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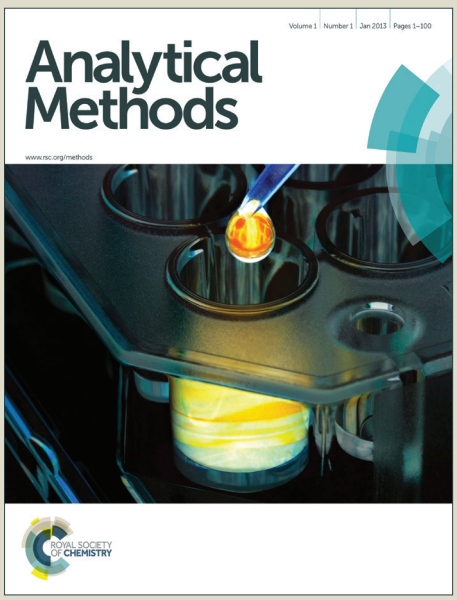

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ARTICLE

Determination of Uronic Acids in Sugarcane Bagasse by Anion-exchange Chromatography using Electrode Modified with Copper Nanoparticles

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Uronic, D-glucuronic and D-galacturonic acids are found in lignocellulosic materials and are known to be used in the food industry and chemical industries. They are present in the fibrous structure of sugarcane bagasse, where they are incapable of being detected owing to the lack of absorption of the chromophore and fluorophore groups in their molecular structure, thus restricting their detection by traditional spectrophotometric methods. The detection is only possible by means of derivatization. In this light, a modified detector with copper nanoparticles by potentiostatic electrodeposition was developed. D-galacturonic and D-glucuronic acids are oxidized irreversibly at potentials of 0.45 and 0.48 V vs Ag/AgCl, respectively in cyclic voltammetry. This modified electrode was used in chromatography with pulsed amperometric detection in wall-jet cell. An anion exchange column, CarboPac PA10, was used for the separation of uronic acids in isocratic conditions, with mobile phase containing 0.1 M NaOH plus 280 mM CH_3COONa. The separation of the acids was found to be complete within 15 minutes. The detection limit was $5.8x10^{-7}$ and $7.3x10^{-7}$ mol^{-1}, the amperometric sensitivity was 3.6 ± $1.8x10^6$ and 1.9 ±1.0 x 10^6 μA L mol^{-1} for D-galacturonic and D-glucuronic acids respectively. The aforementioned method developed was then applied to real samples of hydrolyzate bagasse. The amount of acid found in this sample was 15.8±0.5 g/kg and 12.5±0.5 g/kg for D-galacturonic and D-glucuronic acids respectively. The results demonstrate that the proposed method can be used for the detection of these acids without the need for derivatization given its merits of exerting no interference, having considerable accuracy and relatively shorter run time.

1. Introduction

A biorefinery plant is a facility that integrates biomass conversion processes with the aim of producing fuel, power, and chemical substances from biomass. By producing multiple products, a biorefinery plant can take advantage of the differences in biomass components and maximize the value derived from the biomass feedstock, according to the National Renewable Energy Laboratory (NREL).[1] Biorefinery plants can use various sources of biomass, including lignocellulosic materials, such as wood chips, sawdust, waste containing starch, bagasse, soybean, maize, cassava, among others. In Brazil, the amount of lignocellulosic waste generated annually is approximately 350 million tons[1]. One of the main sources of lignocellulosic material is in sugarcane, which contains cellulose, hemicellulose and lignin amounting to 164.5, 96.25 and 71.05 million tons respectively.[2] Currently, the conversion of lignocellulosic material into fermentable sugars in the sugarcane mill leads to an increase in ethanol production - known as second-generation ethanol, besides other products of interest[2].

The lignocellulosic feedstock biorefinery plant uses biomass for the production of a wide range of products through a combination of technologies. This biorefinery process involves the separation and use of three basic chemical components of lignocellulose, namely: (a) hemicellulose – sugar polymers with five carbon atoms, (b) cellulose – glucose polymers with six carbon atoms, and (c) lignin – phenol polymers[3]. In pectin, which involves the hemicellulose, uronic acids (D-glucuronic acid and D-galacturonic) can be found in percentages ranging from 5.0 to 24.0%, apart from other sugars in lower concentrations. The uronic acids are not metabolized by the Saccharomyces cerevisiae yeast during the production of cellulosic ethanol[5], since this yeast has low affinity for these acids[4]. However, pectins are of great interest to biorefinery because they are a potential source of bioprocesses raw material. The uronic acids that are not metabolized in the production of ethanol can be recovered within the biorefinery process, which may be sold directly for use in the food industry as an acidifying agent while as a surfactant in the chemical industry.

Uronic acids can not be detected owing to the lack of absorption of the chromophore and fluorophore groups in their molecular structure, thus limiting their detection by traditional spectrophotometric methods.[6] Traditional methods for the detection of uronic acids are based on colorimetry using

carbazole-sulfuric acid and meta-hidroxydiphenyl[7,8], derivatizing the uronic acids to be detected. However, derivatization is often affected by the complexity of the matrix, resulting in incomplete derivatization[9]. Several methods have been used for the determination of sugars as well as uronic acids in lignocellulosic waste, including gas chromatography, high-performance liquid chromatography, and capillary electrophoresis.[10,11] However, this method has a long run time, multiple dilutions of samples, use of multiple pumps to run in gradient (and consequently using large amounts of solvents for separation) and requiring the addition of strong hydroxide post-column to minimize baseline drift. Furthermore, for electrochemical detection, the oxidation of these compounds using glassy carbon does not exhibit significant current without modification with copper.[12] Thus, the detection of these uronic acids on modified glassy carbon electrode are of great interest as studies show that it is possible to quantify sugars and their derivatives even at a low concentration. [9,12]

A further advantage of the use of these electrodes lies in the ability of these acids to form complexes with cupric ions[14]. Electrodes modified with copper in strongly alkaline solution have been applied in the oxidation of carbohydrates, sugars and acid derivatives such as alditols, aldonic and uronic. [14,15,16,17] The use of these electrodes for the oxidation of compounds in positive regions (0.5 to 0.6V vs. SCE) have many advantages because it keeps copper in cupric form, reducing the background current and maximizing its use in the oxidation of uronic acids[14]. Use of copper nanoparticles becomes interesting because these structures have four major advantages: better mass transport, catalysis, high effective surface area and improved signal/noise ratio[18]. The use of copper nanoparticles becomes interesting because these structures have four major advantages: better mass transport, catalysis, high effective surface area and improved signal / noise ratio.[18] To date, no work has been reported on the deposition of copper nanoparticles on the surface of glassy carbon electrode (CuNP) for the determination of uronic acids (from sugarcane bagasse) in HPLC. As such, it is factually relevant to detect these acids in sugarcane bagasse, using a method without derivatization, being simple, fast and which does not exert any interference.

