



Original Research Article

Highly Sensitive Voltammetric Determination of Acetaminophen by an Overoxidized Poly (p-aminophenol) Modified Glassy Carbon Electrode

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ABSTRACT

This study aims to develop a promising electrochemical sensor based on polymer film overoxidation following the electrochemical polymerization of p-aminophenol on a bare glassy carbon electrode (GCE) surface for the voltammetric determination of acetaminophen (ACP). Cyclic voltammetry (CV) and scanning electron microscopy (SEM) were employed to characterize the electroanalytical performance and morphology of the modified electrode. The results indicated a significant improvement in electrode sensitivity to ACP after electrochemical polymerization and overoxidation of poly(p-aminophenol). We also investigated the effect of all effective instrumental and experimental parameters on sensor response. The electrode SWV response to ACP within the range 0.07-100.0 $\mu\text{mol L}^{-1}$ with a limit of detection (LOD) of 0.021 $\mu\text{mol L}^{-1}$ was linear under optimized conditions. We also attempted to evaluate the designed sensor selectivity to different interfering species, suggesting no significant interference. The designed sensor was also used to determine ACP in different pharmaceutical preparations and biologic samples with minimal matrix effects, admissible recoveries (99-106), and satisfactory repeatability (1.0-5.3 %RSD). The proposed sensor exhibited admissible repeatability, reproducibility, and stability.

Keywords: Acetaminophen, Square wave voltammetry, Poly 4-Aminophenol, Electrochemical sensor.

Introduction

The use of the electropolymerization method for modification of electrodes with conductive polymers to promote the electrocatalytic features of analytes, ameliorating the electron transfer, and lowering the overpotential has grown dramatically in the field of electrochemical sensors [1-3]. Because despite the drop-casting technique where the reproducibility of the modified electrodes is really poor, in electropolymerization, the characteristics of the created films are completely under the control of the operator, and the thickness of the polymer can be changed easily by altering the electrosynthesis conditions [4-6]. As a result, the prepared electrodes by electropolymerization procedure are more repeatable and reproducible [7, 8].

Among the conductive polymers, poly 4-aminophenol (PAP) has distinguished properties that make it more preferable for sensing purposes. Poly 4-aminophenol has two oxidizable functional groups in its chemical structure (OH and NH₂). Consequently, more reactive sites for interacting with analytes are created on the surface of the polymerized film compared to other polymers like polyaniline [9-11]. Besides, poly-4 aminophenol has high stability, superb electrocatalytic activity, and great antifouling features. Moreover, poly 4-aminophenol can be overoxidized by applying a constant positive potential. In the overoxidation process, the porosity, conductivity, and permselectivity of the polymerized film improved substantially [11]. Besides, by over-oxidization, other reactive oxygen-containing functional groups, including carbonyl and carboxyl, can be generated on the surface of the poly 4-aminophenol film, which can augment the interaction of analyte species with the electrode surface through hydrogen bonding. For the aforementioned excellent features of the overoxidized poly 4-aminophenol (Ox-PAP), this conductive film was used as a modifier for sensitive determination of various analytes, including epinephrine and uric acid, ascorbic acid, dopamine, tryptophan, and glucose [12-14].

In this respect, we investigated the applicability of a modified glassy carbon electrode (Ox-PAP) as an SWV sensor for the electrochemical determination of Acetaminophen (ACP, Fig. 1) for the first time. All of the effective parameters were optimized. The electrochemical behavior of ACP on the modified electrode surface was inspected in detail. In the end, the applicability of the designed sensor at different real samples was also checked out.

