



Lignin-AuNp modified carbon paste electrodes—Preparation, characterization, and applications

Rafael M. Buoro^{a,1,2}, Raphael P. Bacil^{a,1,2}, Robson P. da Silva^{a,1,2}, Luis C.C. da Silva^{a,1,2}, Antonio W.O. Lima^{a,1,2}, Ivana C. Cosentino^b, Silvia H.P. Serrano^{a,*,1,2}

^a Institute of Chemistry of University of São Paulo, Av. Prof. Lineu Prestes, 748, Cidade Universitária, 05508-000 São Paulo, SP, Brazil

^b Instituto de Pesquisas Energéticas e Nucleares-IPEN, Av. Prof. Lineu Prestes, 2242, 05508-000 São Paulo, SP, Brazil

ARTICLE INFO

Article history:

Received 30 November 2012

Received in revised form 14 February 2013

Accepted 15 February 2013

Available online 26 February 2013

Keywords:

Lignin

Gold nanoparticles

Carbon paste modified electrodes

Biological molecules

Voltammetry

ABSTRACT

Carbon paste modified electrodes (CPME) were prepared from composites containing lignin or oxidized lignin adsorbed onto graphite, but no voltammetric signal was observed in $K_4[Fe(CN)_6]$ solutions, probably due to the low conductivity of these composites. Gold nanoparticles were produced on both composites using the reducing properties of the lignin, which was able to reduce Au^{3+} , from $HAuCl_4$ at pH 4.7, although only Au/graphite/lignin_(oxi) composite has shown better characteristics to the electrochemical purposes. There were differences in FTIR spectra between lignin and lignin_(oxi) and TEM images were used to explain the results. The better composite to prepare CPME was that containing 0.2% of gold and 2.5% of oxidized lignin, with the percentages expressed in relation to the graphite mass, and admitting that the Au^{3+} reduction, from the $HAuCl_4$ solution, was quantitative. The proportion of 2:1 (w/w) of Au/lignin_(oxi)/graphite composite to the mineral oil (nujol) was used to prepare the CPME with catalytic properties toward the oxidation of dopamine and ascorbic acid and reduction of nitrite and iodate in the positive potential range.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Lignin is a phenolic macromolecule and the second major component in woods being responsible by their mechanical resistance, attributed to the linkage with carbohydrates, mainly cellulose [1,2]. A highly viscous liquid, called black liquor as well as the main disposal of the cellulose industries, is produced in tons every year and is rich in lignin and lignin derivatives being burned in thermal generators to generate a fraction of the energy consumed in industry. Methods of extraction [2] and applications of this material as an adsorbent of heavy metals in effluents [3,4], carbon source in the synthesis of activated charcoal [2–5] or as a wastewater metal remover in the development of biodegradable resins [6,7] were described. Its chemical properties have been determined by a wide spectrum of analytical techniques which evaluated its thermal [8–13], spectroscopic [8,11–13], and electrochemical characteristics. It was demonstrated that the lignin chemical properties are dependent on the pulp process.

The lignin electrochemical oxidation mechanism is similar to that observed in phenolic models [14,15], as benzoquinone and involves in aqueous medium, an electrochemical–electrochemical (ECE) pathway.

Lignin molecules contain quinone/hydroquinone (Q/HQ) groups and therefore, have antioxidant characteristics, beyond catalytic properties, always desirable for the development of chemically modified electrodes [16–21]. Its application as a reducing agent in materials synthesis has been increasing throughout the years, especially in the field of the nanomaterials synthesis. The application of phenolic compounds in gold and silver nanoparticles synthesis was reported in the literature and involves very quick and easy steps: the heating of a mixture containing humic acid, rich in phenolic groups, and a metallic precursor [22] or, a local pH variation promoted by the neutralization of an acidic mixture of phenol derivatives containing $HAuCl_4$ [22–24]. The electrochemical sensors based on materials containing metal nanoparticles have been widely reported [25–35] and great analytical potential can be exploited [22]. Electrodeposition [22,25,29,30,32] is the most applied methodology when it is desired to create nanostructures at the surface of a solid electrode, such as glassy carbon (GC) or gold (Au) electrodes. The electrode surface is usually pre-modified with a relatively long chain carbon aliphatic molecule containing NH_2 or SH functional groups, surfactants and dithiols, which could establish covalent bonds to the electrode's surface allowing to control the nucleation or the amount of the nanoparticles immobilized

* Corresponding author. Tel.: +55 11 3091 2687/1473.

E-mail addresses: rafbuoro@iq.usp.br (R.M. Buoro), shps@iq.usp.br, silvia.iqusp@gmail.com (S.H.P. Serrano).

¹ Tel.: +55 11 3091 2687/1473.

² ISE member.

onto GC or Au self-assembled monolayers, respectively [26–28]. The use of composite materials is a relevant and versatile alternative synthesis route to the usual methods using citrate reduction or polyalcohol method [22], in which the subsequent immobilization of the nanoparticles formed onto the graphite powder is necessary. Composites formed by nanoparticles (Np) and graphite can be applied in the development of biosensors through the immobilization of redox enzymes [33–35] or can be used as a modified surface electrode in electrochemical sensing of molecules.

This paper demonstrates that lignin, a cheap and abundant reducing agent, can be used for the synthesis of gold nanoparticles directly onto graphite's surface. The method involved the previous oxidation of lignin with hydrogen peroxide in alkaline medium with further impregnation of the oxidized lignin, $\text{lig}_{(\text{oxi})}$, onto graphite by rotoevaporation. The $\text{lig}_{(\text{oxi})}$ /graphite composite was suspended in acetate buffer solution, pH 4.7 and the metallic precursor, HAuCl_4 solution, was added to the mixture. In the last step Au^{3+} was reduced by the $\text{lig}_{(\text{oxi})}$, producing gold nanoparticles with 100 nm of diameter, which were identified by TEM images. FTIR spectrum of $\text{lignin}_{(\text{oxi})}$ showed that thiol groups, which can promote better interaction with gold, were formed and that the conjugation degree in the carbon increased, due to the aligning of the sp^2 orbital hybridized carbons. Additionally, phenol groups were still present after the oxidation process and the reducing properties of the original lignin were maintained, justifying the formation of gold nanoparticles. Carbon paste modified electrodes (CPME) prepared using these composites showed catalytic properties toward the oxidation of ascorbic acid (AA) and dopamine (DA), beyond the reduction of nitrite (NO_2^-) and iodate (IO_3^-) in the positive potential range.

2. Materials and methods

2.1. Reagents and solutions

Graphite (code number 1.04206), hydrochloric acid (100317), hydrogen peroxide (107209), potassium ferrocyanide (1.04984.0500), disodium hydrogen phosphate (1.06586) and sodium nitrite (106544) were acquired from Merck; dimethylformamide (15,481-4) and lignin (370959-100G) from Aldrich; HAuCl_4 (50778-1G) and potassium di-hydrogen phosphate (P0662) from Sigma-Aldrich; mineral oil-nujol (M5904) from Sigma; ascorbic acid (1852) from Mallinckrodt; dopamine hydrochloride (11136) from Avocado and potassium iodate (472565) from Carlo Erba.

