SBA/Nb	730	6.3
SBA/Nb/HpCo	670	6.5

^aPore diameter, obtained from the maxima of the BJH pore distribution curves

Fig. 4 UV-Vis spectra of: (A) ethanolic solution of hematoporphyrin (Hp) and cobalt hematoporphyrin (CoHp), the inset shows their structures and symmetries; (B) solid materials, the inset shows a representation of cobalt hematoporphyrin functionalizing the SBA/Nb/HpCo pores

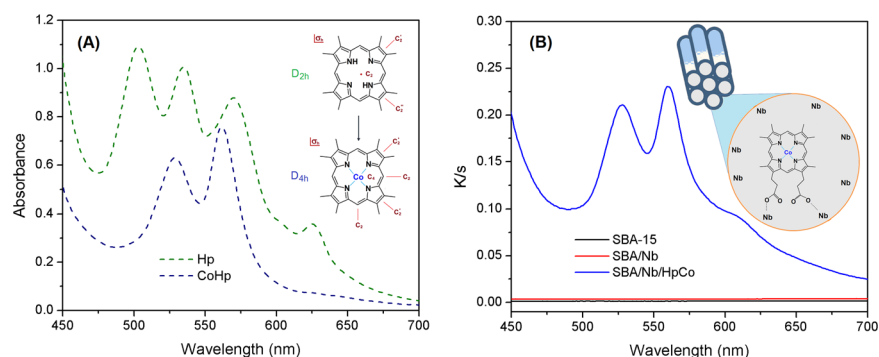
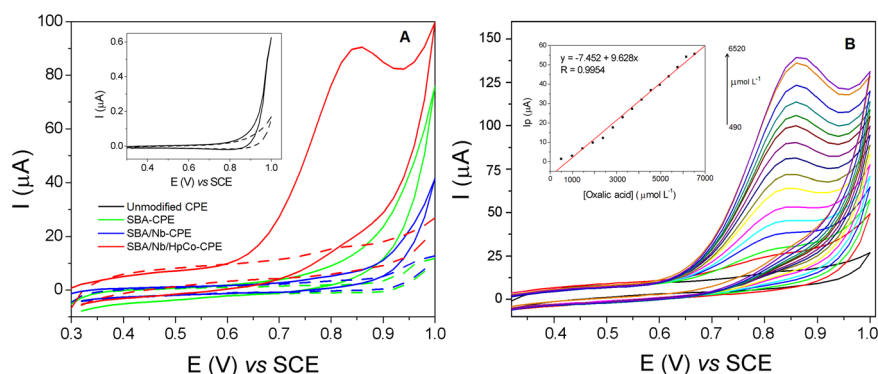


Fig. 5 A Cyclic voltammograms in the absence (segmented line) and in the presence of $4120 \mu\text{mol L}^{-1}$ of oxalic acid (straight line) for unmodified CPE, SBA-CPE, SBA/Nb-CPE and SBA/Nb/HpCo-CPE. B Cyclic voltammograms of SBA/Nb/HpCo-CPE in the presence of

different concentrations (490 and $6520 \mu\text{mol L}^{-1}$) of oxalic acid, using 1 mol L^{-1} of KCl and scan rate of 20 mV s^{-1} ; The Inset to (B) shows the linear correlation between peak current and oxalic acid concentration

Fig. 6 Cyclic voltammograms obtained with SBA/Nb/HpCo-CPE at different scan rates (20–200 $\text{mV}\cdot\text{s}^{-1}$) in the presence of oxalic acid ($4500\ \mu\text{mol}\cdot\text{L}^{-1}$), performed in $1\ \text{mol}\cdot\text{L}^{-1}$ KCl; **A** linear correlation between the current peak intensity (I_p) versus square root of the scan rate ($v^{1/2}$); **B** linear correlation between $\log I_p$ versus $\log v$

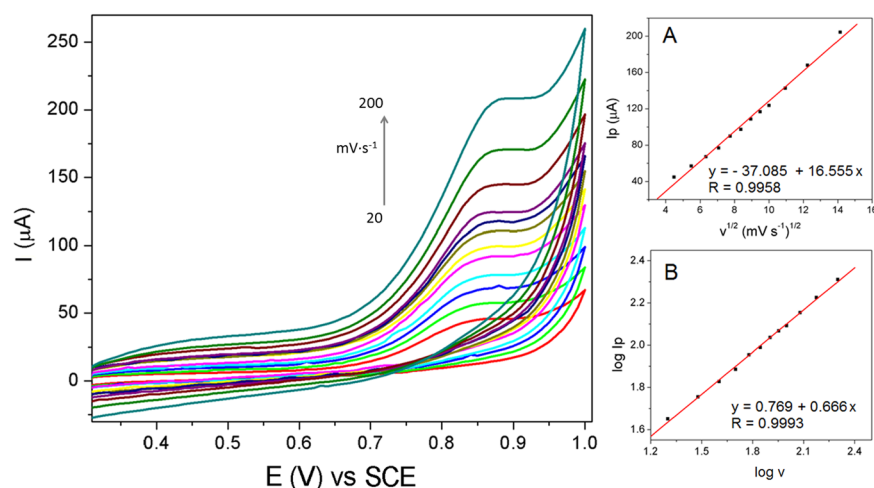
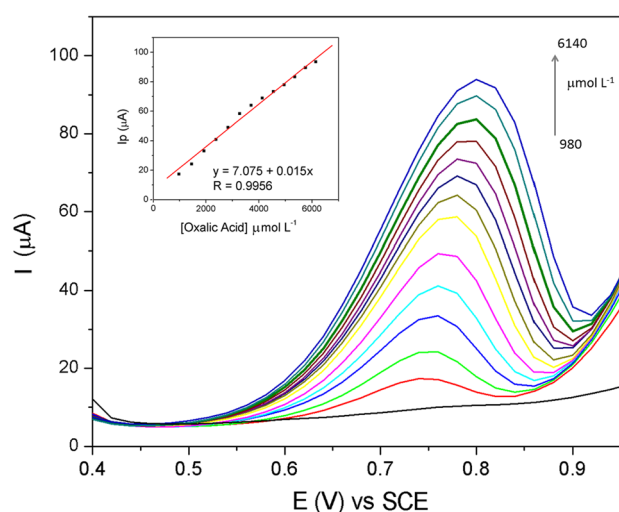


Fig. 7 Differential pulse voltammograms obtained with SBA/Nb/HpCo-CPE, in the presence of oxalic acid, in the concentration range from 980 to $6140\ \mu\text{mol}\cdot\text{L}^{-1}$, using $1\ \text{mol}\cdot\text{L}^{-1}$ KCl as support electrolyte and $v = 20\ \text{mV}\cdot\text{s}^{-1}$. The Inset shows the linear correlation between peak current and oxalic acid concentration

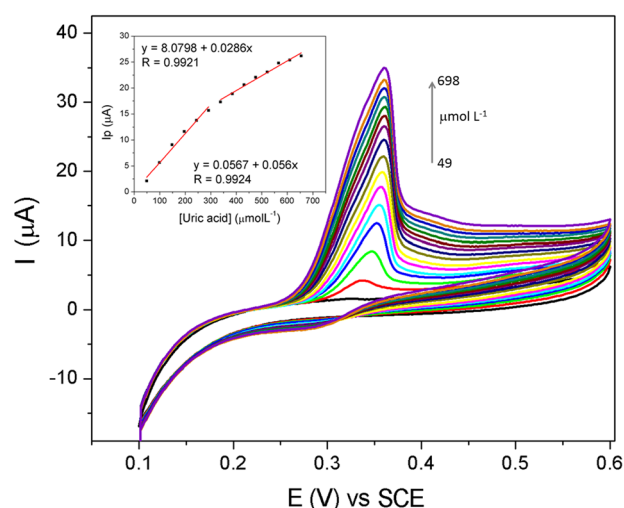


Fig. 8 Cyclic voltammograms obtained with SBA/Nb/HpCo-CPE in the presence of different concentrations of uric acid, in phosphate buffer solution ($0.1\ \text{mol}\cdot\text{L}^{-1}$, pH 6), using $1\ \text{mol}\cdot\text{L}^{-1}$ KCl and $v = 20\ \text{mV}\cdot\text{s}^{-1}$. The inset shows the linear correlation between uric acid concentration and the intensity of the peak current

more reactive. In this way, the textural analysis is in accordance with the XRD data, showing that the successive surface modifications do not produce changes in the ordered pore structure and the texture of materials remains almost unchanged.

The UV-Vis spectra of hematoporphyrin before and after the metalation with cobalt are presented in Fig. 4A. The spectrum of hematoporphyrin presents four bands, assigned as Q bands of the porphyrin ring, associated with the D_{2h} symmetry, while the spectrum of metalated hematoporphyrin shows only two Q bands, which are associated with the D_{4h} symmetry [27–29]. The spectrum of SBA/Nb/HpCo material is depicted in Fig. 4B. Two Q bands are evident in the latter spectrum, identifying the presence of the CoHp in which the porphyrin ring presents

D_{4h} symmetry. The spectra of SBA-15 and SBA/Nb materials show no bands in the visible region (Fig. 4B).

