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Conductive Biofilm Propolis-Based: Electrochemical Determination of Hydroxymethylfurfural in Honey

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Abstract

Honey samples have been widely evaluated regarding the presence of degradation products as an index quality control, for example, hydroxymethylfurfural (HMF) in the presence of carbohydrates. The monitoring of HMF strategy can be a challenge; however, catalyst-based electroanalytical approaches can overcome it. This work aims the use of propolis to promote the anchoring of carbon nanotubes and Ni^{2+} onto the electrode surface, as well as the synthesis of nickel oxyhydroxide. The modified electrode was applied as an electrochemical sensor in the determination of HMF and total carbohydrates, and the quantification of HMF in honey samples. The sensor has shown high sensitivity to successive additions of the evaluated species, presenting LOD of 0.18 and 0.051 mmol L^{-1} for HMF and total carbohydrates, respectively. In the analyses of honey samples, recovery values between 91.8 and 107% of HMF were achieved. Therefore, the proposed modified sensor showed potential for use as a greener alternative for honey quality control.

Keywords Electrochemical sensor · Conductive bio-composite · Bee-glue · Nickel oxyhydroxide · Hydroxymethylfurfural · Honeybee quality control

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Introduction

The presence of toxic species in honey has been monitored over the years, for example, pesticides, herbicides, and heavy metals, as well as the presence of degradation products as an index quality control (da Silva et al. 2016; Souza Tette et al. 2016). One of the most evaluated index control molecules is the hydroxymethylfurfural. 5-Hydroxymethylfurfural (HMF) is an aromatic aldehyde, aromatic alcohol, and a furan ring compound at the same time (Kowalski 2013; Menegazzo et al. 2018). It can be spontaneously formed from different kinds of carbohydrates, through the Maillard reaction (Spano et al. 2006). However, the presence of HMF in carbohydrate-based foods, such as honey, may be an indication of adulteration, maturity, or improper storage of the product (J. W. White 1994; White Jr. and Siciliano 1980). The determination and monitoring of HMF are commonly performed using spectroscopic methods, as suggested by the International Honey Commission (Bogdanov et al. 2002; Spano et al. 2006; White Jr. 1979). On the other hand, this methodology makes use of potentially carcinogenic solvents and presents a systematic positive interference (Besir et al.

2021; Winkler 1955). Therefore, the development of methodologies based on electrochemical devices has become an interesting alternative to spectroscopic methods for the monitoring of HMF. It allows its determination in aqueous matrices without the use of toxic solvents, as well as low consumption of reagents, low generation of waste, and rapid quantification (Ferrari et al. 2021).

In general, the electrochemical determination of HMF has been performed by voltammetric techniques, due to its relatively low cost, simplicity, high sensitivity, fast response, and ease of data acquisition (Compton and Banks 2018; Kaya et al. 2021; Mann and Grunwald 1959). This is usually performed by monitoring the redox process of the direct and irreversible reduction of HMF to bis(hydroxymethyl)furan at negative potential values, less than -1.0 V vs. Ag|AgCl (Reyes-Salas et al. 2006; Salhi et al. 2020). This strategy is attractive from a simplicity point of view; however, some aspects are required such as a wide potential window, low background current, and high sensitivity (Salhi et al. 2020).

The determination of HMF in real samples can also be a challenge, due to the presence of a high concentration of carbohydrates. They can influence the hydrogenation reaction of HMF (Kwon et al. 2015), which is not strongly catalyst-dependent, in comparison to the carbohydrates, such as glucose, sucrose, and fructose (Kwon et al. 2015; Kwon et al. 2013). As alternative to overcome this issue, the use of modifiers with a high catalytic capacity to promote the oxidation process of these carbohydrates and, at the same time, achieve the non-catalytic reaction of HMF at more negative potential values. Among these catalytic species, metallic hydroxide oxides such as cobalt, iron, and especially nickel oxide-hydroxide deserve to be highlighted, being the latter one the most widespread and widely reported in the literature (Arvinte et al. 2010; Hlavay and Polyák 2005; Kalinke et al. 2017; Oliva et al. 1982).

Nickel oxyhydroxide (NiOOH) consists of a high-valence oxyhydroxide species (Ni oxidation state from 2.95 to 3.55), being a highly oxidizing species (Fu et al. 2007). This fact makes NiOOH widely used as an electrode modifier to enhance the sensitivity of the electrode for the determination of some analytes, such as in the non-enzymatic catalysis of carbohydrates and alcohols (Salarizadeh et al. 2022; Woo et al. 2022). Obtaining NiOOH in a stable form can take place chemically by simple mixing of its precursors (Pan et al. 2005). Nevertheless, the electrochemical route is most often performed with the precursor nickel ions and high amounts of OH$^-$ ions in an aqueous solution, or using a Ni metal electrode (García-Cruz et al. 2012). Commonly, this strategy produces thin films on the electrode surface. However, the thickness of these films or their particle size can be difficult to control. An alternative is using sorbent materials with specific sites to immobilize the precursor materials before the electrosynthesis step, which promotes

the NiOOH formation at those sites. An example is the work developed by Kalinke et al. (Kalinke et al. 2017), which used a chemically treated biochar as a modifier in a graphite-based composite electrode. The biochar presented high amounts of superficial oxygenated groups, such as hydroxyl and carboxyl that acted as anchoring sites for the spontaneous immobilization of Ni^{2+} ions, allowing the synthesis of nanostructures of NiOOH. Another approach has demonstrated the excellent capability of biofilm-based on propolis and MWCNTs as an anchoring platform for Ni^{2+} ions (Oliveira et al. 2021). Propolis acted as an immobilization network for the CNTs, which increased the conductivity of the unmodified electrode. In addition, the functional groups present in this material, such as carboxyl ($-COOH$), carbonyl ($-CO$), and hydroxyl ($-OH$), also allowed the anchoring of Ni^{2+} ions, and other metals, without electrical potential application.

