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Cyclic voltammetric investigations on copper α-*N*,*O*-succinated chitosan interactions

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Abstract

Chitin in the α form extracted from crab shells under hot alkaline conditions yielded chitosan with a degree of deacetylation of $80 \pm 1\%$. This was chemically modified with succinic anhydride, following either a homogeneous or a heterogeneous route. The anhydride reaction products in position 2 and 6; and 2, 3 and 6 of the chitosan were characterized by elemental analyses, carbon-13 NMR in the solid state, scanning electron microscopy, infrared spectroscopy and X-ray diffractometry. The second modified chitosan compound better adsorbs copper from aqueous solution and gave a cyclic voltammetric response when divalent copper nitrate was used as a mediator, with high stability in potassium chloride electrolyte solutions. The cyclic voltammetric studies with different scan rates and the potential of the electrode versus pH indicated that the electrode constructed with chitosan-succinate copper ion presents stability for approximately one month, suggesting its use as a biosensor.

Keywords: Chitosan; Succinic anhydride; Cyclic voltammetry

1. Introduction

The natural biopolymer chitin, poly-β-(1→4)-2-acetamide-2-deoxy-D-glucopyranose, extracted from exoskeletons, arthropods, crustaceans, insects and arachnids (Becker, Schlaak, & Strasdeit, 2000; Holme, Foros, Pettersen & Smidsrod, 2001; Kurita, 2001; Rege, Garnise & Block, 2003), is the second most abundant polysaccharide on the planet, losing in quantity only to cellulose (Bathia & Ravi, 2000). Identical polymers can be artificially obtained, after opening the sugar derivative exazoline ring or by biosynthesis from glucose. The derived deacetylated form, chitosan, poly-β-(1→4)-2-amino-2-deoxy-D-glucopyranose, can also be found in the cellular walls of fungi (Chatterjee, Adhya, Guha, & Chatterjee, 2005). Moreover, depending on the chitin source, a high amino group percentage can be found naturally (Roberts, 1992).

Chitin is present in nature in alpha, beta and gamma forms (Tharanathan & Kittur, 2003) with structures related to polymeric ribbon dispositions, in arrangements associated with inherent crystallinity, forms which are originally from different types of living organisms, animal, vegetal or fungus. However, large amounts of this polysaccharide can be extracted from waste organic materials and the gamma structure is rarely investigated. In any case, the chitin functional activity can be seen as a mucopolysaccharide (Focher, Naggi, Torri, Cosani, & Terbojevich, 1992) easily associated with other polysaccharides, proteins, glucans, calcium carbonate and carotenoid pigments, reflecting in physical or chemical macromolecular interactions (Duarte, Ferreira, Marvão, & Rocha, 2002).

The biopolymer chitosan presents peculiar advantages with respect to its attainment, use and reutilization in various research fields. Some derivatives are clear examples of natural materials that contemplate a variety of application areas such as coagulation agents for cheddar cheese (Savant & Torres, 2000), anti-inflammatory activity (Khnal et al., 2001), biosensor for anions (Darder, Colila, & Ruiz-Hitzky, 2003), reduction of industrial residues of divalent cations by their removal (Lima & Airoldi, 2004), biomedical features or in biotechnology (Loke, Lau, Yong, Khor, & Sum, 2000).

A great number of chemical modifications have been carried out on chitin and the chitosan glucopyranoside ring, conferring to these materials surprising possibilities of reactions (Hardy, Hubert, Macquarrie, & Wilson, 2004; Lima & Airoldi, 2003), under homogeneous or heterogeneous synthetic experimental conditions (Grant, Blair, & Mckay, 1990). Chemical chitosan modification in homogeneous conditions with an organic

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anhydride takes place preferentially through amino group acetylation on carbon 2 of the glucopyranoside ring to yield N-acetylation and, in case there is regioselectivity, the reaction also occurs on carbon 6 to give *O*-acetylation. However, the most effective reaction procedure yields simultaneous modifications, causing substitutions on carbons 2, 3 and 6 (Shigemasa, Ishida, et al., 1999). These synthetic routes elucidate the reactivity of the carbons, which decreases in the order C2 > C6 > C3 (Xu, McCarthy, & Gross, 1996).

