



A novel nanostructured poly(thionine)-deep eutectic solvent/CuO nanoparticle film-modified disposable pencil graphite electrode for determination of acetaminophen in the presence of ascorbic acid

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Received: 9 October 2020 / Revised: 12 November 2020 / Accepted: 16 November 2020 / Published online: 7 January 2021
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Abstract

A new electrochemical sensor based on thionine (TH), an electroactive polymer, and CuO nanoparticle (CuONP)-modified pencil graphite electrode (PGE) has been developed. Poly(thionine) (PTH) was formed on the CuO/PGE surface by electropolymerisation in ethaline deep eutectic solvent (DES) containing acetic acid dopant to form PTH_{Ethaline}/CuO/PGE. Cyclic voltammetry, electrochemical impedance spectroscopy, and differential pulse voltammetry were utilized to evaluate the fabrication process, electrochemical properties, and performance parameters of the modified electrodes. The analytical performance of the PTH_{Ethaline}/CuO/PGE was evaluated with respect to linear range, limit of detection, repeatability, and reproducibility for the detection of acetaminophen (APAP) by electrooxidation in the presence of ascorbic acid (AA). Analytical parameters such as pH were optimized. The combined use of PTH and CuONP led to enhanced performance towards APAP due to the large electroactive surface area and synergistic catalytic effect, with a wide linear working range and low detection limit. The reliability of the proposed sensor for the detection of APAP was successfully tested in pharmaceutical samples containing APAP and AA, with very good recoveries.

Keywords Poly(thionine) · Ethaline deep eutectic solvent · CuO nanoparticle · Pencil graphite electrode · Acetaminophen · Ascorbic acid

Introduction

Redox polymers can be used in simple and effective sensing platforms due to their fast electron transfer and electrocatalytic ability. By adjusting electropolymerisation parameters such as electrolyte, pH, and monomer concentration, the desired polymer film with the best properties for electrochemical detection can be obtained [1].

Ionic liquids are widely used as solvents for electropolymerisation in sensing and biosensing applications. However, the increasing demands for non-toxic, eco-friendly, biodegradable green designer solvents, and the low toxicity and easy-to-prepare nature of deep eutectic solvents (DES) have led to a growing interest in their use. In recent years, DES have been used as an alternative to other common

solvents for polymer synthesis, as in many application fields [2]. A number of studies in recent years have indicated that the use of DES is successful in enabling the preparation of polymers with different chemical compositions and morphologies [3].

Due to their excellent mechanical, catalytic, and electrical properties, metal/metal oxide nanoparticles have received much interest in the field of electrochemical sensing [4]. Among transition metal oxide nanoparticles, CuO nanoparticles (CuONP) are good candidates for intervening in electron transfer processes because of copper's multiple oxidation states on oxide dissolution. NP have been widely used in electrochemical sensing, because of their large surface area to volume ratio, easy access to the surface, and strong electrocatalytic ability, and this has been demonstrated by CuONP [5]. Their properties can be altered by introducing chemical or physical modifications.

Acetaminophen (APAP), (*N*-acetyl-*p*-aminophenol or paracetamol), is a common antipyretic analgesic. As a non-steroidal drug, it is used to relieve pains due to migraine and

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tension-type headaches, muscular aches, arthralgia, toothache, and general pain. A precise dosage of APAP is safe, but an overdose can lead to acetaminophen poisoning; it is the most common cause of poisoning worldwide [6]. At higher doses, it can cause liver diseases, kidney problems, skin rashes, inflammation in the pancreas, and hepatic necrosis [7]. Ascorbic acid (AA) can act as an interferent during APAP analysis in many foods, biological fluids, and pharmaceutical preparations. The accurate monitoring of APAP in the presence of AA is thus critical. Therefore, a simple, accurate, low-cost, and rapid method is necessary for the determination of APAP. Different analytical methods available for the analysis of APAP include chromatography [8], flow injection [9], spectrophotometry [10], and electrochemistry [11]. Among these methods, electrochemical sensors, especially disposable electrode-based ones, have attracted more attention in recent years due to their high sensitivity and selectivity, ease-of-use, simple application, low-cost equipment, rapid response, and real-time detection [12, 13].

This study aims to develop electrochemical sensors with the best analytical performance by combining the electrocatalytic properties of poly(thionine) (PTH) redox polymer and the excellent mechanical, catalytic, and electrical properties of CuONP on simple pencil graphite electrode (PGE) supports. For this purpose, to our knowledge for the first time, electrochemical polymerisation of thionine (TH) on CuONP-modified PGE in ethaline DES containing acetic acid dopant was carried out. The combined use of CuONP and PTH led to enhanced analytical characteristics due to synergistic effects. PTH_{Ethaline}/CuO/PGE was electrochemically characterized by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV). The determination of APAP in the presence of AA was successfully carried out by using DPV. Finally, the reliability of the proposed sensor was tested in real samples by determining the amount of acetaminophen in pharmaceutical samples containing APAP and AA.

