An ionic liquid/bismuth film-modified sensor for the electrochemical detection of cefixime

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Abstract: A highly sensitive and simple method based on an ionic liquid/ex situ bismuth film-modified single-use pencil graphite electrode (IL/Bi/PGE) was offered for the electrochemical detection of cefixime (CEF) for the first time. The synergistic effects of the ionic liquid and bismuth film combination as the surface modifier remarkably improved the electrochemical response of the modified sensor. Anodic oxidation of CEF was observed at 0.9 V vs. Ag/AgCl/3 M KCl as one well-defined irreversible oxidation peak. Under optimum experimental conditions, the IL/Bi/PGE exhibited a linear relationship for CEF concentrations in the range of 0.005–0.5 μM ($R^2 = 0.996$) with a detection limit as 0.22 nM with square wave voltammetry. The IL/Bi/PGE had excellent sensor properties to electrochemically detect CEF when compared to the literature. This method was successfully applied for practical use with pharmaceutical samples by standard addition method with high accuracy. These results showed that this simple analytical method can find potential application in the clinical field instead of highly expensive and time-consuming processes.

Key words: Analytical methods, electroanalytical chemistry, ionic liquids, voltammetry, cefixime

1. Introduction

Cephalosporins are antibiotics of β-lactam rings widely used for the therapy of various infections in both humans and animals due to their antibacterial and pharmacokinetic properties. They are among the safest antibiotics for being active against penicillin-resistant bacteria and being feasible for penicillin-allergic patients. Cefixime (CEF) is a semisynthetic third-generation oral antibiotic belonging to the cephalosporin group and works by inhibiting bacterial cell wall synthesis. The general chemical structure of cephalosporins and cefixime is given in Figure 1.

These drugs are available in the treatment of infections against bacteria causing urinary tract infections, gonorrhea, throat infections, laryngitis, pharyngitis, and bronchitis, which are resistant to most commonly used antibiotics.[1,2] The extensive use of CEF in the food and fermentation industry and in veterinary practice as a growth promoter may lead to some health problems due to its accumulation in food products.[2] Residues of these drugs may enter into the human body through the food chain. Considering this wide range of applications of CEF, the development of simple, sensitive, and rapid analytical methods for its determination is of great importance for human health and food quality control analyses.

Numerous analytical methods developed for the detection of CEF can be found in the literature, in-

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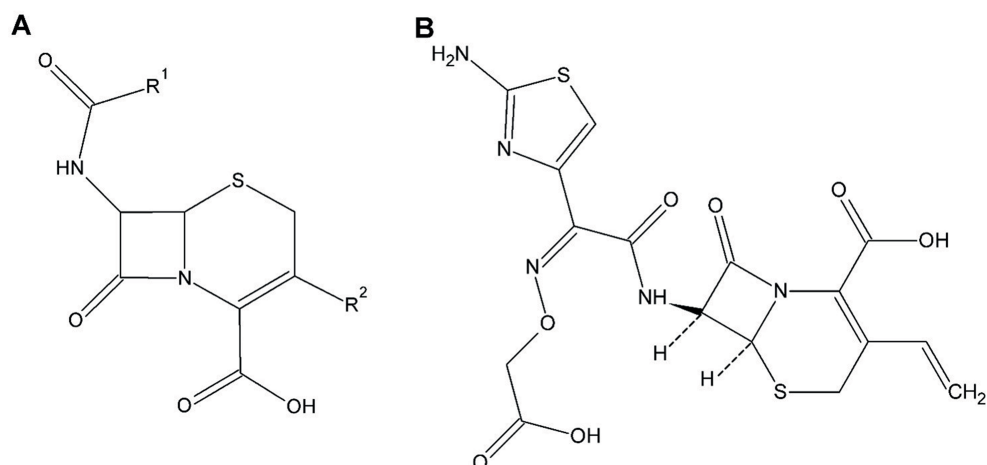*Correspondence: sabaci@hacettepe.edu.tr

Figure 1. A) General chemical structure of cephalosporin, B) chemical structure of cefixime.

cluding spectrophotometry,[3,4] spectrofluorometry,[5,6] capillary electrophoresis (CE),[7] chromatography,[8,9] and spectrometry.[10] Although these methods show high analytical performance, they are relatively time-consuming, complicated, expensive, and limited for on-site analysis. In comparison, electrochemical methods are advantageous owing to their economical instrumentation, quick response, and simplicity with high sensitivity and low detection limits. Furthermore, modifying the electrode surfaces is an effective way for the enhancement of sensitivity and selectivity of the voltammetric method.

