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Preparation and characterization of carboxymethyl cashew gum grafted with immobilized antibody for potential biosensor application

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ABSTRACT

This report details the design of carboxymethylated cashew gum (CG) as a platform for antibody (Ab) immobilization, which can then be used as a biosensor for bacteria detection. The CG was isolated and characterized, followed by conversion to carboxymethyl cashew gum (CMCG). The CMCG film was a viable support for antibody immobilization; it was electrodeposited on gold surface using the cyclic voltammetry technique, applying a potential sweep from -1.0 V to 1.3 V with a scan rate of 50 mV s^{-1} and 10 scans. The COOH groups on the surface of the film were critical in promoting Ab bonding. The immobilization of the Ab was mediated by protein A (PrA) for recognition of the antigen. Voltammetry studies were used to monitor the antibody immobilization. Finally, the analytical response of the CMCG-PrA-Ab system was evaluated with the chronoamperometry technique and was found to detect *Salmonella* Typhimurium bacteria rapidly and efficiently.

1. Introduction

The cashew gum (CG) is an exudate extracted from *Anacardium occidentale* L. tree, a member of the family Anacardiaceae. The CG has received a great deal of attention from researchers, mainly due to its similarity to gum Arabic (viz., molar masses, uronic acid content, and same type of monosaccharides units) as well as its availability and potentially low cost as a by-product of the cashew industry (Paula, Sombra, Cavalcante, Abreu, & De Paula, 2011). The CG from Brazil is a branched acidic heteropolysaccharide of low viscosity, comprising β-D-galactose (72%), α-D-glucose (14%), α-L-arabinose (4.6%), α-L-rhamnose (3.2%) and β-D-glucuronic acid (4.5%) (De Paula, Heatley, & Budd, 1998). However, the proportions of the monosaccharides vary, depending on the seasonality of *A. occidentale*, e.g., source, age of the tree, time of exudation, and climatic conditions (de Paula & Rodrigues, 1995).

The technological and commercial interest in cashew gum comes mainly from its biodegradability and particularly its biocompatibility. There have been numerous studies of CG polysaccharides that explored potentially new applications. Some notable examples include its use in the preparation of nanoparticles for the purpose of transport and delivery of substances (da Silva, Feitosa, Paula, & de Paula, 2009), microcapsule synthesis for use as lipid delivery and storage vehicles (da Silva et al., 2018; Gomez-Estaca, Comunian, Montero, Ferro-Furtado, & Favaro-Trindade, 2016), synthesis of microcapsules for larvicides (Paula et al., 2011), potential use as a chromatographic matrix and bioaffinity ligand for proteins (Lima, da, Lima, de Salis, & de A. Moreira, 2002), new polypyrrole/CG composite grown on gold surface (Castro et al., 2017), CG films with potential application in nanobiomedical devices development (Araújo et al., 2012), and a possible novel platform for enzymes immobilization (Silva, Santiago, Purcena, & Fernandes, 2010).

In some cases, CG in its isolated natural form does not have adequate properties for the desired function, so it is common to employ chemical modification strategies in order to improve its properties. Carboxymethylation is one of most used reactions for polysaccharide derivatization (Heinze & Koschella, 2005; Silva et al., 2004). The product obtained is usually a polyelectrolyte that can be applied in a wide variety of fields, e.g., in the chemical, food, pharmaceutical, and cosmetic industries. Other advantages of carboxymethylation reactions include the ease of processing, the low cost of the chemicals used for modification, and the non-toxicity of the products (Verraest, Peters, Batelaan, & van Bekkum, 1995). Carboxymethylation of cashew gum has been previously reported (Olusola, Toluwalope, & Olutayo, 2014;

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Silva et al., 2004). The CG in its natural form has carboxylic groups in its structure; however, to improve the binding sites for biomolecules, it is necessary to increase the amount of COOH via carboxymethylation.

The detection of bacteria during the production process is a mandatory routine in the food industry, as determined by national and international regulatory entities (Melo et al., 2016). It is a laborious, time-consuming and complex process that often entails a large laboratory facility and expensive materials. In the case of the *Salmonella* genus, there is a need for a pre-enrichment step of the sample that takes around 24 h. The bacterial level determined by international health surveillance authorities for this pathogen is below detection in 25 g or mL of food sample (Melo et al., 2016). Thus, the pre-enrichment step is necessary to raise the population to a level of 10^4 CFU mL^{-1}, considered the detectable lower limit for *Salmonella* detection methods in both rapid tests and conventional culture methods (Lee, Runyon, Herrman, Phillips, & Hsieh, 2015). In contrast, biosensors are recognized as quick and practical analytical tools, especially in the biomedical area. The efficiency of its use for the detection of bacteria in food has already been proven by several studies (Alexandre et al., 2018; Melo et al., 2016, 2018; Poltronieri, Mezzolla, Primiceri, & Maruccio, 2014; Rubab, Shahbaz, Olaimat, & Oh, 2018). In the development of such devices, a convenient platform is desired that allows the integration of the biomolecules to a conductive surface, which captures the analyte detection signal and results in an analytical response.

