Electrochemical Quantification of Eugenol in Clove Extract Using a ZnO-Modified Carbon Paste Electrode

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Abstract
Carbon paste electrode modified with zinc oxide (ZnO) nanoparticles was developed for Eugenol's (Eg) electrochemical detection. Eg's electrochemical behavior was investigated using Cyclic Voltammetry, Electrochemical Impedance Spectroscopy and Square Wave Voltammetry. ZnO-modified electrode exhibited enhanced sensitivity and low limit of detection, enabling Eg's precise quantification. Eg's concentration in the extract was found to be 12.84 µM.

Keywords: chemically modified electrodes; Cyclic Voltammetry; Eugenol; Electrochemical Impedance Spectroscopy; Square Wave Voltammetry.

Introduction•

Natural products have historically been a major source of drugs, and modifying them through semi-synthesis often enhances their bioactivity and stability [1-3]. They exhibit a broad pharmacological potential, including antimicrobial, anticancer, anti-inflammatory, antiviral, antioxidant and neuroprotective properties [4, 5]. Beyond pharmacology, natural products and their derivatives also have applications in cosmetics [6], agriculture [7] and food preservation as natural antioxidants and antimicrobials [8]. Many antibiotics, anticancer agents, and other therapeutics originate from natural compounds, making them an invaluable resource for drug discovery and development [9, 10].

•The abbreviations list is in page 557.

Clove essential oil is a well-known natural product extracted from the cloves of *Syzygium aromaticum* (Giroflier) tree [11], with Eugenol (Eg) as its major component, constituting 75 to 90% of the oil [12]. Eg is a phenylpropene derivative, widely studied for its diverse biological and therapeutic applications, including anticancer, antidiabetic, antileishmanial, antifungal, antibacterial, antiviral, antioxidant and anti-inflammatory properties [13-20]. These pharmacological effects make Eg a highly valuable bioactive compound in the fields of medicine, pharmaceuticals, cosmetics and food preservation [21, 22]. Given the significant biological activity of Eg and its derivatives, their quantification is essential to ensure standardized concentrations in natural extracts, pharmaceutical formulations and various industrial applications. Precise quantification allows for accurate dosing, ensures efficacy and safety, and facilitates quality control in the production and use of Eg-based products.

To achieve Eg accurate quantification in complex natural extracts, electrochemical techniques provide a highly sensitive and selective approach. In this study, a carbon paste electrode (CPE) modified with Zinc Oxide (ZnO) nanoparticles was employed to enhance the electrode's electrochemical properties. ZnO nanoparticles are known for their high surface area, conductivity and catalytic activity [23], which improve sensitivity and selectivity of the electrode for detecting Eg. Various electrochemical techniques, including Cyclic Voltammetry, (CV), Electrochemical Impedance Spectroscopy (EIS) and Square Wave Voltammetry (SWV) were used to analyze Eg's electrochemical behavior. CV was employed to explore its redox properties, while EIS was used to evaluate the electrode's charge transfer resistance and overall electrochemical performance. SWV provided precise quantification of free Eg in the extract, offering reliable and sensitive measurements. The incorporation of ZnO nanoparticles as the modifier significantly enhanced the electrode's performance, making it an effective tool for the rapid and sensitive quantification of Eg in pharmaceutical, cosmetic and food applications.

Materials and methods

Chemicals and reagents

Every chemical utilized was of analytical grade and did not require any additional purification. Zinc chloride ($ZnCl_2$), sodium hydroxide (NaOH), hydrochloric acid (HCl) and graphite powder were purchased from Sigma Aldrich.

Apparatus and instruments

All electrochemical experiments of this study were performed using potentiostat OrigaStat 100, equipped with origamaster5 software. CV and SWV measurements were performed with a three-electrode system, including modified Nano-ZnO/CPE as working electrode, platinum as counter electrode and

saturated calomel as reference electrode. Spectra of ZnO nanoparticles obtained using Fourier Transform Infra-Red Analysis (FT-IR) analyses were performed using a JASCO FT/IR-4600 spectrometer equipped with an ATR (atenuated total reflection) accessory. ATR-FTIR spectra were recorded in the range from 4000 to 600 cm^{-1}, with a resolution of 4 cm^{-1} and an accumulation of 16 scans.