The electrodes of noble metals (Pt, Au) are frequently used as electrochemical detectors[19,20] though in amperometric detection they tend to have low activity during anodic detections of organic substances[20]. The pulsed amperometric detection (PAD) serves as a solution to this problem of activity lost in noble metals alternating anodic and cathodic polarizations in the cleaning as well as the reactivation of the electrode surface. [19,20,21] However, a slow dissolution of the metal particles and higher levels of background noise may occur during the application of the pulse[23]. Hence, it is necessary to search for new electrodes that can be used without loss of activity. The studies using working electrodes modified with copper have shown that several electroactive organic molecules can be determined with good sensitivity in order to improve performance and reduce the undesirable amperometric poisoning.[24,25,26] The copper nanoparticles electrode is as such said to be superior in terms of response range, detection limit and especially stability in comparison to Pt, Au, Ni, Ag and Co.[27]

The anion-exchange liquid chromatography coupled with pulsed amperometric detection has been given considerable attention in the detection of sugars and organic acids[11,12,13]. Separation is bound to occur based on the difference between the ion exchange uronic acids and the stationary phase. The electrochemical detection (ED), which is usually operated in amperometric mode is known to be popular when it comes to the detection of sugars, uronic acids and alditols without derivatization. [9, 22] Furthermore, the method has the advantage of being relatively simpler, with high sensitivity and low detection limit.

This work directs its focus on important areas like renewable energy, and alternative analytical nanoparticles method. In this sense, we sought to develop a modified electrode with copper nanoparticles which is to be utilized as an amperometric detector in an ion exchange liquid chromatography for analyzing the uronic acids, namely D-galacturonic and D-glucuronic without derivatization, with quick separation and without interference from other components in the complex matrix of sugarcane bagasse.

2. Experimental

2.1 Reagents

Solutions were prepared from an analytical-reagent (Sigma-Aldrich, St. Loius, MO, USA) using deionized water from Milli-Q system. The solutions of D-glucuronic acid and D-galacturonic were prepared in alkaline solution (0.1 mol L^{-1} NaOH).

2.2 Measurements

The electrochemical measurements were performed using a potentiostat Autolab PGSTAT30 coupled to a microcomputer that records and stores data obtained using the control software GPES 4.9. Conventional electrochemical cell with three electrodes, reference electrode Ag/AgCl (KCl 3.0 mol L^{-1}), platinum wire auxiliary electrode and glassy carbon (diameter 3.0 mm) as a working electrode.

The chromatographic measurements were performed on a chromatograph-850 Professional IC with a loop of 25 µL with pulsed amperometric detector, connected to MagicNet software, version 2.4. The ion exchange chromatographic column utilized was the DIONEX ® CarboPac PA 10 (250X4 mm I.D.) coupled with guard CarboPac PA 10 column (4x50 mm I.D.). The cell is the amperometric type wall-jet and the electrochemical detector is composed of platinum auxiliary electrode (Pt), palladium (Pd) reference electrode and the electrode modified with CuNP as working electrode. The potentials and time periods for the pulsed amperometric detection were: E_1, +0.45 V for 200 ms; E_2, +0.60V for 50 ms; E_3, −0.05 V for 50 ms.

2.3 Electrodeposition of copper nanoparticles on the surface of the GC.

Before each experimental measurement, the GC electrode was polished with 0.3-µm α-alumina powder on a felt. After polishing, the electrode was washed with deionized water in abundance for the removal of adsorbed particles on its surface. For the electrodeposition of the nanoparticles, 50 mL of a solution was prepared containing 2.0 mmol L^{-1} $CuCl_2$ in 0.1 mol L^{-1} KCl. By chronoamperometry, a potential of -0.40 V for 120 s was applied. After that, the CuNP was put in 0.1 mol L^{-1} NaOH solution and scanned repetitively for 20 cycles under potential range of -0.5 to +0.3 V at 100mVs^{-1} for surface passivation, which means nano-copper oxide was bound to GC surface.

2.4 Preparation of the sample for chromatographic separation

Sugarcane bagasse was obtained from a sugar and alcohol plant in the region of Araraquara – SP/ Brazil. The bagasse hydrolysis follows the methodology based on the National Renewable Energy Laboratory –NREL (Refer to LAP-010 for extraction procedures for isolation and characterization of extractives).[27]

This analysis is referred to as NREL LAP procedures and can be applied to various cellulosic biomass such as corn stover, poplar, bagasse, etc. First, the sugarcane bagasse was placed in a Soxhlet apparatus for the removal of extractives. Initially, water was used for removing aqueous extractives (inorganic materials and nitrogenous material, among others. Inorganic materials may come from both the biomass and any soluble material that is associated with the biomass, such as soil or fertilizer) and then ethanol for the removal of ethanolic extracts (including chlorophyll, waxes or other minor components). The residue was dried at 40°C (overnight), obtaining the dry biomass. Samples of 300 mg of this biomass were transferred to a flask of 250 ml and treated with 3 ml of H_2SO_4 72% (v/v), under vigorous stirring, in a thermostatic bath at 45°C for 60 min. The reaction was halted for the addition of 85 mL of distilled water. For the complete hydrolysis of the remaining oligomers, the flask was sealed and autoclaved at 120°C for 60 min at 1.05 bar. After decompression of the autoclave, the flask was then removed and cooled at room temperature. The reaction mixture was filtered and 20 ml of the hydrolyzate was then transferred to an Erlenmeyer of 50 ml which was subsequently neutralized with $CaCO_3$, filtered with filter 0.45 and 0.22 µm of porosity and finally injected directly into the chromatographic column.