This study proposes the use of carboxymethyl cashew gum (CMCG) as a platform for antibody (Ab) immobilization. We carried out a systematic study, where the polysaccharide from CG was isolated and partially characterized, followed by carboxymethylation to form CMCG. Electrodeposition was then conducted on the gold surface, and antibody immobilization carried out. Finally, the analytical response of the CMCG-Ab assembly was evaluated in the presence of the bacterium *Salmonella* Typhimurium, with the purpose of using the bio-sensing assembly as a food safety tool.

2. Experimental section

2.1. Apparatus and electrodes

Electrochemical measurements were carried out with an AUTOLAB potentiostat (Ecochemie, The Netherlands) using the software Nova 2.0 (Metrohm Autolab B.V., The Netherlands). A 10-mL glass electrochemical cell was utilized in the experiments, consisting of a gold electrode (Φ = 0.02 cm^2) as working electrode, Ag|AgCl|KCl 3 M as reference electrode and a platinum (Pt) wire as counter electrode (Φ = 0.04 cm^2).

2.2. Reagents and biological materials

Horseradish peroxidase (HRP) (250 U mg^{-1}), glutaraldehyde (25%), N-hydroxysuccinimide (NHS), N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), protein A, bovine serum albumin (BSA), hydroquinone, and hydrogen peroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). The culture medium, brain heart infusion agar (BHI agar), brain heart infusion broth (BHI broth), nutrient agar, and nutrient broth were acquired from Difco (Becton, Dickinson and Company, Sparks, MD, USA). All solutions and culture medium were prepared with ultrapure water from Direct-Q® 3 UV purification unit (Millipore Corporation, Billerica, MA, USA).

Salmonella Typhimurium (ATCC 51812) was purchased from Microbiologics (Saint Cloud, MN, USA). The lyophilized strains were activated in BHI broth at 35 °C for 24 h and sub-cultured in BHI agar at 35 °C for 24 h. Cultures were maintained in BHI agar inclined and stored at 4 °C until used.

Stock cultures were maintained in BHI broth supplemented with 25% glycerol at - 80 °C. Polyclonal rabbit antisera, Difco™ *Salmonella* Antiserum Poly A-I & Vi, were properly rehydrated and used as recommended by the manufacturer. The antibodies were purified by precipitation with $(NH_4)_2SO_4$ at 45% saturation (Green & Hughes, 1955), and the concentration was determined by Bradford method (Bradford, 1976). The antibody solutions were prepared by dilution in phosphate buffer saline (PBS) (10 mM). The secondary antibody was conjugated to HRP according to Avrameas (2003).

2.3. Origin and isolation of CG

CG was obtained from exudate of *Anacardium occidentale* L., collected from native trees of the Experimental Field of Embrapa Agroindústria Tropical. The polysaccharide isolation from CG was performed using the methodology previously described by Torquato et al. (2004) with modifications. The exudate was initially triturated in a knives mill. Then, the triturated sample was solubilized in water in the proportion of 300:1 (g/L), filtered and centrifuged (10,000 rpm for 10 min at 25 °C) for residue removal. After removal of the residues, the supernatant was precipitated in 96% ethanol in a ratio of 1:3 (v/v) for 24 h under refrigeration. Ethanol was removed and the precipitate was dried in an air-circulating oven at 60 °C for complete drying. Thereafter, the sample was milled, and the isolated CG was obtained. Finally, the isolated CG was submitted to the freeze-drying process and stored in vacuum sealed packages until use.

2.4. CG characterization

2.4.1. Centesimal composition and phenolic compounds

The humidity determination was performed according to the AOAC Method 934.01 (AOAC, 2005), total nitrogen/protein according to Method 984.13 (AOAC, 2005), ash content according to Method 923.03 (AOAC, 2005), and ethereal extract according to Method 945.38 (AOAC, 2005). The determination of the total phenol content present in the ethanol was made by means of UV/visible spectroscopy (λ = 740 nm) using the Folin-Ciocalteu method with modifications (Sanctis, 2004).

2.4.2. Metal characterization

The sample was subjected to nitric-perchloric acid digestion to obtain the extract according to the procedure described by Miyazawa, Pavan, Muraoka, Carmo, and Melo (2009), followed by quantification of phosphorus, potassium, calcium, magnesium, sulfur, sodium, copper, iron, manganese and zinc by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.4.3. Molar mass by GPC

The molar mass distribution was determined by gel permeation chromatography on a Shimadzu LC-20CE equipment coupled to a refractive index detector (RID-10A). For analysis, a linear polysep column, 300 × 7.8 mm, using $NaNO_3$ (aq) 0.1 mol L^{-1} as the eluent was used. The measurement was made at 30 °C with a flow rate of 1 ml min^{-1} and an injected volume of 50 µL. The molecular mass (M) was converted from elution volume (Ev) with the following relationship: Log M = 14.40967–1.1392 Ev.

2.5. Carboxymethylation of cashew gum

Carboxymethyl cashew gum (CMCG) was prepared following a protocol reported previously by Silva et al. (2004)). The purified gum (5 g) was mixed with 5 ml of deionized water until a homogeneous paste was formed. A 10 M NaOH solution (8.3 mL) was added and the mixture was kneaded for 10 min. After that, monochloroacetic acid (MCA) (2.6 g) was mixed in thoroughly with the paste. The mixture was heated at 55 °C for 3 h. The product was neutralized with 1 M HCl and dialyzed against deionized water until the reagents or salts were eliminated (monitored by water conductivity). Finally, the CMCG was submitted to the freeze-drying process and stored in vacuum-sealed packages.