This present investigation deals with the syntheses and characterization of chitosan chemically modified with succinic anhydride and subsequent copper adsorption properties through coordination by using electrochemical investigations, in order to enlarge future applications in this innovative area, as has been explored for other materials (Lazarin & Airoldi, 2004; Lazarin & Airoldi, 2005).

2. Experimental

2.1. Preparation

2.1.1. N-Succination in position 2

About 3 g of powdered α-chitosan was stirred with 30.0 cm^3 of 1.70 mol dm^{-3} acetic acid at 298±1 K. After pseudo-gel formation 80.0 cm^3 of methanol followed by 1.90 cm^3 of succinic anhydride were added and the solution stirred for 30 h, when the mixture was diluted with 110.0 cm^3 of acetone and left for 12 h under stirring. The solid, named Chitsuc-2, was filtered and washed with ether under vacuum.

2.1.2. N,O-Succination in positions 2 and 6

About 3 g of powdered α-chitosan was stirred with 70.0 cm^3 of a 5% lithium chloride solution in *N*,*N*-dimethylacetamide for 15 min at 298±1 K. To this solution was added 1.20 g of succinic anhydride and stirring was continued for another 30 min. To this mixture was then added dropwise 1.70 cm^3 of triethylamine, with continued stirring for 24 h, when another 30.0 cm^3 of methanol was introduced. After 5 min the solid formed was transferred to doubly distilled water and the pH was adjusted to the 1–2 range with 2.0 mol dm^{-3} hydrochloric acid. The suspension was periodically stirred after sodium hydroxide addition and some drops of 0.10 mol dm^{-3} Na_2HPO_4 solution were added, to maintain the pH in the 8–10 range. The solid was filtered, placed inside a bovine membrane and dialyzed for a period of three days against doubly distilled water and then dried under vacuum to yield Chitsuc-2,6.

2.1.3. N,O-Succination in positions 2, 3 and 6

To 0.50 g of powered α-chitosan was added 500 cm^3 of 45% sodium hydroxide, followed by 0.50 g of sodium dodecylsulfate and the mixture was kept at 277 K. After 6 h the flask was maintained in the refrigerator for 2 days, after which it was stirred and 32.0 cm^3 2-propanol and 0.020 g succinic anhydride were added. The solid formed was dispersed in 1500 cm^3 of methanol, filtered and washed with the same solvent. The product was dissolved in doubly distilled water and the pH was adjusted with drops of 1.0 mol dm^3 hydrochloric acid and dialyzed against doubly distilled water for 3 days. The filtered solid was dispersed in 100 cm^3 of water with 1.0 cm^3 of 0.50 mol dm^{-3} hydrochloric acid for another 15 min, then 50.0 cm^3 of acetone was added. The suspension was centrifuged and the solid was dried under vacuum to yield Chitsuc-2,3,6.

2.2. Copper adsorption

The isotherm of copper adsorption from an aqueous solution was obtained through the batchwise method. For this operation, about 100 mg of Chitsuc-2,3,6 was orbitally shaken for 4 h at 298±1 K, with variable concentrations of the cation, obtained from a 0.050 mol dm^{-3} of copper nitrate solution. The time required to reach equilibrium was first established in a series of adsorptions, wherein the time was varied. After that, the suspension was filtered and samples of the supernatant had their cation concentrations determined by atomic adsorption spectrometry. The number of moles adsorbed (n_f) was determined by applying the equation $n_f = (n_{a\,-}n_s)/m$, where n_a and n_s are the initial and the equilibrium number of moles of the metal in solution and m is the mass of the adsorbent, respectively. The resulting adsorbed material was named Chitsuc-2,3,6-Cu.

2.3. Electrochemistry studies

The modified carbon paste electrode was prepared by mixing 30 mg of the Chitsuc-2,3,6-Cu, 30 mg of graphite and a drop of mineral oil (2.0×10^{-2} cm^3). The paste was deposited into a cavity on the surface of a platinum disk fused at the end of a glass tube with 3 mm inner diameter. The electrode is referred to hereafter as EChitsuc-2,3,6-Cu.