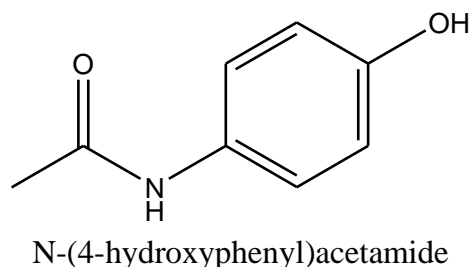


Figure 1. The chemical structure and IUPAC name of ACP

Experimental

Chemicals and Reagents

All employed reagents and components were obtained from Merck (Darmstadt, Germany) or Sigma-Aldrich and utilized without further purification. ACP was purchased from Iran Avandfar Pharmaceutical Company (Tehran, Iran). The stock solution of ACP (1.0×10^{-3} mol L⁻¹) was prepared by double distilled water (DDW). It was attempted to prepare a supporting electrolyte by mixing potassium nitrate (KNO₃) (1.0×10^{-1} mol L⁻¹) and hydrochloric acid (HCl) (1.0×10^{-2} mol L⁻¹) in all electrochemical measurements. ACP pharmaceutical products were bought from a nearby pharmacy for recovery tests. Besides, fresh human serum samples were obtained from the Tehran province blood transfusion organization (Tehran, Iran). DDW was used to prepare all solutions. Serum and pharmaceutical samples were prepared for analysis according to the procedure explained in [15, 16].

Apparatus

The Metrohm 797 VA Computrace Polarograph was used as a base to execute all electrochemical experiments, including CV and SWV. The pH was measured with a Metrohm 827 pH meter (Herisau, Switzerland) with a combined glass electrode. Ag/AgCl (saturated KCl) electrode, GCE, and Pt electrode were all purchased from Azar Electrode Company (Urmia, Iran). A three-electrode GCE-containing system 2 mm in diameter, a modified GCE used as working electrode, platinum electrode used as the counter electrode, and a saturated calomel electrode (SCE) used as a reference electrode were employed. SEM-EDS (MIRA3 TESCAN) was used to evaluate the surface morphology of the developed electrode.

Fabrication of modified electrode

Prior to each measurement, the GCE surface was polished on a polishing cloth with 0.3 μ m alumina slurry for 60 s and underwent ultrasonic cleaning, each for 10 min, with ethanol and re-distilled water. CV was utilized to conduct poly(p-aminophenol) (PAP) electrochemical deposition on GCE in a 5 mol L⁻¹ SDS + 5 mol L⁻¹ P-aminophenol + 1 mol L⁻¹ HCl solution [37]. Polymerization voltammograms were achieved through ten repetitive potential cycles from -0.645 to 1.955 V vs. SCE at a scan rate of 100mV s⁻¹. The PAP/GCE was over-oxidized for 30 s at +1.2 V in 0.1M NaOH solution for a stronger conductive and porous surface. Ultra-pure water was used to wash the modified electrode, referred to as Ox-PAP/GCE.

Results and Discussion

Electrosynthesis and characterization of the polymer film

Figure 2 demonstrates the cyclic voltammograms of p-aminophenol electropolymerization. The modified electrode was donated as PAP/GCE. An irreversible oxidation peak was detected during a CV scan at 1.56 V for p-aminophenol without corresponding cathodic processes during the reverse scan. Furthermore, reduction peaks and quasi-reversible oxidation were identified at \approx +0.67 and +0.31 V, respectively. During the p-aminophenol oxidation process, the peaks may be produced by the intermediate species [17]. The gradual decline of the peak currents of the two anodic peaks and the real increase of the cathodic peak current with its potential shift to more negative values with repetitive CV cycles demonstrated a high polymer content on the electrode surface [18]. The PAP/GCE over-oxidized with + 1.2V for 300 s in 0.1mol L⁻¹ NaOH solution to ensure a more porous surface and higher conductivity. Electrochemical sensor response is a function of its physical morphology. In this respect, the morphology of the electrode surface during the modification process was evaluated by SEM, and the obtained SEM images are presented in Figure 3. As can be seen, after electropolymerization, a thin layer film with a globular structure covered the surface of GCE homogeneously [17]. Then, by overoxidizing the PAP film at high potentials, the coated polymeric substrate changes significantly. As it is obvious from Figure 5B, the surface of the Ox-PAP/GCE electrode is covered by a swollen like a heterogeneous film with randomly distributed large spherical particles and the layer porosity enhanced remarkably in comparison to the PAP/GCE [18].