2.5.1. Attenuated total reflection/Fourier transform infrared (ATR–FTIR)

ATR–FTIR analysis was performed on isolated CG and CMCG to confirm the carboxymethylation reaction. The measurement was made by pressing the electrode against the ATR crystal on a FTIR spectrometer (model FTLA 2000–102, ABB-BOMEN, USA). All spectra were recorded in the range from 600 to 4000 cm^{-1} at 4 cm^{-1} resolution, averaging over 128 scans.

2.5.2. Nuclear magnetic resonance (NMR)

NMR spectra were obtained using a 600 MHz Agilent DD2 instrument (Santa Clara, CA, USA) equipped with a reverse-detection 5 mm internal diameter (HF/15N-31 P) "One-Probe" probe and field gradient in the "z" direction. Samples were prepared by dissolving approximately 10 mg of the CG samples in 0.55 ml D_2O containing 1% sodium trimethylsilyl propionate, (TSP-d_4, v/w). The one-dimensional ^1H spectrum was acquired at 80 °C with a 10 s time between each acquisition, accumulation of 64 transients, with a spectral window of 16 ppm and 32k data points. The one-dimensional ^{13}C spectrum was obtained with a 1 s time between each acquisition, accumulation of 30k transients, with a spectral window of 251.3 ppm and 32k data points. The ^{13}C peaks of the anomeric carbon of cashew gum in the region between 102 and 106 ppm and the carboxyl resulting from carboxymethylation between 165 and 180 ppm were integrated to obtain the relative percentage of both in the sample.

2.6. CG electrodeposition

All the samples were evaluated by cyclic voltammetry technique in 4 mM $K_3[Fe(CN)_6]$ and 1 M KCl solution using a potential that ranged from - 0.30 V to 0.75 V and a scan rate of 100 mV s^{-1}. First, the effect of the use of isolated CG (ICG) versus CMCG was observed for the immobilization of the antibody. Thereafter, the optimal concentration for the electrodeposition of the CMCG film on the gold electrode surface was found. Finally, the impact of the use of protein A in the antibody immobilization was checked.

2.7. Antibody immobilization on CMCG

Initially, the gold electrode surface was cleaned by polishing with alumina (3 μm) for 5 min, followed by immersion in 96% ethanol and sonicated for 5 min in an ultrasonic bath. The cleaned gold surface was modified by CMCG electrodeposition using the cyclic voltammetry technique, applying a potential sweep of -1.0 V to 1.3 V with a scan rate of 50 mV s^{-1} for 10 scans. The modified electrode was immersed in N-hydroxysuccinimide/N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC/NHS) solution (2 mM/5 mM) for 1 h. After washing with PBS buffer (pH 7.4), the electrode was immersed in the protein A solution for 1 h. Finally, the electrode was incubated overnight in an anti-*Salmonella* solution. Protein A and anti-*Salmonella* concentrations were determined during previous optimization studies with the cyclic voltammetry method. The non-specified sites of the modified electrode were blocked with 1% BSA for 1 h.

2.8. Bacteria detection

The analytical response for bacteria detection was obtained by the chronoamperometric technique. Initially, the modified electrode (CMCG-prA-Ab-BSA) was immersed in 100 μL *S.* Typhimurium (10^6 CFU mL^{-1} in 0.1 mol L^{-1} PBS, pH 7.4) for 1 h, and PBS buffer was used as the control. The electrode was incubated with HRP-labeled secondary antibodies for 1 h, forming a sandwich system. After each incubation step, the electrode was rinsed with PBS. The amperometric response was carried out in the electrochemical cell (10 mL) containing 0.1 mol L^{-1} PBS (pH 7.4), 300 mM H_2O_2 and 3 mM hydroquinone. The response was determined by polarizing the gold electrode at -75 mV until a stable baseline (steady state) was reached in 120 s. All measurements were carried out at room temperature.

A scanning electron microscope (SEM; Quanta 450 FEG System: FEI Company, USA) was used to observe the surface morphology of the CMCG-PrA-Ab system before and after the *S.* Typhimurium detection. The images were obtained using a scanning voltage of 20 kV.

2.9. Milk analysis

Skimmed Ultra High Temperature (UHT) milk was purchased from a local market. Milk samples were first spiked with *S.* Typhimurium at a concentration of 10^1 CFU mL^{-1}. The amperometric response was obtained according to Section 2.8 above, after immersion of the immunosensor in the spiked milk samples for one hour at room temperature.

3. Results and discussion

3.1. CG characterization

Materials from plant origin are known to show variation in their compositions because they are susceptible to the influence of geography, biotic and abiotic factors (De Paula & Rodrigues, 1995). Therefore, it is important to characterize the crude cashew gum used in this work.

The following determinations were conducted on the crude cashew gum: centesimal composition, amounts of phenolic compounds, metal profile, and molar mass. The centesimal composition and the phenolic content are summarized in Table 1.