Electrochemical measurements were made using this carbon paste electrode as the working electrode, a saturated calomel (SCE) as reference electrode and a platinum wire as auxiliary electrode. The electrochemical properties were studied by means of the cyclic voltammetry technique with a PAR 273A (EG&G) potentiostat–galvanostat. The experiments were carried out in 0.50 mol dm^{-3} of potassium chloride electrolyte solution under a pure argon atmosphere and the pH was adjusted by adding either hydrochloric acid or sodium hydroxide solutions. The concentrations of different supporting electrolytes were also tested.

2.4. Characterization

^{13}C nuclear magnetic resonance spectra of the solid material were obtained on an AC 300/P Bruker spectrometer at room temperature at 75.5 MHz. A pulse repetition time of 3 s and contact time of 1 ms were used.

Carbon, hydrogen and nitrogen were obtained using a Perkin-Elmer model PE 2400 instrument.

Infrared spectra were performed with a Perkin–Elmer model 1600 FTIR spectrophotometer by using pressed KBr pellets, with 4 cm^{-1} of resolution.

X-ray diffraction patterns were obtained with nickel-filtered Cu Kα radiation on a Shimadzu XD3-A diffractometer (30/20 kV/mA).

The scanning electron microscopic (SEM) images were obtained for samples dispersed on a double-faced conducting tape adhered to an aluminum support. The samples were coated with gold using a low voltage sputtering Balzer MED 020 apparatus, and the measurements were carried out on a JEOL JSM-T300 scanning electron microscope.

The cations from the supernatant solutions were determined through atomic absorption spectroscopy using a Perkin–Elmer Model 5100 atomic absorption spectrometer.

3. Results and discussion

The ^{13}C MAS NMR spectra in the solid state of chitosan modified with succinic anhydride, when present in 2, 2 and 6; 2, 3 and 6 positions are shown in Fig. 1. This is a useful technique to elucidate the effective chemical attachment of the modified agent on alpha chitosan (Lima & Airoldi, 2004). Although, spectra in the solid state do not present a clear sequence of peaks, a suitable distinction about the degree of functionalization can be visualized by considering the carbonyl signals, which differ in complexity as succinic acid increases in the biopolymer chain. Thus, spectrum I, representing chemical substitution in position 2, showed only a carbonyl peak at 175 ppm, in spite of the expected two signals. This fact could be associated with the possible conformation of the chains to give equivalent chemical shifts for carbonyl groups, resulting only one peak. Part II corresponds to the chitosan modified at carbons 2 and 6, as illustrated for the proposed biopolymer in Fig. 1. As evidenced, four carbonyl signals appear in the region near 175 ppm. An increase in complexity was observed in part III that corresponds to simultaneous functionalization at carbons 2, 3 and 6, with indication of a set of peaks, again in the same region.