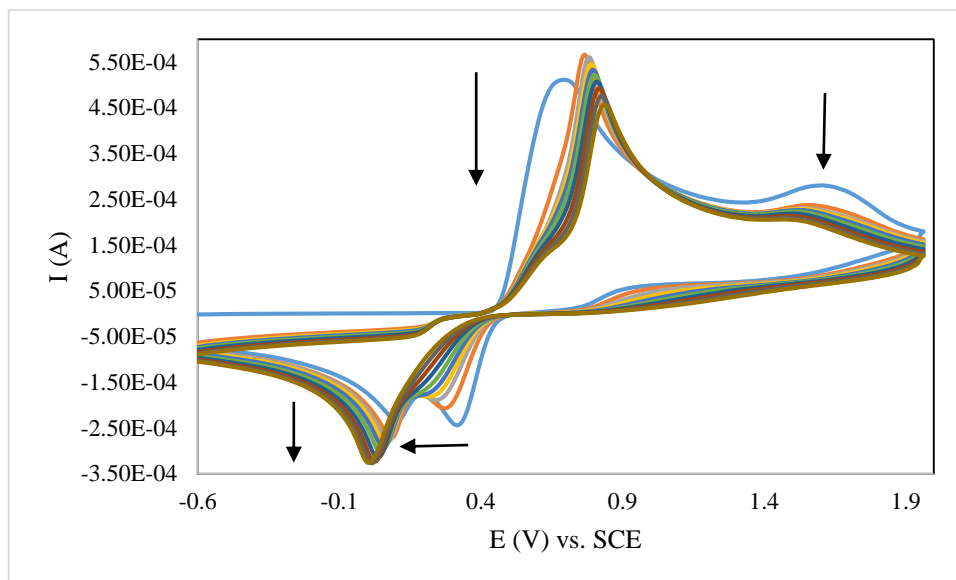


Figure 2. Cyclic voltammogram of p-aminophenol electropolymerization in a 5 mM p-aminophenol monomer + 1 M HCl solution on GCE in the presence of 5 mM SDS at a scan rate of 100 mV s^{-1} . The arrows indicate the trends of current during CVs.