In general, plant gums are amorphous substances containing mostly carbohydrates. The centesimal composition of cashew gum used in this study displayed values close to those reported in the literature, viz., 7.4–11.1 % water, 0.15-0.75% protein, 0.06% lipid, 0.9–1.7 % ash and ca. 95% carbohydrates (Anderson, Bell, & Millar, 1974; De Pinto, Martinez, Mendoza, Ocando, & Rivas, 1995; Lima et al., 2002). In many cases small amounts of nitrogen were detectable that may be traced to the proteinaceous debris arising from enzymes, such as oxidases (peroxidases and polyphenol oxidases), found in cashew gum and generally associated with the response of the plant to infection by pathogens (Rita Marques & Xavier-Filho, 1991). The presence of phenolic compounds was also detected, which are known to be related to the defense mechanisms of plants, and their concentrations in ICG are similar to those reported in the literature (Rita Marques & Xavier-Filho, 1991).

The metals (Table 2) and molar mass (Table 3) associated with the ICG sample were also determined. Metals can interact with enzymes by modifying their activity and thereby interfering with the response of a biosensor. The molar mass data of ICG indicate a distribution of molecular weight with a polydispersity index (PDI) of 2.61.

3.2. Carboxymethyl CG characterization

The ICG was subjected to carboxymethylation reaction (Fig. 1a) in order to increase the amount of carboxylic groups on its structure. This reaction is based on the Williamson synthesis, whereby the polysaccharide alkoxide is reacted with monochloroacetic acid (Ege, 1989) and substituted with carboxymethyl groups. Fig. 1 shows the

Table 1
Centesimal composition and phenolic compounds content of the isolated cashew gum.

Sample	Centesimal composition (%)					Phenolic compounds (mg $100\ g^{-1}$)
ICG[a]	Protein	Water	Ether extract	Ash	Carbohydrate	
	0.76	4.43	0.09	0.63	94.09	143.85

[a] ICG = Isolated cashew gum.

Table 2
Profile of metals determined in the isolated cashew gum sample.

Sample	Metals									
	g Kg^{-1}						mg Kg^{-1}			
	P	K	Ca	Mg	S	Na	Cu	Fe	Zn	Mn
ICG[a]	0.01	1.03	1.46	1.44	0.02	0.21	1	3	1	38

[a] ICG = Isolated cashew gum.

Table 3
Molar mass (g mol^{-1}) of the isolated cashew gum sample.

Sample	Mp[a]	Mn[b]	Mw[c]	PDI[d]
ICG[e]	2.13×10^4	8.16×10^3	2.13×10^4	2.61

[a] Mp = most probable molecular weight.
[b] Mn = number-average molecular weight.
[c] Mw = weight-average molecular weight.
[d] PDI = polydispersity index.
[e] Isolated cashew gum.

carboxymethylation reaction and the FTIR and ^{13}C NMR spectra of starting and modified cashew gum.

ATR-FTIR spectra (Fig. 1b) showed the presence of a band around 1730 cm^{-1} in the ICG spectrum for C═O stretching vibration of the carboxyl moiety of glucuronic acid (De Paula et al., 1998). A substantial increase in the absorbance of C═O vibration was observed in the CMCG spectrum caused by the introduction of more carboxylic groups after the reaction with monochloroacetic acid.

^{13}C NMR spectroscopy is a sensitive tool to evaluate chemical modification of polysaccharides. Fig. 1c gives the ICG and CMCG spectra. ^{13}C NMR spectrum of CMCG showed a carboxyl peak at 178 ppm, as evidence of the carboxymethylation reaction (De Paula et al., 1998). An increase in peak intensities between 85 and 70 ppm attributed to carbons from C-2 to C-5 of monosaccharide residue might be due to CH_2 groups of $-CH_2COOH$ of MCA and also to the shift of primary carbons (C-6) from the region around 60 to 66–69 ppm, after the substitution of $-CH_2COOH$ group on the primary carbon.

3.3. Voltammetry studies for CG electrodeposition

The cyclic voltammogram performed according to the strategies of using CG as a support for immobilization of the antibody are summarized in Fig. 2.

The ICG film was electrodeposited onto the surface of the gold electrode and subsequently exposed to antibody immobilization (ICG-Ab). The same procedure was also applied for CMCG electrodeposition and for antibody immobilization (CMCG-Ab). In the voltammogram for the antibody immobilization step, a greater reduction in the cathodic and anodic peak currents for CMCG-Ab was verified, while in the ICG-Ab voltammogram the peaks had a lower current reduction because there was a smaller hindrance to the flow of electrons. Thus, the cathodic and anodic peak reduction would be related to the amount of antibody molecules immobilized on the surface of CMCG, which was proportionally higher than in ICG.