Experimental

Reagents and solutions

Acetaminophen ($\geq 99.0\%$), L-ascorbic acid, thionine, choline chloride, chitosan from crab shells ($\geq 85.0\%$), ethylene glycol (anhydrous, 99.8%), acetic acid glacial, phosphoric acid (95.0–98.0%), sodium phosphate monobasic monohydrate, sodium phosphate dibasic dihydrate, and copper(II) oxide nanopowder (< 50 nm particle size (TEM)) were supplied by Sigma-Aldrich. Boric acid and sodium hydroxide were purchased from JT Baker. PGE of 1.4 mm in diameter (type 1B)

were bought from Faber Castell. The commercial sample, Paramolan C® Sandoz (Medinfar Consumer Health) was acquired in a local drug store in Portugal.

Ultrapure water obtained from a Millipore Milli-Q Ultrapure Water System (resistivity > 18 M Ω cm) was used in all experiments.

Instrumentation

Electrochemical voltammetric measurements were carried out using an Ivium CompactStat electrochemical analyser (Ivium Technologies, Netherlands). Differential pulse voltammograms were recorded with pulse duration 0.05 s, step potential 2 mV, pulse amplitude 50 mV, and scan rate 5 mV s⁻¹.

EIS measurements were carried out with a Solartron 1250 Frequency Response Analyser coupled to a Solartron 1286 Electrochemical Interface using ZPlot 2.4 software (Solartron Analytical, UK). A frequency range of 65 kHz–0.1 Hz was utilized with a sinusoidal voltage perturbation of amplitude of 10 mV for the EIS measurements, 10 steps per frequency decade.

The electrochemical cell contained a modified PGE as working electrode, an Ag/AgCl (3 M KCl) as reference electrode, and a platinum wire auxiliary electrode. A length of 1 cm of the PGE was exposed to solution in the electrochemical cell (geometric area of 0.47 cm²). A CRISON 2001 micropH-meter was employed for pH measurements.

Pre-treatment of PGE and modification by nanoparticles

Pre-treatment of the PGE is necessary to ensure the reproducibility of the sensor platform. The PGE was immersed in 0.1 M phosphate buffer (pH 7.0), containing 0.1 M KCl and cyclic voltammograms (CVs) were recorded for 10 cycles between -0.3 V and $+1.1$ V at a scan rate of 50 mV s⁻¹ [13].

A mass of 100 mg of chitosan (Chit) was dissolved in 2 M acetic acid and stirred at room temperature until a clear solution was obtained. The optimum nanoparticle amount was dispersed into 1 mL of Chit solution and CuO-Chit composite was sonicated for 5 h. The pre-treated PGE was dipped into this homogenous dispersion and left for 30 min. The prepared CuO/PGE-modified electrode surface was allowed to dry at room temperature.

Preparation of ethaline and polymer-modified electrodes

Earlier studies have shown that ethaline DES is an excellent choice for polymer formation by electrochemical polymerisation [14]. Ethaline is formed owing to the attractive interaction

between the hydrogen bond acceptor choline chloride (ChCl) and ethylene glycol (EG) as the hydrogen bond donor. The eutectic mixture has a molar ratio of 1:2, respectively, of the components. The solid ChCl is first heated to evaporate the excess of water, and then EG is added. The mixture is held at a constant temperature of ~ 60 °C, while continuously stirring until a clear solution is achieved. The ethaline is then cooled down to room temperature.

DES by itself does not have sufficient conductivity for efficient electropolymerisation [14]. Therefore, an ethaline acid solution containing CH_3COOH was used as an ion source for electropolymerisation, and the final concentration was adjusted to 1 M CH_3COOH [3].

For the preparation of the polymerisation solutions, 1 mM TH was dissolved in CH_3COOH -doped DES by heating up to 40 °C under constant stirring for 30 min.

Films of PTH were formed by potential cycling electropolymerisation on CuO/PGE, in the potential range -1.0 to $+0.8$ V vs. Ag/AgCl, during 15 scans, the optimised scan rate being 200 mV s^{-1} . For comparison, PTH film-coated CuO-modified electrodes were also formed in an aqueous solution containing 1.0 mM TH + 1 M CH_3COOH . Characterization of PTH films was performed in Britton-Robinson (BR) buffer solution pH 7.0, by CV in the potential range -0.5 to $+0.7$ V vs. Ag/AgCl, at a scan rate of 100 mV s^{-1} . These optimised experimental conditions were based on previous work [15].

