

Synthesis of Fe(acac)₃ nanoparticles as a modifier of carbon paste electrode for detection of acetaminophen

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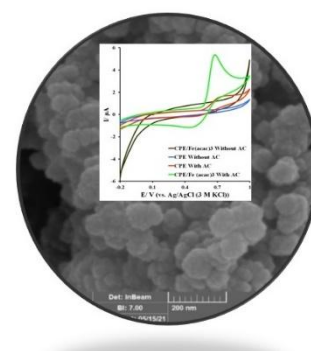
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Abstract

In the present work, a carbon paste electrode modified with tris (acetylacetonato) iron complex (Fe(acac)₃) nanoparticles was used to fabricate a novel electrochemical sensor for the electrochemical determination of acetaminophen (AC). The synthesized Fe(acac)₃ nanoparticles was characterized by Fourier transform infrared (FTIR) spectroscopy and the surface morphology and elemental composition of the fabricated electrode were analyzed by field emission scanning electron microscopy (FESEM) and energy-dispersive X-ray spectroscopy (EDS). Cyclic voltammetry and differential pulse voltammetry methods were used for evaluating the electrochemical behavior of the modified electrode. To optimize the measurement conditions, the effect of various parameters such as pH, deposition time, scan rate, potential, time, and interferences were successfully monitored. The modified electrode enhanced the peak currents relative to the bare carbon paste electrode. Moreover, the proposed electrochemical sensor showed good agreement with previously reported methods and was effectively employed for the quantification of acetaminophen in blood serum.



Keywords: Tris(acetylacetonato)iron nanoparticles, Acetaminophen, Electrochemical sensor, Detection.

Introduction

Acetaminophen (N-acetyl-para-aminophenol, AC) is a foremost antipyretic and analgesic medicine used for the relief of pain and reduce fever associated with headaches, colds, muscle aches, and arthritis. Excessive consumption of AC causes the accumulation of toxic metabolites that may lead to liver toxicity and renal toxicity.¹ The extensive remedial use of this drug gives rise to the need to develop rapid, easy, and sensitive techniques for the detection of AC. Although various methods have been applied for the measurement of acetaminophen,¹⁻⁵ these methods have restrictions such as complex sample pretreatments, high cost, long-time analysis, limited-controlling of the conditions, and possible interfering substances. Besides, low selectivity and sensitivity in some cases make these methods unsuitable for reliable analysis. To address these issues, the electrochemical techniques have fascinated much attention for measurement of acetaminophen due to the rapidity, good sensitivity, suitable selectivity, reasonable accuracy, and precision, and easy preparation process.⁶⁻²²

In the last fifty years, carbon paste electrode (CPE) fabricated

from graphite powder and a pasting liquid is the most acceptable electrode material for the preparation of experimental electrodes and the design of potential sensors, and biosensors.²³⁻²⁵ Low cost, high sensitivity, good detection limit, ease of application, and renewability are some advantages of modified CPE that have made it one of the most widely applied in electroanalysis.²⁶⁻²⁹

Schiff base metal complexes are a class of electrode modifiers that have been used extensively in electrochemical applications such as batteries and photoelectrochemical cells.³⁰ They are also considered a simple and appropriate candidate for catalytic application. Here, a metal complex namely tris(acetylacetonato) iron (Fe(acac)₃) complex with tridentate Schiff base ligand has been used for CPE surface modification. This complex is a deep-red crystalline solid with good solubility in chlorinated and alcoholic solvents.^{31,32} Fe(acac)₃ has been used in electrochemistry as a catalyst for enantioselective oxidation of sulfides to sulfoxides³³ and in the synthetic organic methodology for radical transformations, hydrogen atom transfers, carbometalations, and cross-couplings.³⁴ Several methods are available in the literature for the synthesis and purification of Fe(acac)₃ which the optimal one is