The SBA/Nb/HpCo material was used to modify the CPE (SBA/Nb/HpCo-CPE) in order to verify the viability of its use in the electrochemical determination of oxalic and uric acids. Firstly, cyclic voltammograms were obtained in the absence and in the presence of oxalic acid for unmodified CPE, SBA-CPE, SBA/Nb-CPE and SBA/Nb/HpCo-CPE, using $1\ \text{mol}\cdot\text{L}^{-1}$ KCl solution as electrolyte support. The resulting cyclic voltammograms are shown in Fig. 5A. For all the electrodes, no oxidation peak was detected in the absence of oxalic acid, in the potential range studied. However, for SBA/Nb/HpCo-CPE, in the presence of oxalic acid, a well-defined irreversible anodic peak, which corresponds to its oxidation, was observed at a

Table 2 Comparative performance of different electrodes in the electrochemical determination of oxalic acid

Electrode	Experimental conditions	Electrochemical techniques	Linear range ($\mu\text{mol}\cdot\text{L}^{-1}$)	LOD ($\mu\text{mol}\cdot\text{L}^{-1}$)	Reference
Graphite/Ag/AgCl ^a	H ₃ PO ₄ (0.05 M) $\nu = 350 \text{ mV}\cdot\text{s}^{-1}$	EIS, CV, DPV	10–750	3.7	[31]
TiO ₂ /MWCNTs/GC ^b	HClO ₄ (0.1 M) $\nu = 50 \text{ mV}\cdot\text{s}^{-1}$	CV	100–10000	33	[35]
PdAuNP/graphene ^c	H ₂ SO ₄ (0.2 M) $\nu = 100 \text{ mV}\cdot\text{s}^{-1}$	CV, LSV	5–100	2.7	[36]
Pd/SBA-15/CPE ^d	H ₂ SO ₄ (0.1 M)/0.1 M PBS (pH 6.00) $\nu = 50 \text{ mV}\cdot\text{s}^{-1}$	CV, LSV	10–140	0.4	[37]
PtNP/Glassy Carbon ^e	HClO ₄ (0.1 M) $\nu = 100 \text{ mV}\cdot\text{s}^{-1}$	CV, Amperometry	1.4–595	0.28	[38]
Ag-doped ZSM-5 ^f	PBS (0.1 M) $\nu = 20 \text{ mV}\cdot\text{s}^{-1}$	CV, DPV	16–1800	5.5	[39]
PdNP/rGO ^g	H ₂ SO ₄ (0.1 M) $\nu = 50 \text{ mV}\cdot\text{s}^{-1}$	CV, DPV	50–10,000	50	[40]
AgNRs/Graphene ^h	PBS (0.1 M) $\nu = 50 \text{ mV}\cdot\text{s}^{-1}$	CV	3000–30,000	40	[41]
PdPt/GC ⁱ	HClO ₄ (0.1 M) $\nu = 100 \text{ mV}\cdot\text{s}^{-1}$	CV, Amperometry	10–4700	1	[42]
SBA/Nb/HPCo-CPE	KCl (1 M) $\nu = 20 \text{ mV}\cdot\text{s}^{-1}$	CV, DPV	980–6140	9.94	This work
SBA/Nb/HPCo-CPE ^j	KCl (1 M) $\nu = 20 \text{ mV}\cdot\text{s}^{-1}$	CV, DPV	1456–4128	2.83	This work

LOD Limit of detection, EIS Electrochemical impedance spectroscopy, CV Cyclic voltammetry, DPV Differential pulse voltammetry, LSV Linear sweep voltammetry, ν = scan rate

^aSilver and silver chloride deposited on graphite

^bTiO₂ nanoparticles/multi-walled carbon nanotubes/glassy carbon

^cPalladium nanoparticles on reduced graphene oxide

^dPalladium-doped SBA/carbon paste electrode

^ePlatinum nanoparticles modified glassy carbon electrode

^fSilver incorporated in ZSM-5/carbon paste electrode

^gPalladium nanoparticles on reduced graphene oxide

^hSilver nanorods dispersed on graphene

ⁱPalladium and Platinum/glassy carbon

^jSimultaneous analysis with Uric Acid

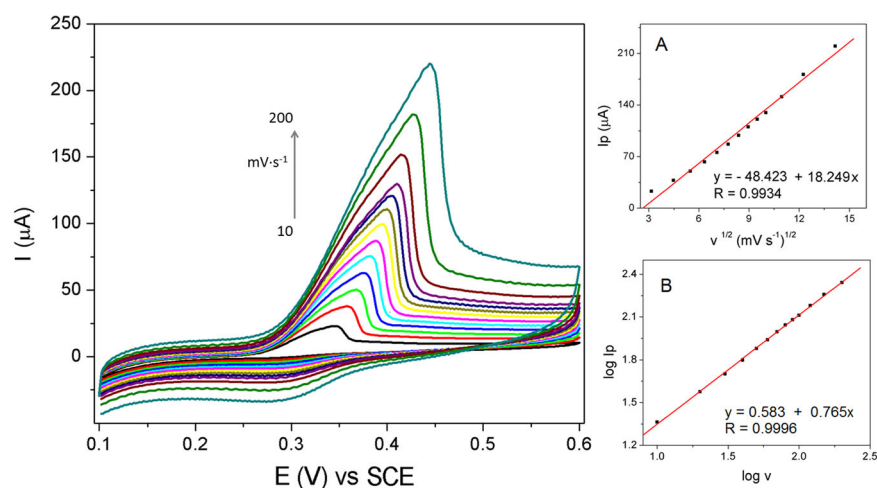
potential near to 0.85 V. For all other electrodes no oxalic acid oxidation peak was observed, in the potential range studied. These results confirm the electrocatalytic properties of the SBA/Nb/HPCo-CPE system. The peak irreversibility is due to the formation of carbon dioxide during the oxidation, which is rapidly released from the electrode's surface, thus making the process irreversible [30, 31].

The cyclic voltammograms of SBA/Nb/HPCo-CPE, obtained by using different oxalic acid concentrations, in the range between 490 and 6520 $\mu\text{mol}\cdot\text{L}^{-1}$, are shown in Fig. 5B. An increase in the intensity of the acid oxalic oxidation peak was observed with increasing oxalic acid concentration and the inset Fig. 5B shows the linear correlation between the anodic peak current and the analyte

concentration, considering only the Faradaic current. In this way, it is revealed that this electrode has potential applicability as a sensor, within the chosen concentration range.

With the purpose of evaluating the electrochemical behavior of the SBA/Nb/HPCo-CPE, in the oxalic acid oxidation, cyclic voltammograms were performed with different scan rates, between 20 and 200 $\text{mV}\cdot\text{s}^{-1}$, in the presence of 1.0 $\text{mol}\cdot\text{L}^{-1}$ KCl solution. The voltammograms are shown in Fig. 6. The linear correlation between the current peak intensity (I_p) and the square root of the scan rate (Fig. 6A) suggests a diffusional mechanism for the electroactive species on the electrode surface. The correlation between $\log \nu$ versus $\log I_p$ was obtained in order to confirm whether the process is really controlled by diffusion. A slope close to 0.5 indicates a

Fig. 9 Cyclic voltammograms obtained with SBA/Nb/HpCo-CPE at different scan rates (10–200 $\text{mV}\cdot\text{s}^{-1}$) in the presence of uric acid ($698\text{ }\mu\text{mol}\cdot\text{L}^{-1}$), using phosphate buffer ($0.1\text{ mol}\cdot\text{L}^{-1}$, pH 6) and $1\text{ mol}\cdot\text{L}^{-1}$ KCl; **A** linear correlation between the current peak intensity (I_p) versus square root of the scan rate ($v^{1/2}$); **B** linear correlation between $\log I_p$ versus $\log v$



diffusional process, while a slope value close to 1.0 indicates that the process occurs by adsorption [32, 33]. As the obtained linear correlation value was 0.666 (Fig. 6B), we can infer that the oxidation process of the oxalic acid on the SBA/Nb/HpCo-CPE is controlled by a diffusion mechanism, because the slope is near 0.5.

Measurements using differential pulse voltammetry techniques, with the SBA/Nb/HpCo-CPE electrode, were performed with concentrations of oxalic acid between 980 and $6140\text{ }\mu\text{mol}\cdot\text{L}^{-1}$. The voltammograms are depicted in Fig. 7, and they show that the anodic peak current increases with increasing oxalic acid concentration. The inset to Fig. 7 shows the linear correlation between the anodic peak current and the oxalic acid concentration. According to the slope of this curve, the sensitivity was estimated as $0.015\text{ }\mu\text{A}\cdot\text{L}\cdot\mu\text{mol}^{-1}$. The limit of detection (LOD) was calculated from $(3 \times \text{SDB}/\text{slope})$ [34], where SDB is the standard deviation of the blank measurements ($n = 10$). The obtained LOD value was $9.94\text{ }\mu\text{mol}\cdot\text{L}^{-1}$.

Table 2 presents the concentration range, the experimental conditions, the applied technique and the LOD for other types of electrodes, which were recently reported for oxalic acid determination. As can be seen, the SBA/Nb/HpCo-CPE presents a LOD value comparable with the values of other electrodes. However, considering the concentration range above $1000\text{ }\mu\text{mol}\cdot\text{L}^{-1}$, the SBA/Nb/HpCo-CPE electrode is among those with the lowest LOD values.

The SBA/Nb/HpCo-CPE was also used in the investigation of the electrochemical behavior of uric acid, using cyclic voltammetry and differential pulse voltammetry. Figure 8 shows the cyclic voltammograms for uric acid in the concentration range from 49 to $698\text{ }\mu\text{mol}\cdot\text{L}^{-1}$, using $1\text{ mol}\cdot\text{L}^{-1}$ KCl and a scan rate of $20\text{ mV}\cdot\text{s}^{-1}$. In the absence of uric acid, no oxidation peak was detected. However, a well-defined and irreversible anodic peak was observed at a potential close to 0.35 V, thus characterizing the oxidation of uric acid by the SBA/Nb/HpCo-CPE.

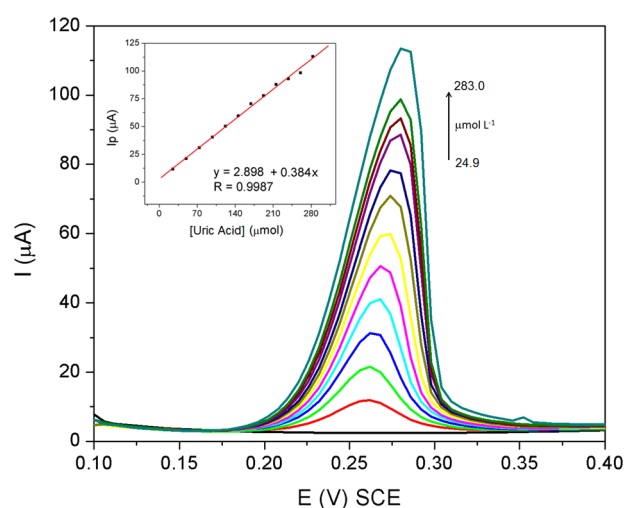


Fig. 10 Differential pulse voltammograms obtained with SBA/Nb/HpCo-CPE in the presence of different concentrations of uric acid, in phosphate buffer ($0.1\text{ mol}\cdot\text{L}^{-1}$, pH 6), KCl $1\text{ mol}\cdot\text{L}^{-1}$ and $v = 5\text{ mV}\cdot\text{s}^{-1}$; The Inset shows the linear correlation between uric acid concentration and peak current intensity

With regard to the inset in Fig. 8, two linear concentration ranges are clearly identified.