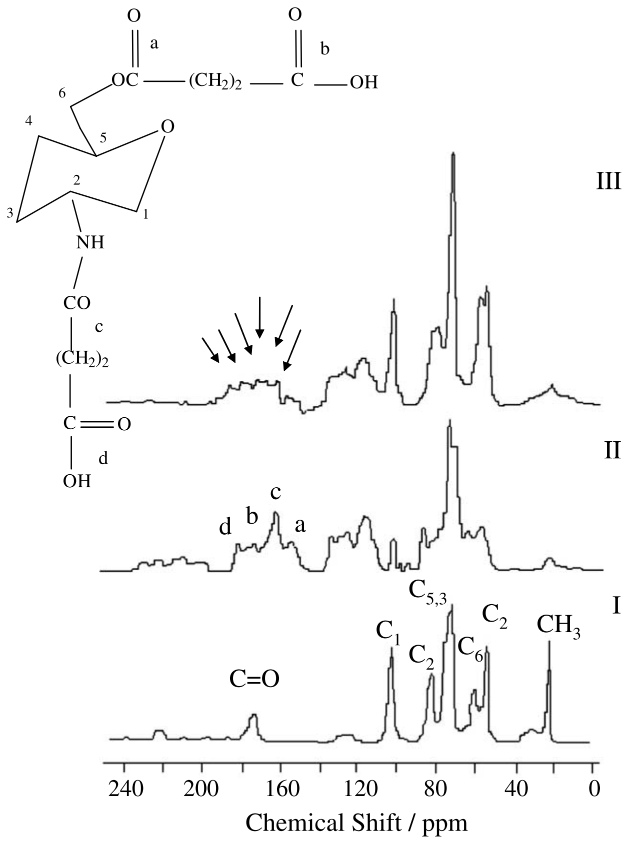

Fig. 1. ^{13}C NMR for chitosans modified at carbons 2 (I), 2,6 (II) and 2,3,6 (III).

Table 1
Crystallinity indices (CrI) for chitin (Chit-X), chitosan (Chitan-X) and the forms modified at carbons 2 (Chitsuc-2), 2,6 (Chitsuc-2,6), 2,3,6 (Chitsuc-2,3,6)

Biopolymer	CrI/%	I_{am}	I_{110}
Chit-X	52	9.3	19.3
Chitan-X	54	9.3	20.3
Chitsuc-2	54	9.3	20.3
Chitsuc-2,6	–	–	18.5
Chitsuc-2,3,6	–	–	21.0

Indices of amorphous (I_{am}) and crystalline (I_{110}) diffractions.

A comparative behavior of crystallinity in these biopolymers can be established by considering the properties associated with cellulose, which is considered as standard. Based on this procedure the indices of relative crystallinity of chitin, chitosan and their derivatives were calculated. Surely, chitin allomorphism or polymorphism (Noishiki, Nishiyama, Wada, Okada, & Kuga, 2003) leads to chitosan and end product derivatives with low crystallinity.

The crystallinity indices are related to relative degree (Struszczyk, 1987), using the angle in the 110 plane and the value corresponds to the index of amorphous material (I_{am}) for each modified chitosan. The calculated values of the crystallinity indices are shown in Table 1. For chitin, chitosan and chitosan modified with succinic anhydride in position 2 the values are very close, indicating that the chemical treatment with sodium hydroxide and the reaction with succinic anhydride did not significantly affect the degree of crystallinity. Such a property was not calculated for the chemically modified compounds Chitsuc-2,6 and Chitsuc-2,3,6 due to the absence of diffraction signals, as illustrated in Fig. 2.

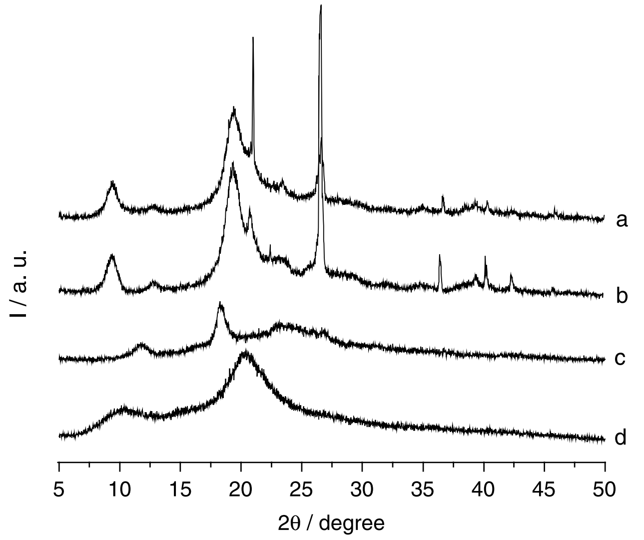

Fig. 2. X-ray diffractometry for chitosan (a) and chitosans modified at carbons 2 (b), 2,6 (c) and 2,3,6 (d).