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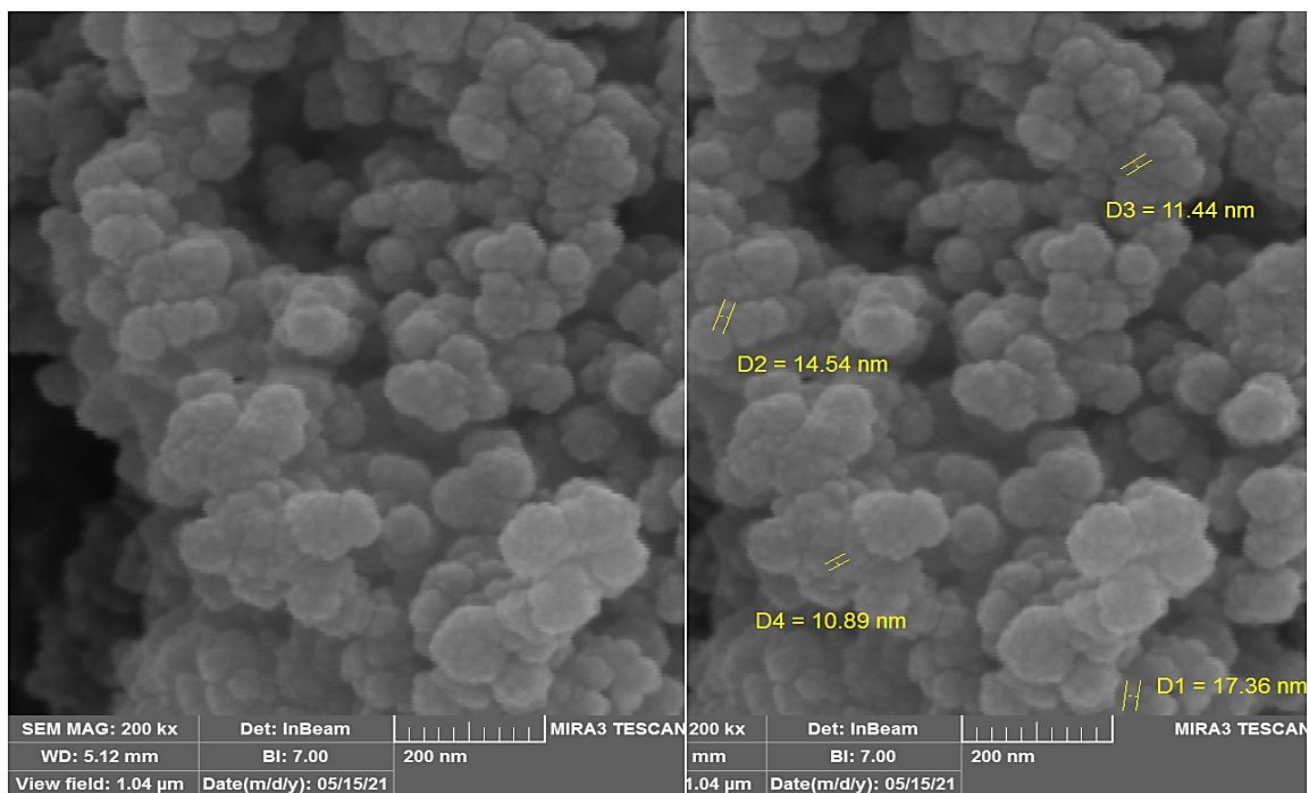


Figure 1. SEM image of the $Fe(acac)_3$ nanoparticles.

direct synthesis from iron(III) hydroxide and acetylacetonate without the use of any buffer.³⁵

In this work, a novel electrochemical sensor based on carbon paste electrode modified with $Fe(acac)_3$ was designed for the determination of acetaminophen with improved sensitivity, selectivity, and detection limit. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to evaluate the electrochemical performance of the prepared electrode and electrochemical sensor.

Experimental

Chemicals

All chemicals were of the analytical grade (A.R.) and were utilized without any cleansing. Graphite powder, paraffin, $FeCl_3 \cdot 6H_2O$, NH_3 , acetylacetonate and ethanol were obtained from Sigma Aldrich company. All experiments were performed with deionized water obtained using a TKACo Smart-2-Pure ultrapure system.

Apparatus

A Philips X'pert Pro MPD XRD system with a graphite-filtered $Cu\ K\alpha$ ($\lambda = 1.5418\text{\AA}$, 40 kV, 20 mA) source was used in the $10^\circ <2\theta < 90^\circ$ window at a 0.021° step size and 0.8 s/point measuring time. Sample morphology was analyzed using Tescan MIRA3 FEG-SEM and FEG-EDAX systems at 30 kV. FT-IR spectra were recorded using KBr disks and a Bruker Tensor 27 instrument. The Autolab PGSTAT 302N electrochemical device with three-electrode configurations was utilized to perform electrochemical quantification. In this system, the CPE acts as the working electrode, the Pt-wire as the auxiliary electrode, and the $Ag/AgCl$ (3M KCl) (Metrohm) as the reference electrode. Metrohm pH meter was utilized to measure the pH of solutions. The NOVA 1.6 software was used to analyze the

obtained electrochemical data.) nanoparticles.