There have been a few works on the electrochemical measurement of CEF. Electrochemical reduction behavior of CEF and its determination was studied at a hanging mercury drop electrode (HMDE) in pharmaceuticals and human urine by Reddy et al.[11] Afkhami et al. examined the electrooxidation behavior of CEF at gold nanoparticles/multiwalled carbon nanotubes/carbon paste electrode (GNPs/MWCPE).[12] The electrooxidation behavior of CEF on a glassy carbon electrode was shown and detection of CEF in real samples was established by Golcu et al.[13] Voltammetric determination of CEF on multiwalled carbon nanotubes decorated with $NiFe_2O_4$ nanoparticles ($NiFe_2O_4$-MWCNTs) by linear sweep voltammetry was studied by Ensafi and Allafchian.[14] Karimian et al. reported a voltammetric sensor based on a molecularly imprinted polymer grafted on multiwall carbon nanotubes for the quantification of CEF.[15] Asadollahi-Baboli et al. represented self-assembly monolayers-modified gold nanoparticles utilizing a screen-printed gold electrode in order to determine CEF in biological fluids.[16] To the best of our knowledge, there are no articles about voltammetric or amperometric determination of CEF utilizing ionic liquids with a bismuth film-modified electrode, which is the main goal of this study.

Among the most commonly used carbon electrodes, disposable pencil graphite electrodes (PGEs) have some advantages in electrochemical studies such as low technology requirements, low cost, good mechanical rigidity, chemical inertness, low background current, ability to work in a wide potential window, ease of modification, miniaturization, and adsorption of analytes.[17,18] Generation of a renewable surface with PGEs is also more facile when compared with solid electrodes (e.g., glassy carbon electrodes, GCEs) requiring polishing steps, which results in good reproducibility. In addition, being disposable, the screen-printed carbon electrode (SPCE) may be an alternative to PGEs, but the total cost of SPCEs is considerably higher than that of PGEs. So far, PGEs have been successfully used for electrochemical analysis of pharmaceuticals due to their

low cost and ease of use as being disposable.[19−25] On the other hand, bismuth-film electrodes (BiFEs) have been widely employed for the quantification of metals and organic molecules in various matrices.[26−29] This is because they have many beneficial properties such as simple preparation, high selectivity and sensitivity, a wide negative potential window, low background current, and well-defined signals.[29−31] Since the toxicity of bismuth is negligible, they have been recognized as environmentally friendly electrodes. In addition, ionic liquids (ILs), composed of organic cations and various anions, have received great attention recently because of their advantages such as high conductivity, good chemical stability, low vapor pressure, and tunable viscosity.[32,33] Due to these properties, ILs are promising materials for constructing sensors and thus represent a new class of conductive modifiers of electrodes for refining electrode sensing performance for trace analysis.[34,35] IL/Bi-modified PGE surfaces have never been used in any electrochemical detection before in the literature.

In the present work, the electrochemical behavior and detection of CEF was examined on the IL (1-butyl-3-methylimidazolium hexafluorophosphate)-modified ex situ bismuth film electrode. The resulting electrode showed good performance in CEF detection. The IL/Bi modification process was analyzed by scanning electron microscopy (SEM) and electrochemical methods such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Next, the analytical performance of the novel sensor was evaluated by square wave voltammetry (SWV) in pharmaceutical samples with high accuracy. The obtained results verified the superiority of IL/Bi film/PGE over other sensors in terms of low cost, simple preparation, fast response, higher sensitivity, and low detection limit for CEF.

2. Results and discussion

2.1. Electrochemical characterization of IL/Bi/PGE

The electrochemical performances of bare PGE and modified electrodes were investigated with $Fe(CN)_6^{3-/4-}$ electro-active probes. Figure 2A displays cyclic voltammograms obtained in 5 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl solution. A pair of redox peaks was monitored at the bare PGE (curve a), while at the Bi film-modified PGE (curve b), slightly increased redox peak currents were obtained. After the modification of Bi/PGE with IL, which has high ionic conductivity, redox peak currents increased and peak to peak separation decreased, as can be seen in curve c. This situation indicates facilitated conductivity and an electron transfer process at the IL/Bi-modified PGE.