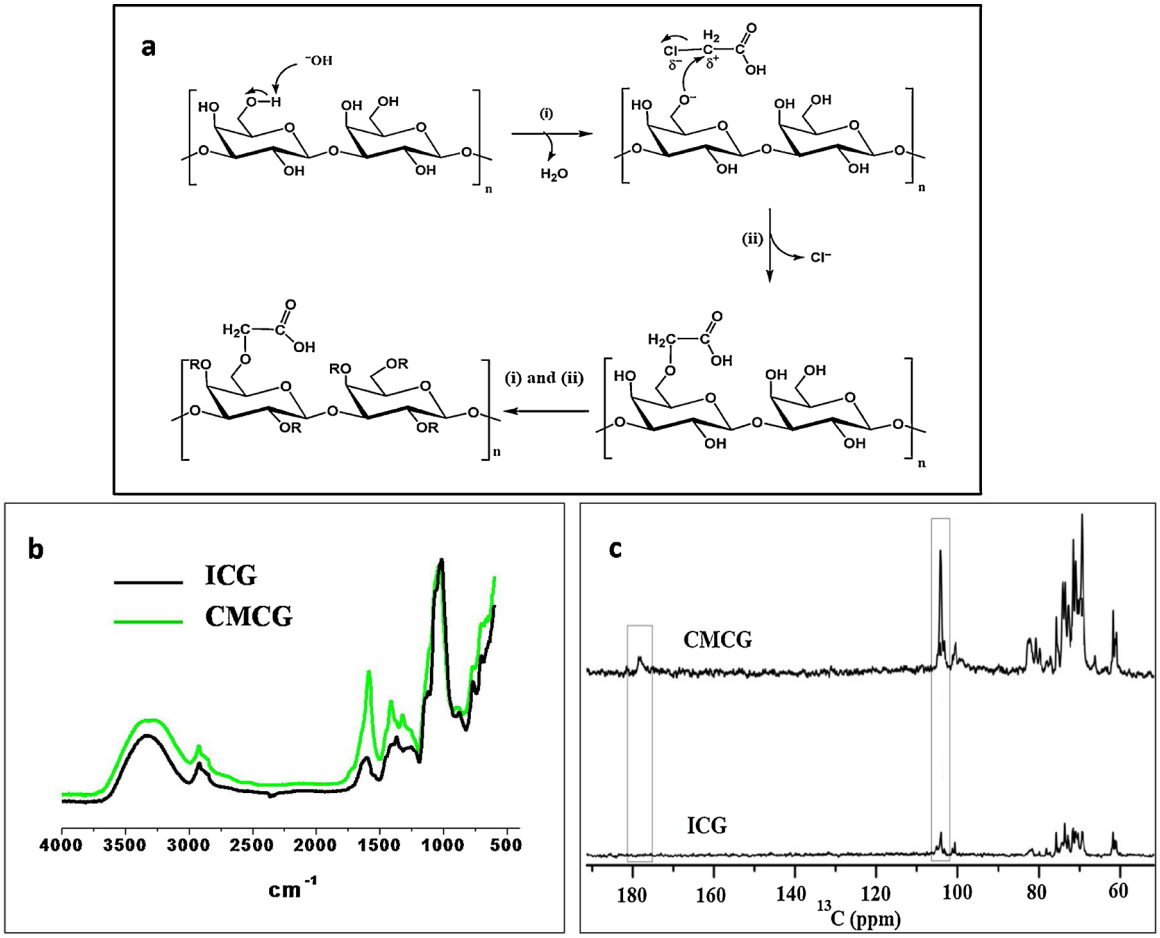

Fig. 1. a) Carboxymethylation reaction for a polysaccharide. R may be H or CH_2COOH group, depending on the progress of carboxymethylation (Silva et al., 2004). b) ATR-FTIR spectra of isolated cashew gum (ICG) and carboxymethyl cashew gum (CMCG). c) ^{13}C NMR spectra in D_2O of ICG and CMCG.