The influence of the scan rate on the electrochemical behavior of uric acid was also investigated by cyclic voltammetry and the results are shown in Fig. 9. An increase in the current peak intensity and also a peak displacement to higher potentials were observed with increasing scan rate. Figure 9A provides information about the uric acid oxidation process, showing a linear correlation between the current peak intensity (I_p) and the square root of the scan rate ($v^{1/2}$). As observed for oxalic acid, this result also suggests that the mechanism of uric acid oxidation on the SBA/Nb/HpCo-CPE surface is controlled by diffusion of electroactive species [33]. However, the linear correlation between $\log I_p$ versus $\log v$, which is presented in Fig. 9B,

Table 3 Comparative performance of different electrodes in the electrochemical determination of uric acid

Electrode	Experimental conditions	Electrochemical techniques	Linear range ($\mu\text{mol}\cdot\text{L}^{-1}$)	LOD ($\mu\text{mol}\cdot\text{L}^{-1}$)	Reference
GO/GCE ^a	PBS (0.1 M, pH 6.5) $\nu = 50 \text{ mV}\cdot\text{s}^{-1}$	CV	20–490	3.45	[43]
RGO-ZnO ^b	PBS (0.1 M, pH 6) $\nu = 50 \text{ mV}\cdot\text{s}^{-1}$ N_2 atmosphere	CV, DPV	1–70	0.33	[44]
Pd/RGO/GCE ^c	PBS (0.1 M, pH 7) $\nu = 0.05 \text{ mV}\cdot\text{s}^{-1}$	CV, DPV	6–470	1.6	[45]
GNP/FTO ^d	PBS (0.1 M, pH 7) $\nu = 100 \text{ mV}\cdot\text{s}^{-1}$	CV, DPV	10–100	0.28	[46]
Au-Cu ₂ O/rGO ^e	PBS (0.1 M), $\nu = 100 \text{ mV}\cdot\text{s}^{-1}$	CV, DPV, EIS	100–900	6.5	[47]
PB/rGO ^f	ABS (0.1 M, pH 6) $\nu = 25 \text{ mV}\cdot\text{s}^{-1}$	CV	40–415	8.0	[48]
NiO/MWCNT ^g	PBS (pH 7) $\nu = 50 \text{ mV}\cdot\text{s}^{-1}$	CV	10–2500	1	[49]
Fe ₃ O ₄ /CPE ^h	PBS (0.1 M, pH 7.5)	CV, EIS	160–2110	80	[50]
3D-ACNT ⁱ	PBS (0.01 M, pH 7.4) $\nu = 50 \text{ mV}\cdot\text{s}^{-1}$	CV	100–1000	1	[51]
SBA/Nb/HpCo-CPE	PBS (0.1 M, pH 6) KCl (0.1 M) $\nu = 20 \text{ mV}\cdot\text{s}^{-1}$	CV, DPV	25–350	0.17	This work
SBA/Nb/HpCo-CPE ^j	PBS (0.1 M, pH 6) KCl (0.1 M) $\nu = 20 \text{ mV}\cdot\text{s}^{-1}$	CV, DPV	98–260	0.14	This work

LOD Limit of detection, EIS Electrochemical impedance spectroscopy, CV Cyclic voltammetry, DPV Differential pulse voltammetry, LSV Linear sweep voltammetry, ν = scan rate

^aUricase onto graphene oxide/glassy carbon electrode

^bGraphene zinc oxide/glassy carbon electrode

^cPalladium on reduced graphene oxide/glassy carbon electrode

^dgraphene nanoplatelet-modifiedfluorine-doped tin oxide electrode

^eAu-Cu₂O nanoparticles/reduced graphene oxide

^fPrussian blue/reduced graphene oxide

^gNiO/multi-walled carbon nanotube

^hMagnetite modified carbon paste electrode

ⁱ3D-aligned carbon nanotube

^jSimultaneous analysis with Oxalic Acid

shows a slope of 0.765. This value of slope, between 0.5 and 1.0, indicates that the process is controlled simultaneously by diffusion and adsorption [32]. This adsorption can be explained by the high porosity of the electrodes containing nanostructured SBA and by the presence of the Nb₂O₅, which presents Lewis acid sites that can interact with the uric acid [12, 14].

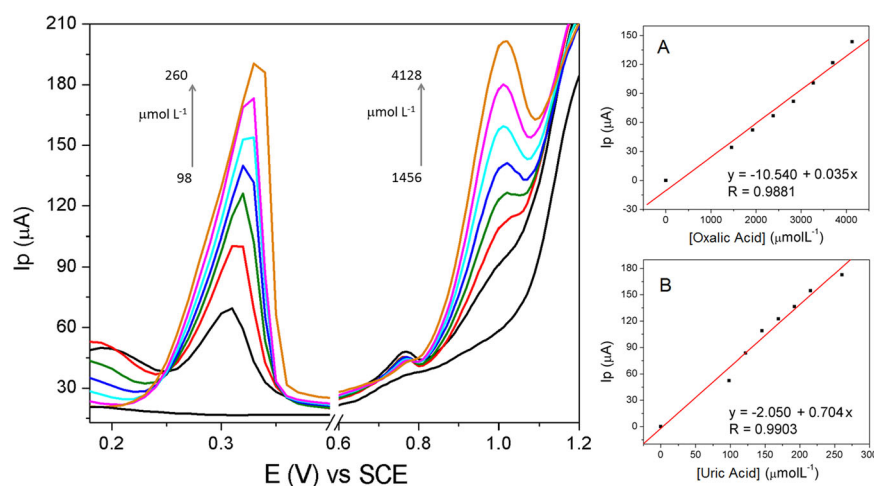
Differential pulse voltammograms were obtained with SBA/Nb/HpCo-CPE for uric acid, by varying its concentration in the range from 24.9 to 283.0 $\mu\text{mol}\cdot\text{L}^{-1}$. The voltammograms are depicted in Fig. 10 and they show that the anodic peak current increases with increasing uric acid concentration. The inset to Fig. 10 shows a linear correlation between the anodic peak current and the uric acid concentration, within the applied concentration range. The sensitivity was determined from the slope of the calibration curve, whose value was

0.384 $\mu\text{A}\cdot\text{L}\cdot\mu\text{mol}^{-1}$. The calculated LOD was 0.17 $\mu\text{mol}\cdot\text{L}^{-1}$. Therefore, compared with the oxalic acid, the SBA/Nb/HpCo-CPE showed higher sensitivity toward uric acid.

Table 3 presents a comparison of the performance of different electrodes recently used for the determination of uric acid with that of SBA/Nb/HpCo-CPE. As can be seen in Table 3, the electrode developed in this work showed a lower LOD value when compared with other electrodes already reported. Therefore, the electrode proposed in this work showed high sensitivity in the determination of uric acid over a wide linear concentration range. These results are promising and indicate that this electrode can be used as a sensor to determine uric acid.

The reproducibility of the SBA/Nb/HpCo-CPE was evaluated using four similar electrodes. The cyclic voltammograms were made using 9.8 $\text{mmol}\cdot\text{L}^{-1}$ of oxalic acid and the

Fig. 11 Simultaneous analysis via differential pulse voltammetry for oxalic and uric acids using the SBA/Nb/HpCo-CPE, in phosphate buffer ($0.1 \text{ mol}\cdot\text{L}^{-1}$, pH 6) and KCl ($1 \text{ mol}\cdot\text{L}^{-1}$ and $v = 20 \text{ mV}\cdot\text{s}^{-1}$; **A** linear correlation between oxalic acid concentration and peak current intensity; **B** linear correlation between uric acid concentration and peak current intensity



obtained standard deviation was 12.38%. In addition, the SBA/Nb/HpCo-CPE was evaluated using one electrode and $9.8 \text{ mmol}\cdot\text{L}^{-1}$ of oxalic acid. The standard deviation for six cycles, after 1 min of stirring and 2 min of rest, was 5.91%.

Taking into account the considerable oxidation peak separation between the two analytes, simultaneous analyses of oxalic and uric acids were also performed by differential pulse voltammetry, using SBA/Nb/HpCo-CPE, KCl $1 \text{ mol}\cdot\text{L}^{-1}$ and a scan rate of $20 \text{ mV}\cdot\text{s}^{-1}$. The voltammograms, shown in Fig. 11, exhibit an increase in peak current after consecutive additions of oxalic acid and uric acid. Figure 11A shows the linear correlation between peak intensity versus oxalic acid concentration, within the range from 1456 to $4128 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$; whereas Fig. 11B presents the linear correlation for uric acid, in the concentration range between 98 and $260 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$. Based on these linear regressions, in the presence of both analytes, the sensitivities increased to 0.035 and $0.704 \text{ }\mu\text{A}\cdot\text{L}\cdot\mu\text{mol}^{-1}$ for oxalic acid and uric acid, respectively. In this way, the calculated LOD values in the simultaneous evaluation of oxalic acid and uric acid have been decreased to 2.83 and $0.14 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$, respectively. These results indicate that the developed electrode (SBA/Nb/HpCo-CPE) allows the simultaneous evaluation of oxalic acid and uric acid concentrations with high sensitivity.

4 Conclusions

A high surface area mesoporous SBA-15 silica material with highly ordered and interconnected pore structure was obtained and successfully modified with niobium oxide (SBA/Nb). Afterwards, a further modification with CoHp was performed (SBA/Nb/HpCo). XRD and N_2 adsorption-desorption isotherms show that the modified material maintains the well-ordered pore structure as well as the textural properties of the SBA-15 silica material. The modified mesoporous material was used to manufacture a

CPE (SBA/Nb/HpCo-CPE), which was applied to the evaluation of oxalic and uric acids by using cyclic voltammetry and differential pulse voltammetry, in a wide linear range of concentration. When compared with other reported electrodes, the modified CPE developed in this work presents the lowest LODs for uric acid. In addition, it allows the simultaneous evaluation of both analytes with high sensitivity, which makes it a very promising system for its applicability as a sensor for oxalic and uric acids.

Acknowledgements The authors thank CMM (Centro de Microscopia e Microanálise—UFRGS) and CNANO (Centro de Nanociência e Nanotecnologia—UFRGS) for the use of microscope and XRD equipment. The authors also thank the CBMM (Companhia Brasileira de Mineração e Metalurgia), which provided the NbCl_5 compound.