Synthesis of ZnO nanoparticles

$ZnCl_2$ and NaOH were used as precursors to synthesis ZnO nanoparticles. The following chemical equation was applied to calculate concentration ratio between NaOH and $ZnCl_2$:

$$ZnCl_2 + 2NaOH \longrightarrow Zn(OH)_2 + 2NaCl \quad (1)$$

To fully dissolve $ZnCl_2$, a magnetic stirrer was used to continuously stir a aqueous $ZnCl_2$ solution (0.5 M). In the same way, a 1 M aqueous NaOH solution was also prepared and then was added dropwise for 20 min. The mixed solution obtained after the complete addition of NaOH was left under constant stirring for 2 h, then sealed and stored overnight. After filtering the resultant solution, the precipitate was repeatedly cleaned with distilled water and methanol before being dried for 12 h at 100 °C. To create a nano-sized ZnO, the dried precipitate was calcined for 4 h at 400 °C.

Preparation of bare CPE and modified CPE

Using a mortar and pestle, 0.1 g ZnO nanoparticles and 0.9 g graphite powder were manually mixed to create modified CPE. The aforesaid mixture was then combined with 0.5 mL paraffin, and mixed for 30 min, to create a consistently wet paste. The paste was then put into a syringe tube that had a 2 mm internal radius. To establish electrical contact, a copper wire was pushed inside the tube. The same procedure was used to manufacture unmodified CPE, without adding ZnO nanoparticles.

Synthesis of Eg hydrazide

Clove essential oil is extracted from the cloves of Giroflier tree, with Eg being its main compound. Eg hydrazide was derived from clove essential oil, and its complete synthesis was carried out as stated by [24].

Results and discussion

Characterization of ZnO-NP synthesis

ZnO nanoparticles were characterized using FT-IR, as shown in Fig. 1. The peak at 418 cm^{-1} is attributed to Zn-O stretching vibrational mode of ZnO. The other peaks at 1652 and 3413 cm^{-1} may be due to O-H bending and stretching frequency of H_2O molecules. The peak at 1129 cm^{-1} is assigned to C-O stretching. FT-IR data is similar to other data obtained in literature [25, 26].

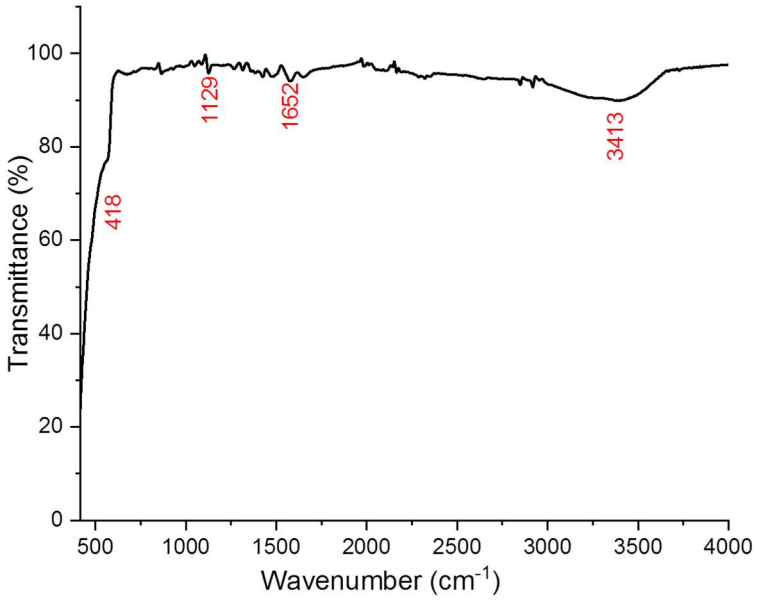

Figure 1: FTIR spectra of synthesized ZnO nanoparticles.