Therefore, the main goal of this work consists of the use of the Propolis/MWCNTs biofilm to promote the incorporation of Ni^{2+} ions into specific immobilization sites present on propolis and, subsequently, the synthesis of NiOOH. In this context, the modified electrode with the catalyst material was applied as a voltammetric sensor in the electroanalytical evaluation of HMF and total carbohydrates, as well as the quantification of HMF in wild honey samples allowing the monitoring of the honeybee quality.

Experimental

Materials and Solutions

For the biofilm obtaining, multi-walled carbon nanotubes (MWCNTs) were purchased from Aldrich (Milwaukee, WI, USA), the propolis extract 25% (w/v) from Apis Flora (Ribeirão Preto, SP, Brazil), and the isopropyl alcohol PA from Dinâmica (Indaiatuba, SP, Brazil). The sodium hydroxide (NaOH, 98%) from Dinâmica (Indaiatuba, SP, Brazil) was used as a supporting electrolyte. For the electrosynthesis of NiOOH, nickel acetate 98% (Sigma-Aldrich) was used as Ni^{2+} ions source. All aqueous solutions were prepared with ultrapure water (resistivity ≥ 18.0 MΩ cm) using a Milli-Q system (Merck Millipore–Merck Group–Burlington, MA, EUA).

Preparation of Conductive Propolis-Based Biofilm

The conductive biofilm based on propolis and MWCNTs was prepared by homogenizing both components and isopropyl alcohol (5.0 mL) as a dispersant using a Vortex (Vixar–Plymouth, MN, USA) for 5.0 min. The biocomposite (Propolis/MWCNTs) concentration was 0.14 mg mL^{-1} propolis and 0.10 mg mL^{-1} MWCNTs, following previous

Scheme 1 Preparation steps of the biocomposite electrode (Propolis-MWCNTs/GCE) and electrochemistry synthesis of NiOOH after Ni^{2+} ions spontaneous sorption
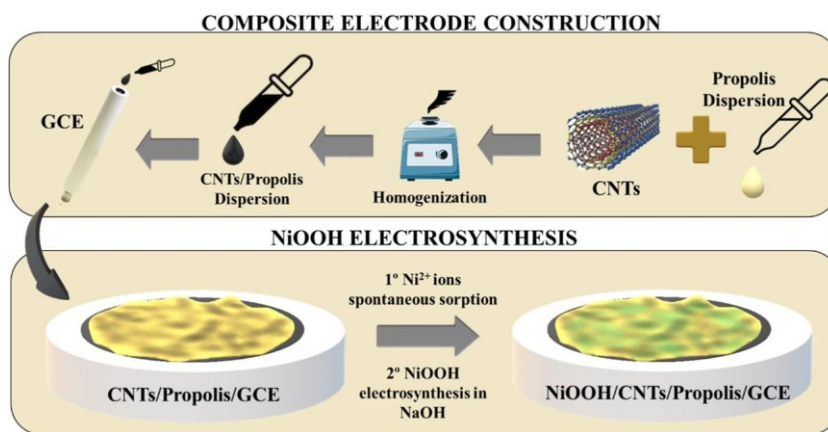
work (Oliveira et al. 2021) in which 5.0 µL of biocomposite dispersion was placed by drop-casting on the glassy carbon electrode surface (GCE, Ø = 5.0 mm) and dried at room temperature (around 25 °C) during 5.0 min.

NiOOH Electrochemical Synthesis

All electrochemical analyses were performed using an array of three electrodes: the Propolis-MWCNTs/GCE was used as the working electrode, a platinum wire as the auxiliary electrode, and the Ag|AgCl|3.0 mol L^{-1} KCl was used as a reference electrode. For these measurements, a potentiostat/galvanostat PGSTAT204 (Metrohm Autolab–Utrecht, Netherlands) was used, managed by NOVA 2.1 software.

The electrochemical synthesis of NiOOH was performed by cyclic voltammetry (CV) using the Propolis-MWCNTs/GCE with anchored Ni^{+2} on its surface, as presented in Scheme 1 (Oliveira et al. 2021). Ni^{+2} ions were immobilized on the proposed electrode surface by spontaneous adsorption using aqueous solutions containing 0.01 mol L^{-1} NiCl$_2$ for 5.0 min under an open potential circuit condition. After the preconcentration step, the electrochemical synthesis of NiOOH on the Propolis-MWCNTs/GCE surface (NiOOH/Propolis-MWCNTs/GCE) was carried out in an electrochemical cell with 10.0 mL of 0.50 mol L^{-1} NaOH under conditions previously optimized: CV from 0.0 to +0.8 V, for 30 scans at 100 mV s^{-1} (Oliveira et al. 2021).