Funding The work was financially supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, FAPERGS (Fundação de Amparo à Pesquisa do estado do Rio Grande do Sul) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Consent to participate All authors agree to participate in this work.

Consent for publication All authors agree on the content and its publication.

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Research Papers

Application of NiFe₂O₄ nanoparticles towards the detection of ovarian cancer markerI. Maghiani^a, L.V. Souza^b, L. Bach-Toledo^b, A.M. Faria^b, P.P. Ortega^c, R.A.C. Amoresi^{a,d}, A. Z. Simões^{a,*}, T. Mazon^{b,*}^a School of Engineering and Sciences, São Paulo State University (UNESP), Guaratinguetá, São Paulo, Brazil^b Centro de Tecnologia da Informação Renato Archer, CTI, Rod. D. Pedro I, KM 143.6, 13069-901 Campinas, SP, Brazil^c Center for Development of Functional Materials (CDMF), Federal University of São Carlos (UFSCar), São Carlos, São Paulo, Brazil^d Federal University of ABC (UFABC), Center for Engineering, Modeling and Applied Social Sciences, Av. Dos Estados, 5001, 09210-580, Santo André, SP, Brazil

ARTICLE INFO

Keywords:

Nanostructures

Oxides

Chemical synthesis

Magnetic properties

Electrochemical properties

ABSTRACT

In this work, we explored the potential application of NiFe₂O₄ magnetic nanoparticles in sensing platforms due to their cost-effectiveness and good magnetic properties. The spinel-type metal oxide was prepared using the conventional hydrothermal synthesis method. The structural, morphological, and magnetic properties of the as-synthesized NiFe₂O₄ nanoparticles were discussed. The nanoparticles were functionalized with cystamine, glutaraldehyde, and PVDF and then modified with the CA 125 antibody to obtain the immunosensor. FTIR and electrochemical analyses reveal immobilization of the antibody on the surface of the NiFe₂O₄ magnetic nanoparticles. The immunosensor developed in this work was made on a printed circuit board, allowing it to be used as a point-of-care device. The immunosensor showed excellent stability, reproducibility, specificity, and selectivity. Our findings are of great significance for developing novel, high-performance point-of-care electrochemical biosensors to be applied in detecting the CA 125 antigen, a widely used biomarker for the prognosis of ovarian cancer.

1. Introduction

Ovarian cancer (OC) is one of the most lethal gynecological diseases and the fifth leading cause of death in women [1]. Family history stands out among the relevant risk factors, while obesity and hormone replacement therapy pose moderate risks. Recent studies show that the 5-year survival rate in early stages (I and II) is around 90 %, dropping to 20–40 % in advanced ones (III and IV) [2]. This decrease is due to the absence of specific symptoms, often leading to a late diagnosis. Poor prognosis in advanced stages is mainly related to the resistance of cancer cells to conventional chemotherapy [1]. Therefore, screening ovarian cancer in an early stage can enhance treatment success and increase the survival rate [3]. The CA 125 antigen is a widely used biomarker for the prognosis of ovarian cancer. It is a mucin, that is, it is a surface antigen on a high molecular weight glycoprotein, expressed in OC epithelial cells, tissues of the mesothelial cells of the tubes, endometrium, and endocervical epithelium. Also, it is found in the blood of patients with ovarian cancer, being present in more than 90 % of patients with advanced epithelial OC (stage II, III, and IV) and 50 % of patients with

early OC stages [4]. Studies indicate that while acceptable levels of CA 125 in the body are less than 35 U/mL (units per milliliter), about 82 % of women with ovarian cancer in advanced stages have a higher level of CA 125 in the blood. In comparison, only 1 % of healthy women have concentrations exceeding 35 U/mL [5,6]. Despite disagreements about the direct or indirect role of CA 125 in diagnosing OC [7,8], existing results demonstrate a connection between high levels of this biomarker and OC [5,6], showing that the presence of CA 125 indicates this disease. Therefore, the development of accurate and rapid analytical methods for the detection of CA 125 is essential.

Ultrasound is one of the most traditional methods for ovarian cancer detection, which can be Transabdominal Ultrasound (UTA) or Transvaginal Ultrasound (UTV). In this technique, the diagnosis is via images through the observation of irregularities in the vaginal canal, cervix, uterus, fallopian tubes, and ovaries. However, the method has limitations, such as the low energy of the sound wave, which can prevent its penetration through bones and specific organs, and the reduction of image resolution as the sound beam penetrates deep tissue layers [9]. Given this, ultrasound is insufficient for a reliable early diagnosis due to

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Received 6 November 2023; Received in revised form 12 February 2024; Accepted 8 April 2024

Available online 9 April 2024

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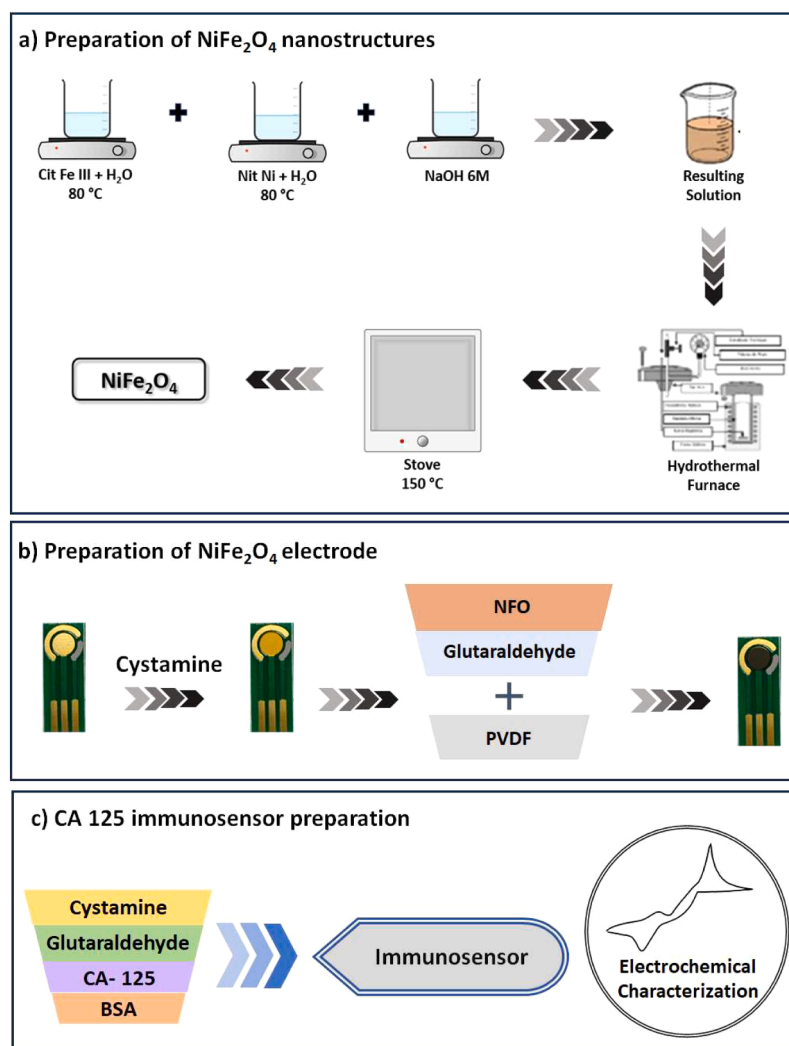


Fig. 1. (a) Schematic illustration of the synthesis of the NiFe_2O_4 NPs; (b) Preparation of NiFe_2O_4 electrode; (c) Immunosensor fabrication process for the CA 125 detection.

its limited sensitivity and inability to provide specific and functional information [10]. As a result, alternative methods for early screening have been studied in the specialized literature. In this sense, biosensors emerge as a low-cost technology for fast and accurate diagnosis since they have specificity, detection speed, and portability [11]. Depending on their transduction method and biorecognition molecule, there are several categories and subcategories of biosensors. The main transduction methods for detecting cancer biomarkers are the electrochemical, optical, and gravimetric [12]. Different materials have been tested as biosensors for the CA 125 protein detection. For example, Gasparotto et al. [13] developed an electrochemical biosensor based on ZnO nanorods (NRs)-Au nanoparticles (NPs) hybrids via the hydrothermal and sputtering methods and built on glass substrates with gold electrodes and silver counter electrodes. The authors reported good reproducibility, stability, and high sensitivity due to the ZnO NRs-Au NPs nanohybrid matrix, which provides an appropriate surface for the immobilized anti-CA 125 antibody, preserving its active sites. Attia et al. [14] developed an optical biosensor consisting of a thin film of Ni-Pc doped on a PS matrix for the CA 125 antibody detection, resulting in a highly sensitive biosensor. In another work, Abou-Omar et al. [15] built an optical biosensor based on a thin sol-gel film containing gold nanoparticles coated with Schiff-based ligands quickly and precisely to detect the CA 125 biomarker. Li et al. [16] used the hydrothermal method to synthesize an electrochemical biosensor based on molybdenum disulfide

supported on a metal-organic framework (CuBTC@MoS_2) decorated with gold nanoparticles (AuNPs), which exhibited an excellent electrochemical performance with fast ions and electrons transferring and excellent specificity.

Electrochemical biosensors based on nanomaterials stand out due to their high sensitivity, specificity, simplicity, and inherent miniaturization, which make them low-cost and allow a fast analysis [13]. Several nanomaterials, especially nanostructures of semiconductor oxides, can be used as matrices for the immobilization of biomolecules for electrochemical biosensors since they have a high surface-to-volume ratio combined with electron transport properties, high catalytic efficiency, and good adsorption capacity [17]. Nickel ferrite (NiFe_2O_4) nanostructures are promising for biosensing applications since their surfaces are susceptible to the immobilization of biomolecules. Bimetallic oxides have been widely investigated in bioanalysis, electrocatalysis, and gas sensing due to their intrinsic mixed valence, drastically improving their conductivity and catalytic activity. Nickel ferrite (NiFe_2O_4 , or NFO) is a ceramic material with an inverse spinel-type crystalline structure, low magnetic coercivity, chemical stability, mechanical hardness, and excellent electrochemical performance [18]. In addition, among other magnetic materials, it has relatively high electrical resistivity and reasonable costs, which allow a wide variety of applications in various electronic devices [18]. Different methods have been employed in the literature to synthesize NiFe_2O_4 , such as solid-state reaction, sol-gel,

conventional and microwave-assisted hydrothermal synthesis, precipitation, combustion, chemical reduction, and others [19]. Among these methods, the conventional hydrothermal synthesis yields powders with high purity, high crystallinity, average particle size from 3 nanometers to micrometers depending on the synthesis parameters (time, temperature, pH, etc.), narrow particle distribution, and good stoichiometric control at low temperatures. Particle size and morphology can be controlled by changing synthesis parameters. Conventional hydrothermal synthesis also presents an excellent cost-benefit ratio, as it involves a closed system (without environmental problems) and with high energy efficiency. Another essential advantage of hydrothermal synthesis is that the purity of the obtained powders significantly exceeds the purity degree of the starting materials. This occurs because hydrothermal crystallization is a self-purification process, during which the growth of crystals/crystallites tends to reject the impurities present in the growth environment [20–24]. Therefore, manipulating the surface of NiFe_2O_4 nanostructures to produce more selective and sensitive biosensors can enable significant advances in screening diseases that are difficult to detect by conventional methods.