3. Results and discussions

3.1 Morphological characterizations

After the electrodeposition of copper nanoparticles on the glassy carbon electrode, the surface morphology was studied by scanning electron microscope (SEM), which is shown in Fig.1. The image shows a uniform electrodeposition of nanoparticles with a homogeneous distribution on the glassy carbon surface. A histogram of the size distribution of the nanoparticles was calculated by counting 200 particles where it was observed that 61% of the copper oxide nanoparticles size was in the range of up to 90 nm (Fig.2). These nanoparticles have a large specific surface area, high surface reaction activity and efficient transmission channel for analyte molecules to reach the active sites[26], while improving the stability and detectability of the electrode to be used in HPLC-PAD.

3.2 Electrochemical behavior of uronic acids

The uronic acids do not exhibit significant current in the unmodified glassy carbon electrode[16]. When the GC electrode is modified with copper nanoparticles, the peak of uronic acid oxidation can be observed. As D-galacturonic and D-glucuronic acids are stereoisomers, they are known to have the same connectivity yet different spatial arrangements. In addition to that, they are found to have similar electrochemical behavior, which can be proven by analyzing the oxidation potential of the electrode using cyclic voltammetry CuNP. The D-galacturonic acid oxidized at the potential of 0.45 V, whereas the D-glucuronic underwent oxidation at the potential of 0.48 V at a scan rate of 50 mVs^{-1} (Fig.3).

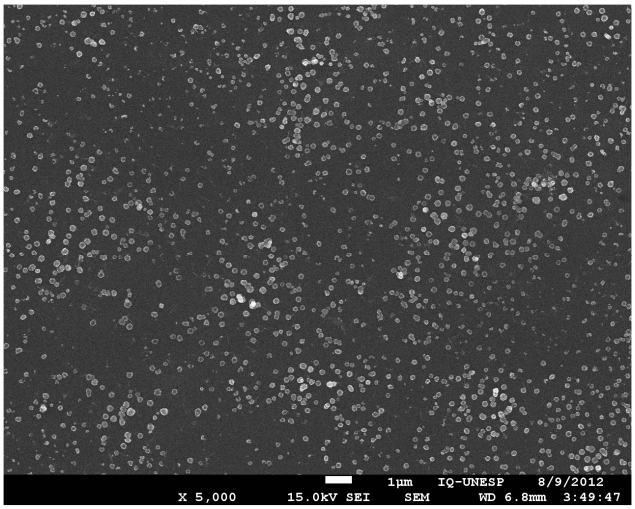

Fig. 1. SEM images of nano-copper oxide electrodeposited on GC surface.

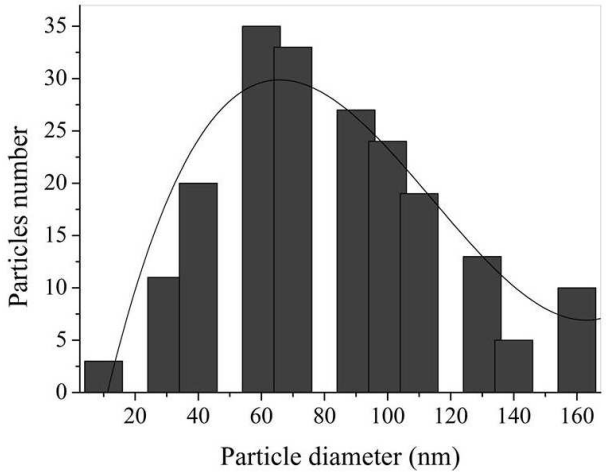

Fig. 2. Particle size histogram of the nano-copper oxide.

The anodic peak current showed linear dependency with the scanning speed in the range of 10 to 100 mVs^{-1} (R= 0.997). This behavior is indicative that the process is controlled by adsorption[32]. The system is considered irreversible, exhibiting only the anodic peak which tends to shift with increasing scanning speed.

The literature reports the formation of formic acid following the oxidation of D-galacturonic acid using vanadium (V).[29] Similar behavior is expected for the electrochemical oxidation of D-galacturonic acid and D-glucuronic acid catalyzed by copper oxide nanoparticles.

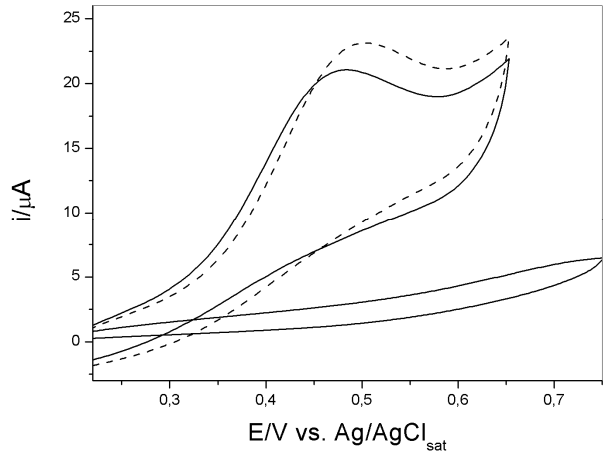

Fig. 3. Cyclic voltammograms of 5.0 mmol L^{-1} D-galacturonic (-) and D-glucuronic (--) acids in the presence of 0.1 mmol L^{-1} NaOH on modified electrode (CuNP), υ = 50 mVs^{-1}.

3.3 Stability of the modified electrode

The study of the stability of the copper nanoparticles modified electrode was carried out by chronoamperometry. Figure 4 shows the response of the CuNP electrode in D-glucuronic acid solution. After a period of 300 s to stabilize the current, $1.0x10^{-3}$ $molL^{-1}$ of D-glucuronic acid was added and supporting electrolyte of 0.1 mol L^{-1} NaOH. The response time between the addition of D-glucuronic acid and response of the electrode was 2.48 s and remained stable during 700 s, indicating the stability of the electrode. The response time for D-galacturonic acid was similar.