Structural Characterization of NiOOH/Propolis-MWCNTs/GCE

The structural characterization for the Propolis-MWCNTs composite film was performed by scanning electron microscopy (SEM), using a Thermo Scientific Prisma E scanning electron microscope with colorSEM technology (Waltham, MA, EUA). The images were recorded using two different aluminum sample holders, at 180° and 90°, for two different purposes. For the evaluation of

the presence of NiOOH, the Propolis-MWCNTs dispersion was dropped (5.0 µL) and dried at room temperature (25 °C) on a glassy carbon plate (1.0 cm × 1.0 cm × 0.1 cm), and it was submitted to the NiOOH synthesis step. The glassy carbon plates, in the absence and presence of NiOOH, were placed on the 180° aluminum sample holder to perform the analysis. For the composite film thickness evaluation, the dispersion was directly placed (5 layers of 5.0 µL) on the 90° aluminum sample holder.

Voltammetric Performance of NiOOH/Propolis-MWCNTs/GCE

The analytical performance of the NiOOH/Propolis-MWCNTs/GCE was evaluated by simultaneous monitoring of glucose (1.0−30.0 mmol L^{-1}) and HMF (30.0 mmol L^{-1}) by CV. For this, a 10.0 mL electrochemical cell was employed with 0.10 mol L^{-1} NaOH as a supporting electrolyte (presence of OH$^-$) (Kalinke et al. 2019), and a potential range from +1.0 to −1.8 V, at 100 mV s^{-1}.

For an initial and exploratory evaluation of total carbohydrates (absence of HMF), cyclic voltammograms from 0.0 to +0.8 V at 100 mV s^{-1} were performed in the simultaneous presence of glucose, fructose, and sucrose, all from 2.5 to 25.0 mmol L^{-1}, using 0.10 mol L^{-1} NaOH as the supporting electrolyte.

For the selection of the best electrochemical technique for the determination of total carbohydrates and HMF, linear sweep voltammetry (LSV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV) techniques were performed. The voltammetric measurements were carried out in a wide range of potential to allow the monitoring of the Ni^{2+}/Ni^{3+} redox process (carbohydrates catalyst reaction) and HMF reduction process: from +1.0 to −1.8 V, at 100 mV s^{-1}, in 0.10 mol L^{-1} NaOH in the presence of different concentrations for all species (0.10−4.3 mmol L^{-1}).

Analytical Performance

The electrode calibration (analytical curve) was carried out by the addition of different aliquots of standard solutions of HMF and total carbohydrates, resulting in a concentration range of 0.050 to 16.0 mmol L^{-1} for all analytes. The measures were obtained by LSV from +1.0 to −1.8 V, at a scan rate at 100 mV s^{-1}, in 0.10 mol L^{-1} NaOH, using a potentiostat/galvanostat PGSTAT204 (Metrohm Autolab–Utrecht, Netherlands), managed by NOVA 2.1 software.

## Monitoring of HMF in Honey Sample

The detection of HMF in wild *Apis mellifera* honey samples was performed by the standard addition method using the LSV technique and applying the same parameters used for the analytical performance analysis (+1.0 to −1.8 V, at 100 mV s^{-1}). Initially, two aliquots of 1.0 g (from the original wild honey) were weighed and diluted in two different beakers in the proportion of 1:10 in NaOH 0.10 mol L^{-1} (with the presence of 2.0 and 4.0 mmol L^{-1} after addition of standard solution of HMF). Next, the analysis of the prepared samples was carried out, and later, they were spiked with four known concentrations of HMF previously prepared; and between each addition, the sample analysis was performed. Sample 1 (sample initially containing 2.0 mmol L^{-1}) was spiked to final concentrations of 0.3, 0.5, 0.7, and 1.0 mmol L^{-1} (disregarding the initial concentration of the sample). Sample 2 (sample containing 4.0 mmol L^{-1} initially) was spiked to final concentrations of 0.5, 1.0, 1.5, and 2.0 mmol L^{-1} (disregarding the initial concentration of the sample).

Results and Discussion

Electrochemical Behavior of NiOOH/Propolis-MWCNT/GCE

After the Ni^{2+} adsorption step, under open circuit potential conditions, the electrochemical synthesis of NiOOH was performed by CV during 30 cycles (100 mV s^{-1}) in 0.5 mol L^{-1} NaOH. The cyclic voltammograms are shown in Figure S1, as well as the correlation curve between the anodic peak current intensity (I_{pa}) and the number of cycles. The voltammetric profile observed in Figure S1A shows a stable redox pair between +0.43 and +0.35 V (after 15 cycles), which relates to the oxidation reaction of nickel hydroxide, Ni(II)/Ni(III) (Ni(OH)$_2$) into NiOOH and the respective reduction reaction (Reaction 1), as mentioned in previous works (Kalinke et al. 2019; Zhang et al. 2011). The I_{pa} and I_{pc} current stabilization was observed after 15 cycles (Figure S1B), which suggests no more significant amounts of

NiOOH would be formed after this point. Therefore, this synthesis condition was used for the next steps.