Electrochemical behaviour of Eg on Nano-ZnO /CPE electrode

Electrochemical interaction between Eg and nano-ZnO in a 0.1 M HCl solution was studied by CV. Fig. 2 shows CV of CPE and nano-ZnO/CPE with 2 µmol/L Eg, at a scan rate (SR) of 80 mV/s, in a potential range from -2.0 to + 1.0 V. It is clearly seen that there is no current signal for Eg redox at CPE electrode. However, an oxidation peak appeared at 0.319 mA, at a potential of -0.53 V, in the presence of Eg in the electrolyte. One can then conclude that the electrode has a high electrochemical performance, due to active sites available on its surface, which allows Eg to access large internal surfaces and improve adsorption onto nano-ZnO surface [27]. This means that the electrode was successfully used as sensor for Eg detection.

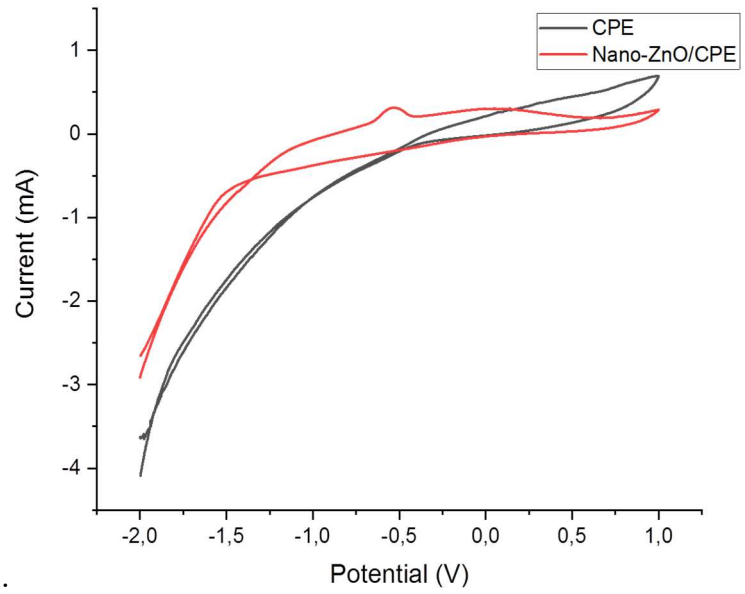

Figure 2: CV of CPE and nano-ZnO /CPE in the presence of 2 µM Eg in a 0.1 M HCl solution (pH 3.5), at a SR of 80 mV/s.

Electrochemical Impedance Spectroscopy

EIS was used to analyze surface characteristics of the modified electrode over a frequency range from 100 mHz to 1 kHz. Impedance spectrum generally shows a semi-circular region at high frequency, indicating charge transfer resistance, and a linear region at low frequency, associated with Warburg resistance. Fig. 3 presents EIS Nyquist diagrams of electrodes with 2 µmol/L Eg.

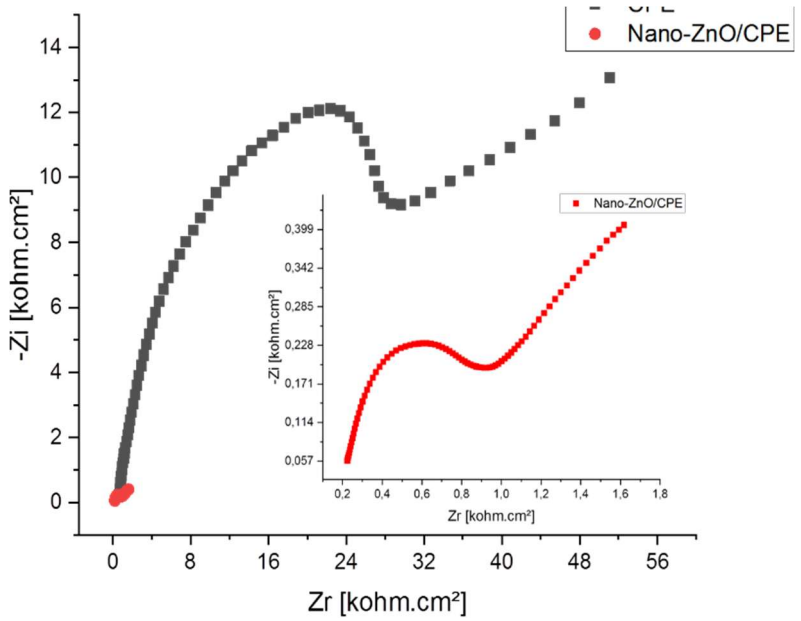

Figure 3: Nyquist plots of CPE and nano-ZnO/CPE with 2 µM Eg in a 0.1 M HCl solution (pH 3.5).