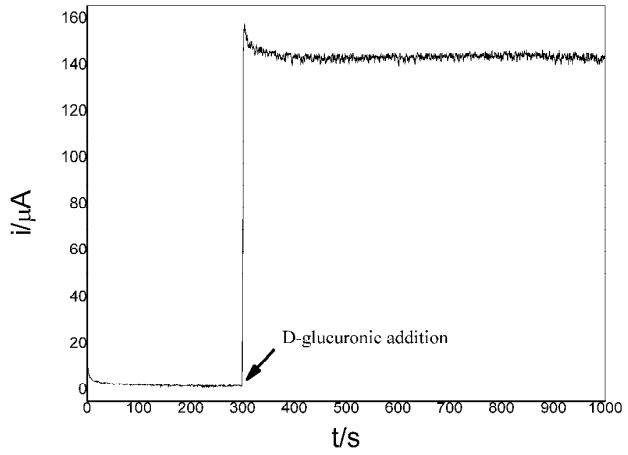

Fig. 4. Chronoamperometric response to 1.0 mol L^{-1} of D-glucuronic acid in alkaline medium during the period of 1000 s, potential of 0.55 V.

3.4 Optimization of chromatographic parameters

In alkaline medium, electrodes modified with copper nanoparticles exhibited a good electrochemical activity for the oxidation of uronic acids.[14] For this reason, CuNP electrode was used as an electrochemical detector in thin-layer cell to detect uronic acids by HPAEC. Under such circumstances, an anion exchange column (CarboPac PA10) was used for the separation of the compounds, using isocratic eluent (simpler and with good baseline stabilization), without the need for derivatization. Direct current amperometric detection was not used largely due to the fact that the adhesion of oxidation products occurs on the electrode surface, leading to the passivation of the electrode surface thereby resulting in poor reproducibility.[9,10,20] Thus, the pulsed amperometric detection was used, which has advantages in complex matrices, more sensitivity, speed and ensures the stability of the response for a prolonged time

Owing to high affinity of organic acids toward the stationary phase, for the chromatographic separation mode, the anion exchange solution requires stronger conditions than those employed with carbohydrates and alditols.[11,12,22,23] In general, the sequence of elution of the matrix components is related to the pKa value. The uronic acids have the lowest pKa value due to the carboxylic group in their molecular structure, thus enabling them to interact more strongly in the column by increasing the retention time in comparison with the sugar compounds.[17] In this sense, for the purpose of decreasing the retention time of uronic acids, concentrations of acetate ions (buffer solution) were added in the alkaline mobile phase for the isocratic elution of uronic acids in order to obtain lower retention times, maintaining satisfactory resolution of the chromatographic bands. The effect of the concentration of acetate ions in the retention time was also evaluated. Here, a standard mixture of 1.0 mmol L^{-1} D-glucuronic and D-galacturonic acid was prepared in 0.1 mol L^{-1} NaOH for isocratic elution with mobile phase containing 0.1 mol L^{-1} NaOH and concentrations of CH_3COONa, in the range of 8.0 to 280 mmol L^{-1}. The flow rate was maintained at 1.0 mL min^{-1} and pulsed amperometric detection at 0.45 V vs. Pd. The retention times and peak width at half height for each concentration of CH_3COONa were evaluated for each peak (D-glucuronic acid and D-galacturonic).

With the increasing concentration of acetate ions in the mobile phase, the order of elution of the analyte investigated was found to remain always the same. However, as expected, the retention times were significantly reduced. For the concentration of 80 mmol L^{-1} of CH_3COONa, the retention time was 38.38 min for the D-galacturonic acid and 50.65 min for the D-glucuronic acid. However, when the concentration of CH_3COONa was 280 mmol L^{-1}, the retention time was found to decrease to 8.73 min for the D-galacturonic acid and 11.53 min for the D-glucuronic acid. A value less than 0.1 mol L^{-1} of NaOH concentration in the mobile phase is not recommendable because the retention times are considerably longer given that the mobile phases with concentrations of NaOH higher than 0.2 mol L^{-1} have already resulted in poor resolution. Therefore, for analytical applications in electrode modified with nanoparticles, the alkaline eluent of 0.1 mol L^{-1} NaOH was chosen to compose a mobile phase, with appropriate retention time, resolution, the magnitude of the amperometric response and minimum noise. As such, the mobile phase to proceed with the chromatographic studies was 0.1 mol L^{-1} NaOH plus 280 mmol L^{-1} CH_3COONa, with appropriate retention time, resolution, the magnitude of the amperometric response and minimum noise.

Uronic acids are detected by measuring the electrical current generated through their oxidation on the surface of CuNP electrode. The products of this oxidation reaction can poison the surface of the electrode and as such it has to be cleaned between measurements. PAD thus employs a sequence of three potentials. The first potential (E_1) is where the uronic acids are oxidized. The second potential, E_2, should be more positive to enable it oxidize all the species present on the

electrode surface and prevent them from being adsorbed. The third potential, E_3, should be low enough to reduce the oxidized surface of the electrode and increase the selectivity and stability of the electrochemical signal. As the electrode was formed from copper oxides, not more than the negative potential of -0.05 V was applied for only 50 ms. The potential of 0.45 V vs. Pd chosen to detect uronic acids presented higher detectability. So, the potentials and time periods for the pulsed amperometric detection were: E_1, +0.45 V for 200 ms; E_2, +0.60 V for 50 ms; E_3, −0.05V for 50 ms.

Therefore, the optimum conditions for the separation of uronic acids (found in the supplementary data) were: isocratic elution containing 0.1 mol L^{-1} NaOH plus 280 mmol L^{-1} CH_3COONa with the detection potential of 0.45V vs. Pd and flow rate of 1.0 mL min^{-1}.The chromatographic parameters such as dead time (tm), retention time (tr), adjusted retention time (tr'), resolution (Rs), number of theoretical plates (N), separation factor (α) and retention factor (k), are within the expected results for good chromatographic performance [33] and are shown in Table 1.