All reagents were used as received and the solutions were prepared using deionized water from a reverse osmosis device (Gehara Co., model OS10LX ultra-pure system, water resistivity $\geq 18 \text{ M}\Omega \text{ cm}$).

2.2. Lignin oxidation [36–39]

Adequate quantity of lignin (500 mg) was solved into 50.0 mL of 0.1 mol L^{-1} NaOH solution containing 3% (v/v) of hydrogen peroxide. The mixture was kept under vigorous stirring and heating at 60°C for 2 h. After cooling to room temperature, 0.1 mol L^{-1} HCl was added until the lignin quantitative precipitation. The solid oxidized lignin, $\text{lig}_{(\text{oxi})}$, was isolated by centrifugation process, re-suspended in deionized water and centrifuged again to remove any excess of HCl and/or H_2O_2 . The last step was repeated 2 times and the resultant solid was dried in oven at 60°C for 12 h.

2.3. Preparation and optimization of the composites

Preparation of the composites containing lignin and graphite; $\text{lignin}_{(\text{oxi})}$ and graphite and; gold nanoparticles (AuNps), $\text{lignin}_{(\text{oxi})}$

and graphite and optimization of the composites containing gold nanoparticles (AuNps), $\text{lignin}_{(\text{oxi})}$ and graphite are as follows: To obtain a graphite free of superficial functional groups it was pre-treated with $\text{HCl } 0.1 \text{ mol L}^{-1}$ solution under vigorous stirring for 12 h followed by a vacuum filtration. After this procedure, the graphite was submitted to a thermal treatment under 400°C for 2 h, to remove any volatile component adsorbed superficially, and was kept in oven at 200°C for 24 h. Enough amount of solid lig or $\text{lig}_{(\text{oxi})}$ was weighed and added to the graphite in a variable (w/w) proportions and the mixture was suspended in 10.0 mL of DMF. After adequate homogenization, the solvent was eliminated by rotoevaporation under reduced pressure, and the lignin or $\text{lignin}_{(\text{oxi})}$, soluble in DMF, was re-precipitated onto the graphite.

The solid material was re-suspended in acetate buffer 0.1 mol L^{-1} , pH 4.7, and adequate volume of a 3.4 mmol L^{-1} HAuCl_4 in $\text{HCl } 0.1 \text{ mol L}^{-1}$ solution was quickly added to the mixture, which was kept under vigorous stirring for 15 min. No pH variation was observed after the addition of the HAuCl_4 solution and AuNps were formed under action of the lignin previously incorporated onto the graphite. The solid material was filtrated, washed with deionized water and dried in oven for 80°C . Table 1 presents the composition of the composites obtained and the respective carbon paste modified electrodes (CPME), which were prepared using these materials.

The composition of CPME containing lignin or oxidized lignin adsorbed onto graphite was not optimized since no or bad response was observed in $\text{K}_4[\text{Fe}(\text{CN})_6]$ solutions, probably due to the low conductivity of these materials.

To optimize the gold content in the composite 2 the following gold percentages were used: 0.1% (composite 2a; CPME 2a), 0.2% (composite 2b; CPME-2b); 0.4% (composite 2c; CPME-2c) and 0.8% (composite 2d; CPME-2d), with the percentages also being expressed as function of the graphite mass.

In order to evaluate, separately, the lignin, $\text{lignin}_{(\text{oxi})}$, and gold nanoparticles' effects on the voltammetric profile of the CPME, composites containing only gold nanoparticles on graphite were also prepared.

Gold nanoparticles were prepared according to the Turkevich method [40]. Briefly: 1.0 mL of 0.1 mol L^{-1} HCl containing 3.4 mmol L^{-1} HAuCl_4 , was mixed with 2 mL of 0.1% (w/w) citrate solution. The pH of the mixture was adjusted to 6.0, using 0.1 mol L^{-1} sodium hydroxide solution, and the volume was completed to a 20.0 mL with deionized water. The final solution was heated and maintained at the boiling point for 15 min, and after removal of the heating, it was stirred for another 15 min. After that, 500 mg of graphite, pre-treated as described before, was subsequently added to the mixture, which was stirred for 12 h. After centrifugation, the material was washed twice with deionized water and subsequently dried at room temperature. The proportion gold/graphite was the same used at composite 2b. The AuNp carbon paste electrode (AuNpCPE) was prepared as described below.

2.4. Preparation of carbon paste modified electrodes (CPME)

The CPME were prepared by mixing the desired composite and mineral oil in a proportion of 2:1 (w/w). The body of the electrode consists of a Teflon tube, 7 cm in length, drilled from side to side by a brass stem to make the electrical contact. One of the extremities of the brass stem was lowered toward the extremity of the Teflon tube in order to produce a cavity with a diameter of 3 mm where the composite was added while the other extremity was used for the connection to the potentiostat.

Table 1

Mass, percentage of the composite components, expressed as function of the graphite mass, and denomination of the correspondent carbon paste modified electrode (CPME) – variation of the lig and lig_(oxi) mass on the composite compositions.

Composite	CPME	Graphite (mg)	Lig _(oxi) (mg)	HAuCl ₄ (mg)	Mineral oil (mg)
1	1	500	5.0 (1.0%)	0.5 (0.1%)	250
2 ^a	2 ^a	500	12.5 (2.5%)	0.5 (0.1%)	250
3	3	500	25.0 (5.0%)	0.5 (0.1%)	250
Composite	CPME	Graphite (mg)	Lig (mg)	HAuCl ₄ (mg)	Mineral oil (mg)
4	4	500	5.0 (1.0%)	0.5 (0.1%)	250
5	5	500	12.5 (2.5%)	0.5 (0.1%)	250

^a Optimized condition.

The percentages were expressed as function of the graphite mass, and the Au content was calculated admitting quantitative reduction of the metal added.

2.5. Apparatus

A JEM-2100 microscope (Cs 1.4 mm, resolution 0.25 nm) at 200 kV was used to obtain the transmission electron microscopy (TEM) images, which were recorded with a slow scan CCD camera (Gatan SC1000 ORIUS). The samples were dispersed in isopropyl alcohol and introduced onto a holey carbon film on a 400 mesh Cu grid.

2.5.1. FTIR – Fourier transform infrared spectroscopy

The FTIR spectra were registered using Bomem MB100 equipment, briefly: it used 1 mg of sample to 100 mg of dried KBr; the mixture was macerated in a mortar of agate and pressed into 13 mm diameter pastillator at a pressure of 7 metric tons. In the spectral range of 4000–400 cm⁻¹, the resolution was 4 cm⁻¹ in 64 scans.