In this work, we aim to fulfill two objectives: to comprehend (i) the mechanisms associated with magnetism on NiFe_2O_4 nanoparticles and (ii) the mechanisms behind detecting the CA 125 antigen for the early diagnosis of ovarian cancer using electrochemical biosensors. Thus, we report a well-controlled synthesis of NiFe_2O_4 nanoparticles using the conventional hydrothermal (CH) synthesis. We discussed the structural, morphological, and magnetic properties of the as-synthesized NiFe_2O_4 nanoparticles. Our findings are of great significance for developing novel, high-performance electrochemical biosensors for the CA 125 antigen detection, a widely used biomarker for ovarian cancer prognosis.

2. Experimental procedures

2.1. Synthesis of the NiFe_2O_4 nanoparticles

NiFe_2O_4 nanoparticles (NPs) were synthesized using the conventional hydrothermal (CH) synthesis method, as illustrated in Fig. 1a. First, iron III citrate (99 % purity, Sigma Aldrich) and nickel nitrate hexahydrate (99 % purity, Éxodo Científica) were dissolved in deionized water in separated beakers at 80 °C and under constant magnetic stirring. Then, the two solutions were transferred to a single beaker and homogenized. After that, a basic medium (NaOH , ~ 6 M) was slowly added to the solution, under constant stirring, until pH 12 was reached. The resulting solution was poured into a Teflon autoclave, which was sealed and inserted into a conventional hydrothermal synthesis furnace at 150 °C for 24 h. After the synthesis, the autoclave cooled to room temperature naturally. The resulting suspension was washed four times (three minutes at 7000 rpm) with deionized water, neutralizing the pH. A final wash was performed with ethanol. Then, the red/brown precipitate was dried in an oven at 150 °C for 16 h and subsequently ground into a fine powder using an agate mortar.

2.2. Preparation of the NiFe_2O_4 electrode

The electrochemical sensor board was constructed on a commercially available 0.8 mm-thick printed circuit board (PCB). The PCB is composed of FR-4 woven fiberglass and a flame-retardant epoxy resin. The PCB contains three electrodes with an ENIG (Electroless nickel immersion gold) surface finish. The working electrode (WE) and the counter electrode (CE) were made of gold from the ENIG surface finish, and the reference electrode (RE) was of silver/silver chloride (Ag/AgCl) deposited by screen printing. To prepare the RE, a silver/silver chloride ink was deposited along with heat treatment at 60 °C. The WE was modified with the as-synthesized NiFe_2O_4 NPs, as illustrated in Fig. 1b. For this, 0.01 g of the NiFe_2O_4 NPs were added to a 50 % glutaraldehyde (Glut) solution and kept under constant stirring overnight. After that,

5% m/m of polyvinylidene fluoride (PVDF) was added to the NiFe_2O_4 /Glut mixture. The PVDF was used as a plasticizer to improve the fixing process of the NiFe_2O_4 NPs on the working electrode. At the same time, the WE was incubated with 20 mM Cystamine dihydrochloride (Cys) (Sigma Aldrich) at 120 °C for 15 min. Finally, 5 μL of the NiFe_2O_4 /Glut emulsion was deposited onto the Cys-WE and incubated at 60 °C for 24 h to promote the crosslinking with the amine-terminated monolayer.

2.3. CA 125 immunosensor preparation

The anti-CA 125 antibody (Abcam, Cambridge, MA, USA) was immobilized onto the surface of the NiFe_2O_4 NPs using a solution containing 20 mM Cys and 2.5 % Glut (Fig. 1c). The antibody was diluted in buffered phosphate saline (PBS) at pH 7.4 to a concentration of 1:1000. Then, a 25 μL aliquot of this antibody/PBS solution was dropped on the working electrode's surface and incubated overnight at 4 °C inside a humid chamber. After incubation, 30 μL of BSA 1 % (bovine serum albumin) was dropped on the working electrode's surface and incubated for 30 min at room temperature to avoid nonspecific reactions with other proteins.

2.4. Characterization methods

X-ray diffractometry (XRD) was carried out in a DMax/2500 PC (Rigaku) instrument to investigate the phase of the nanoparticles, using $\text{CuK}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) from 20° to 70° A confocal Raman spectrometer (T64000, Horiba) with an exposure time of 30 s, accumulation of 10 spectrums, and laser source of 633 nm was used to characterize the NiFe_2O_4 powder. The morphology of the NiFe_2O_4 nanoparticles was investigated using a model Mira 3 Tescan field-emission gun scanning electron microscope (FEG-SEM) operated at an acceleration voltage of 5 kV for SE detector with energy-dispersive X-ray spectroscopy (Brookfield) and 30 kV for STEM detector. Fourier-transform infrared (FTIR) spectroscopy was performed using an attenuated total reflectance (ATR) accessory of the Perkin Elmer model Spectrum 100 spectrometer. The FTIR spectra were recorded in transmission mode in the 650 – 4000 cm^{-1} range to investigate the nature of the chemical bonds formed during the immunosensor preparation.

Dot Blot assays were used to test the specificity of the CA recombinant antibody-antigen binding. Two microliters of the CA 125 recombinant protein were dotted onto a nitrocellulose membrane. The dots were made with 1.5 $\mu\text{g.mL}^{-1}$ and 0.15 $\mu\text{g.mL}^{-1}$. The membrane was then incubated overnight at 4 °C with a 1:5000 anti-CA125 antibody. The blots were subsequently incubated with an HRP-conjugated secondary antibody, and immunoreactive dots were used with the enhanced chemiluminescence method.

2.5. Electrochemical measurements

Electrochemical measurements were performed using a portable potentiostat Sensit Smart (Palm Sens) instrument connected to a laptop via the PStace software. The analytical responses of the immunosensor were evaluated by electrochemical measurements using cyclic voltammetry (CV) and Differential Pulse Voltammetry (DPV). The assays were performed by scanning the potential from –0.5 to 0.5 V at the scan rate of 100 mV.s^{-1} and recorded in an electrolytic solution of 10 mmol.L^{-1} $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ and a KCl solution (0.1 M) as mediator. Electrochemical impedance spectroscopy was realized in Open Circuit Potential (OCP) with 64 different potential frequencies from 50,000 to 1 Hz with an amplitude of 0.01 V applied. The immunosensors' reproducibility, repeatability, calibration curve, selectivity, and specificity were evaluated. The calibration curve of the immunosensor was performed by incubating with CA 125 isolated molecules in different concentrations (from 8.5 to 70 U/mL) for 10 min at room temperature, followed by washing with PBS buffer. Selectivity and specificity were performed by incubating the CA 125 immunosensor with Albumin or

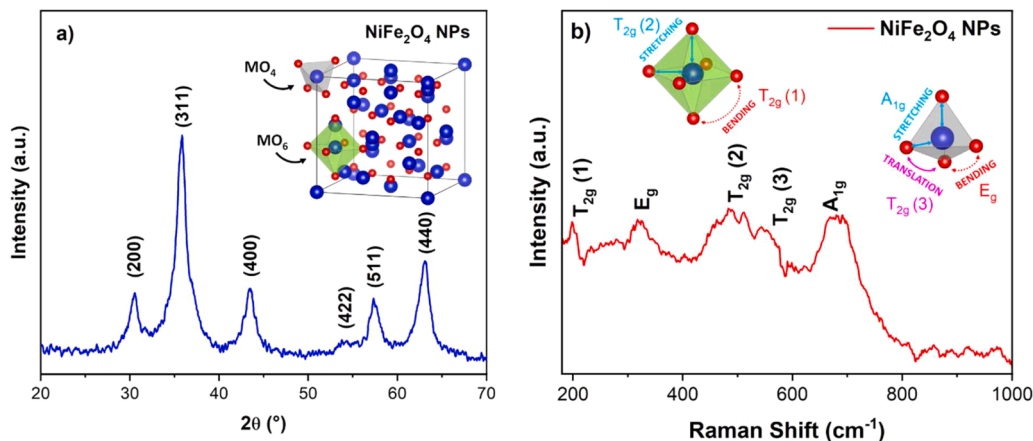


Fig. 2. XRD pattern (a) and Raman spectrum (b) of the NiFe_2O_4 nanoparticles.

VEGF-A. The concentrations of albumin and VEGF-A were 3.5 g/dL and 0.1 ng.mL⁻¹, respectively.

3. Results and discussion

3.1. Characterization of the NiFe_2O_4 nanoparticles

Fig. 2a depicts the XRD pattern of the NiFe_2O_4 nanoparticles synthesized via the conventional hydrothermal method. All diffraction peaks were indexed according to the JCPDS 89-4927 form [25], and no peaks associated with secondary phases were found, confirming the formation of a pure NiFe_2O_4 phase. The main diffraction peaks are located at $\sim 36^\circ$ (311), $\sim 63^\circ$ (440), and $\sim 30^\circ$ (220), corresponding to the cubic spinel structure. It is worth noting that the XRD pattern shows a higher crystallinity of the NiFe_2O_4 nanoparticles compared to other results in the literature, which also frequently exhibit secondary phases [26–31]. The absence of secondary phases can be attributed to the high energy supplied to the system in the conventional hydrothermal reactor, causing the atoms to move with greater intensity, making it difficult to form a secondary phase and thus favoring the formation of the desired phase [24]. According to Vinaykumar et al. [32] and Kombaiah et al. [25], a better crystallization for NiFe_2O_4 can be achieved via calcination, though temperatures as high as 1000 °C are required.