Table 1. Parameters for the chromatographic separation of D-galacturonic acid and D-glucuronic[a]

Analyte	t_m	t_r	t_r'	R_s[b]	N	α[c]	k[d]
galacturonic	1.60	8.72	7.12	4.04	3238	1.40	4.45
glucuronic	1.60	11.55	9.95	2.07	3441		6.22

[a] Experimental conditions: Isocratic separation of D-galacturonic acid, 8.75 min and D-glucuronic acid. Mobile phase 0.1 mol L^{-1} NaOH plus 280 mmol L^{-1} CH_3COONa with the detection potential of 0.45 V vs. Pd and flow rate of 1.0 ml min^{-1}.
[b] $Rs= 2*(t_r')/(w_1+w_2)$, where w_1 and w_2 is the peak width at half height (galacturonic and glucuronic) [33]
[c] α= kb/ka, where kb is the distribution constant for the species more strongly retained and ka is the constant for the species less retained, which is eluted faster. [33]
[d] $k=t_r'/t_m$. [33]

The variation retention times for the D-galacturonic acid during chromatographic detection was 0.45% and 0.52% for D-glucuronic acid. The variation of these retention times, according to literature,[34] can be up to 3.0%, which shows that the times have good reproducibility.

3.5 Studies major interferences present in the sample

The method may be subject to interference from compounds of the matrix during the chromatography analysis. The main electroactive interferents are: D-(+)-glucose, D-(+)-xylose, D-(+)-mannose and D (-) -arabinose. First, a standard solution of concentration 1.0 mmol L^{-1} was prepared with the interferent together with the uronic acids and subjected to chromatographic analysis as shown in Fig. 5. The sugar compounds co-eluted near the solvent front during a period of 2.02 min. Thus, the presence of a relatively high concentration of such electroactive compounds had no apparent effect on the peaks of the uronic acids under investigation.

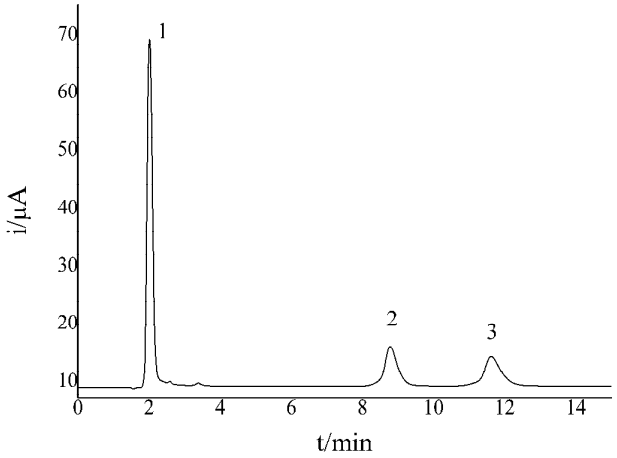

Fig. 5. Isocratic separation chromatogram of standard solution (1) D-(+)-glucose, D-(+)-xylose, D-(+)-mannose, D-(-)-arabinose, (2) D-galacturonic acid, and (3) acid D-glucuronic in CuNP detector. Mobile phase 0.1 mol L^{-1} NaOH plus 280 mmol L^{-1} CH_3COONa, detection potential of 0.45V vs. Pd and flow rate of 1.0 ml min^{-1}.

3.6 Concentration Study of the Uronic Acids

After the optimization of the chromatographic conditions, analytical curves were constructed to evaluate the analytical parameters of the technique which can be found in the supplementary data. Standard solutions were prepared at concentrations ranging from 1.0 $x10^{-7}$ mol^{-1} to 1.0 $x10^{-4}$ mol^{-1} where the linear region, detection limit (LOD), detection quantification (LOQ) and sensitivity of amperometry(Sa) were all evaluated. The linear range was 3.0 $x10^{-6}$ to 8.0 $x10^{-5}$ mol^{-1} for D-galacturonic acid with LOD of 5.8 $x10^{-7}$ mol^{-1}, LOQ of 1.9 $x10^{-6}$ mol^{-1} and Sa of 4.9 $x10^6$ μA L mol^{-1}. For the D-glucuronic acid the values obtained included linear range of 3.0×10^{-6} to 8.0 $x10^{-5}$ mol^{-1} with LOD of 7.3 $x10^{-7}$ mol^{-1}, LOQ of 2.4 $x10^{-6}$ mol^{-1} and Sa of $3.1x10^6$μA L mol^{-1}. The amperometric sensitivity was studied in three different CuNP electrodes in order to check the reproducibility of detection where the values obtained were 3.6 ± 1.8 x 10^6 μA L mol^{-1} for D-galacturonic acid and 1.9 ± 1.0x 10^6 μA L mol^{-1} for D-glucuronic acid. These results demonstrated that three electrodes of CuNP used for the pulsed amperometric detection had standard deviation which did not interfere with the method.

3.7 Determination of uronic acids present in the bagasse of sugarcane.

The separation and detection of uronic acids in sugarcane bagasse can be of great interest to the biorefinery process since these compounds can be used in the food (as acidifiers) and chemical (as surfactant) industries. They can also be chemically or enzymatically transformed into substances with higher added value such as meso-galactaric (polyesters production) and L-galactonic (cosmetics, food, cleaning products).[35] In order to demonstrate the easy and simple nature of the use of this analytical method in practical applications, 300 mg samples of sugarcane bagasse were subjected to hydrolysis, neutralization and filtration processes as described in section 2.4. The samples were injected into the chromatograph in optimized conditions and the identification of the chromatographic peaks was based on the retention time specific to each analyte which was confirmed by the addition of standard solution. Figure 6 shows the chromatogram for the sample where due to the high oxidation current of the other components which co-eluted in 2.02 min, the peaks uronic acids were found not to be visible, the chromatogram (b) shows a magnification of the acids retention time.

The standard addition method was used to determine the concentration of uronic acids present in the sample which consists of adding known concentrations of the substance of interest in the sample. These samples within which the standard solution is inserted are used to obtain quantities of the substance in the sample through their respective areas obtained. The point where the line cut the ordinate axis represents the peak area of the substance which is being determined, without any added standard. The straight line extrapolation set in the x-axis is equivalent to the concentration of analyte. The standard addition method is laborious, though it stands out to be especially important when the sample is very complex, as is the case of sugarcane bagasse. The curve for the method of standard addition for the D-galacturonic has regression equation, $y= 1.84x10^{6}x + 50.33$, with linear correlation of 0.996. The D-glucuronic acid has regression equation, $y= 9.97x10^{5}x + 13.08$, with linear correlation of 0.988. By the extrapolation of the line, the concentration of 14.3 g/kg was found for the D-galacturonic acid and 13.6 g/kg for the D-glucuronic acid. The standard additions were performed on three different CuNP electrodes in order to verify the repeatability of detection. The concentration of D-galacturonic acid found was 15.8 ± 0.5 g/kg while that of D-glucuronic acid was 12.5 ± 0.5 g/kg.