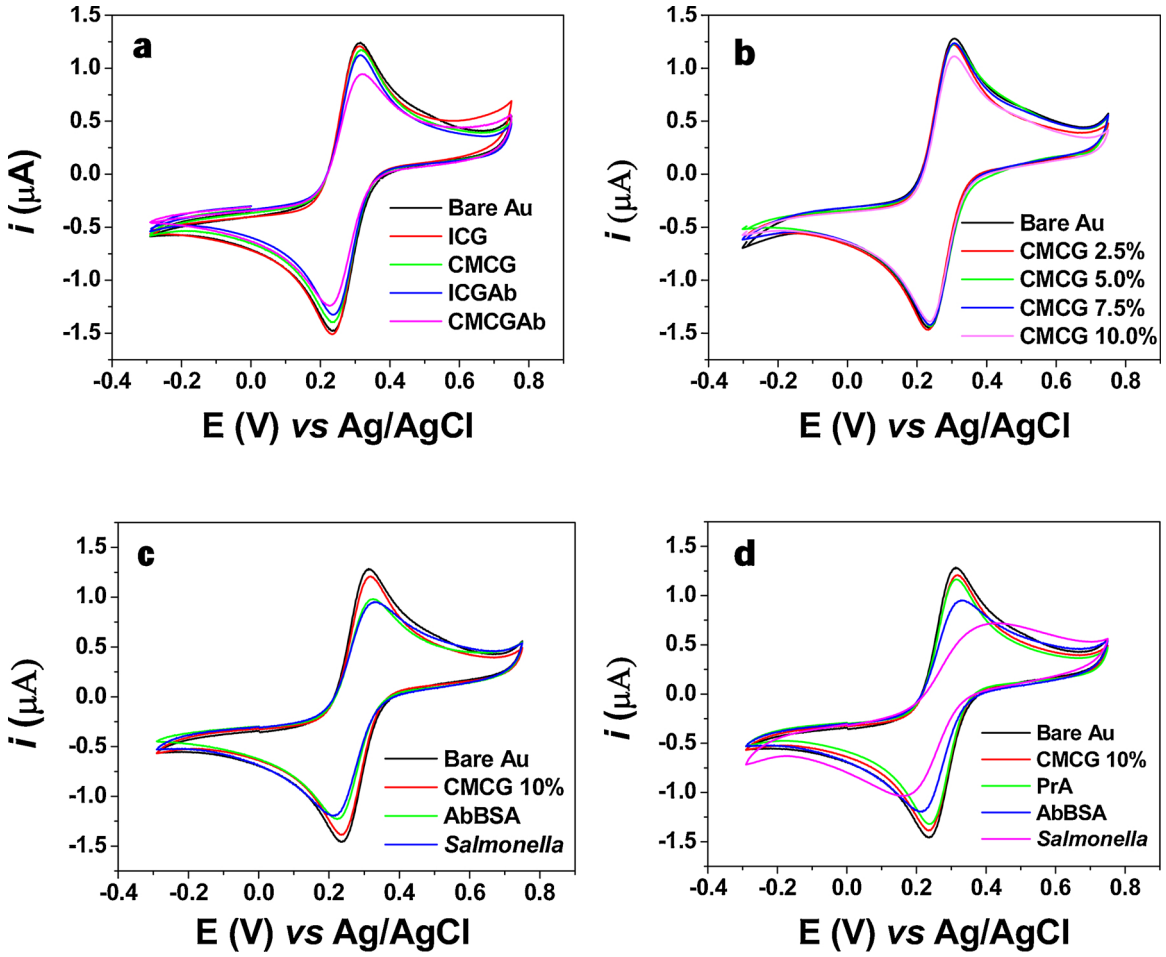

Fig. 2. a) Cyclic voltammograms in 4 mM $K_3[Fe(CN)_6]$ and 1 M KCl solution at each step of ICG and CMCG electrodeposition and antibody immobilization (ICG-Ab and CMCG-Ab). b) Cyclic voltammograms in 4 mM $K_3[Fe(CN)_6]$ and 1 M KCl solution of CMCG electrodeposition in different concentrations (2.5–10 %). c) Cyclic voltammograms in 4 mM $K_3[Fe(CN)_6]$ and 1 M KCl solution at each step of biosensor assembly without protein A, d) assembly with protein A.

This result corroborates the data presented in Fig. 1, where it was observed in the CMCG a greater amount of COOH clusters, a result promoted by the reaction of carboxymethylation. The carboxymethylation of natural polymers has been shown to increase the efficiency of immobilization of biomolecules on their surfaces and seems to be a promising technique for the development of analytical devices with CG-based platforms (El-Sheikh, 2010; Hebeish & Khalil, 1988); in addition, it increases the adsorption of the CG film on the gold surface (Paik, Han, Shin, & Kim, 2003), thus improving its performance as a support for biomolecules immobilization on metallic surfaces. The activation of COOH groups (to become COO^-) on the surface and the Coulombic interaction between this functional group and the amino group of the biomolecules (NH_2) was facilitated via the EDC/NHS treatment (Ricci, Adornetto, & Palleschi, 2012). The voltammograms of Fig. 2a show clearly that the use of CMCG for the immobilization of the antibody was more efficient than the use of ICG; thus, our remaining studies were focused on CMCG only.

Different concentrations of CMCG were electrodeposited on gold surface and their voltammograms are shown in Fig. 2b. When electrodeposited on the surface, the gum film promotes the reduction of the electric current of the anodic and cathodic peaks of the voltammograms as evidence of the modification of the gold surface. An estimate of the percentage coverage of the surface (θ) can be made with the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ probe using Eq. (1):

$$\theta = 1 - (Ipmodified\ electrode/Ipcleaned\ electrode) \times 100 \quad (1)$$

where Ip is the electric current peak of the modified and cleaned electrode obtained from voltammograms shown in Fig. 1b. By varying the peak current, we can estimate the surface coverage (Alexandre et al., 2018). In view of the different concentrations tested, the result that best illustrated the modification of the gold surface was the 10% CMCG because it was the concentration with the best coverage, estimated at 5.7% on the surface of the gold electrode. Good surface coverage is generally related to efficient immobilization of biomolecules as observed in previous studies (Alexandre et al., 2018; Melo et al., 2018).

The optimum experimental conditions for the immobilization of the antibody (Ab) molecules on the 10% CMCG film were investigated next. Fig. 2c gives the voltammograms of the biosensor assembly without protein A, and Fig. 2d gives the voltammograms of the assembly using protein A molecule in order to facilitate the orientation of the Ab immobilization. In the absence of protein A, there was a minimum reorganization of the *Salmonella* antigen; this probably occurred because the COOH groups of the CMCG were indistinctly bound to the Ab molecule and possibly blocked the Fab (the fragment antigen-binding) region of the Ab. This is evidenced by the voltammogram in the presence of *Salmonella* being practically in the same position of the Ab voltammogram (Fig. 2c). This behavior demonstrates the importance of protein A for both antibody immobilization and antigen interaction. In Fig. 2d, the voltammogram of *Salmonella* shows smaller peak currents than the antibody voltammogram. Such a reduction indicates that the bacterium *Salmonella* is bound to the antibody. This happens because the protein A binds specifically to the Fc region (the crystallizable fragment) of Ab, leaving the Fab region free for antigen recognition.