The synthesis of $Fe(acac)_3$ cube-like nanoparticles

We added an amount of 4 g of $FeCl_3 \cdot 6H_2O$ to 6 mL distilled water. Then, 9 mL of NH_3 solution was added to reaction container dropwise through constant stirring. The mixture was heated to $50^\circ C$. Afterward, we filtered the precipitation of iron hydroxide with water to remove the left chloride. Next, the moist iron hydroxide with 12 mL of acetylacetonate was transferred to beaker (the inset of beaker was covered with cotton) and was heated up in to 35 min to reach $80^\circ C$. After it cooled down, the large and red crystals of acetylacetonate were washed down and dried. Eventually, the recrystallization process with Reflux. And at the temperature of $80^\circ C$ was performed.

Fabrication of the CPE/ $Fe(acac)_3$ nanoparticles

A mixture with a weight ratio of 80:20 %w/w prepared from graphite powder and binder oil (paraffin oil) was used to fabricate the CPE. After homogenization, the resulting blend was carefully compressed in a glass cell with an inner diameter of 1.43 mm and a height of 6 cm. The electrical contact was made through a Cu-wire. The electrode surface was refreshed each time with soft sandpaper. CPE modified CPE/ $Fe(acac)_3$ nanoparticles powder was prepared.^{27,36}

Preparing of the real sample

Serum sample were employed to prepare real samples. For this means, 3 mL of methanol was added to the collected serum sample, and the precipitated proteins were separated using centrifuge at 1000 rpm. The top layer was filtered again with 0.45- μm Millipore filter paper so that the resulting solution was free of protein.²⁷