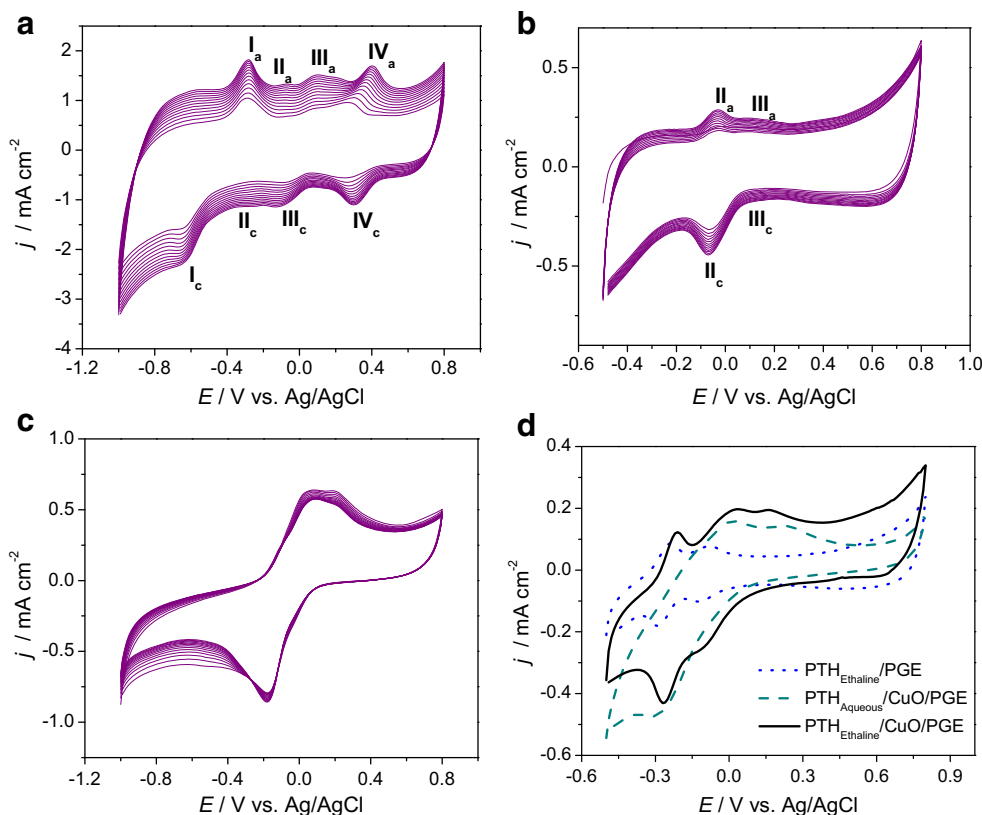
Results and discussion

Effect of CuONP electrode surface modification on PTH film growth

In order to investigate the effect of electrode surface modification on the growth of the polymer films, TH was electrochemically polymerized onto a bare PGE and CuONP-modified PGE surface by potential cycling in acid-doped ethaline DES (Fig. 1a and b) at 200 mV s^{-1} , as optimised previously for PTH on carbon nanotube-modified glassy carbon electrodes [15]. The best-defined polymerisation CV curves are with $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$ (Fig. 1a) which show four well-defined redox couples. For $\text{PTH}_{\text{Ethaline}}/\text{PGE}$ (Fig. 1b), there is one well-defined redox couple, the other expected pair of redox peaks not being visible. For comparison, Fig. 1c shows PTH-modified CuO/PGE prepared in aqueous solution, at the same scan rate. Electropolymerisation on CuO/PGE in ethaline DES leads to more pronounced increases of anodic and cathodic peak currents with each cycle, than of $\text{PTH}_{\text{Ethaline}}/\text{PGE}$ and $\text{PTH}_{\text{Aqueous}}/\text{CuO}/\text{PGE}$.

PTH is an electroactive polymer with heterocyclic nitrogen atoms, nitrogen bridges, and free amino groups [16]. The mechanism of electropolymerisation involves the formation of a cation-radical species upon electrooxidation because of the positively charged sulphur of PTH [17]. The interaction of copper ions with the secondary amine of the

Fig. 1 Potential cycling electropolymerisation of TH (concentration 1 mM) in ethaline or aqueous solutions, scan rate 200 mV s^{-1} , to form (a) $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$, (b) $\text{PTH}_{\text{Ethaline}}/\text{PGE}$, and (c) $\text{PTH}_{\text{Aqueous}}/\text{CuO}/\text{PGE}$. (d) CV curves of $\text{PTH}_{\text{Ethaline}}/\text{PGE}$ (---), $\text{PTH}_{\text{Aqueous}}/\text{CuO}/\text{PGE}$ (—), and $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$ (—) in 0.1 M BR buffer solution (pH 7.0) at 100 mV s^{-1}



redox polymer is also described in the literature [18]. For the electropolymerisation of TH, strong electrostatic interactions between CuONP and PTH can be attributed to Cu acting as a catalytic site, [19], some Cu^{2+} being formed owing to the low pH in the polymerisation medium. Moreover, the sulphur atom of PTH can form a Cu–S bond with copper ions so that CuO nanoparticles can be stabilized on the polymer [20].