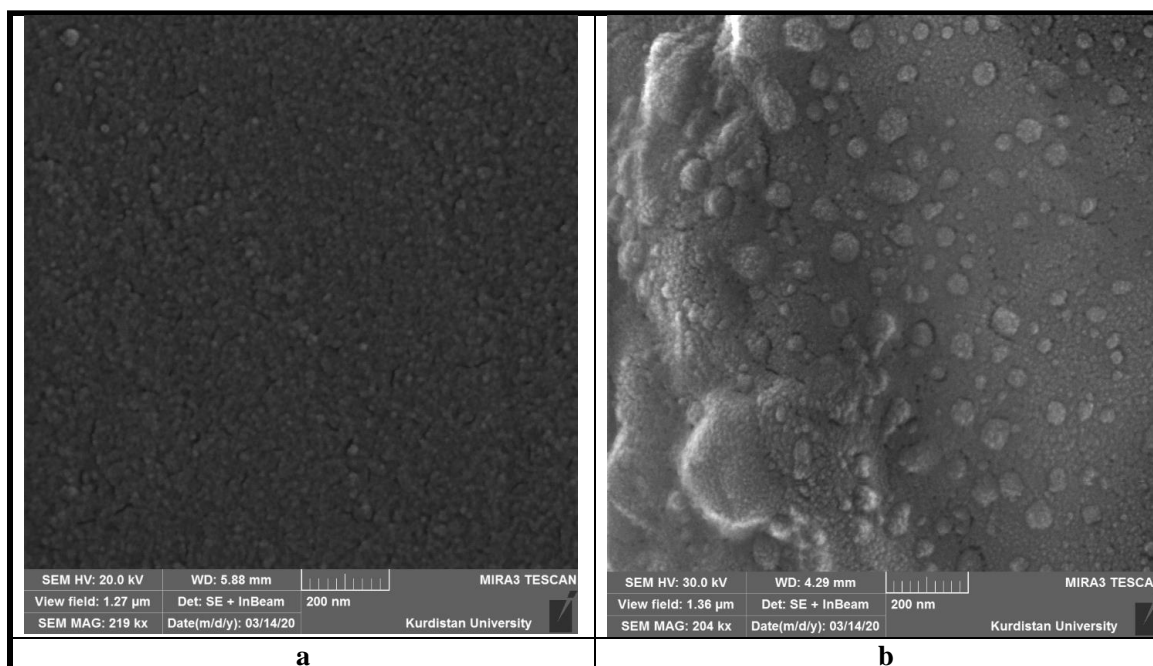


Figure 3. The SEM images of PAP/GCE (a) and Ox-PAP/GCE (b)

Optimization of effective experimental parameters

Measurement of the current dependency has been examined to assess the effect of all chemical instrumental parameters, such as pH, accumulation time and potential, pulse amplitude, voltage step, and frequency, on the SWV response [19]. These parameters were optimized to achieve a peak signal-to-noise ratio (PSNR), whose results obtained are shown in Table 1. The impact of the pH and accumulation potential on anodic stripping peak current was investigated within the pH and potential ranges of 1-10 and -0.500 to 0.200 V versus SCE under the optimal conditions above. As illustrated in Table 1, the maximum peak current was reached at an accumulation potential of 0.055 V versus SCE and pH solution of 2.0. Thus, 0.055 V versus SCE and pH=2.0 were chosen as an accumulation potential and working solution in the process. Also tested in the range of 0-300 s was the dependency of the maximum stripping peak current on accumulation time. The stripping peak current rose in proportion from 0 to 100 s under the other optimal conditions. Thus, the peak current was constant, and 100 s was chosen for accumulation time. Other beneficial parameters such as pulse amplitude, phase voltage, and frequency on SWV response were also tested. The optimal values of 55 mV, 9 mV, and 100 Hz were selected for the parameters above, respectively.

Table 1. The optimum values of the studied instrumental parameters

Parameter	Range studied	Optimum value
pH	1-10	2
Accumulation potential (V)	-0.5 to +0.2	+0.055
Accumulation time (S)	0-300	100
Pulse amplitude (mV)	10-100	55
Voltage step (mV)	1-15	9
Frequency (Hz)	25-140	100

Analytical parameters of the sensor

Under the optimal conditions alluded to above, the SWV procedure was used for extremely sensitive ACP measurement. An ACP calibration curve was achieved from at least three replicate measurements on average (Figure 4). As indicated, the peak current linearly rose with a rise in concentration from 0.07 to 100 $\mu\text{mol L}^{-1}$. The ACP concentration (C_{ACP}) and peak current are linearly correlated with two linear equations of $I(\mu\text{A}) = 107.94 C_{\text{ACP}} (\mu\text{mol L}^{-1}) - 0.8468$ ($R^2 = 0.9921$) and $I(\mu\text{A}) = 10.731 C_{\text{ACP}} (\mu\text{mol L}^{-1}) + 10.897$ ($R^2 = 0.9986$) within the range 0.07-1.00 $\mu\text{mol L}^{-1}$ and 1.00-100.00 $\mu\text{mol L}^{-1}$, respectively. The LOD was estimated as 0.021 $\mu\text{mol L}^{-1}$, according to IUPAC ($3S_b/m$).