$$Ni(OH)_2 + OH^- \rightarrow NiOOH + H_2O + e^- \qquad (1)$$
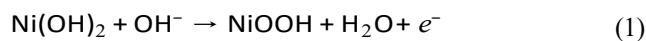
The appearance of the redox pair discussed above confirms the presence of NiOOH on the composite film surface after the electrochemical synthesis step. In addition, it is also possible to see structural differences on the surface of the material by comparing SEM images before and after the NiOOH synthesis. Figure 1 shows SEM images obtained for the glassy carbon plate containing the Propolis-MWCNTs composite film before (Fig. 1A–C), and after the NiOOH synthesis (Fig. 1D–F) at different magnifications. By the analysis of both images obtained at 52× magnification, it is difficult to identify significant differences between the surfaces before (Fig. 1A) and after NiOOH synthesis (Fig. 1B). However, by amplifying them in the lighter regions, where there is a greater presence of MWCNTs, it is possible to notice the appearance of different structures on the surface modified with NiOOH (Fig. 1E–F) (Kim et al. 2018), which it is not observed on the electrode without the synthesis step (Fig. 1B–C). Due to technical limitations, it was not possible to perform the chemical mapping of the surface and the chemical composition of the composite film surface. However, we believe that these structures are either NiOOH and/or Ni(OH)$_2$, since all electrode surfaces were properly washed with deionized water after each modification step to remove the presence of salts and supporting electrolytes. Regarding the film thickness, images of the Propolis-MWCNTs composite film were obtained on an aluminum substrate arranged at 90°. SEM images were also used to estimate the thickness of the proposed Propolis-MWCNTs biofilm on the GCE surface, as shown in Figure S2. A homogeneous dispersion has been observed over the entire border of the substrate. An average thickness of 2.9±0.7 μm was obtained for 5 layers of the film deposited on the electrode surface. This suggests that the thickness of the 1-layer film on the GCE is probably in the order of hundreds of nanometres or a few micrometers, approximately 0.58 μm.

Electrochemical Evaluation for Total Carbohydrates and HMF

First of all, it is important to highlight that the best composition of the biocomposite film has already been evaluated in a previous work (Oliveira et al. 2021). In that work, electrochemical analysis by CV and electrochemical impedance spectroscopy (EIS) were performed, and the best electrochemical performance was obtained for the biocomposite containing 0.14 mg mL^{-1} propolis and 0.10 mg mL^{-1} MWCNTs. Therefore, after the electrochemical synthesis, the modified electrode (NiOOH/Propolis-MWCNTs/GCE) was
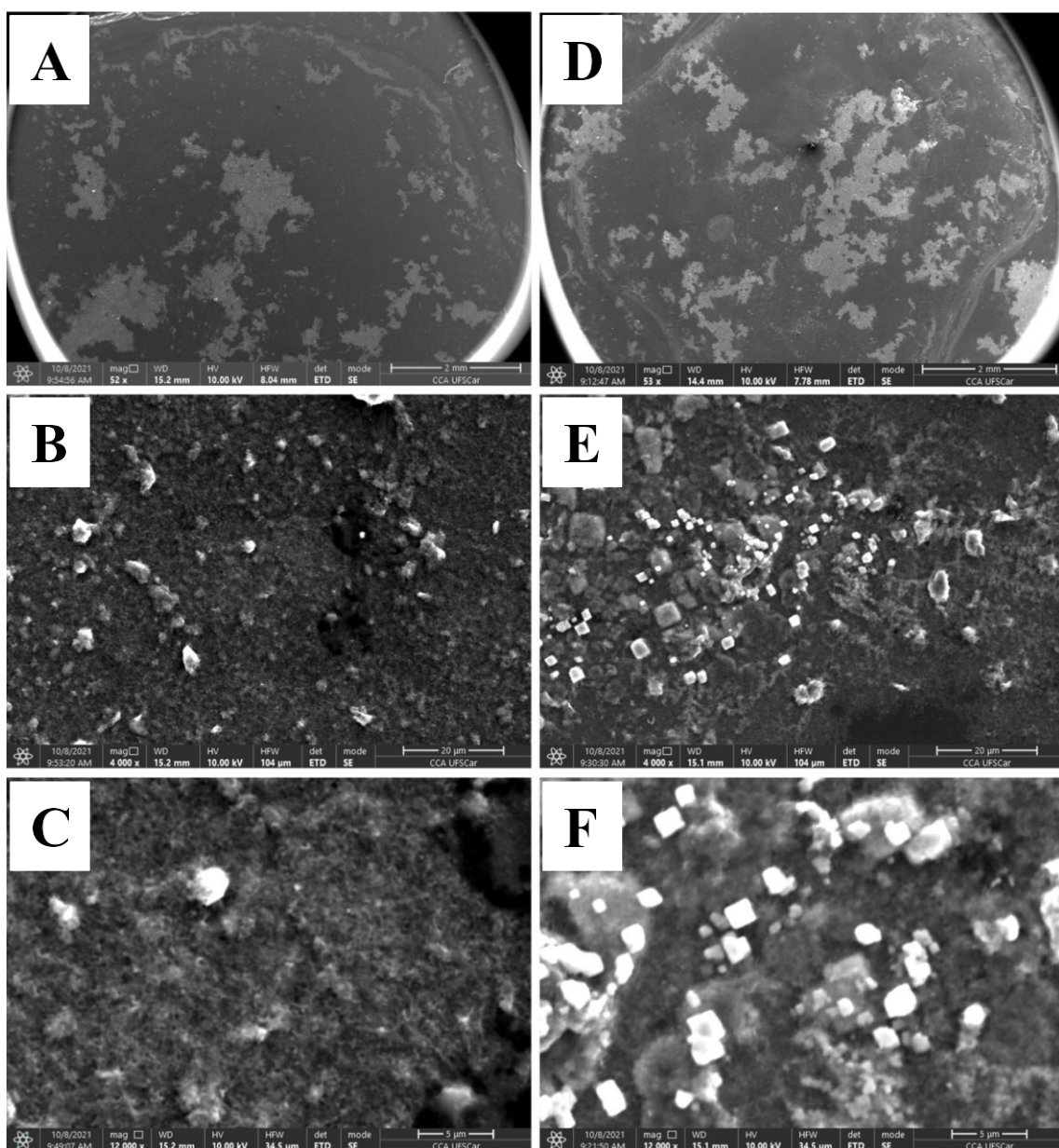
Fig. 1 SEM images obtained for the Propolis-MWCNTs composite film (**A**–**C**) before and (**D**–**F**) after the NiOOH synthesis with the magnifications of (**A**, **D**) 52×, (**B**, **E**) 4000×, and (**C**–**F**) 12,000×