For PTH electrodeposited on CuONP/PGE in CH_3COOH -doped DES, four redox couples were observed in the polymerisation CVs (Fig. 1a) as mentioned above. The first redox couple is attributed to Cu(I)/Cu(0) oxidation/reduction (I_a/I_c) and the other redox couple to Cu(II)/Cu(I) oxidation/reduction (IV_a/IV_c). This observation was confirmed by the CV of CuONP/PGE in ethaline DES (Supplementary Information (ESM), Fig. S1) and also reported in the literature [21, 22]. The redox couple (II_a/II_c) is assigned to monomer oxidation/reduction and the couple (III_a/III_c) to polymer oxidation/reduction. The peak current increases with the number of cycles, indicating the growth of the polymer film [23]. From the polymerisation profiles in Fig. 1a–c, it is clearly seen that PTH film electrodeposited on CuO/PGE in aqueous medium leads to a low polymer film growth rate after the first cycle, while the film deposited in CH_3COOH -doped DES continues to form, reflected by the continuing substantial increase in the peak currents, which are also of higher magnitude. The anodic peak current III_a of the 15th cycle is 0.75 mA cm^{-2} for $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$, 0.20 mA cm^{-2} for $\text{PTH}_{\text{Ethaline}}/\text{PGE}$, and 0.10 mA cm^{-2} for $\text{PTH}_{\text{Aqueous}}/\text{CuO}/\text{PGE}$. According to these results, it is concluded that $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$ is the best electrode platform for the polymerisation of TH. Because they provide catalytic sites for the growth of monomer, CuONP play an important role in the polymerisation of TH and furnish an increased surface area [19, 24].

CVs at $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$, $\text{PTH}_{\text{Ethaline}}/\text{PGE}$, and $\text{PTH}_{\text{Aqueous}}/\text{CuO}/\text{PGE}$ were recorded in 0.1 M BR buffer (pH 7.0) at a scan rate of 100 mV s^{-1} (Fig. 1d). The CVs of PTH films electrodeposited in ethaline DES have the same two redox couples. PTH electrodeposited in aqueous solution exhibits only one redox couple; the same tendency was also confirmed during electropolymerisation. Due to more electrodeposited PTH film than on $\text{PTH}_{\text{Ethaline}}/\text{PGE}$, $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$ presents higher peak currents and, additionally, the redox couples shift to more positive potentials (Fig. 1d). To evaluate the effect of nanoparticle loading on the polymerisation process to form $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$, different CuO–Chit dispersions were prepared by adding 1.0, 2.0, and 3.0 mg CuONP to 1 mL of Chit. The CVs obtained in 0.1 M BR buffer (pH 7.0) at 100 mV s^{-1} (ESM Fig. S2) show that anodic and cathodic peak currents increase on increasing the CuONP loading from 1.0 to 2.0 mg/mL and then decrease. The optimum concentration was thus determined as 2.0 mg mL^{-1} for further experiments. The decrease in the peak current at high

CuONP amounts could be due to the proximity of the CuO nanoparticles preventing monomer diffusion towards the polymer nucleation sites [24].

Electrochemical characterization of PTH

Effect of pH

The electrochemical behaviour of PTH is highly dependent on pH because of the presence of amino groups in PTH [25]. DPV was used to evaluate the effect of pH at $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$. As shown in Fig. 2a, on increasing the pH from 3.0 to 8.0, the anodic peak potential (E_{pa}) shifts to more negative values with a linear dependence on pH [11]. The equation for the variation of peak potential with pH for $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$ was $E_{pa} \text{ (V)} = 0.42 - 0.059 \text{ pH}$ with a correlation coefficient of 0.9938, the slope being close to the theoretical value of 59 mV pH^{-1} for an equal number of protons and electrons (Fig. 2b). The peak width at half height, $W_{1/2}$, was approximately 45 mV within the pH range studied; therefore, it can be deduced that two electrons, together with two protons, are involved in the electrochemical oxidation reaction [26], in agreement with PTH studies in the literature [27]. In Fig. 2, the maximum peak current was obtained at pH 7.0, which was selected as optimum pH.

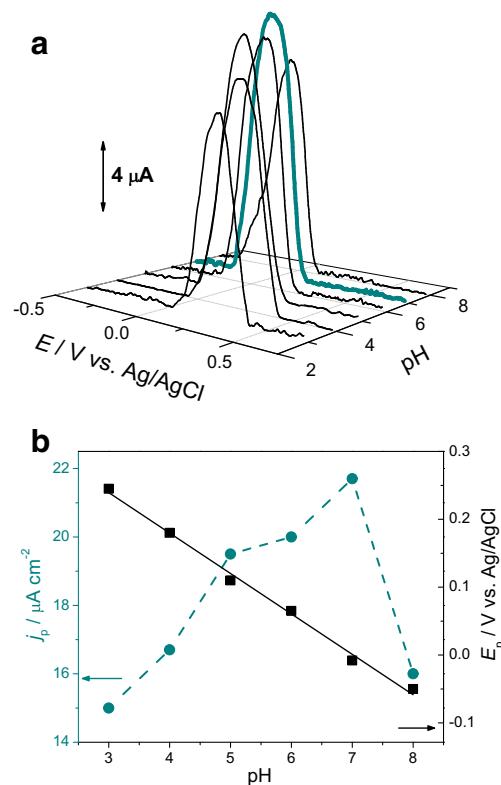


Fig. 2 a Differential pulse voltammograms for the oxidation of $\text{PTH}_{\text{Ethaline}}/\text{CuO}/\text{PGE}$ in 0.1 M BR buffer at pH 3.0 to 8.0. b The effect of pH on the anodic peak potential, E_{pa} (■), and peak current, j_{pa} (●)

Effect of scan rate

In order to assess the influence of scan rate on the electrochemical behaviour of PTH_{Ethaline}/CuO/PGE, the CVs of the modified electrode were obtained in 0.1 M BR buffer (pH 7.0) at different scan rates (Fig. 3). All peak currents increased linearly with scan rate (10–100 mV s⁻¹) suggesting a surface-controlled electrochemical mechanism [28].