2.5.2. Voltammetric measurements

Voltammetric measurements were carried out with a potentiostat/galvanostat PGstat 20 Autolab (Ecochemie) coupled to the stirrer device IME663 interface. Data treatment was done using the software NOVA version 1.6. A conventional three-electrode system was adopted. The working electrode was the above described CPMEs while the auxiliary and reference electrodes were platinum wire and Ag/AgCl, KCl_(sat), respectively. All electrochemical measurements were performed in a 20 cm³ electrochemical cell at room temperature. All pH measurements, when necessary, were done using a pH-meter model 654 and a combined glass electrode, model 6.0203.100 (OE), both from Metrohm.

3. Results and discussions

3.1. Electrochemical characterization of the modified electrodes

As stated earlier, CPME containing lignin or oxidized lignin adsorbed onto graphite showed no response in K₄[Fe(CN)₆] solutions, probably due to the low conductivity of the composites. To increase this conductivity, preliminary studies were carried out, but in no optimized conditions, to evaluate the possibility of using the reducing properties of lignin, previously adsorbed onto graphite, to produce gold nanoparticles.

The electrochemical behavior of carbon paste electrode (CPE), Lig-CPE³ and CPME prepared from the composite containing Au, lignin and graphite (Au-Lig-CPE)⁴ was evaluated using a 5.0 mmol L⁻¹ [Fe(CN)₆]⁴⁻ in 0.1 mol L⁻¹ phosphate buffer solution (PBS), pH 6.0, as an electrochemical probe.

Fig. 1 shows the cyclic voltammograms, recorded in 0.1 mol L⁻¹ KCl pH 6.0, at a carbon paste electrode (CPE); and at carbon

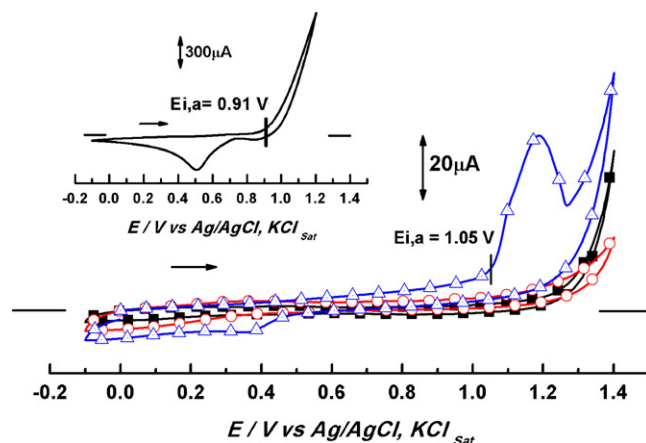


Fig. 1. 1st cyclic voltammograms obtained in 0.1 mol L⁻¹ KCl, pH 6.0, at CPE (■); lig-CPE (○) and Au-lig-CPE (Δ). Inset: Cyclic voltammogram recorded at AuE in 0.1 mol L⁻¹ KCl, pH 6.0. Experimental conditions: E_i = -0.1 V; E_λ = +1.2 V (for AuE) and +1.4 V (for lig-CPE and Au-lig-CPE); E_f = -0.1 V and ν = 100 mV s⁻¹.

paste modified electrodes using the composites containing lignin adsorbed onto graphite (lig-CPE) and lignin adsorbed onto graphite, containing gold, Au-lig-CPE.

As can be seen from Fig. 1, the gold oxidation peak was observed only at Au-lig-CPE and disappeared when the supporting electrolyte, KCl, was substituted PBS, which does not act as complexing agent of Au³⁺ (Fig. 2). The working potential range of the Au-lig-CPE

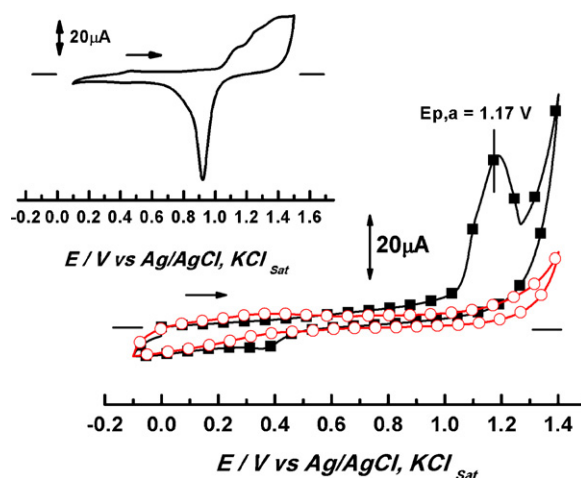


Fig. 2. 1st cyclic voltammograms obtained at Au-lig-CPE in 0.1 mol L⁻¹ KCl solution, pH 6.0 (■) and in 0.1 mol L⁻¹ PBS, pH 6.0 (○). Inset: cyclic voltammogram obtained at AuE in 0.1 mol L⁻¹ PBS pH 6.0. Experimental conditions: E_i = -0.1 V, E_λ = +1.4 V, E_f = -0.1 V and ν = 100 mV s⁻¹.

³ Lig-CPE: lignin: 12.5 mg of lig; 500 mg of graphite and 250 mg of mineral oil.

⁴ Au-Lig-CPE: 12.5 mg of lig; 500 mg of graphite; 4.0 mg of H₂AuCl₄ and 250 mg of mineral oil.

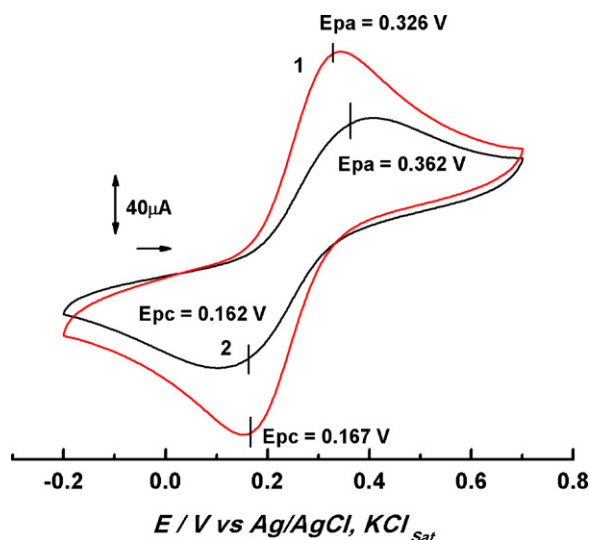


Fig. 3. Cyclic voltammograms obtained in 0.1 mol L⁻¹ PBS, pH 6.0 containing 5.0 mmol L⁻¹ [Fe(CN)₆]⁴⁻ at CPME-2 (1) and CPME-5 (2). Experimental conditions: $E_i = -0.2$ V, $E_x = +0.7$ V, $E_f = -0.2$ V and $\nu = 100$ mV s⁻¹.

in PBS was expanded to +1.4 V, while this potential was limited to about 1.0 V at a solid gold electrode, AuE (Fig. 2, inset).