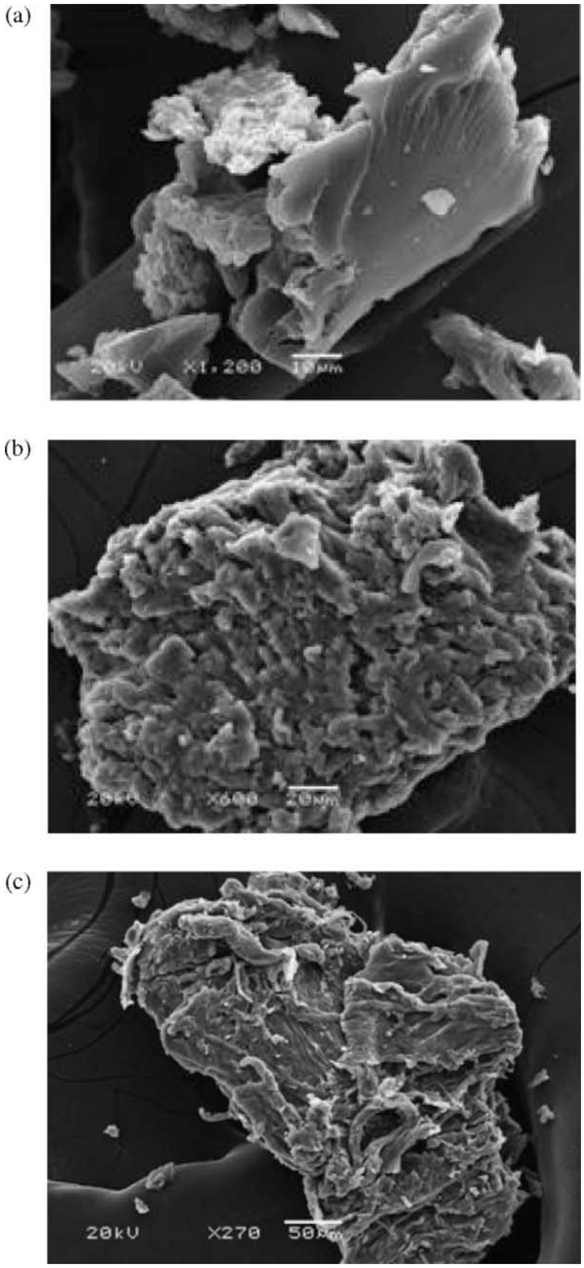

Fig. 3. Scanning electron microscopy for chitosans modified in positions: 2 (a), 2,6 (b), and 2,3,6 (c).

The micrographs for the chitosans modified with succinic anhydride are shown in Fig. 3, with a significant similarity in their morphologies, by considering the compounds having 2,6 and 2,3,6 positions modified, even though they were synthesized through different routes. While the first was prepared by a heterogeneous method, the other followed the synthetic route based on a homogeneous method. Both micrographs present more irregular surfaces than the chitosan modified only in position 2.

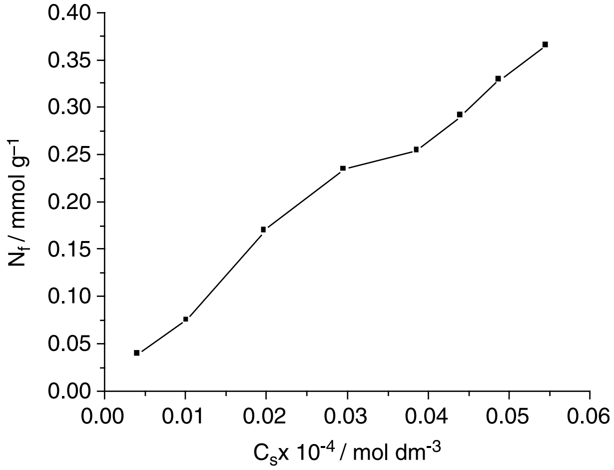

Fig. 4. Isotherm of adsorption of copper on Chitsuc-2,3,6 indicating (N_f) values versus concentration (C_s).

The isotherms involving copper ion adsorption gave similar behavior, with an increase in adsorption without establishing a defined plateau, but following a tendency of increasing the number of moles adsorbed with increases in the cation concentration, as illustrated by Chitsuc-2,3,6 in Fig. 4. This adsorption process confirms that the synthesized chitosans have properties of retaining cations as the original characteristics of the raw material were chemically organofunctionalized.