In all cases, two redox couples were observed. For the redox couple (I_a/I_c) $j_{pIa} = 0.71 v + 0.002$ ($r^2 = 0.9960$) and $j_{pIc} = -0.67 v - 0.050$ ($r^2 = 0.9853$), and for the redox couple (II_a/II_c) $j_{pIIa} = 0.62 v + 0.006$ ($r^2 = 0.9917$) and $j_{pIIc} = -0.64 v - 0.059$ ($r^2 = 0.9959$), where the current densities (j) are expressed in mA cm⁻² and the scan rates (v) are in V s⁻¹.

Electrochemical impedance spectroscopy

EIS was used to characterize the interfacial properties of PTH_{Ethaline}/CuO/PGE, PTH_{Aqueous}/CuO/PGE, and PTH_{Ethaline}/PGE with polymer films electrodeposited at 200 mV s⁻¹. Figure 4a presents the impedance spectra of different modified electrodes at -0.25 V vs. Ag/AgCl (in 0.1 M pH 7.0 BR buffer).

Spectra were fitted with the electrical equivalent circuit in Fig. 4b-1, except for PTH_{Ethaline}/PGE (Fig. 4b-2). The circuit contains the cell resistance, R_0 , in series with two parallel

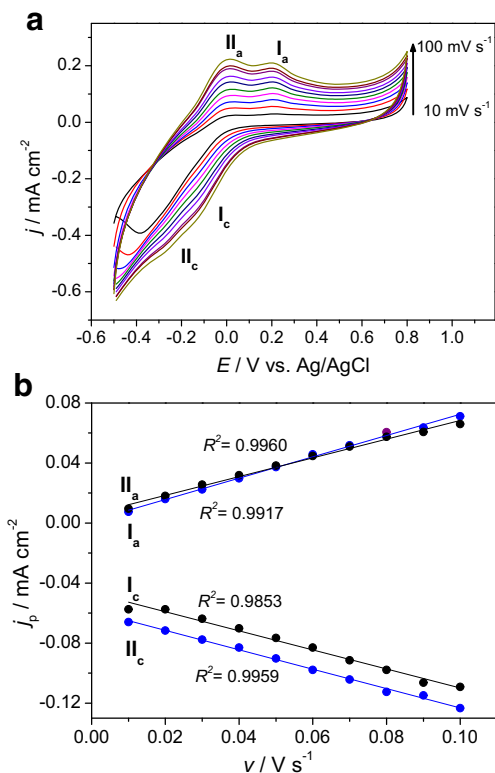
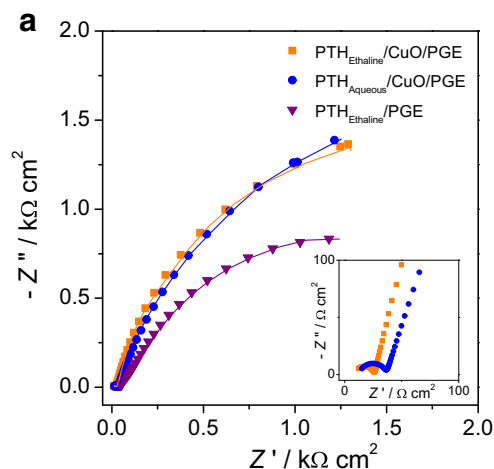
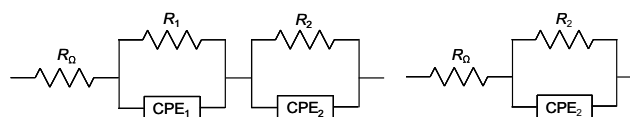


Fig. 3 **a** CVs of PTH_{Ethaline}/CuO/PGE in 0.1 M BR buffer (pH 7.0) at scan rates from 10 to 100 mV s⁻¹. **b** Plots of peak current vs. scan rate



b-1



b-2

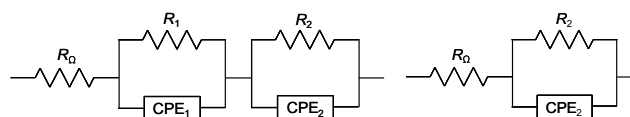


Fig. 4 **a** Complex plane impedance spectra of PTH_{Ethaline}/CuO/PGE, PTH_{Aqueous}/CuO/PGE, and PTH_{Ethaline}/PGE in 0.1 M BR buffer (pH 7.0) at -0.25 V vs. Ag/AgCl. **b** The electrical equivalent circuits used to fit the spectra

combinations of a constant phase element, CPE, and a resistance. The features in the high frequency region that appear for PTH_{Aqueous}/CuO/PGE and PTH_{Ethaline}/CuO/PGE can be attributed to the interface electrode/CuO modifier film and inside the film, modelled by a parallel R_1 and CPE₁. The semicircles at lower frequencies that appear for all three architectures are attributed to the modifier film/solution interface, described by R_2 and CPE₂. The CPE is expressed as a non-ideal capacitance with $CPE = [(C_i \omega)^\alpha]^{-1}$; the values of the CPE exponent (α) can vary between 0.5 and 1.0 corresponding to porous and to completely uniform and smooth electrode surfaces, respectively [27]. The values of the electrical equivalent circuit elements obtained by fitting the spectra are given in Table 1.