Earlier studies have shown that oriented antibodies on the electrode

surface produced improved specificity and sensitivity for the *Salmonella* Typhimurium detection (Gopinath, Tang, Citartan, & Chen, 2014). Danczyk et al. (2003) compared the antibody functional behavior using different immobilization methods and concluded in their studies that protein A is able to orient the antibodies, allowing for greater antigen capture per antibody present on the surface. By improving the immobilization of primary antibodies on the surface, the bio-device becomes more cost-effective by reducing the amount of antibodies that are blocking the surface rather than capturing antigen. In our research group, successful experiments have been previously conducted through the use of protein A in the development of *Staphylococcus aureus* toxin immunosensor (Pimenta-Martins et al., 2012) and detection of *Salmonella* bacteria (Alexandre et al., 2018; Melo et al., 2018).

3.4. Bacteria detection

Our biosensor's ability to detect the presence of *S.* Typhimurium was verified using the chronoamperometry technique. The results are summarized in Fig. 3.

The chronoamperometry technique was used to verify the analytical response of the CMCG-PrA-Ab system in the presence of *S.* Typhimurium (10^6 CFU mL^{-1}). The measurements were conducted in triplicates. Fig. 3a shows the typical chronoamperograms representing the baseline signal, the control (PBS), and measurements of *S.* Typhimurium in the range of concentrations tested. The baseline represents the noise of the electrochemical system, being obtained from the CMCG-PrA-Ab system in the electrochemical cell with PBS without hydrogen peroxide and hydroquinone. In the *S.* Typhimurium chronoamperograms, a fast degradation of H_2O_2 was observed in the first 20 s. After that, the current stabilized, indicating that most of the hydrogen peroxide present in the system had been consumed by the HRP enzyme. It is clear in Fig. 3a and b that the CMCG-PrA-Ab system developed was effective in detecting *S.* Typhimurium bacteria in the PBS buffer.

Additionally, the immunosensor presented a limit of detection (LOD) of 10 CFU mL^{-1} for S. Typhimurium; this was lower than the previous limit reported by Melo et al. (2016). The LOD is one of the most important performance parameters of a biosensor, and it demonstrates the device's ability to detect the lowest analyte concentration in a sample. The LOD was obtained from Eq. (2) where $\bar{x}$ is the average of multiple determinations, t is the student distribution factor, and SD is the standard deviation.

$$LOD = \bar{x} + t(n-1,\ 1-\alpha)^* SD \quad (2)$$

Moreover, the detection time was 125 min, which included the incubation time for recognition of antigen in the PBS, the time for the binding of HRP-labeled antibody, and the time for amperometric measurement. This result makes the current system a promising bio-device to be used as a viable method for *Salmonella* detection in the food industry, especially for the rapid emergency screening of contaminated samples.

Furthermore, the CMCG-PrA-Ab system was studied by scanning electron microscopy (SEM) before and after the recognition of the antigen and binding of the HRP-labeled antibody. *S.* Typhimurium cells were clearly observed on the surface as further evidence of the antigen-antibody interaction (Fig. 3c), again demonstrating the effectiveness of the detection system. These results confirm the utility of cashew gum in the development of bioelectronic devices as demonstrated earlier by