Cyclic voltammetry experiments using the carbon paste electrode modified with Chitsuc-2,3,6-Cu was carried out. An electrode modified only with Chitsuc-2,3,6 provides no current peaks, as shown in Fig. 5a. Fig. 5b presents the cyclic voltammetry curve obtained for a carbon paste electrode modified with Chitsuc-2,3,6-Cu. In this case, a current peak is seen with a midpoint potential at $E_m = -0.25$ V, that

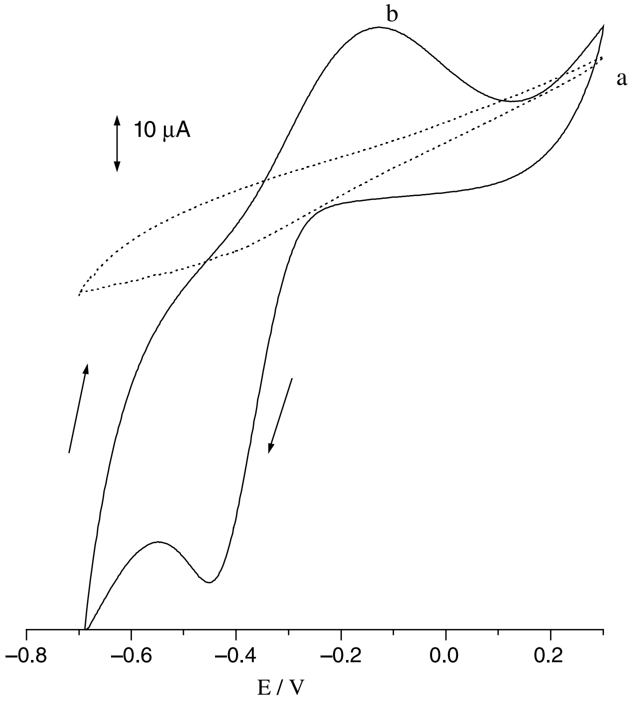

Fig. 5. Cyclic voltammograms of Chitsuc-2,3,6 (a) and Chitsuc-2,3,6-Cu (b), both under argon. Supporting electrolyte: 1.0 mol dm^{-3} KCl. Scan rate: 10 mV s^{-1}.

corresponds to the potential of oxidation of Cu(I) to Cu(II) (Landoll, 1982).

Cycling the potential in a series of experiments tested the stability of the electrode. The results indicate that the peak current intensities do not decrease, which is in agreement with having copper strongly bonded to Chitsuc-2,3,6, and it is not released to the solution phase under the operating conditions. This behavior was confirmed by carrying out the cyclization for at least one hundred redox cycles.

Cyclic voltammograms obtained at different scanning rates indicate that the value of ΔE_p ($\Delta E_p = E_{pa} - E_{pc}$) increases at higher rates, as shown in Fig. 6. This is an indication that the kinetics of electron transfer on the electrode surface is not sufficiently fast, as a consequence of the matrix having considerable resistance. The correlation of the peak current, I_{pa} and I_{pc}, against $v^{1/2}$ (v is the scan rate) is linear, as shown in the insert part of Fig. 6, which is very similar to a diffusion controlled process. Since, the electroactive species is strongly bonded to the matrix, as shown earlier, this mechanism may be explained by transport of the ions of the supporting electrolyte to and from the electrode surface for charge compensation (Lazarin & Airoldi, 2004, in press).

An investigation of the influence of the electrolyte was made by varying the concentration of saline solution (0.10, 0.50 and 1.0 mol dm^{-3}). These results indicate that the supporting electrolytes are not interacting with the matrix surface and produce no significant change in the midpoint potential. Dependence of the potential in relation to pH for the EChitsuc-2,3,6-Cu electrode was not observed in the interval studied (6.0–12.0) as shown in Fig. 7. However, chitosan protonation occurs at pH 3, a condition in which it is supposed that a gel is formed. A remarkable feature is that this new electrode does not show significant changes in response over a month of use, demonstrating good chemical stability.