The high frequency combination R_1/CPE_1 that appears for PTH_{Aqueous}/CuO/PGE and PTH_{Ethaline}/CuO/PGE is attributed to the presence of the CuONP coated with PTH and reflects the behaviour of the modifier film as well as the interface with the electrode support. The low values of R_1 , around 20 Ω cm² and CPE₁ of $\sim 4 \mu F$ cm⁻² s ^{α -1}, are consistent with this. A fitting value of the CPE exponent of 0.85–0.90 was obtained. The R_2 values of the modified electrodes increase in the order PTH_{Ethaline}/PGE (2.53), PTH_{Ethaline}/CuO/PGE (3.57), and PTH_{Aqueous}/CuO/PGE (4.33) k Ω cm², based on electrode substrate geometric area. In the presence of CuO, the active area will be greater which explains the larger values of R_2 . It also shows that using ethaline as polymerisation medium, the R_2 value is smaller than in aqueous solution, implying faster electron transfer, in harmony with the CV in Fig. 1D that shows

Table 1 Values of electrical equivalent circuit elements obtained by fitting the spectra in Fig. 4a to the circuit in Fig. 4B

Modified electrode	R_1/Ω cm^2	$\text{CPE}_1/\mu\text{F cm}^{-2}$ $\text{s}^{\alpha-1}$	α_1	$R_2/\text{k}\Omega$ cm^2	$\text{CPE}_2/\text{mF cm}^{-2}$ $\text{s}^{\alpha-1}$	α_2
PTH _{Ethaline} /PGE	–	–	–	2.53	0.54	0.85
PTH _{Ethaline} /CuO/PGE	18.0	4.1	0.90	3.57	0.60	0.90
PTH _{Aqueous} /CuO/PGE	24.3	3.1	0.85	4.33	0.62	0.85

the best performance for PTH_{Ethaline}/CuO/PGE. This can be attributed to a synergistic catalytic effect between CuO and PTH, and an increase of electroactive surface area [29, 30]. As would be expected, the change in R_2 in the presence of CuONP is accompanied by an increase in CPE_2 in the platform, with CPE exponents of 0.85–0.90.

Analytical characteristics of the modified electrodes

Electrochemical oxidation of ascorbic acid and acetaminophen at PGE sensors

DPV was employed to evaluate the electrochemical performances of AA and APAP in 0.1 M BR buffer (pH 4.0) (Fig. 5). As shown in Fig. 5(a), the oxidation peaks of AA and APAP at the bare PGE in DPV appear at approximately 0.28 V and 0.58 V, respectively. When PTH films on PGE are formed in ethaline DES (Fig. 5(b)), and PTH film-coated CuO/PGE in aqueous solution (Fig. 5(c)), the oxidation signals of AA and APAP are higher than that at the bare electrode. For CuO/PGE (Fig. 5(d)), the shift in peak potential is less than with PTH films. However, at PTH_{Ethaline}/CuO/PGE in Fig. 5(e), AA and APAP have peak potentials of 0.08 and 0.50 V, both less positive values than at PGE and at all the other modified electrodes, indicating a synergistic electrocatalytic effect. The anodic peak currents of AA and APAP at PTH_{Ethaline}/CuO/PGE are 6.0 and 5.6 times higher, respectively, than those at PTH_{Aqueous}/CuO/PGE, showing the benefits

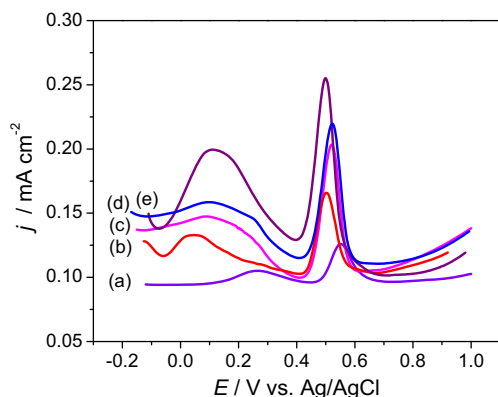


Fig. 5 DP voltammograms at (a) bare PGE, (b) PTH_{Ethaline}/PGE, (c) PTH_{Aqueous}/CuO/PGE, (d) CuO/PGE, and (e) PTH_{Ethaline}/CuO/PGE in 0.1 M BR buffer solutions (pH 4.0) containing 0.2 mM AA and 0.1 mM APAP

of using acid-doped ethaline DES as polymerisation medium in relation to aqueous solution.