evaluated for the simultaneous monitoring of glucose and HMF. First, an exploratory curve, by CV analysis, was carried out in the absence and presence of glucose from 0.001 to 0.030 mol L^{-1} (Fig. 2A). A linear behavior was observed in the anodic peak current intensity at around +0.35 and +0.50 V. Thus, it is possible to suggest that the redox couple Ni(II)/Ni(III) present on the electrode surface allowed the EC catalyst oxidation of glucose, which is one of the most important honey components and a carbohydrate widespread evaluated in literature (Doner 1977; Rahman et al. 2010; Wilkins and Atanasov 1996). The mechanism of the reaction

is shown in the following Reaction 2 (El-Nagar et al. 2017; Rocha et al. 2020).

NiOOH + glucose → Ni(OH)$_2$ + glucolactone   (2)
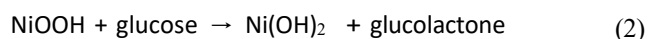
In Fig. 2A, CV measurements were also performed in 0.10 mol L^{-1} NaOH at 100 mV s^{-1} in the presence of different concentrations of glucose. Two oxidation processes were observed around +0.5 V, one in the forward scanning direction and another in the reverse direction, at close potential
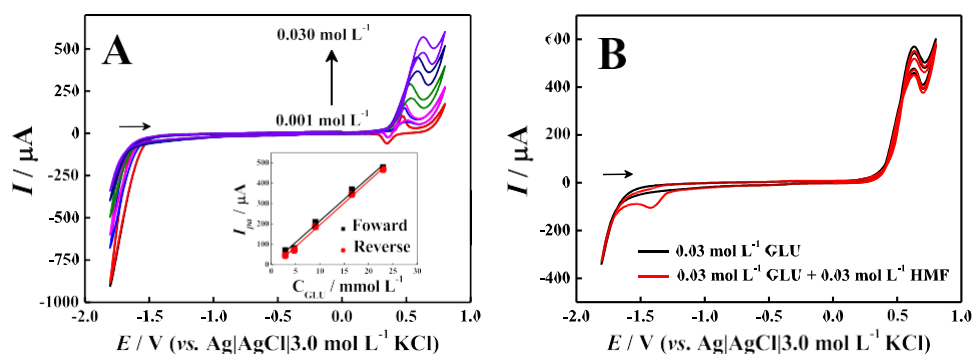
Fig. 2 (**A**) CV obtained for NiOOH/Propolis-MWCNTs/GCE in the absence (blank–black line) and presence of different concentrations of glucose (0.001–0.030 mol L⁻¹), (insert) Correlation curve between I_{pa} and glucose concentration (linear concentration range from 0.001 to 0.22 mol L⁻¹) obtained in Fig. 2A voltammograms for forward (black points) and reverse (red points) scanning directions. and (**B**) CV obtained for NiOOH/Propolis-MWCNTs/GCE after the addition of 0.030 mol L⁻¹ HMF. Conditions: 0.10 mol L⁻¹ NaOH supporting electrolyte at 100 mV s⁻¹

values, which is better demonstrated in Figure S3 (Jafarian et al. 2009). Forward and reverse peak intensities increased after successive addition of glucose with the same sensitivity, as observed in insert graphic. It means that both process could be used for glucose monitoring. After the addition of glucose, an increase in the oxidation peak of the Ni(II)/Ni(III) reaction around +0.50 V was observed. The anodic process observed during the reverse scan is due to the regeneration of more Ni(III), since the applied potential is positive enough for that, allowing the glucose to continue being oxidized on the electrode surface (Almutairi et al. 2022). On the other hand, the decrease/disappearance of the reduction peak (Ni(III)/Ni(II)) around +0.35 V was also evident. This behavior occurs by the regeneration of Ni(OH)₂ species after the glucose oxidation step, which decrease the amount of Ni(III) species available on the electrode surface (Jafarian et al. 2009).