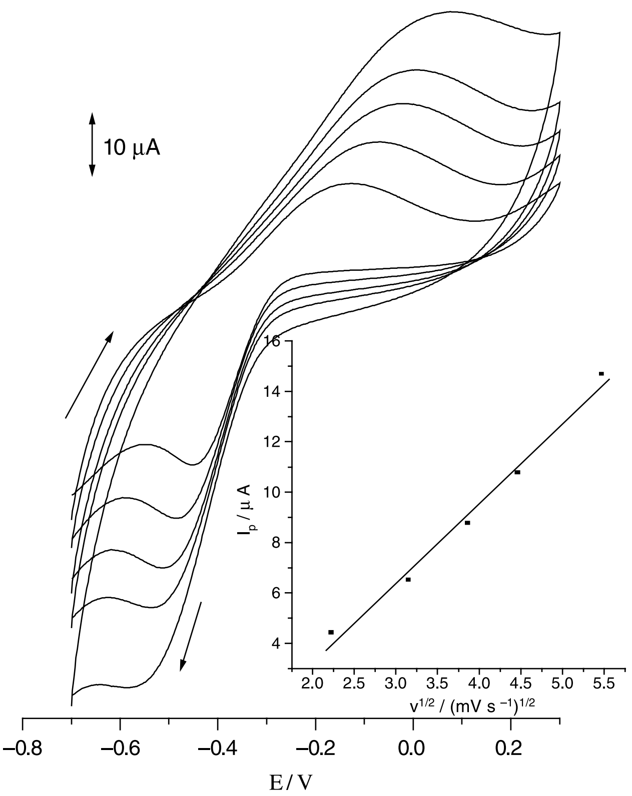

Fig. 6. Cyclic voltammograms obtained at different scan rates (5, 10, 15, 20, 30 mV s^{-1}), under argon. The inserted figure represents the peak current, I_p versus the square root of the scan rate, $v^{1/2}$ for the Chitsuc-2,3,6-Cu electrode. Supporting electrolyte: 1.0 mol dm^{-3} KCl.

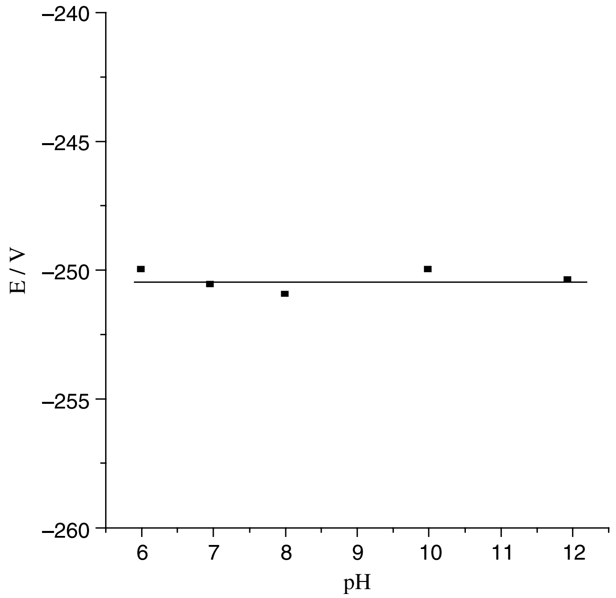

Fig. 7. Potential against pH for Chitsuc-2,3,6-Cu electrode. Supporting electrolyte: 1.0 mol dm^{-3} KCl and scan rate: 10 mV s^{-1}.

4. Conclusion

Amongst the three synthetic chemically modified chitosans, only the matrix Chitsuc-2,3,6 was stable from the operational point of view, showing it to be a useful material to adsorb copper from aqueous solution. An electrode prepared from this matrix demonstrated stability in the pH range from 6.0 to 12.0, in 1.0 mol dm^{-3} potassium chloride at a scan rate of 10 mV s^{-1}. The new electrode did not show significant changes in response over a month of use, demonstrating good chemical stability. All these electrode characteristics demonstrated that they can be promising candidates for further application as a biosensor.

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