The semicircle corresponding to the modified electrode is significantly smaller compared to CPE, indicating that the surface modified by nano-ZnO had a superior conductivity, which confirms results obtained by CV. Furthermore, Nyquist plots of EIS shows a Warburg line. This indicates that Eg reaction on the electrode surface was governed by a diffusion phenomenon [28].

Experimental variable optimization

SR effect

To analyze reaction kinetics of each metal at modified CPE, the effect of SR (ν) ranging from 40 to 140 mV/s on oxidation peak current (I_{pa}) was studied. As shown in Fig. 4-A, redox I_{pa} consistently increased with higher SR. As Fig. 4-B demonstrates, plot of I_p logarithm as a function of SR logarithm shows a slope value of 0.51, which approached ideal value for a diffusion-controlled reaction (0.5), confirming Eg oxidation by diffusion mass transport in nano-ZnO/CPE [29].

The pH solution effect on electrode response was investigated in the pH range 2.5–5.5. Fig. 5 shows that I_p decreased as the solution pH increased. This

reduction resulted from Eg hydrolysis, which became more important at higher pH levels [30]. Ph of 2.5 was considered the best one for Eg determination.

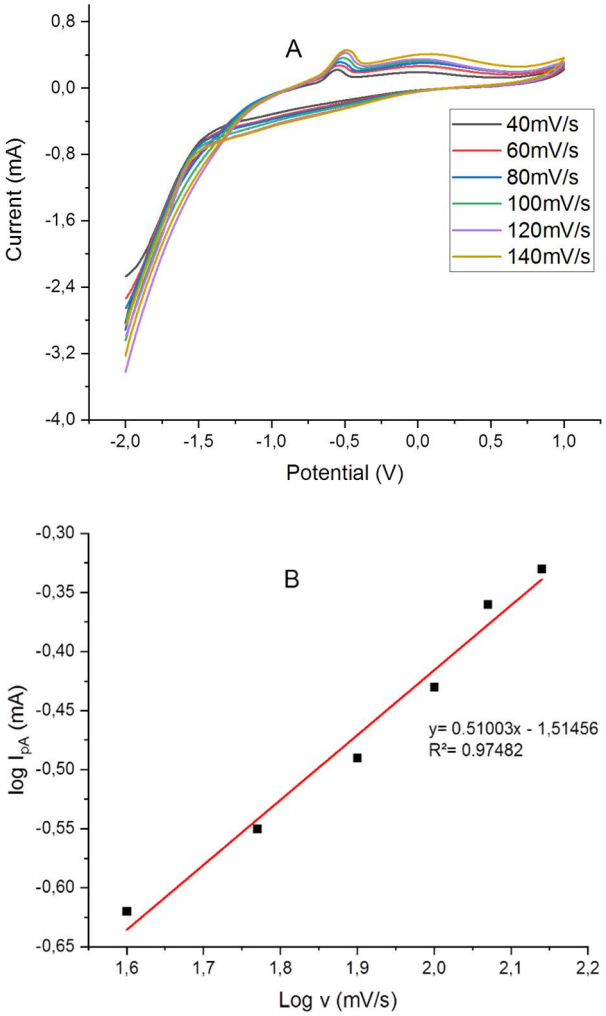

Figure 4: (A) Nano- ZnO/CPE CV in 0.1 M HCl solution (pH= 3.5) with 2 µM Eg at various SR: 40, 60, 80, 100, 120 and 1400 mV/s^{-1}; **(B)** Plot of log I_{pa} versus log of SR.