Other authors who have separated and quantified organic acids and carbohydrates in lignocellulosic matrices had run time between 25 and 44 minutes using gradient and mobile phase comprising of up to 3 components. In some works, the use of a post-column was needed to stabilize the baseline and/or derivatization with p-Aminobenzoic Acid.[36,37,38] The amounts of uronic acids in other lignocellulosic materials detected by amperometry using gold electrode are dependent on the raw material under analysis.

After performing the method of standard addition, the accuracy was evaluated. To this end, the recovery method was used for each uronic acid. Three different concentrations of acid were injected into the sample as shown in Table 2. The average recovery for D-galacturonic acid was 94.1%, with a coefficient of variation (CV) of 9.0% whereas for D-glucuronic acid the average recovery was 99.4% with a coefficient of variation of 3.4%. These tests show that the method can be considered suitable for the detection of acids.

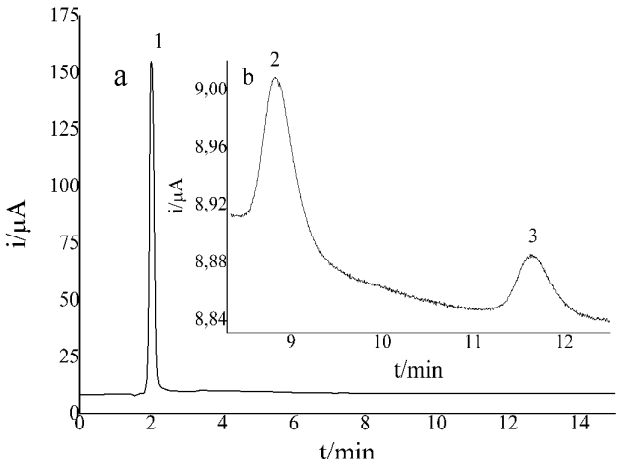

Fig. 6. Chromatogram for sample hydrolyzed of sugarcane bagasse. In (a) the peak (1) shows oxidation of the sample components, 2.02 min and (b) magnification of the retention times for (2) D-galacturonic acid 8.82 min and (3) D-glucuronic acid, 11.67. Mobile phase 0.1 mol L^{-1} NaOH plus 280 mmol L^{-1} CH_3COONa, detection potential of 0.45 V vs. Pd and flow rate of 1.0 ml min^{-1}.

Table 2. Percent recovered in HPLC technique for the D-galacturonic and D-glucucronic acid[b]

D-galacturonic/ mol L^{-1}	Amount found/ mol L^{-1}	Recovery %
$2.5x10^{-5}$	$2.1\ x10^{-5}$	84.8
$5.0\ x10^{-5}$	$4.8\ x10^{-5}$	96.0
$1.0\ x10^{-5}$	$1.0x10^{-4}$	101.5
D-glucuronic		
$2.5x10^{-5}$	$2.5\ x10^{-5}$	101.9
$5.0\ x10^{-5}$	$4.7x10^{-5}$	95.5
$1.0\ x10^{-5}$	$1.0x10^{-4}$	100.9

[b] Experimental conditions as in Table 1.

Conclusions

The uronic acids present in lignocellulosic material (sugarcane bagasse) can be used in the chemical and food industries once recovered within the concept of biorefinery. With these new technologies, we can add value to what was previously wasted by the sugar and alcohol plants. These acids can not be detected owing to the lack of chromophore and fluorophore groups in their molecular structure and the currently used methods are found to only quantify the total amount of uronic acids present in the sample with the use of

derivatization. By virtue of that a detector modified with oxide copper nanoparticles electrodeposited on the surface of the glassy carbon was developed in order to detect these acids and improve the sensitivity. This modified electrode was used in chromatography with pulsed amperometric detection because the electrochemical reaction occurs only during a short period of time, reducing problems of passivation which produces poor reproducitibility. The separation of the acids was complete within 15 min. The method was applied to real samples of hydrolyzate bagasse and the value found in this sample was 15.8±0.5 g/kg and 12.5±0.5 g/kg for D-galacturonic and D-glucuronic acids respectively. The results demonstrate that the proposed method can be used for the detection of these acids without the need for derivatization, having the advantage of exerting no interference, with considerable accuracy. In addition to that, it seems the method can also be applied to other lignocellulosic matrices.

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Notes and references

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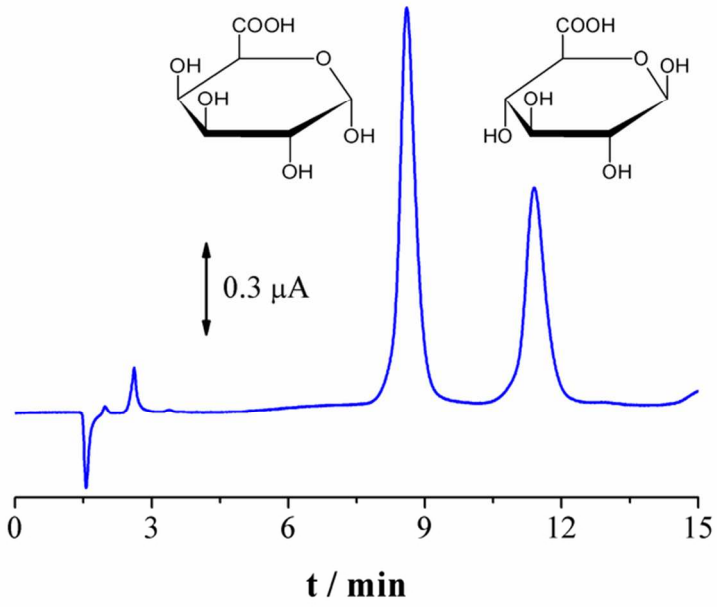