Scherrer's equation was used to calculate the average crystallite size (D) of the NiFe_2O_4 nanoparticles, as shown in Eq. (1):

$$D = K\lambda / \beta \cos\theta \quad (1)$$

where K is the Scherrer constant (0.94), λ is the wavelength (1.5406 Å), β

is the full-width at half maximum (FWHM) for (311), and θ is the Bragg angle for (311). The estimated crystallite size from Eq. (1) was 6.2 nm, indicating the sample is nanometric. Also, it is possible to calculate the interplanar spacing for the (311) ($d_{(311)}$) peak using Bragg's equation (Eq. (2)):

$$d_{(311)} = \lambda / 2\sin\theta_{(311)} \quad (2)$$

The interplanar spacing estimated from Eq. (2) for the (311) reflection was 2.511 Å, which agrees with the reported values in the literature [33,34].

Raman spectroscopy was carried out to investigate the phase and short-range structural disorder of the crystals (Fig. 2b). NiFe_2O_4 has an Fd3m space group in which half of the Fe^{3+} cations are located on tetrahedral A-sites. In contrast, the rest of Fe^{3+} and Ni^{2+} cations are distributed over the octahedral B-sites [35]. Among the possible vibrational groups for NiFe_2O_4 , five of them are Raman active ($A_{1g} + E_g + 3 T_{2g}$). Octahedral and tetrahedral modes can be distinguished in the Raman spectrum by their Raman shift: vibrational modes below 600 cm^{-1} generally correspond to the movement of oxygen atoms in MO_6 octahedra, while those above it correspond to the movement of oxygen atoms in MO_4 tetrahedra. The Raman modes at about 200, 330, 500, 580, and 700 cm^{-1} were assigned to the $T_{2g}(1)$, E_g , $T_{2g}(2)$, $T_{2g}(3)$, and A_{1g} modes, which are in agreement with the literature [36–38], confirming the formation of the spinel structure of NiFe_2O_4 without secondary phases. The inversion level of the structure and the laser wavelength are known to cause minor shifts in the position of these modes [37,39]. Also, the broader nature of the modes indicates some level of disorder in the short range. The A_{1g} mode is assigned to the

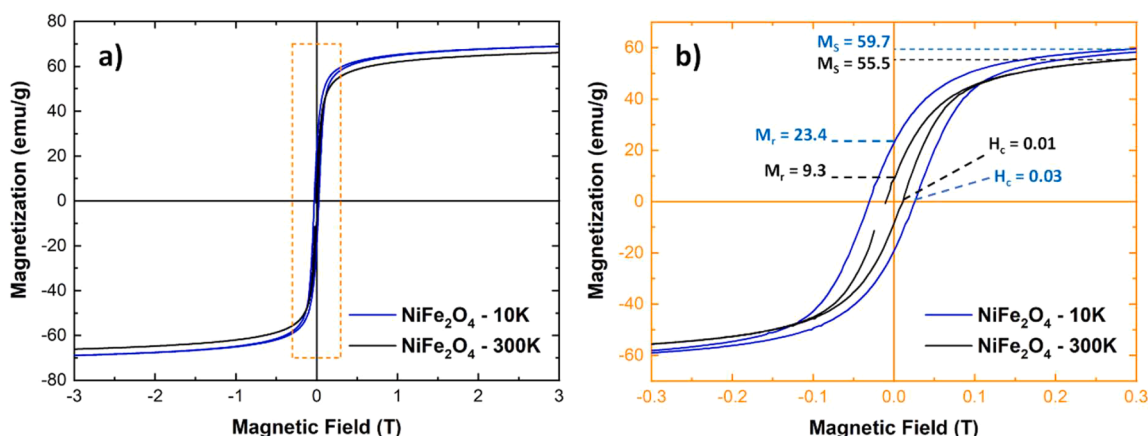


Fig. 3. Magnetic hysteresis curves of the NiFe_2O_4 nanoparticles at 0 K and 300 K (a) and zoomed section containing the magnetic properties.

Table 1Magnetic properties of the NiFe_2O_4 nanoparticles at 10 K and 300 K.

Temperature	M_r (emu/g)	M_s (emu/g)	H_c (T)
10 K	23.4	59.7	0.03
300 K	9.3	55.5	0.01

symmetric stretching of O in MO_4 and E_g to the bending of O concerning the metal in MO_4 [35]. The three T_{2g} modes were attributed to the asymmetric bending of oxygen (T_{2g} (1)), asymmetric stretching of Ni-O or Fe-O (T_{2g} (2)), and translational movement of tetrahedra (T_{2g} (3)) [36].

Fig. 3 shows the B vs. M curves measured at 10 and 300 K in the $-3 \text{ T} \leq B \leq 3 \text{ T}$ magnetic field range. The saturation magnetization (M_s), remaining magnetization (M_r), and coercive field (H_c) were obtained from the hysteresis loops and are shown in Table 1. The zoomed section in Fig. 3b highlights the central region of the hysteresis loop ($-0.3 \text{ T} \leq B \leq 0.3 \text{ T}$). The M_s did not change significantly with temperature, but the M_r and H_c decreased about 60 % upon increasing the temperature from 10 K to 300 K. Such decrease is expected and well reported in the literature, ascribed to the dominance of thermal activation energies over exchange interactions [40]. At 0 K, the shape of the hysteresis loop indicates that the NiFe_2O_4 NPs are ferromagnetic. At 300 K, the opening of the hysteresis loop reduced, yet it did not completely disappear; therefore, it should not be indicative of superparamagnetic behavior [41]. Moreover, small variations of the saturation magnetization values as a function of measuring temperature can be explained by the exchange interaction between tetrahedral and octahedral sub-lattices occupied by Ni^{2+} ions on both the A- and B-sites.

The hopping process between iron and nickel ions of variable oxidation states at oxygen sites is desirable in biosensors. In this inverse spinel structure, a magnetic dipole is induced when a magnetic field is applied, and there will be a net alignment of magnetic moments. After removing the external magnetic field, the magnetic nanoparticles

randomly orient and return to their ground state. The random orientation of the spins can cause high analytical signals and sensitivity in biosensors by enhancing mass transport, leading to more electroactive interaction sites.

The morphology of the NiFe_2O_4 powder was investigated through SEM. Fig. 4 (a–c) indicates that the particles have an irregular shape with variable sizes. We can see small nanoparticles, with sizes around 10–20 nm, randomly distributed on the surface of the particles with sizes around 2 μm . The growth of the NiFe_2O_4 in the conventional hydrothermal synthesis suggests that the concentration of OH^- in the reaction medium leads to an increase in the effective contact between nuclei, reducing the total energy of the system and suppressing the crystal growth for oriented crystallization. Unlike most wet synthesis techniques, which usually use conductive heating, conventional hydrothermal synthesis is a self-purification process during which the growth of crystals/crystallites tends to reject the impurities in the growth environment. That results in multiple nucleating sites, which avoids crystal overgrowth and tends to form fine nanoparticles. The NiFe_2O_4 NPs were deposited onto the WE using cystamine and glutaraldehyde as linkers to evaluate their performance towards immobilizing the anti-CA 125 antibody. Fig. 4d and 4e show the micrographs of the WE, revealing the formation of a uniform and homogeneous NiFe_2O_4 film. The spherical-shaped particles in Fig. 4d are attributed to the PVDF added during the powder deposition onto the WE to ensure the film's stability. The images show that modifying the electrode surface with cystamine, NiFe_2O_4 , glutaraldehyde, and PVDF resulted in a stable film, providing an excellent surface to immobilize the anti-CA125 antibody.

The NiFe_2O_4 powder was characterized by energy dispersive spectroscopy (EDS) analysis to map the presence of nickel, iron, and oxygen in the sample (Table S1 and Fig. S1). From Table S1, the NiFe_2O_4 material presented 48 wt% iron, 28 wt% nickel, and 23 wt% oxygen, proving the formation of the NiFe_2O_4 . Elemental mapping also confirms that the sample contains iron (purple), oxygen (green), and nickel (red). These results corroborate with the XRD and Raman results regarding the

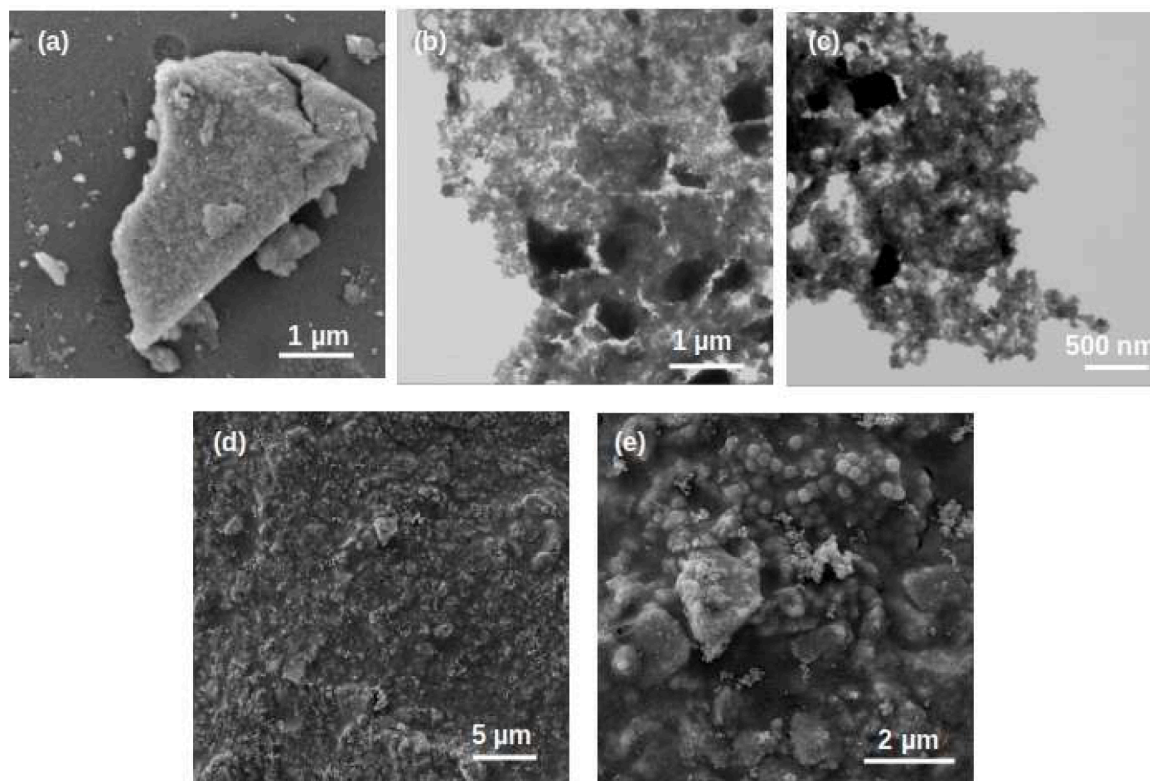


Fig. 4. SEM and STEM images of the (a, b, c) NiFe_2O_4 nanoparticles and the (c, d) NiFe_2O_4 film on the working electrode.