Effect of pH

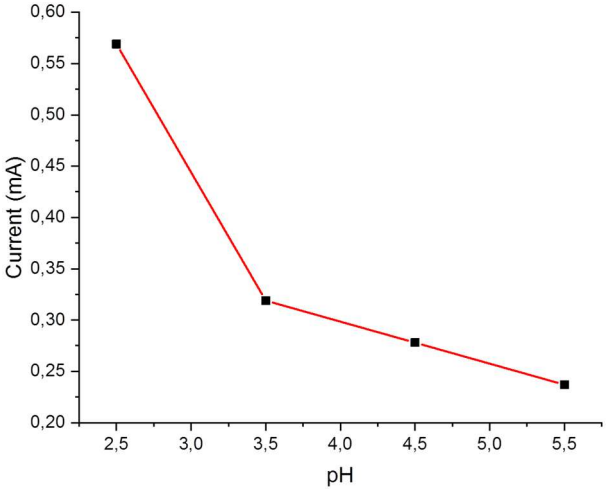

Figure 5: Effect of pH on I_p of Eg at nano-ZnO/CPE in a 0.1 M HCl solution with 2 µM Eg.

Accumulation time effect

The impact of AT for Eg detection was studied in the range from 0 to 5 min, in a 0.1 M HCl solution (pH 2.5) with 2 µM Eg. Fig. 6 shows that I_p increased in the first min, then I_{pa} intensity decreased, due to the electrode surface saturation [31]. Thus, 1 min was chosen as optimum accumulation time for Eg determination.

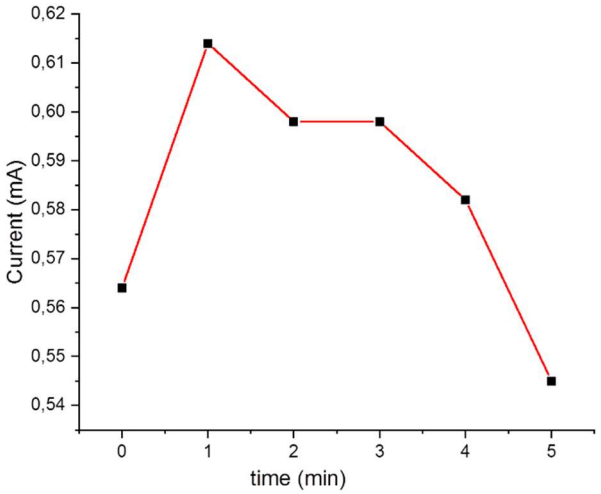

Figure 6: Effect of accumulation time on I_p of Eg at Nano-ZnO/CPE electrode in 0.1 M HCl solution with 2 µM Eg (pH 2.5).

Concentration effect

SWV at different Eg concentrations (Fig. 7 (A)) shows that current intensity increased with higher concentrations, which demonstrates a linear relationship ($R^2 = 0.99$), as shown in Fig. 7 (B)).

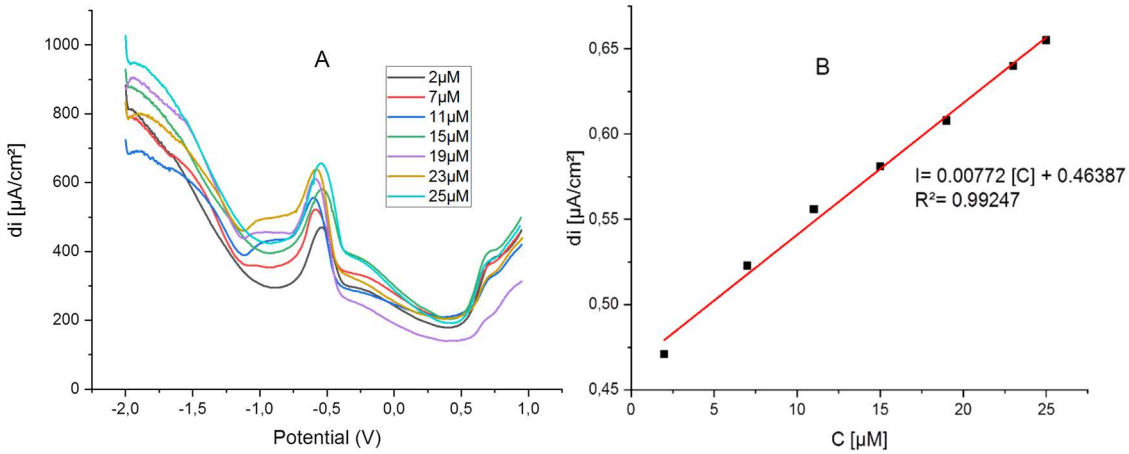

Figure 7: (A) SWV of nano-ZnO /CPE for the determination of Eg in a 0.1 M HCl solution (pH= 2.5); **(B)** calibration graph corresponding to peak of Eg.