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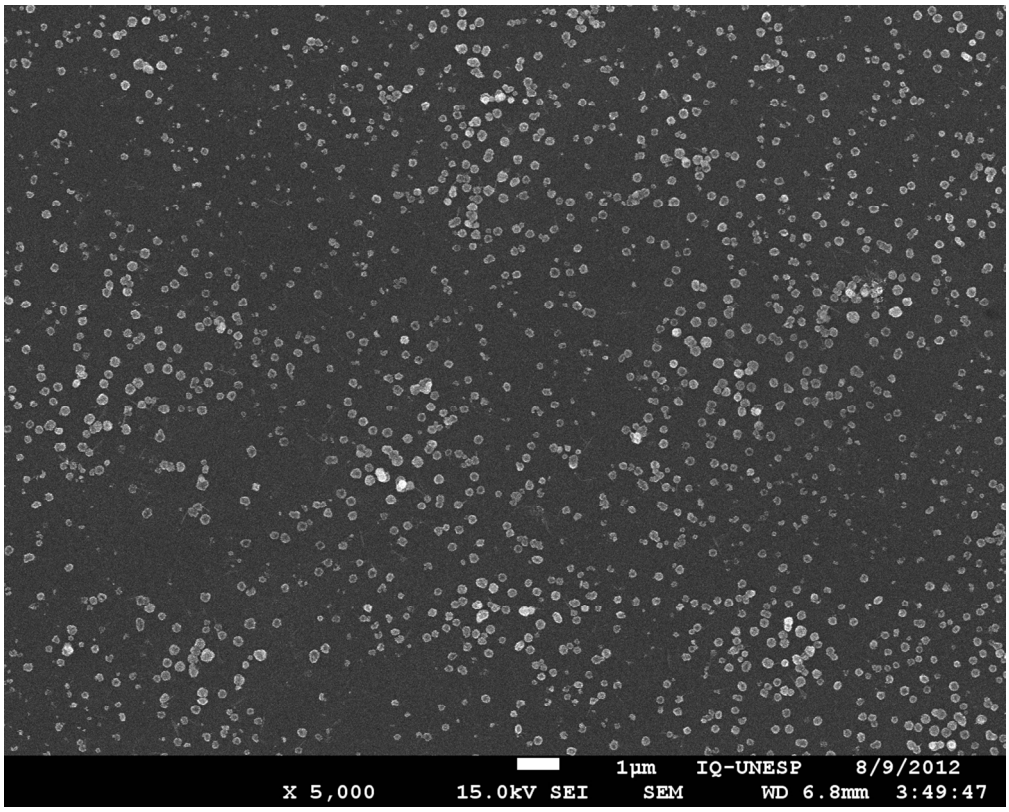

SEM images of nano-copper oxide electrodeposited on GC surface.
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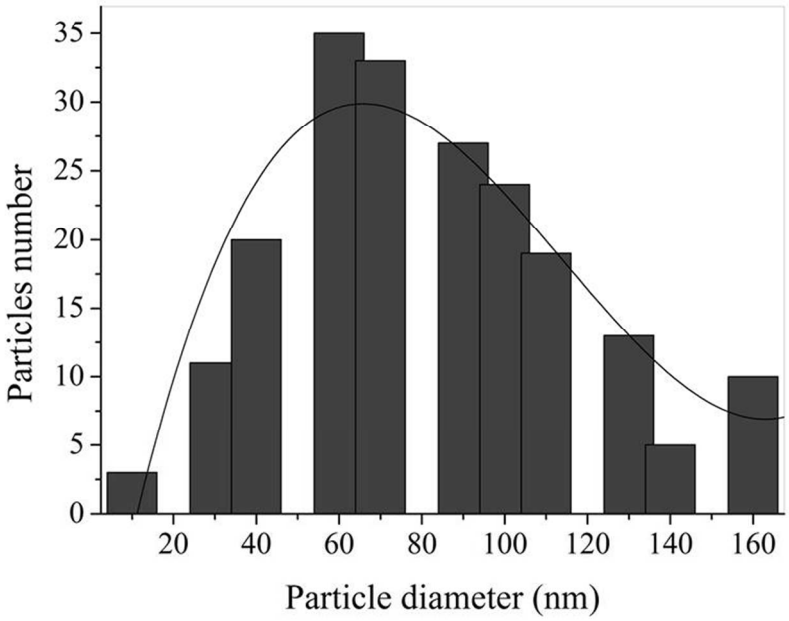
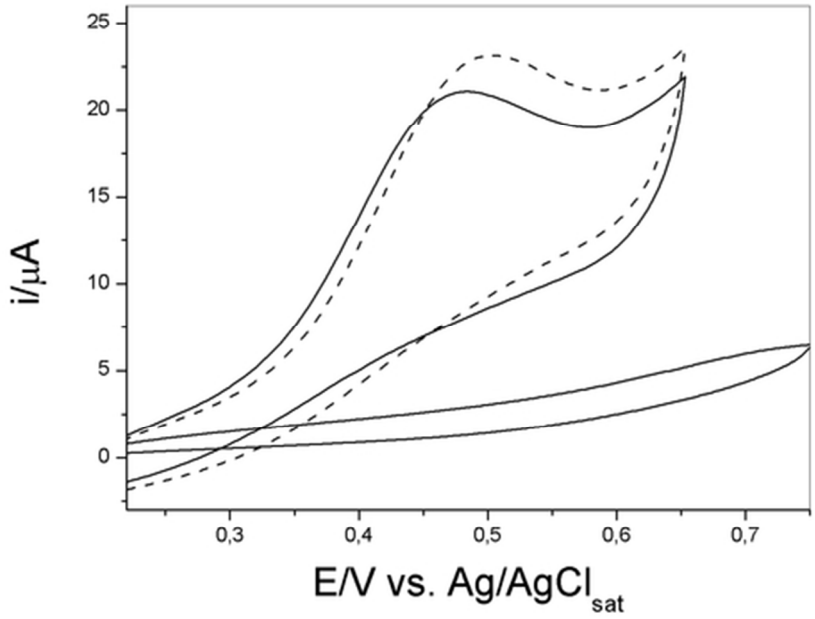
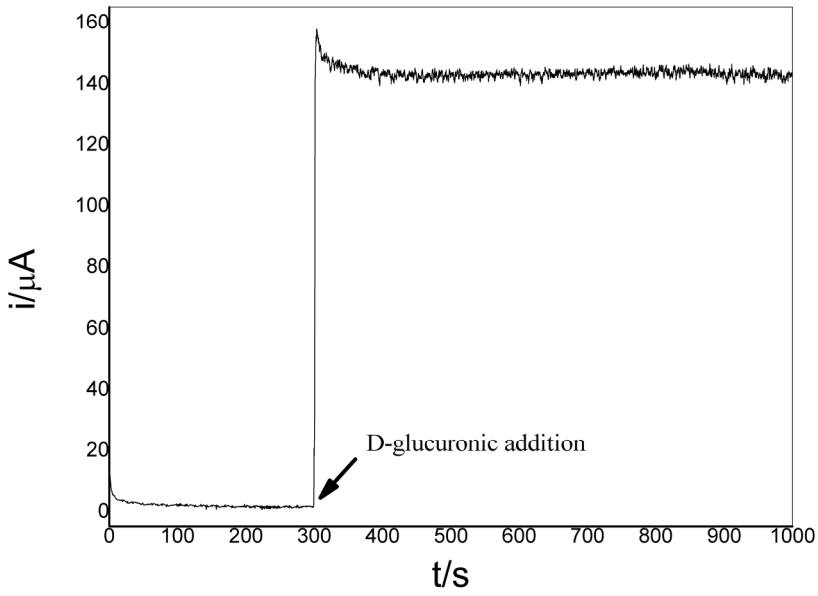