After the experimental evidence that Au³⁺ had been reduced by lignin adsorbed onto the graphite, CPME were prepared using the composites described on Table 1. Initially, the contents of gold, graphite and mineral oil were fixed while the lignin or lignin_(oxi) was varied. CPME-2, containing lignin_(oxi) presented better response in a 0.1 mol L⁻¹ phosphate buffer solution (PBS), pH 6.0, containing 5.0 mmol L⁻¹ [Fe(CN)₆]⁴⁻, used as an electrochemical probe, than CPME-5, containing no oxidized lignin (Fig. 3).

It is possible to conclude from data presented in Fig. 3 that the lignin oxidation state is an important experimental parameter to be considered when this material is used for electroanalytical applications. In fact, the experimental conditions previously used in the literature, in which potentials of about +0.8 V [19] and +1.0 V [17] were reached during the electrochemical preparation of the lignin modified electrodes, already indicated this possibility.

CPME-1, CPME-3, CPME-4 and CPME-5 showed higher ΔE_p ($E_{pa} - E_{pc}$) and lower current levels than CPME-2 in a 0.1 mol L⁻¹ PBS, pH 6.0, containing 5.0 mmol L⁻¹ [Fe(CN)₆]⁴⁻, and were discarded.

To understand the role of the lignin_(oxi) in the electrochemical behavior of the CPME, a FTIR spectra of lignin and lignin_(oxi) were obtained and compared (Fig. 4).

As can be seen from Fig. 4, the vibrational spectrum of the lignin_(oxi) showed important structural differences in relation to the vibrational spectrum of the lignin. In the lignin_(oxi) a large band at 2530 cm⁻¹, attributed to S–H stretches, appeared simultaneously to the increasing of the band at 600 cm⁻¹, which corresponds to the C–S stretches and therefore, an indicative of thiol groups formation exists after the oxidation process which could, following Pearson's principle, promote better interaction with gold.

Furthermore there was a significant increase in the band corresponding to conjugated carbonyl groups at 1685 cm⁻¹ and a decrease in the OH stretching at 3400 cm⁻¹, which increases the degree of conjugation of the molecule by aligning of the orbitals from sp² hybridized carbons, besides maintenance of the phenol groups present in the oxidized lignin, detected by the C–O phenol stretching at 1220 cm⁻¹.

The oxidation reaction decreased the aromaticity of lignin, evidenced by decrease in the intensity of bands corresponding to C=C aromatic stretching at 1511 and 1470 cm⁻¹, without a

significant change in the intensity of the band corresponding to C=C at 1631 cm⁻¹, which can be associated with both, asymmetric stretch of double aromatic bonds or the asymmetric stretch of the conjugated double bonds. Hydrolysis of the aliphatic ethers occurred, as can be seen by the decrease of the intensity of absorption at 1270 cm⁻¹, but there was a formation of alkyl aryl ethers, detected by the appearance of the band at 1140 cm⁻¹.

All the three facts, the appearance of the C–S stretches, indicative of thiol groups formation, which can promote better interaction and stabilization of the AuNp formed; the increase in the conjugation degree of the molecules, providing higher conductivity at the material; the maintenance of the phenolic groups in the lig_(oxi), which can act as reducing groups, explain the better response of the CPME-2 (Table 1 and Fig. 3), and justify the maintenance of the lignin reducing properties, even after a previous oxidation step with hydrogen peroxide in alkaline medium.

After optimization of the amount of lignin_(oxi) to be incorporated into the composite, the content of graphite, lignin_(oxi) and mineral oil, present in CPME-2, was kept constant and the gold content was varied as described in the experimental section and the better composite to prepare the CPME was the composite 2b (data not shown).

Considering that lignin_(oxi) maintained its reducing properties, TEM images were used to verify if the gold reduction occurred with the formation of nanoparticles and in fact, gold nanoparticles were formed and their morphology was dependent of the gold content in the composite (Fig. 5).

An irregular morphology was observed for the gold nanoparticles present in the composite 2b (0.2% in gold) (Fig. 5A and C), as compared with the morphology of the nanoparticles present in the composite 2c (0.4% in gold), Fig. 5B and D which presented a more spherical shape.

In the composite 2b, the AuNps formed do not have very definite morphology and the different energies, existent on the irregular AuNps surface, can improve the catalytic and conductive behavior of the CPME-2b [41,42]. On the other side, the AuNps formed on the composite 2c, have a more spherical shape with a more homogeneous superficial energy distribution, and therefore the improvement of the catalytic and conductive behavior can be limited.

To elucidate whether the voltammetric performance of the CPME-2b was related to the presence of lignin_(oxi), gold nanoparticles or the synergistic action of both, the voltammetric profile of a carbon paste modified electrode containing only gold nanoparticles adsorbed on graphite, (AuNp-CPE), was also evaluated using [Fe(CN)₆]⁴⁻ as an electrochemical probe, and the results compared with those obtained at lig_(oxi)-CPE* and CPME-2b (Fig. 6).

As can be seen from Fig. 6, the electrochemical behavior of the CPME-2b can be attributed to a synergistic action of lignin_(oxi) and gold nanoparticles and the effect was similar to that observed at a gold nanocluster/overoxidized-polypyrrole composite modified glassy carbon electrode [29].

3.2. Applications

Taking into account the data and discussions made earlier, the CPME-2b was chosen to evaluate the oxidation of reducing agents, dopamine (DA) and ascorbic acid (AA) and oxidizing agents (NO₂⁻ and IO₃⁻).

Fig. 7A shows the cyclic voltammograms obtained at CPE and CPME-2b in 0.1 mol L⁻¹ PBS (pH 6.0) containing 5.0 mmol L⁻¹ DA. The oxidation peak potential shifted about 87 mV to less positive values. This behavior can be attributed to the DA catalytic oxidation by the phenol/quinone groups from lignin_(oxi) immobilized at CPME-2b surface or to gold nanoparticle, which can act as an electron promoter [29]. DA oxidation peak potential at

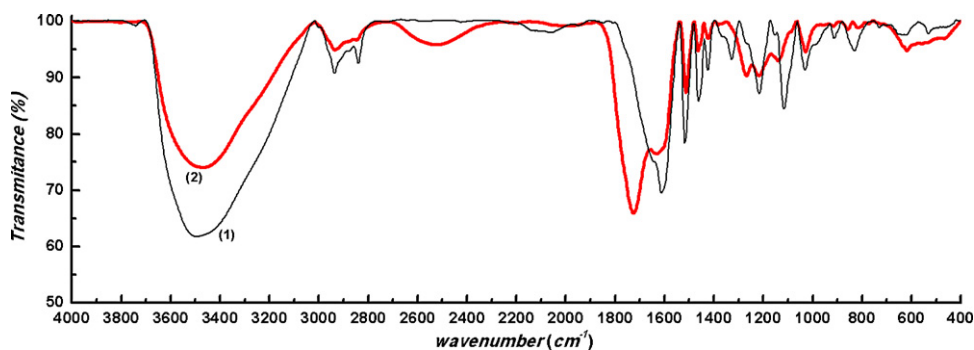


Fig. 4. FTIR spectra: lignin (1) and lignin_(oxi) (2).