Figure 2B shows the obtained CV for the proposed electrodes in the presence of 0.03 mol L⁻¹ glucose before and after the addition of 0.03 mol L⁻¹ HMF. For the obtained CV in the presence of HMF, a reduction process of around −1.35 V was also observed. This redox process consists of the reduction of 5-hydroxymethylfurfural into 2,5- dihydroxymethylfurfural, which involves two electrons and two protons (Reyes-Salas et al. 2006). On the other hand, this peak showed no significant interference in the signals observed for the glucose oxidation. In this way, the monitoring of the HMF reduction process can be carried out, even in the presence of glucose.

CV measurements after successive additions using a solution containing glucose and HMF (2.5−25 mmol L⁻¹) were taken place to evaluate a possible simultaneous determination of both analytes. CV and the current intensity correlation curves are shown in Fig. 3. As observed in Fig. 3B–C, linearity for both species was obtained, under the employed

Fig. 3 (**A**) Cyclic voltammograms obtained for NiOOH/Propolis-MWCNTs/GCE in the simultaneous presence of glucose (GLU) and HMF (2.5–25 mmol L⁻¹). Correlation curves between (**B**) I_{pa} and glucose concentration, and (**C**) I_{pc} and HMF concentration. Conditions: 0.10 mol L⁻¹ NaOH as supporting electrolyte at a scan rate of 100 mV s⁻¹
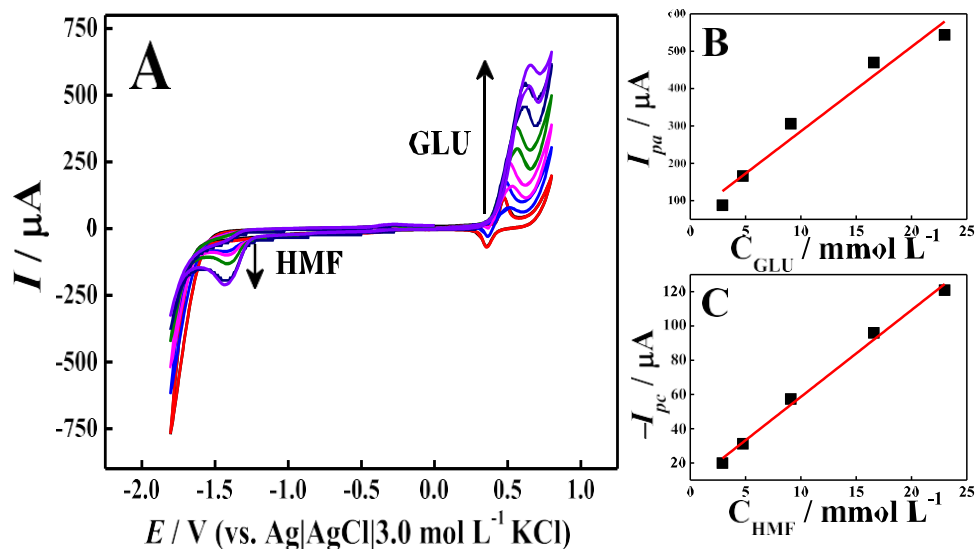
conditions. This demonstrates the potentiality of the proposed electrode for the monitoring of glucose and HMF, allowing for honey sample analysis, for example.

However, most honey samples are composed of several organic species, predominately 38.4 wt% fructose, 30.3 wt% glucose, and 1.3 wt% sucrose (Ball 2007). Therefore, a CV study was carried out in the presence of different concentrations (2.5–25 mmol L^{-1}) of glucose, fructose, and sucrose, individually (Figure S4A-C), as well as the peak current correlation curves for each species are shown in inset curves into respective voltammograms. The results demonstrate that the electrode is sensitive to the three evaluated carbohydrates. The increasing signal in the presence of fructose and sucrose occurs in the same potential region when in the presence of glucose, at around +0.50 V. According to other reported works (Kumar et al. 2020; Reim and Van Effen 1986), this suggests the presence of the same type of chemical interaction between the NiOOH and these species, as well as amines and alcohols, more precisely between the high-valence oxyhydroxide species and their hydroxyl groups, followed by a dehydrogenation process. This implies that a thorough determination of a honey sample should take place in the presence of the three investigated species, using the total carbohydrate term.

## Electroanalytical Performance of NiOOH/Propolis-MWCNTs/GCE

The analytical curve was performed using a solution containing total carbohydrates and HMF. Firstly, the influence of the type of monitoring technique on the electrochemical response at different concentrations of the species was evaluated. CV, LSV, DPV, and SWV techniques were used for this proposal. Total carbohydrates and HMF were added

simultaneously in a concentration range from 0.10 to 4.3 mmol L^{-1}. LSV, DPV, and SWV measurements were performed in the potential range from +1.0 to −1.8 V since it would allow both the oxidation of total carbohydrates in the direction of negative potential values, as well as the reduction of the HMF. Scanning in the opposite direction would make it impossible to monitor the reduction peak of HMF. All voltammograms and the dependency correlation between peak current and concentration of the species for NiOOH/Propolis-MWCNTs/GCE can be seen in Figure S5.

The analyses carried out using different voltammetric techniques suggest that it is feasible to monitor the HMF reduction faradaic process in a similar concentration range for both. The highest sensitivity was observed for DPV and LSV techniques. However, considering the monitoring of the total carbohydrate oxidation signal, the correlation between the peak current signal and the concentration was not possible by DPV and SWV techniques. Therefore, for the analytical curve and the other analytical studies, the LSV technique was used, which presented a better performance in terms of sensitivity for both species after a comparative study with CV, DPV, and SWV (Table S1).