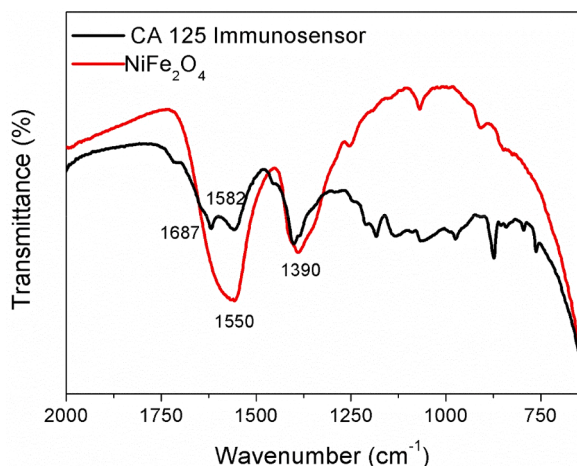


Fig. 5. ATR-FTIR spectra of NiFe_2O_4 and CA 125 immunosensor.

preparation of nanoparticles with the composition NiFe_2O_4 .

Fourier-transform infrared spectroscopy (FTIR) was performed to assess the immobilization of the anti-CA 125 antibody on the surface of the NiFe_2O_4 immunosensor (Fig. 5). The absorption spectrum in the

infrared region of the NiFe_2O_4 nanoparticles shows bands at 1550 cm^{-1} , attributed to H—O—H bending vibration modes due to adsorbed H_2O on the surface of the nanoparticles [42]. The absorption band at 1390 cm^{-1} is attributed to the stretching vibration of N—O in nitrate ions, indicating the presence of nitrates in the sample [43]. Due to our FTIR instrument's limitation, the spectrum region below 650 cm^{-1} (generally assigned to the stretching vibration of metal-oxide bonds (Fe—O, Ni—O)) was not investigated. Regarding the immunosensor spectra, two absorption bands at 1656 and 1582 cm^{-1} are observed, which can be attributed to the amide groups I and II of the antibody protein [44]. Thus, this confirms the immobilization of the anti-CA 125 antibody on the surface of the NiFe_2O_4 immunosensor. The other bands in the spectrum are related to the chemical interaction of the antibody with Cys, Glut, and PVDF, which are used in the immobilization process of the antibody on the surface of the electrode [45–46].

3.2. CA 125 immunosensor characterization

The anti-CA 125 antibody was immobilized on the NiFe_2O_4 surface by adding 20 mM Cys and 2.5 % Glut. This step used Cys as a cross-linking agent between the carbonyl (C=O) and hydroxyl (O—H) groups from the prepared Glut-modified NiFe_2O_4 WE with the aldehydic groups. The aldehydic groups from Glut are highly reactive and can form covalent bonds with the amine group from the anti-CA 125 antibody.

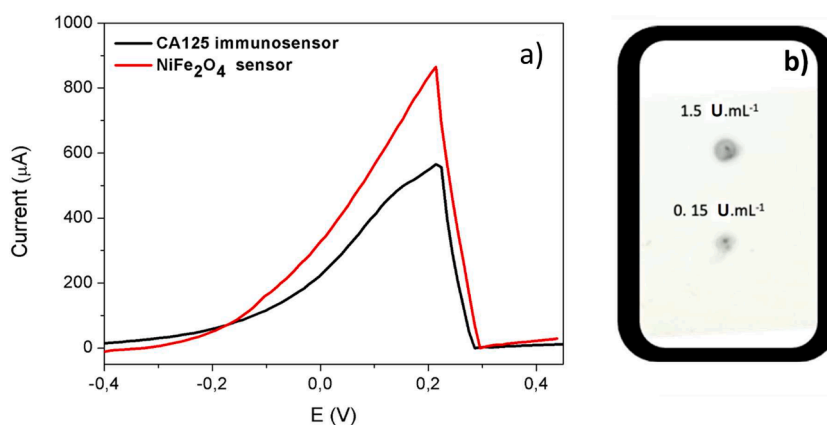


Fig. 6. (a) Differential Pulse voltammograms (DPVs) were obtained for different bare-sensor boards. In black, DPV represents the bare board sensor and red immunosensor. All CVs were performed in the presence of $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ (10 mM) in KCl (0.1 mol.L^{-1}), with a scan rate of 100 mV.s^{-1} . (b) Dot blot analysis.

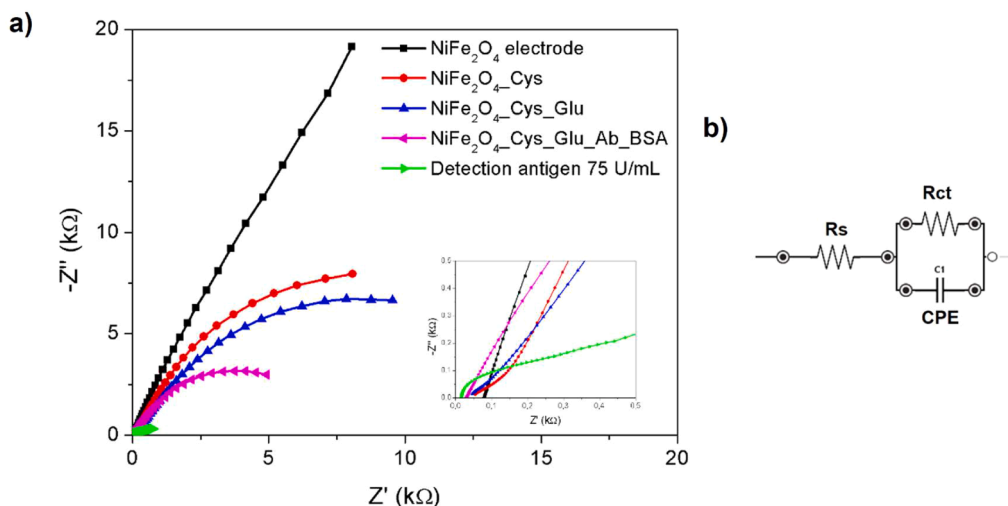


Fig. 7. The Nyquist plot (a) and the corresponding Randles circuit (b).

Table 2

Values of the equivalent circuit fitting for the experimental data from Fig. 7.

Samples	Rs (Ω)	Cdl (F)	Rct (k Ω)
NiFe ₂ O ₄ _Cys	81.27	9.900E-6	15.9
NiFe ₂ O ₄ _Cys_Glu	70.26	7.900E-6	14.1
NiFe ₂ O ₄ _Cys_Glu_AB_BSA	50.00	1.450E-5	0.665
Detection antigen 75 U/mL	15.80	5.000E-6	0.200

To prove the immobilization of the anti-CA 125 antibody on the NiFe₂O₄ WE through Cys and Glut, differential pulse voltammogram (DPV) analyses (Fig. 6a) were performed for the NiFe₂O₄ sensor and NiFe₂O₄ sensor containing the anti-CA 125 antibody (named CA 125 immunosensor). A decrease in the anodic peak current (I_{pa}) of around 30 % occurs with the addition of the anti-CA 125 antibody. The smallest I_{pa} value for the CA 125 immunosensor can be attributed to the insulating characteristics of the antibody. These results prove that the anti-CA 125 antibody was efficiently immobilized onto the NiFe₂O₄ surface. Another fundamental issue in obtaining electrochemical immunosensors is ensuring the capacity of the specific anti-CA125 antibody to link specifically with the CA 125 isolated molecules. In this work, the ability of the anti-CA 125 antibodies to bond specifically with the CA 125 molecule was first proved via a dot blot assay performed for two different concentrations of antigen (1.5 U/mL and 0.15 U/mL). The dot blot results show the presence of dots for both concentrations tested, indicating that the anti-CA 125 antibody can effectively detect the CA

125 isolated molecule (Fig. 6b).

All impedance measurements were carried out at open circuit potential (OCP); under these conditions, the resistance to electron transfer decreased after each surface modification step (Fig 7). The NiFe₂O₄ electrode shows capacitive behavior without resistance to charge transfer [47]. The random orientation of the spins can enhance mass transport, leading to more electroactive interaction sites. The decrease of the resistance in the presence of the CA125 antibody can be explained by the positively charged amino groups in this protein. The redox probe ([Fe(CN)₆]⁴⁻/[Fe(CN)₆]³⁻) with a negative charge is attracted by the positive charge of the CA 125 antibody, increasing its concentration close to the electrode surface (double layer electrical). The conductivity at the electrode surface increases (resistance decreases) regardless of the additional antibody mass on the electrode. The electrochemical experimental data were fitted (Table 2) to a modified Randles equivalent circuit consisting of the solution resistance (Rs), the charge transfer resistance (Rct), and Cdl is a constant-phase element (CPE) describing the double layer capacitance.