CPME-2b was observed at 0.302 V, value which is about 79 and 51 mV more positive than those observed at amine-terminated monolayer of cystamine containing gold nanoparticles [27] and gold nanocluster/overoxidized-polypyrrole composite modified glassy carbon electrode [29], respectively, and coincident with the value found at carbon paste gold nanoparticle sensor [30].

Fig. 7A also shows that at CPME-2b, ΔE_p ($E_{pa} - E_{pc}$) decreased from 292 to 125 mV in comparison to CPE. This behavior is common at a gold nanostructured material and generally is related to the increase of the electron transfer kinetics [30,41] producing, simultaneously, an increasing current.

However, it may be considered that DA molecule could be anchored on the gold nanoparticles surface by the amino group,

which directs the molecule to the electrode surface and in this case, a pre-concentration effect and an increase of the electron transfer kinetics could be also observed. This effect was not expected for the ascorbic acid and in fact, the AA oxidation peak potential was +0.302 V at CPME-2b as compared to +0.389 V observed at CPE, although the current levels were comparable on both electrodes (Fig. 7B).

Even considering that CPME-2b has a diameter which is two times bigger than that for the gold electrode used in the literature, the oxidation current levels obtained in 1.0 mmol L⁻¹ AA, pH 6.0, at CPME-2b were much higher than those obtained at pH 7.4 [17], and in this case the gold nanoparticles can make the difference. On the other side, the selective determination of DA in the presence

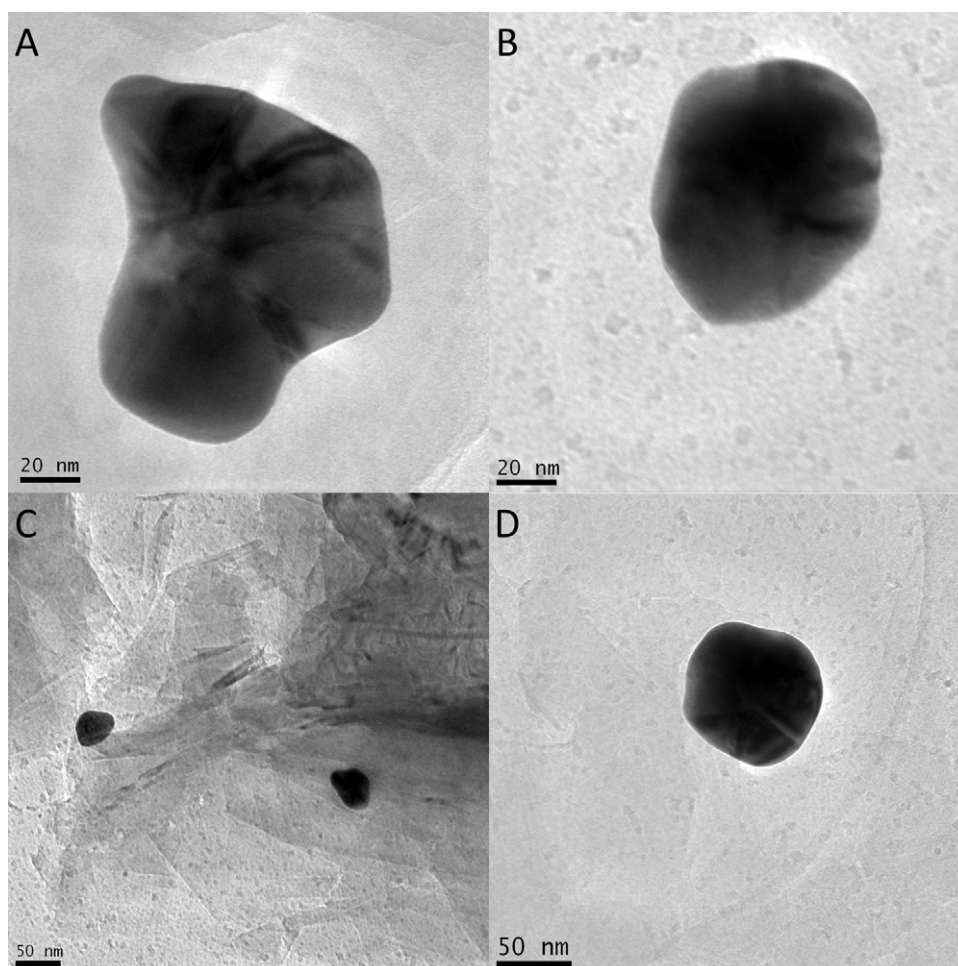


Fig. 5. TEM images of: the composite 2b (A and C) and the composite 2c (B and D) at decreasing order of magnification. The particle diameter dimension is about 100 nm.

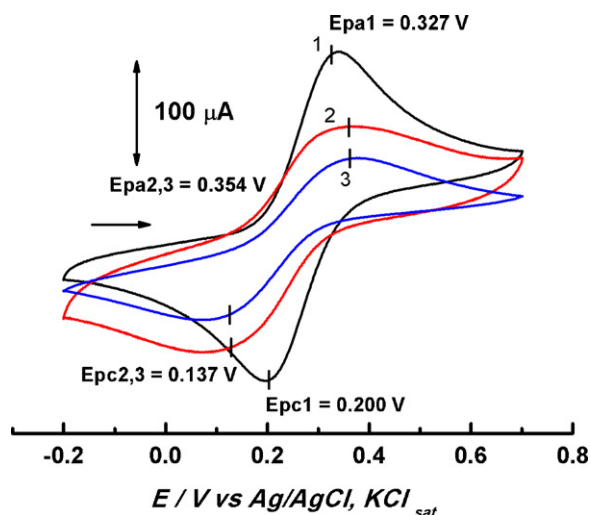


Fig. 6. 3rd cyclic voltammograms obtained at CPME-2b (1), $\text{lig}_{(\text{oxi})}\text{-CPE}^*$ (2) and AuNp-CPE (3) in 0.1 mol L^{-1} PBS, pH 6.0 containing 5.0 mmol L^{-1} $[\text{Fe}(\text{CN})_6]^{4-}$. Experimental conditions: $E_i = -0.2$, $E_\lambda = +0.7$, $E_f = -0.2$ V and $\nu = 100 \text{ mV s}^{-1}$. * $\text{Lig}_{(\text{oxi})}\text{-CPE}$: 12.5 mg of oxidized lignin; 500 mg of graphite and 250 mg of mineral oil.

of AA, as obtained at amine-terminated monolayer of cystamine containing gold nanoparticles [27], is not possible at CPME-2b.