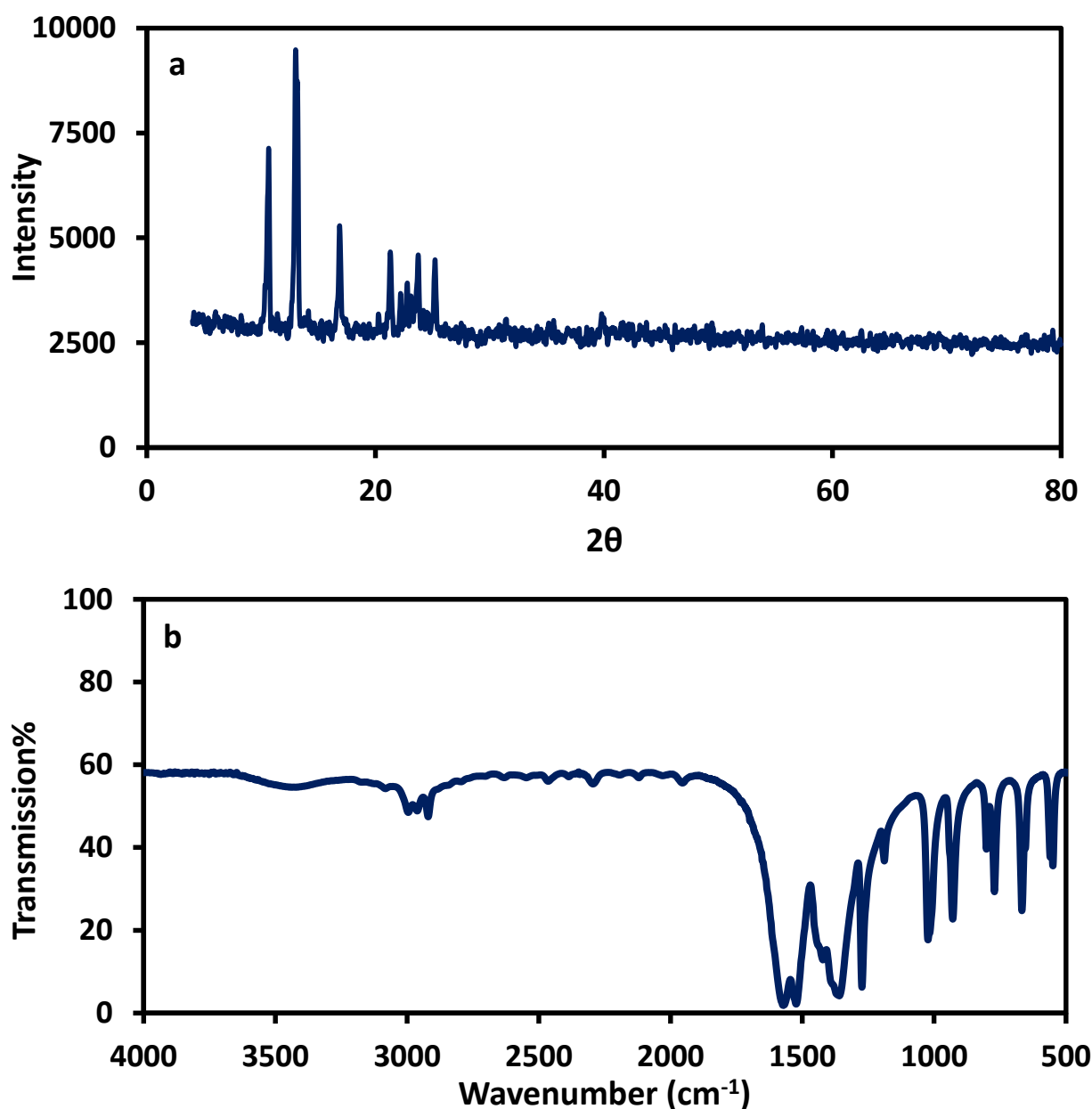


Figure 2. (a) XRD patterns and (b) FT-IR spectrum of $\text{Fe}(\text{acac})_3$ nanoparticles.

Results and discussion

Characterization of the $\text{Fe}(\text{acac})_3$ nanoparticles

The morphology of the ferric acetylacetonate spherical-like has been examined by FE-SEM image (Figure 1). According to the Figure 1, it is seen that the products composed of ferric spherical-like structures with average size of about 10-20 nm.

XRD patterns of $\text{Fe}(\text{acac})_3$ spherical-like nanoparticles are shown in Figure 2a. According to this figure, crystal structure of $\text{Fe}(\text{acac})_3$ is match with its crystal standard. The hexagonal phase of $\text{Fe}(\text{acac})_3$ diffraction peaks are indexed to planes (221), (000), (222), and (121) at $2\theta = 27.6, 28.6, 29.8,$ and 33.0 (space group $P63mc$, JCPDS No. 00-011-0864) with the calculated cell parameters of $a = b = 3.2539 \text{ \AA}$ and $c = 5.2098 \text{ \AA}$.

The formation of $\text{Fe}(\text{acac})_3$ was characterized by FT-IR as shown in Figure 2b. It can be seen that the $\text{Fe}(\text{acac})_3$ exhibits the

strong peak at $1572, 1524, 1359,$ and 1273 cm^{-1} that is assigned to the C=O, C=C, C-H, and C=C-H groups, respectively. In addition, the the stretching vibrations of the COO and C-O groups in $\text{Fe}(\text{acac})_3$ nanoparticles was observed by the peaks at $1620, 1421,$ and 1051 cm^{-1} , respectively.

Electrochemical behavior of AC at the CPE and $\text{Fe}(\text{acac})_3$ nanoparticles

The efficiency of CPE/ $\text{Fe}(\text{acac})_3$ for detection of AC was evaluated using cyclic voltammetry method (CV) method. Figure 3 shows the CVs at the CPE and CPE/ $\text{Fe}(\text{acac})_3$ nanoparticles in the phosphate buffer solution (pH 3.0) over the range of -0.2 to 1 V at the scan rate of 50 mV s^{-1} in the presence and absence AC (50 \mu M). It can be seen that CPE/ $\text{Fe}(\text{acac})_3$ exhibited greater anodic peak current in comparison to the unmodified CPE. The peak current on the CPE/ $\text{Fe}(\text{acac})_3$ nanoparticles has been about 3.2 fold greater than that on the unmodified CPE, reflecting that $\text{Fe}(\text{acac})_3$ nanoparticles