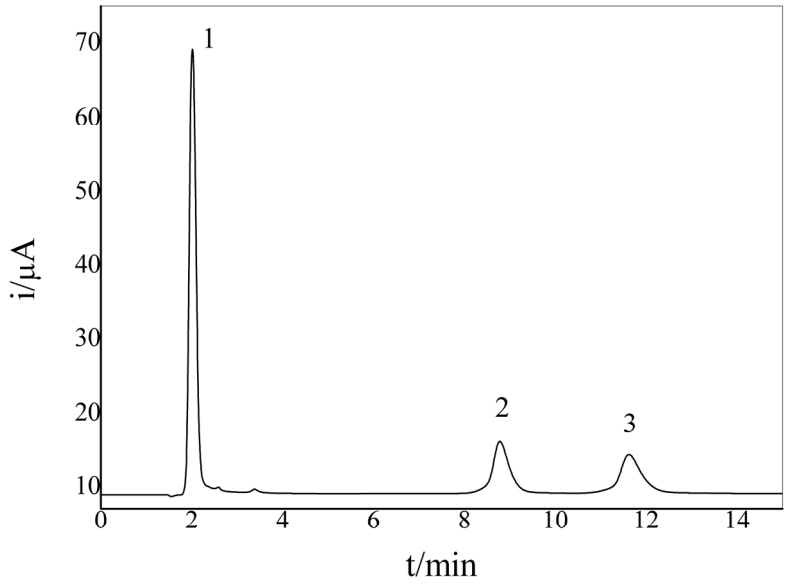
Particle size histogram of the nano-copper oxide.
254x179mm (96 x 96 DPI)



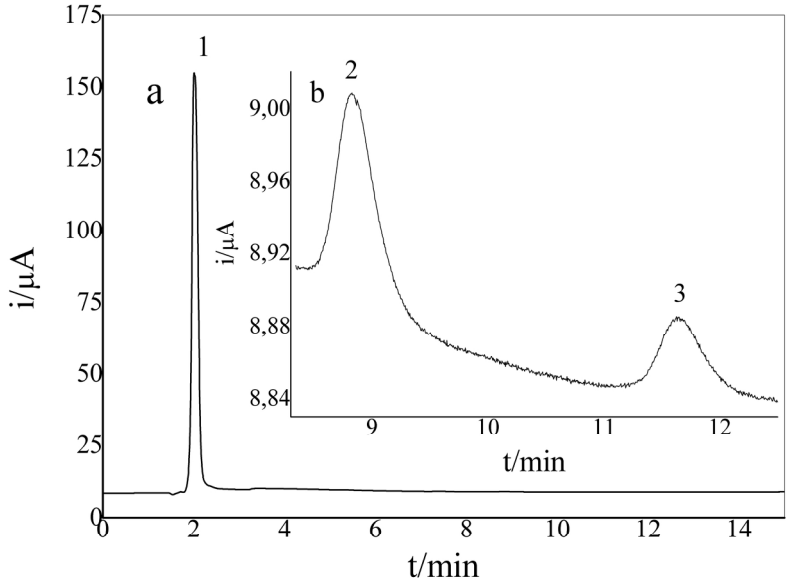
Cyclic voltammograms of 5.0 mmol L^{-1} D-galacturonic (-) and D-glucuronic (--) acids in the presence of 0.1 mmol L^{-1} NaOH on modified electrode (CuNP), v = 50 mVs^{-1}
25x17mm (600 x 600 DPI)



Chronoamperometric response to 1.0 mol L^{-1} of D-glucuronic acid in alkaline medium during the period of 1000 s, potential of 0.55 V.
100x72mm (600 x 600 DPI)



Isocratic separation chromatogram of standard solution (1) D-(+)-glucose, D-(+)-xylose, D-(+)-mannose, D-(-)-arabinose, (2) D-galacturonic acid, and (3) acid D-glucuronic in CuNP detector. Mobile phase 0.1 mol L^{-1} NaOH plus 280 mmol L^{-1} CH_3COONa, detection potential of 0.45V vs. Pd and flow rate of 1.0 ml min^{-1}.
96x67mm (600 x 600 DPI)



Chromatogram for sample hydrolyzed of sugarcane bagasse. In (a) the peak (1) shows oxidation of the sample components, 2.02 min and (b) magnification of the retention times for (2) D-galacturonic acid 8.82 min and (3) D-glucuronic acid, 11.67. Mobile phase 0.1 mol L^{-1} NaOH plus 280 mmol L^{-1} CH_3COONa, detection potential of 0.45 V vs. Pd and flow rate of 1.0 ml min^{-1}
96x67mm (600 x 600 DPI)