The catalytic activity for the reduction of NO_2^- and IO_3^- [32,33] on nanostructured surfaces is well reported in literature and the

composite prepared showed catalytic activity in acidic medium (H_2SO_4) among both species (Fig. 7C and D), indicating a good applicability of the prepared material in the region of positive potential.

It is also notable that the synergism among gold nanoparticles and lignin produces a catalytic effect toward the reduction of NO_2^- when compared to the gold nanoparticle/poly(3-methylthiophene) modified glassy carbon electrode, which presented electrocatalytic activity toward the oxidation of nitrite to nitrate in PBS, pH 4.0 [32]. Although Au nanoparticles are stabilized by thiol groups in both modified electrodes, the quinone/hydroquinone groups could act as an electron donor toward the reduction of nitrite explaining the different behavior of the gold surfaces. Because of this behavior, usually copper surfaces or copper nanostructures are used in the development of electrochemical sensors for the reduction of NO_2^- [43,44] as long as copper acts as an electron donor. In the CPME-2b, it was observed a current discharge toward nitrite reduction starting at positive potential range, in comparison to -0.33 V observed at CuNp/Thiol/Au modified electrode [43] and -0.05 V at Cu_{nano}/CNTs-Nf/GCE [44]. In both cases, the nitrite reduction process showed a peak shape instead a current discharge. The literature also presents that IO_3^- is frequently determined in WO_3 modified electrodes at low concentration levels [45,46] and therefore CPME-2b is an alternative to those surfaces. The discharge current for IO_3^- at CPME-2b starts at $+0.3$ V, value which is comparable to $+0.2$ V obtained at WO_3/Pt modified electrode microarrays [46], but it was an improvement toward the value of 0.0 V, obtained at WO_3/GCE electrode or -0.2 V, obtained at a AuNp/poly(3-methylthiophene) [33,45], respectively. The presence of the quinone/hydroquinone,

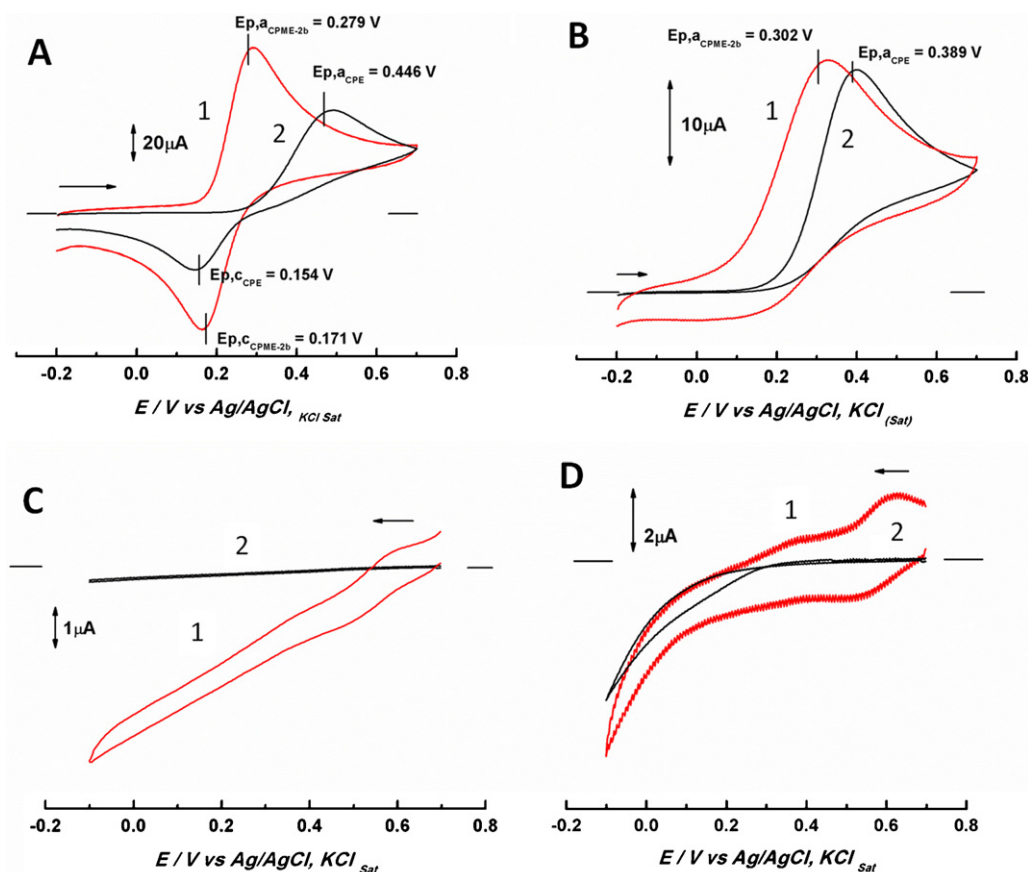


Fig. 7. (A) Cyclic voltammograms obtained in 0.1 mol L^{-1} PBS, pH 6.0, containing 1.0 mmol L^{-1} DA at CPME-2b (1) and CPE (2). Experimental conditions: $E_i = -0.2$ V, $E_\lambda = +0.7$ V, $E_f = -0.2$ V and $\nu = 100 \text{ mV s}^{-1}$. (B) Cyclic voltammograms obtained in 0.1 mol L^{-1} PBS, pH 6.0, containing 1.0 mmol L^{-1} AA at CPME-2b (1) and at CPE (2). Experimental conditions: $E_i = -0.2$ V, $E_\lambda = +0.7$ V, $E_f = -0.2$ V and $\nu = 20 \text{ mV s}^{-1}$. (C) Cyclic voltammograms obtained in 0.5 mol L^{-1} H_2SO_4 solution containing 1.0 mmol L^{-1} NO_2^- at CPME-2b (1) and CPE (2). (D) Cyclic voltammograms obtained in 0.5 mol L^{-1} H_2SO_4 containing 0.1 mmol L^{-1} IO_3^- at CPME-2b (1) and CPE (2). Experimental conditions: $E_i = +0.7$ V, $E_\lambda = -0.1$ V, $E_f = +0.7$ V and $\nu = 20 \text{ mV s}^{-1}$.