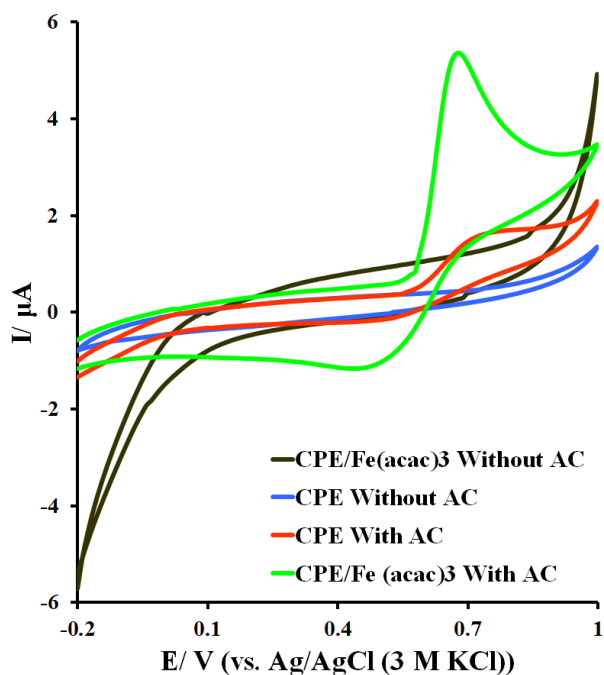


Figure 3. CVs of CPE and CPE/Fe(acac)₃ in 0.1 M phosphate buffer solution (pH=3.0) containing 50 μM AC at a scan rate of 50 mV s⁻¹

would improve the electrochemical sensitivity of the CPE/Fe(acac)₃ to determine AC.

The effective surface area of the electrode (*A*, cm²) was calculated from obtained data of CV in K₃[Fe(CN)₆] (5 mM) with a diffusion coefficient (*D*, Cm² s⁻¹) of 7.5 × 10⁻⁶ using Equation 1:³⁷

$$I_{pa} = 2.69 \times 10^5 n^{3/2} AC_0 D^{1/2} \nu^{1/2} \quad (1)$$

where, *n* refers to the electrons transferred in the oxidation and reduction process of ferrocyanide, *C* points to the ferrocyanide

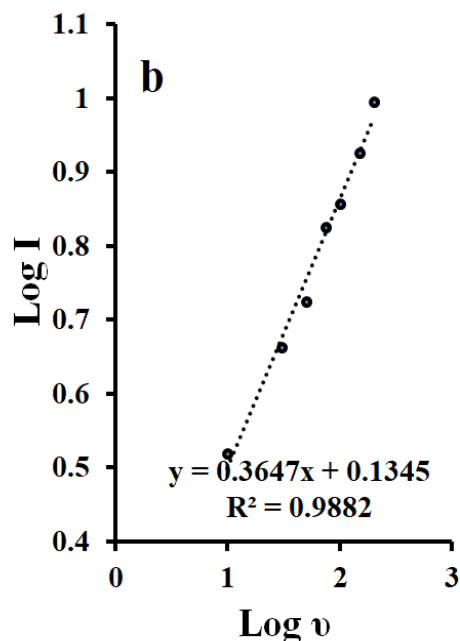
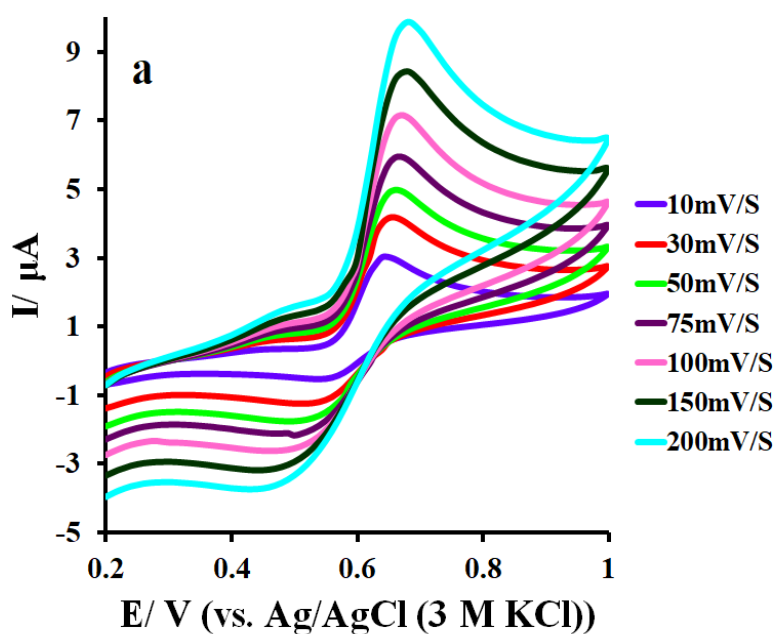
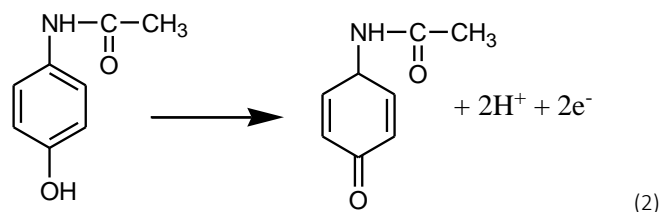


Figure 4. (a) CVs of AC on the CPE/Fe(acac)₃ surface at various scan rates in the range of 10–200 mV s⁻¹ in the phosphate buffer solution (pH 3.0), (b) Log *I_p* versus Log *ν*.

concentration (5 × 10⁻⁹ mol cm⁻³) and *ν* implies the scan rate. The CPE/Fe(acac)₃ (0.045cm²) surface area is larger than that of CPE (0.028 cm²) which can be related to the higher electrical conductivity and larger specific surface area of the compound.