LS voltammograms recorded for the analytical curve are shown in Fig. 4A, using a concentration range of 0.050 to 16.0 mmol L^{-1} for all analytes, as well as peak current correlation curves for total carbohydrate in Fig. 4B, and HMF in Fig. 4C. The analytical curves demonstrated the feasibility of using the proposed electrode as a sensor for monitoring carbohydrates and HMF. Linear responses between the peak current intensity and the species concentrations ($R^2 >$ 0.99) were observed for each faradaic process at concentrations of 0.35–11 mmol L^{-1} for HMF, I_{pc} (μA) = −3.12(0.2) − 7.43(0.2) C$_{HMF}$ (mmol L^{-1}); and 0.070–11 mmol L^{-1} for total carbohydrates I_{pa} (μA) = 13.7(1.2) + 18.8(0.6)

Fig. 4 (**A**) LS voltammograms obtained using the NiOOH/Propolis-MWCNTs/GCE for the determination of different concentrations of total carbohydrates and HMF in supporting electrolyte. Peak current correlation curves for (**B**) total carbohydrates and (**C**) HMF. Conditions: 0.10 mol L^{-1} of NaOH as supporting electrolyte at a scan rate of 100 mV s^{-1}
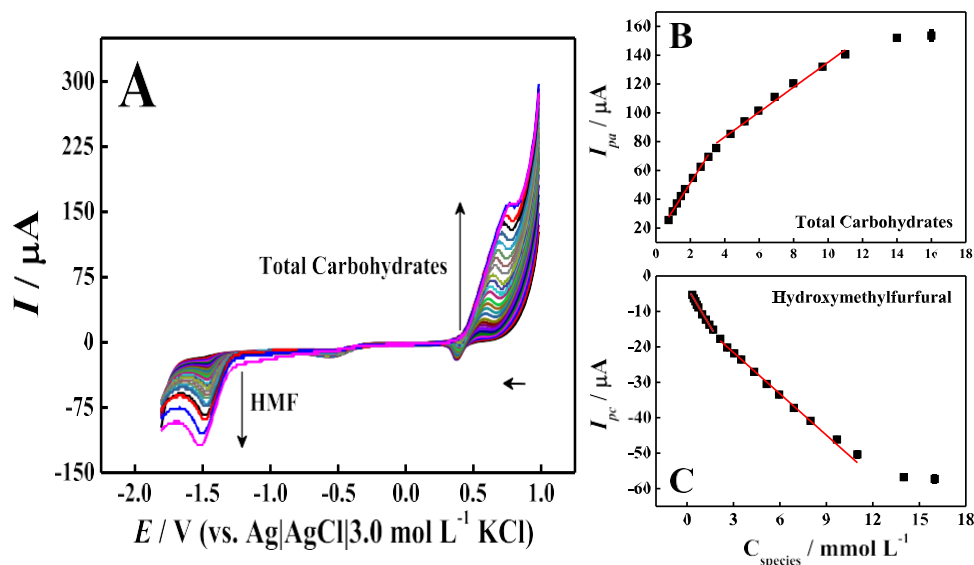
$C_{\text{Total Carbohydrates}}$ (mmol L^{-1}). In addition, limits of detection (LOD) of 0.18 mmol L^{-1} and 0.051 mmol L^{-1} were achieved for HMF and total carbohydrates, respectively.

As proof of concept of the effectiveness of the proposed sensor for HMF, the wild honey samples were properly diluted in 0.10 mol L^{-1} NaOH and analyzed by LSV by the standard addition method. Figure 5 shows the correlation curves of the currents obtained by LSV and the concentration of HMF added to the samples. It is important to highlight that, for a better evaluation of HMF in honey, concentrations of 2.0 and 4.0 mmol L^{-1} HMF were used. Therefore, in this condition, the total carbohydrate concentration in the honey sample is above the previously calibrated concentration range. As a result, the analytical evaluation in honey was carried out only by monitoring different amounts of HMF. Regarding the analytical evaluation of honey samples, it was possible to recover values close to the two initial concentrations added to the sample (2.0 and 4.0 mmol L^{-1}), with recovery values of 91.8 and 107%.

One of the most important points in this work, is the use of propolis as an immobilization agent, both for the conductive nanomaterial and redox mediator. From the electrochemical application point of view, the determination of HMF in honey, even in high amount of total carbohydrates, can be performed in a cheaper and simpler way than the commonly used techniques, such as chromatographic

techniques. However, the electrochemical sensor needs to present some important properties, such as high electrical conductivity, low background current, and chemical affinity with the evaluated species. This fact can become a challenge; however, it was overcome in this work by the combination of MWCNTs with NiOOH through into the propolis. The difficulty in obtaining these required properties can be observed by the low number of works that use this determination strategy. Some of these works have been summarized in Table 1, comparing their electroanalytical performance with the present work.