The stability of the CA 125 immunosensor was tested by performing five consecutive voltammograms (Fig. 8a) by CV. The immunosensor showed excellent stability with a variation coefficient of 4.42 %. The first scan was not considered for the variation coefficient calculation because the system is entering equilibrium. The CA 125 immunosensor reproducibility was also analyzed by CV in triplicate, obtaining a variation coefficient of 9.79 % (Fig. 8b), indicating the excellent

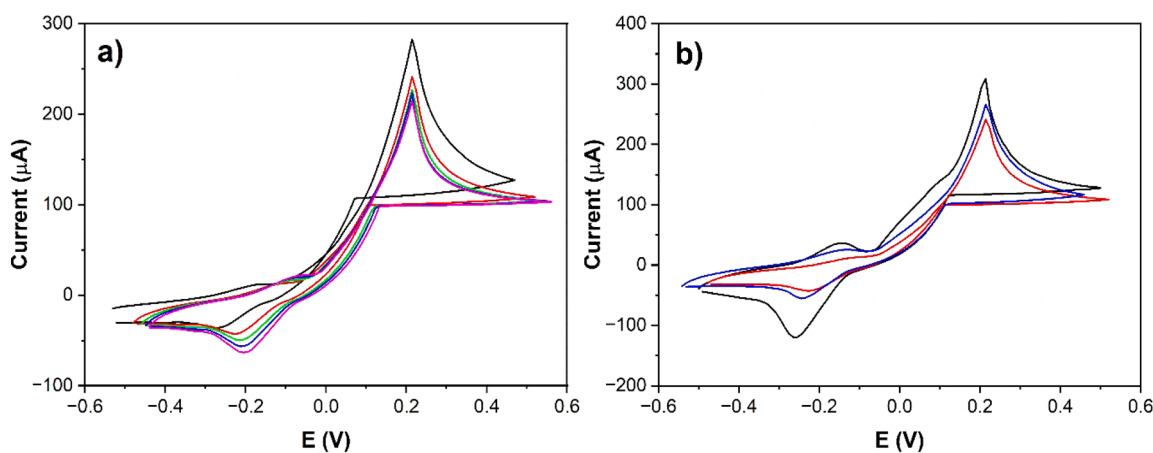


Fig. 8. (a) Five consecutive CVs of CA 125 immunosensor demonstrating immunosensor stability. (b) VC representing three different CA 125 immunosensors showing reproducibility of immunosensors. Electrochemical measurements were performed in the presence of K₃[Fe(CN)₆]/K₄[Fe(CN)₆] (10 mM) in KCl (0.1 mol.L⁻¹), with a scan rate of 100 mV.s⁻¹.

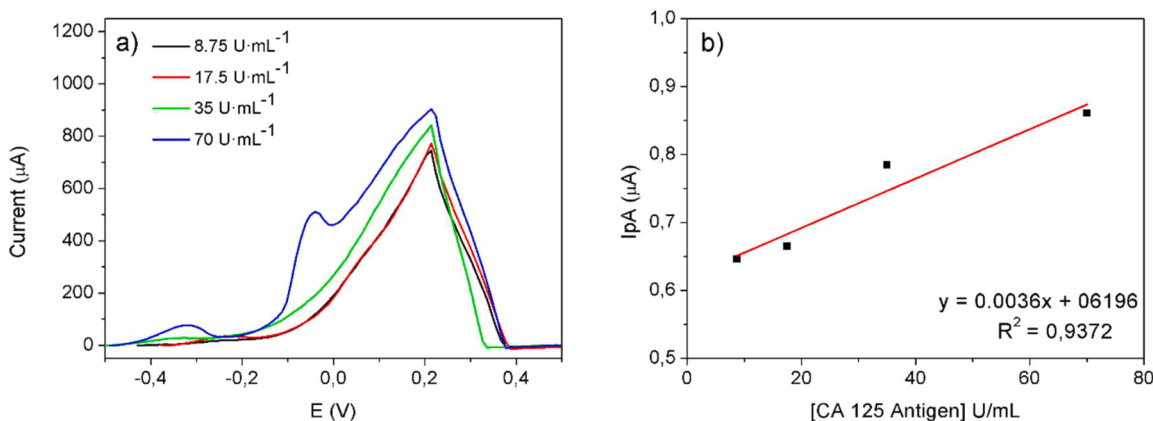


Fig. 9. (a) DPVs for immunosensors with different CA 125 antigen concentrations. (b) Linearity of calibration curve. R² = 0.94; n = 4. All DPVs were performed in the presence of K₃[Fe(CN)₆]/K₄[Fe(CN)₆] (10 mM) in KCl (0.1 mol.L⁻¹), with a scan rate of 100 mV.s⁻¹.

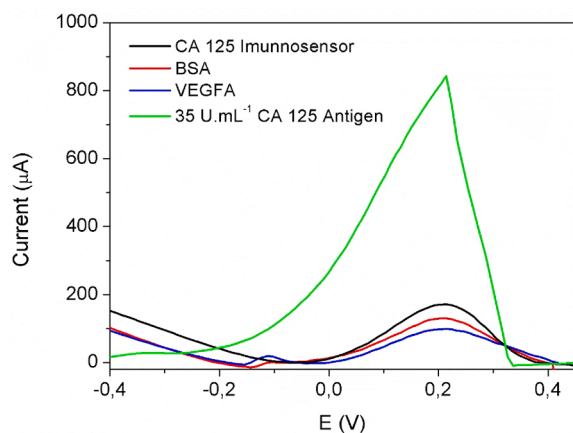


Fig. 10. CA 125 immunosensor incubated with PBS containing BSA (red line) and VEGF-A (blue line). All DPVs were performed in the presence of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (10 mM) in KCl (0.1 mol.L^{-1}), with a scan rate of 100 mV.s^{-1} .

reproducibility of our immunosensor.

3.3. Immunosensor response to CA 125

The WE was incubated at room temperature for 60 min with CA 125 isolated molecules in different concentrations (8.5 to 70 U/mL). This concentration range was chosen considering the reference value of CA 125 in serum, which is 35 U/mL. The calibration curve obtained by DPV of the CA 125 immunosensor is shown in Fig. 9. The peak current value rises as the CA 125 concentration increases, indicating an increase in the density of negative charge in the immunosensor due to the CA 125 isolated molecules, showing a negative charge density. The correlation coefficient (R^2) obtained from the calibration curve was ~ 0.94 , and the limit of detection (LOD) was less than 8.5 U/mL. These values indicate that $NiFe_2O_4$ is an excellent new material for detecting CA 125 antigen in the human body.

3.4. Specificity and selectivity of the immunosensor

The selectivity and specificity of the immunosensor were tested against Albumin (BSA) and VEGF-A, respectively (Fig. 10). Albumin is the main protein present in human serum, and it could be an interferent component during the immunosensor measurements. VEGF-A is a protein found in the human body and its concentration increases during inflammatory processes and cancer. So, the cross-reaction of the CA 125 immunosensor with these two components present in human serum should be avoided to ensure the selectivity and specificity of the test. Fig. 10 shows no increase in I_{pa} in the presence of 3.5 g.dL^{-1} Albumin (the optimum concentration of Albumin present in human serum) or 0.1 ng.mL^{-1} VEGF-A (the reference value of VEGF-A in the human serum). An increase of the I_{pa} is observed just in the presence of the CA 125 antigen, evidencing the excellent selectivity and specificity of the

immunosensor.

The results obtained were compared with other studies of different biosensors for the CA 125 detection, as shown in Table 3. The advantage of the biosensor developed in this work is that it is the only one made on a printed circuit board, a cheaper technology than the ones used in other sensor bases. Furthermore, it presented a LOD within the levels for detection of deregulated CA 125, and the correlation coefficient obtained through the calibration curve was 0.94, being close to other values reported in the literature. Therefore, $NiFe_2O_4$ -based immunosensor is a promising device for detecting CA 125.

4. Conclusions

$NiFe_2O_4$ magnetic nanoparticles were synthesized using the conventional hydrothermal synthesis method and applied as a working electrode in electrochemistry sensing platforms to detect CA 125, a biomarker of OC. The as-synthesized $NiFe_2O_4$ nanoparticles crystallized in the spinel-type structure without secondary phases. Although the $NiFe_2O_4$ nanoparticles are agglomerated, the deposition in the WE using cystamine and glutaraldehyde as linkers ensures the formation of a uniform, homogeneous, and stable $NiFe_2O_4$ film. The immobilization of the anti-CA 125 antibody on the $NiFe_2O_4$ sensor board was proved by FTIR and CV results. The variation coefficient of the reproducibility of the immunosensor was 9.79 %. The immunosensor showed a low limit of detection (LOD) (less than 8.5 U/mL) with a correlation coefficient (R^2) around 0.94. The immunosensor showed excellent selectivity in the presence of Albumin (the main protein of the serum) and specificity against VEGF-A (another cancer biomarker). The immunosensor developed in this work was made on a printed circuit board, allowing it to be used as a point-of-care device. The low LOD, excellent selectivity, and specificity indicate that $NiFe_2O_4$ nanoparticles are an excellent new material for detecting CA 125 antigen in the human body.

CRedit authorship contribution statement

I. Maghiani: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **L.V. Souza:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **L. Bach-Toledo:** Formal analysis, Writing – review & editing. **A.M. Faria:** Formal analysis, Writing – review & editing. **P.P. Ortega:** Formal analysis, Writing – review & editing. **R.A. C. Amoresi:** Formal analysis, Writing – review & editing. **A.Z. Simões:** Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing. **T. Mazon:** Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Talita Mazon reports equipment, drugs, or supplies was provided by State of Sao Paulo Research Foundation. Alexandre Zirpoli Simoes reports equipment, drugs, or supplies was provided by State of Sao Paulo

Table 3

Biosensors used for the diagnosis of ovarian cancer.

Transduction	Material	Obtaining method	LOD	R^2	Reference
Optical	Thin film of Ni-Pc doped on PS matrix	Emulsion polymerization without emulsifier with slight modification	$1 \times 10^{-4} \text{ U/mL}$	0.99	[48]
Electrochemical	Mesoporous carbon doped with nitrogen	Versatile nanoemulsion assembly	0.027 U/mL	0.998	[49]
Optical	Au nanoparticles coated with Schiff-based ligands	Sol-gel	1.45 U/mL	0.999	[50]
Electrochemical	ZnO (NRs)-Au (NPs) nanohybrids	Pechini method followed by microwave-assisted hydrothermal synthesis	2.5 ng/ μL		[13]
Electrochemical	CuBTC@MoS ₂ nanocomposites and gold nanoparticles (AuNPs)	Hydrothermal synthesis	2.5 ng/ μL	0.998	[51]
Electrochemical	$NiFe_2O_4$	Conventional hydrothermal synthesis	8.5 U/mL	0.94	This work

Research Foundation. Luana V Souza reports financial support was provided by National Council for Scientific and Technological Development. Aline Macedo Faria reports financial support was provided by National Council for Scientific and Technological Development. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

Acknowledgements

This work was financially supported by CNPq (444303/2018-9) and FAPESP (CEPID - CDMF 2013/07296-2). Research supported by CTI's Open Labs - Multiple Users and Shared Facilities, CTI Renato Archer, research institution from MCTI.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.materresbull.2024.112835](https://doi.org/10.1016/j.materresbull.2024.112835).

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