Electrochemical behavior of AC at CPE/Fe(acac)₃ surface were examined by recording CVs of AC in pH 3.0 at various scan rates. Figure 4a shows the CVs of AC at CPE/Fe (acac)₃ surface at various scan rates. As seen, *I_{pa}* is enhanced by raising the scan rate from 10 mV s⁻¹ to 200 mVs⁻¹, which results in a shift in the *E_p* of AC to more positive values. Also, the linear relationship between *I_{pa}* and scan rate (Figure 4b) was indicated that the electrochemical performance of AC at CPE/Fe(acac)₃ surface is dominated by the diffusion process.

AC electrochemical behaviors depended on the solution pH. Therefore, the various solutions with pH values from 2.0 to 9.0 (0.1 M phosphate buffer solution) were prepared and its effect on redox AC at the CPE/Fe(acac)₃ surface was investigated (Figure 5). The results demonstrated greater advantages of the pH=3 condition for redox AC at the surface of CPE/Fe(acac)₃. Consequently, pH=3.0 has been selected as the best pH during further investigations. Figure 5c gives a linear correlation between *E_p* and the pH of the solution. The obtained slope value of 54 mV/pH is near the predicted amount of Nernst, indicating that the number of protons and electrons involved or conveyed in electrochemical processes is similar.³⁸ The proposed mechanism is presented Equation 2:



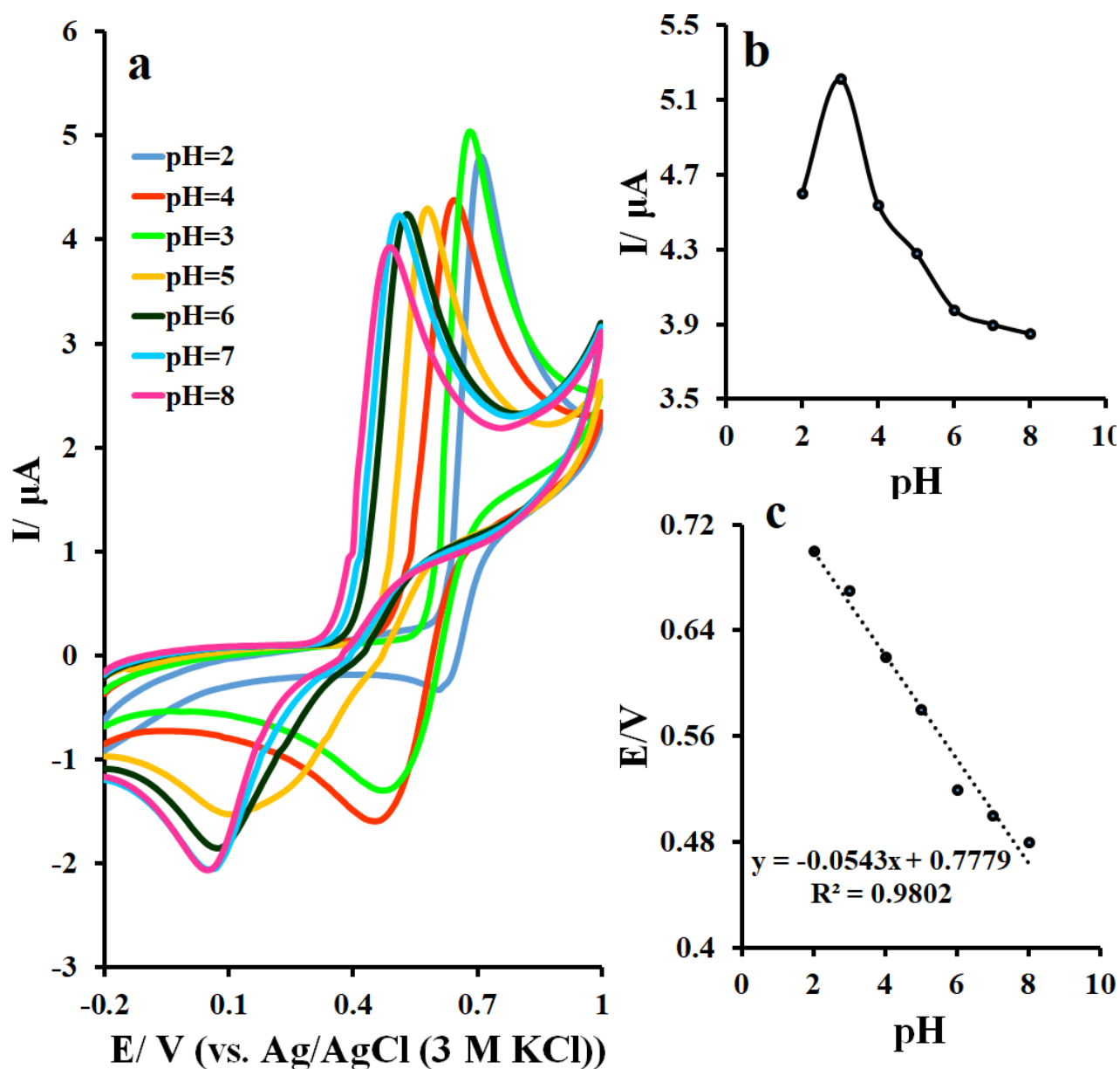


Figure 5. (a) CVs of the CPE/Fe(acac)₃ in 0.1 M phosphate buffer solution having 50 μM AC with different pH values in a range of 2, 3, 4, 5, 6, 7 and 8, (b) *I*_p versus pH and (c) *E*_p versus pH.

Table 1. A comparison between different electrochemical sensors for AC determination.

Modified electrodes	Analytical Method	Linear range (μM)	LOD (μM)	Ref
AuNP-PGA/SWCNT	DPV	8.3-145	1.18	41
ERG/GCE	DPV	5-800	1.2	42
ERG/Ni ₂ O ₃ -NiO/GCE	DPV	0.04-100	0.02	43
SWCNT-GNS/GCE	DPV	0.05-64.5	0.03	44
Nafion/TiO ₂ -GR/GCE	DPV	1-20	0.21	45
Fe ₃ O ₄ -PDDA-G/GCE	DPV	0.1-100	0.03	46
CPE/Fe(acac) ₃ nanoparticles	DPV	5-500	1.6	This Work