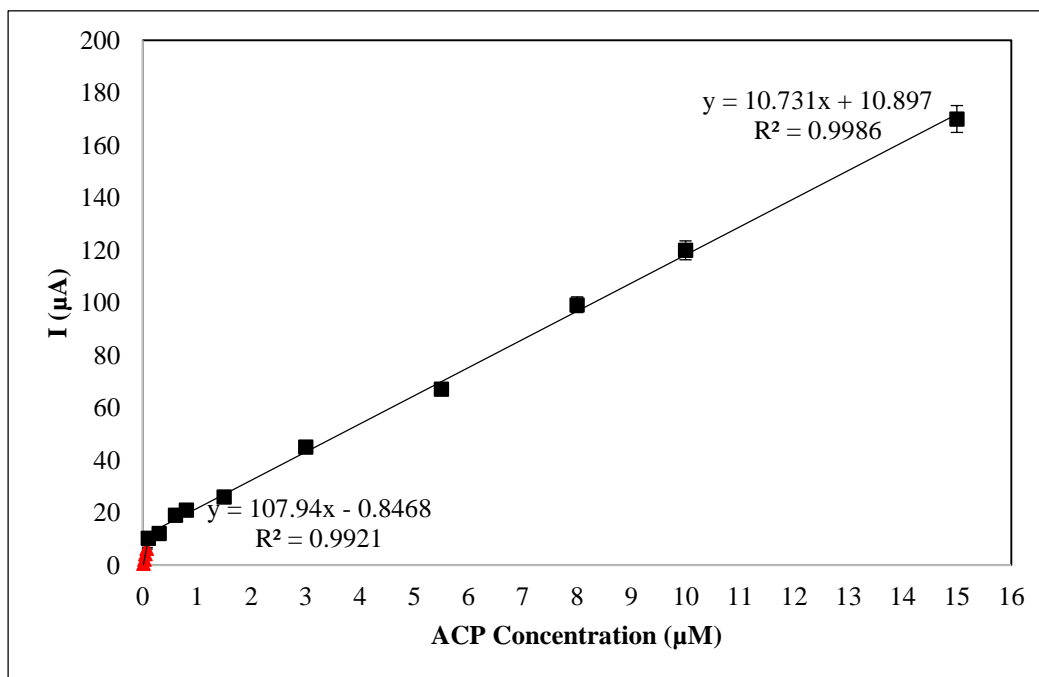


Figure 4. Plot of SWV oxidation peak current as a function of ACP concentrations

The selectivity of the proposed sensor

The selectivity of an analytical technique has an important effect on the accuracy of the obtained results. In this respect, the effects of 30 various species on the analytical response of the designed sensor were examined. Thus, a 0.10 $\mu\text{mol L}^{-1}$ solution of ACP in the supporting electrolyte was prepared. Different amounts of the interfering species were added to the solution. The

voltammogram of the sample was measured in the presence of other interfering species. The tolerance limit, defined as the maximum volume of the interfering species causing an error not higher than $\pm 5\%$ in the peak current of ACP, was calculated for the studied interfering species. The obtained results are presented in Table 2. The results indicate that the designed electrode showed an admissible selectivity towards ACP over a wide range of compounds that coexist with ACP in pharmaceutical samples, biological specimens, and some medicines prescribed simultaneously with ACP.

Table 2. The influence of some interfering species on the measurement of ACP ($0.1 \mu\text{mol L}^{-1}$) by the proposed method

Interfering species	Tolerance limit
Cl^-	1500
Mg^{2+} , Fe^{3+} , Fe^{2+} , Na^+ , Ca^{2+}	1000
F^- , Mn^{2+} , Cu^{2+} , Co^{2+}	800
Glucose, Lactose, Fructose, Maltose, Sucrose	600
Ibuprofen, Urea	500
L-Cysteine, L-Arginine	300
Ascorbic acid, Diclofenac	100
Levodopa, Dopamine, Mefenamic acid, Naproxen	80
Codeine, Meloxicam, Caffeine	50
Oxycodone, Sumatriptan	15