The effect of pH on the oxidation of AA and APAP

The influence of pH on the oxidation peak currents of AA and APAP was evaluated by DPV in BR buffer solution in the pH range pH 2.0 to 7.0. The optimum pH value was found to be 4.0, giving the widest peak separation and the highest peak currents in the simultaneous determination of AA and APAP. With increasing pH values, the anodic peak potentials (E_{pa}) of AA and APAP shift to less positive values, indicating the participation of protons in the oxidation reaction [31]. The linear equations obtained for E_{pa} variation with pH are $E_{\text{pa}}(\text{AA}) = -0.056 \text{ pH} + 0.3773$ and $E_{\text{pa}}(\text{APAP}) = -0.058 \text{ pH} + 0.7678$; see the plots in Fig. S3 (see ESM). It can be concluded that for AA and APAP on PTH_{Ethaline}/CuO/PGE,

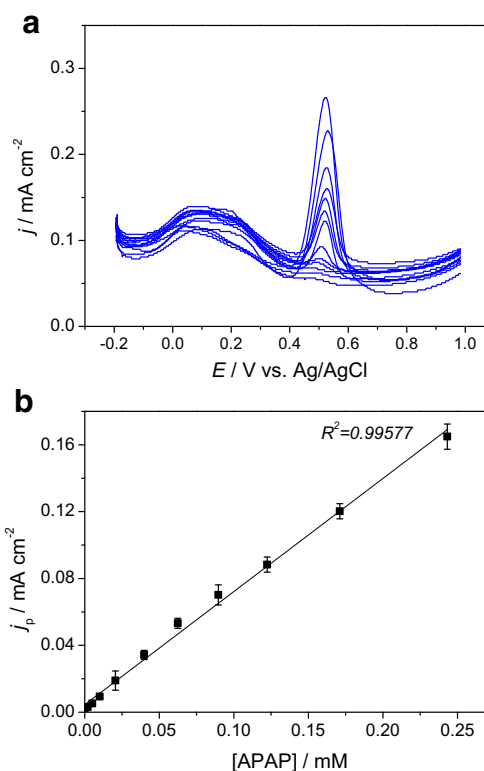


Fig. 6 a DP voltammograms for increasing concentrations of APAP up until 0.25 mM in the presence of 0.2 mM AA. b Plot of the peak current as a function of APAP concentration

the number of electrons and protons is equal, in agreement with literature reports on other modified electrode platforms [11].

Performance parameters

The determination of APAP by electrooxidation in the presence of AA under optimised experimental conditions was performed using the PTH_{Ethaline}/CuO/PGE by DPV (Fig. 6a). Aliquots of APAP were added to a buffer solution containing AA, and a linear calibration curve of the anodic peak current of APAP was constructed, with a correlation coefficient of 0.9958 (Fig. 6b). The LOD and LOQ were found to be 0.05 μM and 0.17 μM , respectively, using literature equations as in [32], respectively. The calibration plot follows the linear equation: $\Delta j \text{ (mA cm}^{-2}\text{)} = +0.0045 + 0.68 \text{ [acetaminophen] (mM)}$.

The analytical performance of the sensor was compared with literature reports during the last 5 years (Table 2). The proposed sensor shows better or comparable performance analytical parameters (limit of detection and linear working range) than other APAP sensors recently reported in the literature. Compared to most other reported APAP sensors, the detection limit at PTH_{Ethaline}/CuO/PGE is very low; see

Table 2. There are a few studies with lower detection limit [7, 11, 41–43]; however, the working ranges are narrower than here [7, 11] or the sensor fabrication is more complicated [41–43].

The repeatability and reproducibility of the PTH_{Ethaline}/CuO/PGE were studied by measuring the anodic peak current in 0.2 mM APAP solution in BR buffer (pH 4). In order to evaluate the repeatability of the sensor, five successive calibration curves were plotted with the same electrode, and a relative standard deviation (RSD) was calculated using the slopes of these calibration curves. The RSD value was found as 5.2% at PTH_{Ethaline}/CuO/PGE towards APAP, which showed the high repeatability of the proposed sensor. The reproducibility was evaluated by five sensors fabricated the same way. The RSD was 2.0%, indicating that the fabrication of the sensor was highly reproducible.

Analysis of real samples

The sensor with the best analytical performance, PTH_{Ethaline}/CuO/PGE, was used for the detection of acetaminophen in a pharmaceutical product. For the real sample analysis, Paramolan C® Sandoz (each envelope of Sandoz contains

Table 2 Comparison of analytical performances of voltammetric sensors for the determination of APAP