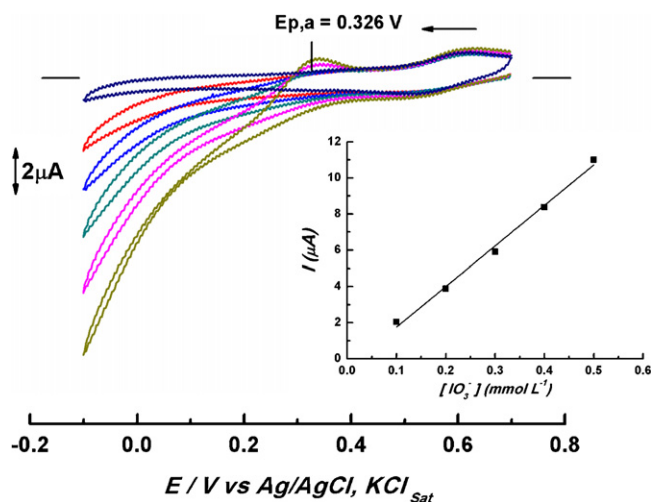


Fig. 8. Cyclic voltammograms obtained at CPME-2b in 0.5 mol L⁻¹ H₂SO₄ containing 0.1, 0.2, 0.3, 0.4 and 0.5 mmol L⁻¹ IO₃⁻. Experimental conditions: E_i = +0.7 V, E_λ = -0.1 V, E_f = +0.7 V and ν = 20 mV s⁻¹. Inset: iodate analytical curve: I (μA) = -0.485 μA + 22.4 [IO₃⁻] (mmol L⁻¹); R = 0.992.

acting as an electron donor group, allied to the AuNp interaction with iodine atom in IO₃⁻, can be a possible explanation to the catalysis observed at CPME-2b.

For higher concentrations of IO₃⁻ (>0.4 mmol L⁻¹) an oxidation peak in the positive potential was observed after iodate reduction (Fig. 8). The initial proposition is the electrochemical oxidation of the iodate reduction product to I₂, which suffers strong adsorption on gold surfaces [19] and therefore can be oxidized to hypoiodite, IO⁻. It must be emphasized that it is not possible to determine IO₃⁻ with CPE due to lack of stability of baseline through potential sweeps, including line intersection of these various points in the potential sweep.

4. Conclusions

CPME, prepared from composites containing lignin or oxidized lignin adsorbed onto graphite, show no or bad voltammetric signal in K₄[Fe(CN)₆] solutions, probably due to the low conductivity of these composites. Nevertheless, the reducing properties of the lignin were used to incorporate gold nanoparticles in the lignin or lignin_(oxi)/graphite composites to increase the conductivity of these materials.

Both composites, lignin/graphite and lignin_(oxi)/graphite adsorbed onto graphite were able to reduce Au³⁺, from HAuCl₄ at pH 4.7, but the conductivity of the composite containing Au/graphite/lignin_(oxi) showed the better characteristics to the electrochemical purposes. FTIR spectra from lignin and lignin oxidized showed that, in the last case, the spectral changes were compatible with the formation of thiol groups, which could promote better interaction with gold and an increase in the conductivity of the composite and maintenance of the phenol groups, which could be able to reduce Au³⁺ to Au with formation of nanoparticles, identified by TEM images.

The better composite to prepare CPME was that containing 0.2% of gold and 2.5% of oxidized lignin, with the percentages expressed in relation to the graphite mass, and admitting that the Au³⁺ reduction, from the HAuCl₄ solution, was quantitative. The proportion of 2:1 (w/w) of Au/lignin_(oxi)/graphite composite to the mineral oil (nujol) was used to prepare the CPME with catalytic properties toward the oxidation of dopamine and ascorbic acid and reduction of nitrite and iodate in the positive potential range.

Acknowledgments

The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2009/18223-0), Conselho Nacional de Pesquisa (CNPq) and Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support. They would like to thank Prof. Dr. Pedro Henrique Camargo Cury from Chemistry Institute of the University of São Paulo for the valuable discussions and suggestions. Also they would like to thank the Referee of the ECA for his questioning, which was fundamental at improving of this manuscript.

References

- [1] M.P. Pandey, C.S. Kim, Lignin depolymerization and conversion: a review of thermochemical methods, *Chemical Engineering and Technology* 34 (2011) 29.
- [2] Suhas, P.J.M. Carrott, M.M.L. Ribeiro Carrott, Lignin – from natural adsorbent to activated carbon: a review, *Bioresource Technology* 98 (2007) 2301.
- [3] X. Guo, S. Zhang, X. Shan, Adsorption of metal ions on lignin, *Journal of Hazardous Materials* 151 (2008) 134.
- [4] A. Demirbas, Heavy metal adsorption onto agro-based waste materials: a review, *Journal of Hazardous Materials* 157 (2008) 220.
- [5] E. Gonzalez-Serrano, T. Cordero, J. Rodríguez-Mirasol, J.J. Rodríguez, Development of porosity upon chemical activation of Kraft lignin with ZnCl₂, *Industrial and Engineering Chemistry Research* 36 (1997) 4832.
- [6] G. Sun, H. Sun, Y. Li, B. Zhao, N. Zhu, K. Hu, Comparative study on the curing kinetics and mechanism of a lignin-based-epoxy/anhydride resin system, *Polymer* 48 (2007) 330.
- [7] Y. Matsushita, S. Wada, K. Fukushima, S. Yasuda, Surface characteristics of phenol–formaldehyde–lignin resin determined by contact angle measurement and inverse gas chromatography, *Industrial Crops and Products* 23 (2006) 115.
- [8] Q. Liu, S. Wang, Y. Zheng, Z. Luo, K. Cen, Mechanism study of wood lignin pyrolysis by using TG-FTIR analysis, *Journal of Analytical and Applied Pyrolysis* 82 (2008) 170.
- [9] S. Tsujiyama, A. Miyamori, Assignment of DSC thermograms of wood and its components, *Thermochimica Acta* 351 (2000) 177.
- [10] R.C. Korosec, B. Lavric, G. Rep, F. Pohleven, P. Bukovec, Thermogravimetry as a possible tool for determining modification degree of thermally treated Norway Spruce wood, *Journal of Thermal Analysis and Calorimetry* 98 (2009) 189.
- [11] A. Tejado, C. Pena, J. Labidi, J.M. Echeverria, I. Mondragon, Physico-chemical characterization of lignins from different sources for use in phenol–formaldehyde resin synthesis, *Bioresource Technology* 98 (2007) 1655.
- [12] M. Bartkowiak, R. Zakrzewski, Thermal degradation of lignins isolated from wood, *Journal of Thermal Analysis and Calorimetry* 77 (2004) 295.
- [13] S. Kuboab, J.F. Kadlaa, Thermal decomposition study of isolated lignin using temperature modulated TGA, *Journal of Wood Chemistry and Technology* 28 (2008) 106.
- [14] T.A. Enache, A.M. Oliveira-Brett, Phenol and para-substituted phenols electrochemical oxidation pathways, *Journal of Electroanalytical Chemistry* 655 (2011) 9.
- [15] P. Janeiro, I. Novak, M. Seruga, A.M. Oliveira-Brett, Electroanalytical oxidation of p-coumaric acid, *Analytical Letters* 40 (2007) 3309.
- [16] R.M. Buoro, R.P. Silva, R.P. Bacil, A.W.O. Lima, T. Rabóczkay, L.C. Cides da Silva, S.H.P. Serrano, Electrochemical characterization and evaluation of the analytical potentialities of glassy carbon electrodes modified with extracted lignin from black (Kraft) liquor, *ECS Transactions* 43 (2012) 119.
- [17] G. Milczarek, Preparation and characterization of a lignin modified electrode, *Electroanalysis* 19 (2007) 1411.
- [18] G. Milczarek, Lignosulfonate-modified electrode for electrocatalytic reduction of acidic nitrite, *Electroanalysis* 20 (2008) 211.
- [19] G. Milczarek, Preparation, characterization and electrocatalytic properties of an iodine/lignin-modified gold electrode, *Electrochimica Acta* 54 (2009) 3199.
- [20] G. Milczarek, Lignosulfonate-modified electrodes: electrochemical properties and electrocatalysis of NADH oxidation, *Langmuir* 25 (2009) 10345.
- [21] A. Chen, E.I. Rogers, R.G. Compton, Abrasive stripping voltammetric studies of lignin and lignin model compounds, *Electroanalysis* 22 (2010) 1037.
- [22] C.M. Welch, R.G. Compton, The use of nanoparticles in electroanalysis: a review, *Analytical and Bioanalytical Chemistry* 384 (2006) 601.
- [23] Sirajuddin, A. Mechler, A.A.J. Torriero, A. Nafady, C.-Y. Lee, A.M. Bond, A.P. O'Mullane, S.K. Bhargava, The formation of gold nanoparticles using hydroquinone as a reducing agent through a localized pH change upon addition of NaOH to a solution of HAuCl₄, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 370 (2010) 35.
- [24] Y. Lee, T.G. Park, Facile fabrication of branched gold nanoparticles by reductive hydroxyphenol derivatives, *Langmuir* 27 (2011) 2965.
- [25] N.F. Atta, A. Galal, S.M. Azab, Determination of morphine at gold nanoparticles/Nafion carbon paste modified sensor electrode, *Analyst* 136 (2011) 4682.
- [26] L. Wang, J. Bai, P. Huang, H. Wang, L. Zhang, Y. Zhao, Self-assembly of gold nanoparticles for the voltammetric sensing of epinephrine, *Electrochemistry Communications* 8 (2006) 1035.