Table 1 shows some recent works that reported low LOD for the determination of HMF. However, it is important to highlight that these methods were applied for non-simultaneous determination, just in the presence of HMF in different samples. Some of them make use of more sensitive electrochemical techniques, as is the case of the works by Upan et al. (Upan et al. 2022) and Francisco et al. (Francisco et al. 2022), who carried out the determination of HMF by SWV and DPV, respectively. Despite low LODs, they have performed the HMF determination with LDRs presenting one decimal place at most. On the other hand, the present work achieved the LDR of two decimal places, allowing the monitoring of a wider range of samples at different concentration levels. Another point is that only the work developed by Ye et al. (Ye et al. 2022) investigates the possibility of

Fig. 5 Representative LS voltammograms obtained using the NiOOH/Propolis-MWCNTs/GCE for wild honey samples in the presence of a standard solution of (**A**) 2.0 mmol L^{-1} and (**B**) 4.0 mmol L^{-1} HMF. (Insert) Peak current correlation for HMF. Conditions: 0.10 mol L^{-1} NaOH as supporting electrolyte at a scan rate of 100 mV s^{-1}
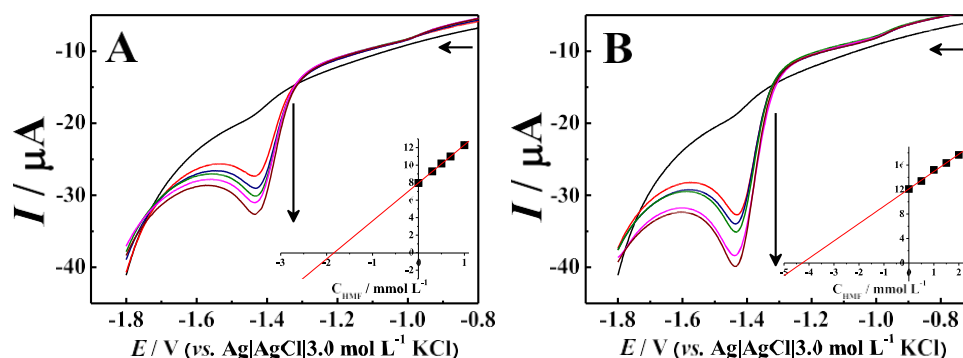
Table 1 Electroanalytical performance of the NiOOH/Propolis-MWCNTs/GCE for the determination of HMF compared to other similar works in the literature

Sensor	Sample	LDR (mmol L^{-1})	LOD (mmol L^{-1})	Sensitivity (µAL mmol^{-1})	Voltammetric Technique	Ref
GCE	Water	0.001–0.010	0.0006	–	CV	(Salhi et al. 2020)
AgMDs/SPCE	Honey	0.02–0.80	0.008	111	SWV	(Upan et al. 2022)
GCE-MIP	Coffee	0.01–0.05	0.003	–	DPV	(Francisco et al. 2022)
Cu-Ni MP/CuE	Vinegar, beer, rice wine, Chinese baijiu, and honey	0.40–10	0.004	0.98	LSV	(Ye et al. 2022)
NiOOH/Propolis-MWCNTs/GCE	*Honey*	*0.35–11*	*0.180*	*7.43*	*LSV*	*This work*

AgMDs silver microdendrites, *SPCE* screen-printed carbon electrode, *MIP* molecularly imprinted polymer, *Cu-Ni MP* copper-nickel bimetallic microparticle, *CuE* copper electrode

monitoring other organic species, in a non-simultaneous way. The authors also used oxyhydroxides as catalysts to promote the oxidation of HMF, in this case, Ni and Cu alloy onto the electrode surface. Comparatively, the electrochemical performance of the NiOOH/Propolis-MWCNTs/GCE showed similar LDR and sensitivity about seven times higher. Hence, the proposed method presents potentiality for the simultaneous determination of HMF and total carbohydrates using a greener material as an immobilizing agent, with similar electrochemical performance to other works.

Conclusion

In this work, we demonstrate the monitoring of glucose, fructose, and sucrose (total carbohydrates) by the electrocatalytic mechanism provided by the presence of NiOOH redox mediator immobilized on the composite biofilm based on propolis and MWCNTs. The presence of MWCNTs increased the sensitivity of the unmodified electrode, which also allowed the monitoring of the HMF reduction signal, even in the presence of total carbohydrates. Therefore, the proposed sensor was able to perform the determination of species and the quantification of HMF in honey samples. Due to this strategy requiring a short analysis time, a small volume of reagents, modifiers, and waste, and the use of propolis as an immobilizer agent for the MWCNTs and the NiOOH, the proposed method can be considered a greener alternative in comparison to other commonly used sensors and methods, following the main principles of the green chemistry.

Author Contribution Paulo Roberto de Oliveira: investigation, methodology, conceptualization, validation, data curation, writing—review and editing. Luiz Ricardo Guterres e Silva: methodology, data curation, writing—review and editing. Cristiane Kalinke: conceptualization, validation, data curation, writing—review and editing. Alejandro Garcia-Miranda: conceptualization, validation, writing—review and editing. Jai Prakash: conceptualization, validation, data curation, writing—review and editing. Yangping Wen: conceptualization, validation, data curation, writing—review and editing. Roberta Cornélio Ferreira Nocelli: conceptualization, validation, data curation, writing—review and editing. Juliano Alves Bonacin: conceptualization, validation, data curation, writing—review and editing. Craig E. Banks: conceptualization, validation, data curation, writing—review and editing. Bruno C. Janegitz: conceptualization, visualization, supervision, project administration, resources, funding acquisition, writing—review and editing

Declarations

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