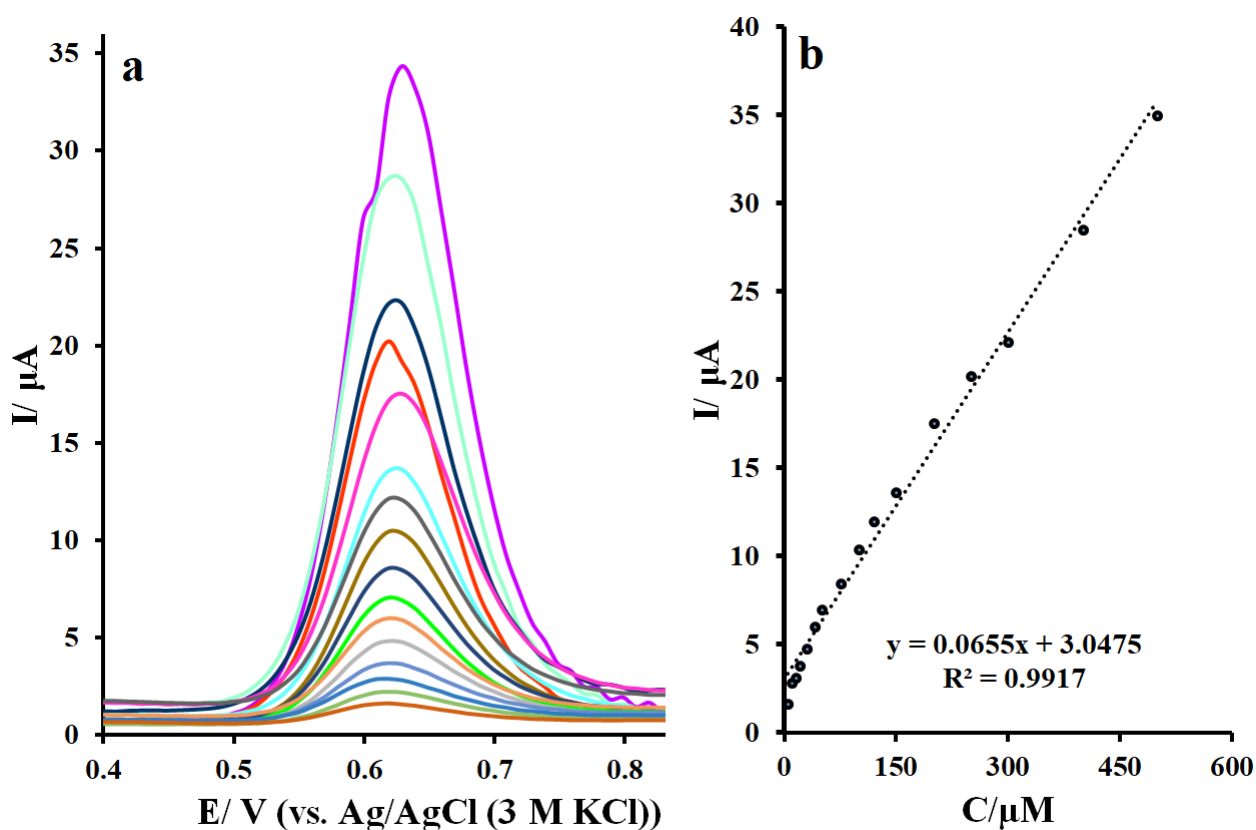


Figure 6. (a) DPVs of the CPE/Fe(acac)₃ nanoparticles in the presence of 50.0-500.0 μM AC (b) the corresponding linear relationships between the current and different concentrations of the AC.

Due to multiple benefits like higher sensitivity, lower background currents, and more acceptable peak resolutions, the DPV method was used to quantitate AC. Figure 6 presents a diagram of the DPV response of the various concentrations of AC (5.0-500.0 μM) on the CPE/Fe(acac)₃ surface. As seen, the final calibration curve is linear from 5.0 to 500.0 μM. Moreover, the detection limit (based on 3S/m)^{39,40} and sensitivity is about 1.66 μM (S/N=3) and 0.0655 μA μM⁻¹, respectively. The detection limit of this method (1.66 μM) is comparable compared to the other methods. Comparison of the suggested system with the reported electrochemical techniques for AC analysis is in Table 1.

To estimate the efficiency and suitability of the modified electrode for measuring AC in blood serum samples at CPE/Fe(acac)₃ surface, the standard addition method was used. For this purpose, different concentrations of AC in real samples were analyzed by the sensor. The obtained samples were diluted up to five-fold using PBS (pH 3.0), and DPV analysis was performed under optimal conditions. The results were presented in Table 2. The results showed a good recovery percentage and demonstrated the voltammetric method's reliability in determining AC in real biological samples using CPE/Fe(acac)₃ without any matrix interference.

The reproducibility of the CPE/Fe(acac)₃ nanoparticles was examined by DPV measurements of ten sensors. The relative standard deviation (RSD) calculated for the DPVs responses (I_{pa}) was 4.2 %, which indicated the good reproducibility of the CPE/Fe(acac)₃ nanoparticles. Also, the analytical repeatability of the sensor was confirmed by a low RSD value (3.7 %) resulted from three performed analyses utilizing one CPE/Fe(acac)₃ nanoparticles.

The selectivity of the technique was checked out by examining the change in AC signal during the addition of electroactive foreign species (Ca²⁺, PO₄³⁻, NO₃⁻, Mg²⁺, CO₃²⁻, Cl⁻, NH₄⁺, ascorbic acid, uric acid). The results did not show an apparent change in the signal, indicating that the method was selective and appropriate for measuring drugs in complicated matrixes.

Conclusion

In the present study, a novel electrochemical sensor based on carbon paste electrode modified with tris(acetylacetonato)iron complex (Fe(acac)₃) nanoparticles was fabricated for enhancing the electrochemical sensing responses of AC. The CV results showed that the peak currents of AC were outstandingly enhanced with the addition of Fe(acac)₃ nanoparticles to carbon paste electrode (CPE).

Table 2. Determination of the AC in human serum samples at CPE/Fe(acac)₃.

Solution	Spiked (μM)	Obtained (μM)	Recovery (%)	RSD % (n=3)
Serum sample	10.0	9.8	98	2.76
	30.0	30.2	100.6	2.14
	100.0	98.1	98.1	1.56

The increased surface area and electron mobility rate of the modified electrode support the observed current augmentation. Under the optimal condition obtained from optimization of the effective parameters, CV and DPV of AC present good selectivity without any interference, lower limit of detection, and acceptable linearity range. Besides, the constructed sensor revealed high stability, quick response, ease of use, and good reproducibility making the proposed method fascinating, appropriate, and reliable for standard analysis of AC in blood serum.

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