- [27] C. Retna Raj, T. Okajima, T. Ohsaka, Gold nanoparticle arrays for the voltammetric sensing of dopamine, *Journal of Electroanalytical Chemistry* 543 (2003) 127.
- [28] Z. Yang, G. Hu, X. Chen, J. Zhao, G. Zhao, The nano-Au self-assembled glassy carbon electrode for selective determination of epinephrine in the presence of ascorbic acid, *Colloids and Surfaces B: Biointerfaces* 54 (2007) 230.
- [29] J. Li, X. Lin, Simultaneous determination of dopamine and serotonin on gold nanocluster/overoxidized-polypyrrole composite modified glassy carbon electrode, *Sensors and Actuators B* 124 (2007) 486.
- [30] N.F. Atta, A. Galal, F.M. Abu-Attia, S.M. Azab, Carbon paste gold nanoparticles sensor for the selective determination of dopamine in buffered solutions, *Journal of the Electrochemical Society* 157 (2010) F116.
- [31] G. Hu, Y. Guo, S. Shaoa, Simultaneous determination of dopamine and ascorbic acid using the nano-gold self-assembled glassy carbon electrode, *Electroanalysis* 21 (2009) 1200.
- [32] X. Huang, Y. Li, Y. Chen, L. Wang, Electrochemical determination of nitrite and iodate by use of gold nanoparticles/poly(3-methylthiophene) composites coated glassy carbon electrode, *Sensors and Actuators B* 134 (2008) 780.
- [33] I. Willner, B. Willner, R. Tel-Vered, Electroanalytical applications of metallic nanoparticles and supramolecular nanostructures, *Electroanalysis* 23 (2011) 13.
- [34] J.M. Pingarrón, P.Y. Sedeño, A.G. Cortés, Gold nanoparticle-based electrochemical biosensors, *Electrochimica Acta* 53 (2008) 5848.
- [35] L. Aguí, J. Manso, P.Y. Sedeño, J.M. Pingarrón, Amperometric biosensor for hypoxanthine based on immobilized xanthine oxidase on nanocrystal gold-carbon paste electrodes, *Sensors and Actuators B* 113 (2006) 272.
- [36] P. Maziero, M.O. Neto, D. Machado, T. Batista, C.C.S. Cavalheiro, M.G. Neumann, A.F. Craievich, G.J.M. Rocha, I. Polikarpov, A.R. Gonçalves, Structural features of lignin obtained at different alkaline oxidation conditions from sugarcane bagasse, *Industrial Crops and Products* 35 (2012) 61.
- [37] G. Tong, T. Yokoyama, Y. Matsumoto, G. Meshitsuka, Analysis of progress of oxidation reaction during oxygen-alkali treatment of lignin. 1: Method and its application to lignin oxidation, *Journal of Wood Science* 46 (2000) 32.
- [38] A. Mancera, V. Fierro, A. Pizzi, S. Dumarçay, P. Gérardin, J. Velásquez, G. Quintana, A. Celzard, Physicochemical characterization of sugar cane bagasse lignin oxidized by hydrogen peroxide, *Polymer Degradation and Stability* 95 (2010) 470.
- [39] C. Bonini, M. D'Auria, R. Ferri, Singlet oxygen mediated degradation of lignin – isolation of oxidation products from steam-exploded lignin from pine, *Photochemical & Photobiological Sciences* 1 (2002) 570.
- [40] J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot, A. Plech, Turkevich method for gold nanoparticle synthesis revisited, *Journal of Physical Chemistry B* 110 (2006) 15700.
- [41] B.K. Jena, C. Retna Raj, Morphology dependent electrocatalytic activity of Au nanoparticles, *Electrochemistry Communications* 10 (2008) 951.
- [42] S. Liu, J. Yu, H. Ju, Renewable phenol biosensor based on a tyrosinase-colloidal gold modified carbon paste electrode, *Journal of Electroanalytical Chemistry* 540 (2003) 61.
- [43] W.Y. Ko, W.H. Chen, C.Y. Cheng, K.J. Lin, Highly electrocatalytic reduction of nitrite ions on a copper nanoparticles thin film, *Sensors and Actuators B* 137 (2009) 437.
- [44] S. Yang, X. Zeng, X. Liu, W. Wei, S. Luo, Y. Liu, Y. Liu, Electrocatalytic reduction and sensitive determination of nitrite at nano-copper coated multi-walled carbon nanotubes modified glassy carbon electrode, *Journal of Electroanalytical Chemistry* 639 (2010) 181.
- [45] O. Ordeig, C.E. Banks, F.J. Del Campo, F.X. Munõz, R.G. Compton, Electroanalysis of bromate, iodate and chlorate at tungsten oxide modified platinum micro-electrode arrays, *Electroanalysis* 18 (2006) 1672.
- [46] J.R.C. da Rocha, T.L. Ferreira, R.M. Torresi, M. Bertotti, An analytical application of the electrocatalysis of the iodate reduction at tungsten oxide films, *Talanta* 69 (2006) 148.