Modified electrode	Other analytes present	Linear range/ μM	LOD/ μM	Sample	Ref.
CNTCE	AA, PP	0.1–3.5	0.04	Human serum	[33]
P-ASP/TOHS/MWCNT/CPE	LD	0.1–300	0.07	Human urine	[9]
GPE/LFOR	AA, TRP	7.0–300	0.842	Pharmaceutical, biological	[34]
TCP- <i>Sa</i> /CPE	DA, TYR	1–46 and 46–90	0.2	Pharmaceutical	[35]
Fc-S-Au/CNC/graphene/GCE	AA, DA, UA	0.5–46 and 46–275	0.10	Human serum, urine	[36]
GPtNPs-GCE	AA, DA	5–71.5 and 91.5–1130.5	5	–	[37]
GO-XDA-Mn ₂ O ₃	AA	1–1000	0.056	Human urine	[38]
ErGO/GCE	AA, CAF	0.2–4.4	0.25	Pharmaceutical	[39]
PEDOT/h-CN/GCE	AA	1–10 and 10–50	0.49	Pharmaceutical	[40]
CeO ₂ /CNT/GCE	AA, DA, UA	0.01–900.0	0.004	Pharmaceutical, biological	[41]
ZKAKC/GCE	AA	0.01–20	0.007	Human urine	[7]
AuNP-PGA/SWCNT	AA	50–300	15	Pharmaceutical	[6]
3DCu _x O–ZnO/PPy/ErGO/GCE	AA, DA, TRP	0.033–400	0.010	Human serum	[42]
SEP/MWCNTs/PGE	AA, ASA	0.059–60	0.018	Pharmaceutical	[11]
MWCNTs/ZnCrFeO ₄ /CPE	AA, CO	0.1–368	0.009	Pharmaceutical, biological	[43]
Co ₂ Fe ₂ O ₄ /GCE	AA, CAF	0.2–4.4	0.267	Pharmaceutical, beverage	[44]
PTH _{Ethaline} /CuO/PGE	AA	0.07–250	0.05	Pharmaceutical	This work

CNTCE, carbon nanotube ceramic electrode; P-ASP, poly-aspartic acid; TOHS, TiO₂ hollow spheres; MWCNT, multi-walled carbon nanotubes; GPE, graphite paste electrode; LFOR, Lewatit® FO36 resin; TCP-*Sa*, meso-tetra(4-carboxylphenyl)porphyrin; CPE, carbon paste electrode; Fc-S, ferrocene derivative; CNC, carbon dots; GCE, glassy carbon electrode; GPtNPs, platinum nanoparticle-decorated graphene; GO, graphene oxide; XDA, 1,4-xylenediamine; ErGO, reduced graphene oxide; PEDOT, poly(3,4-ethylenedioxythiophene); h-CN, g-C₃N₄ nanofibers; ZKAKC, ZnCl₂-KOH-activated kelp carbon; PGA, polyglutamic acid; PPy, polypyrrole; SEP, nanosepiolite clay; AA, ascorbic acid; ASA, acetylsalicylic acid; CAF, caffeine; CO, codeine; DA, dopamine; LD, levodopa; PP, phenylephrine; TRP, tryptophan; TYR, tyrosine; UA, uric acid

APAP 500 mg and AA 250 mg) was supplied from a local drug store. The drug sample was dissolved in 250 mL distilled water. A total of 10 μ L of the prepared solution was added to the electrochemical cell containing 2 mL of BR buffer solution. The APAP content in the drug sample was found to be 0.503 ± 0.03 g ($n = 3$) with PTH_{Ethaline}/CuO/PGE sensor. An average recovery of $103.5 \pm 2.5\%$ was obtained. It can be concluded that there is no difference between the results obtained with the declared value and the presented sensor at a t test confidence level of 95%. The average apparent recovery shows that it is an accurate and simple method for the direct determination of APAP in the presence of AA.

Conclusions

Electrochemical polymerisation of TH at CuONP-modified PGE in ethaline DES containing acetic acid dopant was successfully achieved. For the first time, to our knowledge, a CuONP-based electrode was used for the polymerisation of TH. The PTH_{Ethaline}/CuO/PGE platform was characterized using CV, DPV, and EIS. The bare electrode and CuONP-modified electrode were compared to investigate the effect of modified electrode composition on the growth of the polymer film. This showed that the CuONP played an important role in the electropolymerisation of TH and supplied a surface suitable for polymerisation. The use of phenazine polymers with CuO nanoparticles as a nanocomposite film in sensing provides a synergistic interaction, as well as showing the properties of each nanomaterial leading to a higher APAP and AA current response. The PTH_{Ethaline}/CuO/PGE showed a low detection limit of 50 nM and wide linear range for the detection of APAP in the presence of AA using DPV. The proposed sensor was also successfully tested for the determination of APAP in pharmaceutical samples containing APAP and AA, demonstrating its potential in the development of sensors for the analysis of APAP in different types of sample containing AA.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00216-020-03078-6>.

Funding B. Dalkiran thankfully acknowledges the Scientific and Technological Research Council of Turkey (TUBITAK 2219) for a post-doctoral fellowship. The authors thank Fundação para a Ciência e a Tecnologia (FCT), Portugal, project PTDC/QEQ-QAN/2201/2014, in the framework of Project 3599-PPCDT, co-financed by the European Community Fund FEDER, and CEMMPRE, project UIDB/EMS/00285/2020 by FEDER funds through the program COMPETE – Programa Operacional Factores de Competitividade, and by national funds through FCT.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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