The sensor's sensitivity was confirmed by limit of detection (LoD) ($3S_b/S$) and limit of quantification (LoQ) ($10S_b/S$) calculations, where S_b is standard

deviation of six blank measurements and S is the slope of calibration plot [32]. The results obtained are presented in Table 1.
A calibration curve equation (I= 0.00772 [C] + 0.46387) was obtained from the standard curve of current as function of concentration. This equation was then used to quantify Eg in the extract. Eg was obtained with various concentrations in the range from 2 to 25 µM.

Table 1: LoQ and LoD values for Eg at Nano-ZnO /CPE.

Concentration	LoQ	LoD
2–25 µM	5.86 µM	1.76 µM

Quantification of free Eg on the extract

To quantify the concentration of free Eg in the extract, a solution was prepared by dissolving 1 g extract in 50 mL of a 0.1 M HCl solution, ensuring optimal solubilization of Eg in an acidic medium. The analysis was performed using SWV, over a potential range from -2 to 1 V. Fig. 8 shows I_p of 0.563 mA, attributed to free Eg, at a potential of -0.6 V, confirming its presence in the sample. Using previously obtained calibration curve equation, free Eg concentration in 1 g extract was found to be 12.84 µM. This value provided an estimate of free Eg content in the extract, helping assess the extraction process efficiency.

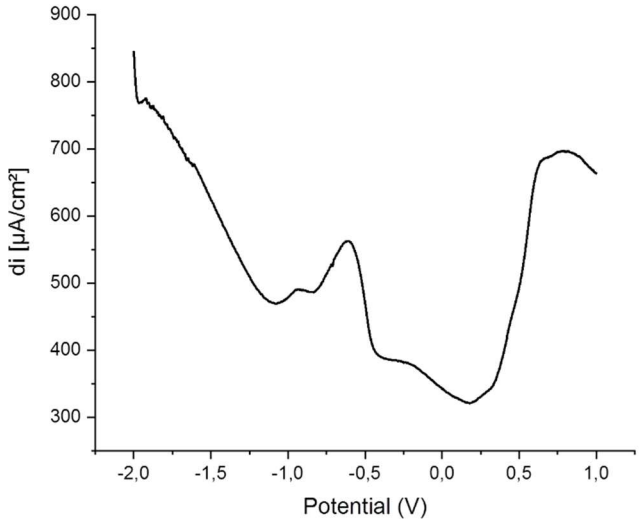

Figure 8: SWV of nano-ZnO/CPE for the quantification of free Eg in 1 g extract dissolved in a 0.1 M HCl solution.

Conclusion

In this study, ZnO nanoparticles were synthesized and characterized by FT-IR, then used for modifying CPE. Nano-ZnO/CPE was developed for Eg's electrochemical detection, and its performance was successfully evaluated by

CV, EIS and SWV. The sensor revealed high sensitivity, low detection limit and precise quantification of Eg.

Authors' contributions

Mohammed Ellaite, Sofia Kerouad, Issam Forsal: conceived and designed analysis; collected data; inserted data; wrote original draft; project administration. **Bahija Rebbah**: contributed to conceptual development; performed analysis; contributed to interpretation of results. **Wisal Kotmani**: conceived and designed analysis; edited and formatted paper. **Mohammed Mbarki**: literature review and editing; collected data. **Abderrahim El Haib**: contributed to interpretation of results; resources and validation.

Abbreviations

ATR: atenuated total reflection
CPE: carbon paste electrode
CV: cyclic voltammetry
Eg: Eugenol
EIS: electrochemical impedance spectroscopy
FTIR: Fourier transform infrared spectroscopy
HCl: hydrochloric acid
I_p: peak current
LoD: limit of detection
LoQ: limit of quantification
NaOH: sodium hydroxide
SR: scan rate
SWV: square wave voltammetry
$ZnCl_2$: zinc chloride
ZnO: zinc oxide

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