Repeatability, reproducibility, and stability of the modified electrode

SWV measurements for the $0.10 \mu\text{mol L}^{-1}$ ACP solution were used to test the repeatability, reproducibility, and stability of Ox-PAP/GCE. For investigating the repeatability of the proposed sensor, five different solutions of ACP with the same concentration were prepared, and their peak current was measured. Then the relative standard deviation (RSD%) was calculated, which was 3.43%. While the modified electrode reproducibility was explored by measuring the peak current

of one ACP solution with five different electrodes, the RSD% was 4.91% for five measurement assays. To test the electrode stability, the electrode was stored for 30 days at room temperature in the lab. SWVs were recorded and compared to SWVs achieved before storage [17]. The findings revealed only minor changes in the peak current and the excellent repeatability, reproducibility, and stability of the modified electrode.

Applicability of the proposed sensor for determination of ACP in human serum samples and pharmaceutical preparation

ACP was determined in serum and pharmaceutical samples to show the capabilities of the modified electrode to assess ACP in real samples. As previously mentioned, the SWV techniques were utilized upon sample preparation and the necessary dilution measures for determining ACP in human serum samples and pharmaceutical preparations. Table 3 and Table 4 display the results. The average outcomes of the three determinations for ACP pharmaceutical preparation were close to the values on the labels. A recovery test was carried out to verify the accuracy and applicability of the technique examined in pharmaceutical preparations for ACP determination in the human serum sample. The findings of a recovery test in Tables 3 and 4 show that ACP can be determined in both human serum samples and prescription formulations using the modified electrode.

Table 3. Determination results of ACP in pharmaceutical samples (n=3)

Sample	Labelled (mg)	Found (mg)	Recovery%	RSD%
Tablet	500	480.2 ± 24.55	96.04	4.91
Ampoule	150	146.1 ± 5.34	97.40	3.56
Syrup	120	124.99 ± 6.16	104.16	5.13
Drop	100	102.89± 3.21	102.89	3.21
Suppository	75	74.11± 2.91	98.81	3.88
Soft gel	325	323.61± 13.10	99.57	4.03
Capsule	325	327.12± 11.54	100.65	3.55

Table 4. Determination results of ACP in biologic specimens (n=3)

Sample	Added (μM)	Found (μM)	Recovery%	RSD%
Serum	---	ND	---	---
	0.050	0.049 ± 0.002	97.74	4.08
	0.80	0.834 ± 0.04	104.28	5.12
	10.0	9.630 ± 0.50	96.30	5.19
Urine	---	ND	---	---
	0.050	0.053 ± 0.001	106.98	1.88
	0.80	0.770 ± 0.03	96.25	3.89
	10.0	10.310 ± 0.41	103.10	3.97
Saliva	---	ND	---	---
	0.050	0.049 ± 0.002	98.04	4.08
	0.80	0.841 ± 0.02	105.16	1.68
	10.0	10.153 ± 0.45	101.53	4.43

Conclusion

In this study, a novel, rapid, and simple voltammetric sensor was developed for determining ACP in human serum samples and pharmaceutical preparations. For this purpose, p-aminophenol was electropolymerized on a GCE surface and then overoxidized by applying a constant potential in a basic solution to increase the porosity and conductivity of the polymer film. The influence of various parameters such as pH, accumulation potential, accumulation time, frequency, voltage step, and pulse amplitude was optimized to obtain the highest sensitivity towards ACP. Under optimum conditions, the electrode response was linear to ACP concentration within the range of 0.07 to 100 $\mu\text{mol L}^{-1}$ with a LOD of 0.021 $\mu\text{mol L}^{-1}$. The proposed electrode showed an eminent selectivity towards ACP. It was successfully applied for determining ACP in the human serum and pharmaceutical specimens with acceptable recovery values.

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