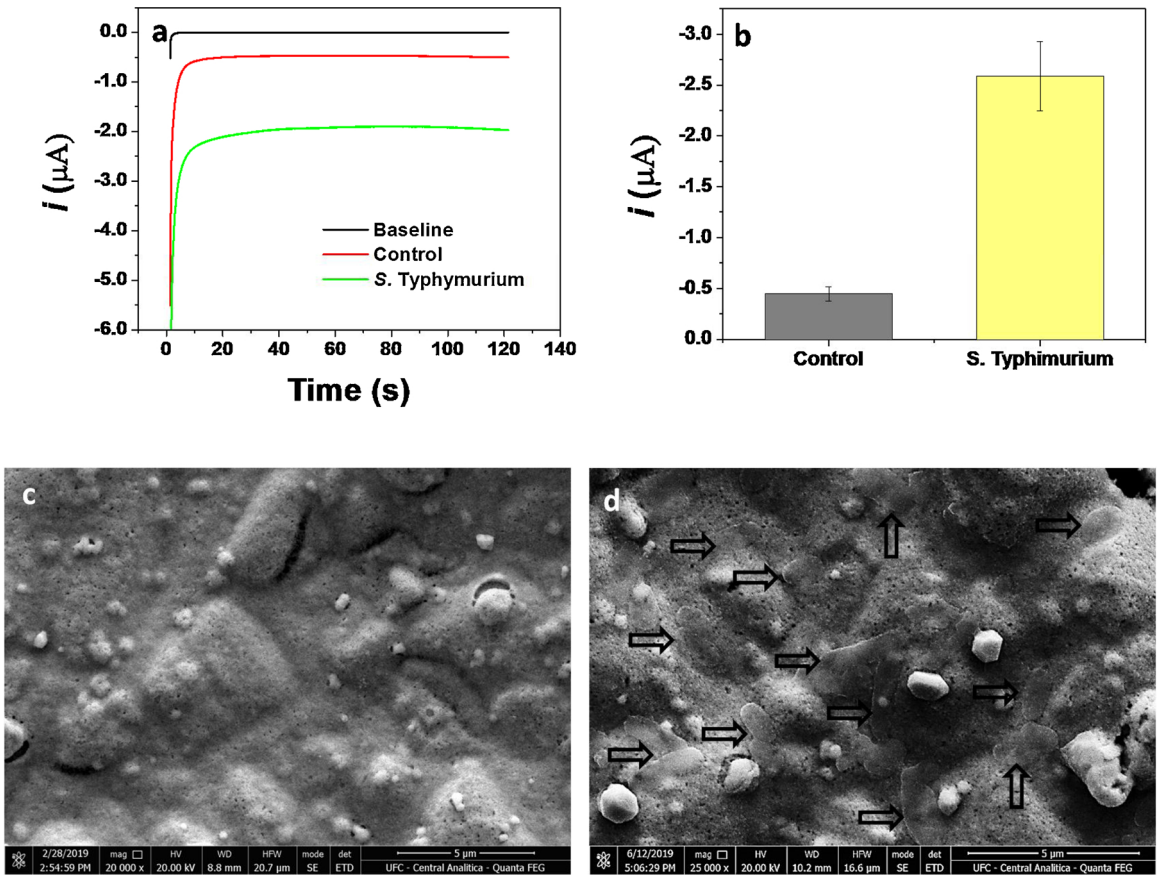

Fig. 3. Current response of the biosensor. a) Chronoamperometric data obtained for baseline, control (PBS), and three measurements of *S.* Typhimurium 10^6 CFU mL^{-1} in PBS solution (pH 7.4) containing 3 mM hydroquinone and 300 mM H_2O_2. b) Graphical representation of the analytical response. The amperometric measurements were determined by polarizing the electrode at −75 mV until a stable baseline (steady state) was reached in 120 s. c) Scanning electron photomicrograph of the CMCG-PrA-Ab system. The arrows indicate the *S.* Typhimurium captured by the antibody immobilized on the CMCG film surface.

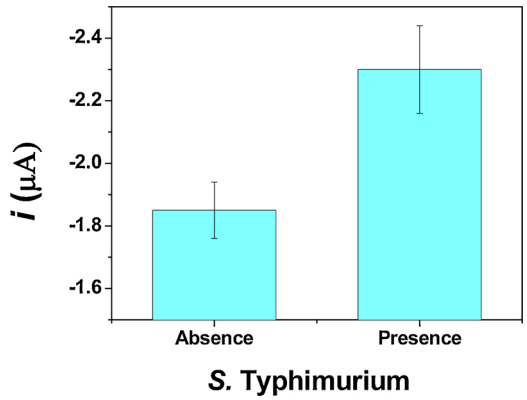

Fig. 4. Amperometric response of the CMCG-based immunosensor the absence and in the presence (10^1 UFC mL^{-1}) of *S.* Typhimurium in skimmed milk samples. Results were obtained in 10 mM PBS buffer (pH 7.4) in the presence of H_2O_2 (300 mM) and hydroquinone (3 mM), with 75 mV potential for 120 s.

Araújo et al. (2012) who developed a device for the detection of dopamine and by Silva et al. (2010) who immobilized the peroxidase enzyme on the cashew gum.

3.5. Application in milk samples

The feasibility of the CMCG-based immunosensor to detect bacterial contamination in a complex food matrix was evaluated in the absence and the presence (10^1 UFC mL^{-1}) of *S.* Typhimurium in skimmed milk samples (Fig. 4).

The analysis was performed in a detection time of 125 min. It is important to note that most commercial alternative methods for *Salmonella* detection in food (such as immunology assays, nucleic acid tests, and miniaturized biochemical assay) require a 24 -h pre-enrichment step in order to elevate the *Salmonella* concentration to 10^4 - 10^5 CFU mL^{-1}, which is the limit of detection for those procedures (Lee et al., 2015). The device developed in this study is very rapid because it does not require sample pre-enrichment and thus can provide the desired result in about 2 h. Furthermore, in view of automated integration and portability of the device, this methodology represents a simple, fast, easily handled, and accurate tool for bacterial detection in order to ensure food safety.

4. Conclusions

One of the thrusts in our laboratories is to develop biosensors for food applications, e.g., the design of self-assembled thiol monolayers for *Salmonella* detection (Alexandre et al., 2018; Melo et al., 2018). The present work represents an example of the use of a polysaccharide extracted from renewable sources for the development of bio-devices, substituting synthetic polymeric platforms with the cashew gum derivative. Thus, a new biosensor has been designed based on the CMCG platform, where a polyclonal antibody (Ab) was immobilized on CMCG with the help of protein A (PrA). This biosensor is capable of detecting the presence of the *S.* Typhimurium bacteria dispersed in PBS buffer. This result opens up a range of possibilities for the use of CG and its derivatives to immobilize biomolecules that will function as bioreceptors in the future development of biosensors and bio-devices for rapid tests for various analytes.

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