EIS was also used to monitor the impedance changes during the modification. Figure 2B shows the Nyquist plots of bare PGE, Bi/PGE, and IL/Bi/PGE in the presence of $Fe(CN_6)^{3-/4-}$, respectively. The charge transfer resistance (R_{ct}) is associated with the diameter of the semicircle in the Nyquist plot. It was clear that the R_{ct} value of the bare PGE (422.3 ± 17.03 Ω) was larger than those of the ex situ bismuth film-modified PGE (199.1 ± 10.79 Ω), indicating that the modified surface showed higher conductivity than bare PGE due to the metal feature of bismuth (Figure 2B, b). Introduction of IL on the surface can also distinctively reduce the R_{ct} by accelerating the transfer rate with its high ionic conductivity and electrocatalytic ability. By means of these features of IL, the most obviously change in R_{ct} was found after modification of the electrode with IL that showed the smallest diameter of the semicircle (35.0 ± 4.865 Ω).

SEM was used in order to investigate the surface properties of the developed sensor. Low and high magnification images are shown in Figures 3a–3f.

The images of bare PGE (Figures 3a and 3d) showed irregular and separated graphite layers. The bare
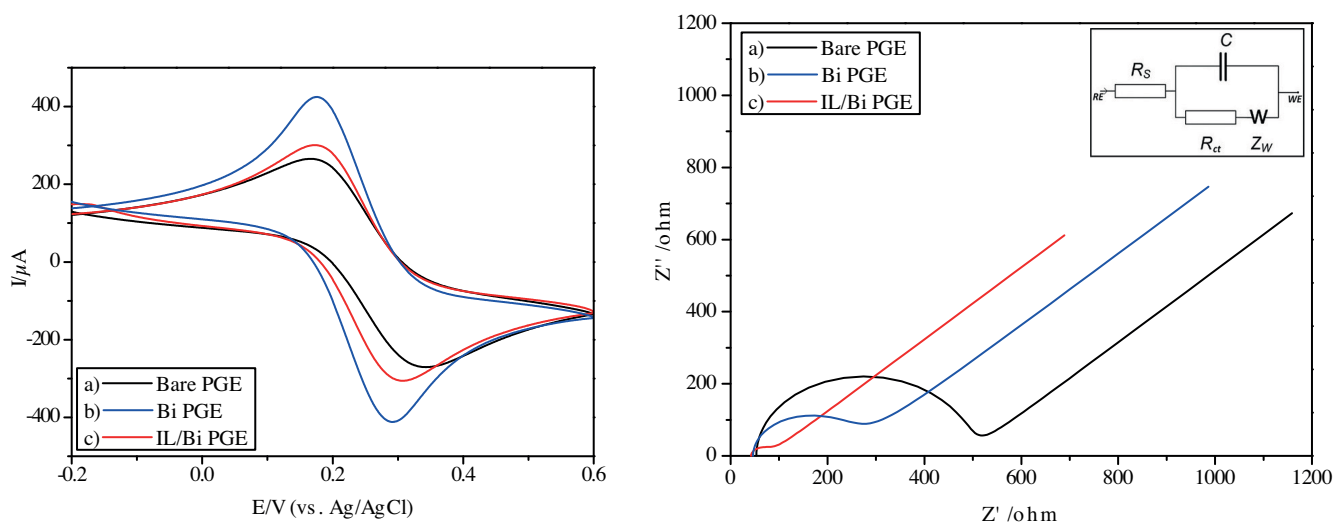
Figure 2. A) Cyclic voltammograms: a- PGE, b- Bi-film/PGE, c- IL/Bi-film/PGE. B) Nyquist diagrams: a- PGE, b- Bi-film/PGE, c- IL/Bi-film/PGE in 5 mM $Fe(CN)_6^{3-/4-}$, 0.1 M KCl (E: 0.2 V; frequency: 100,000–0.1 Hz) (inset: Randles equation).

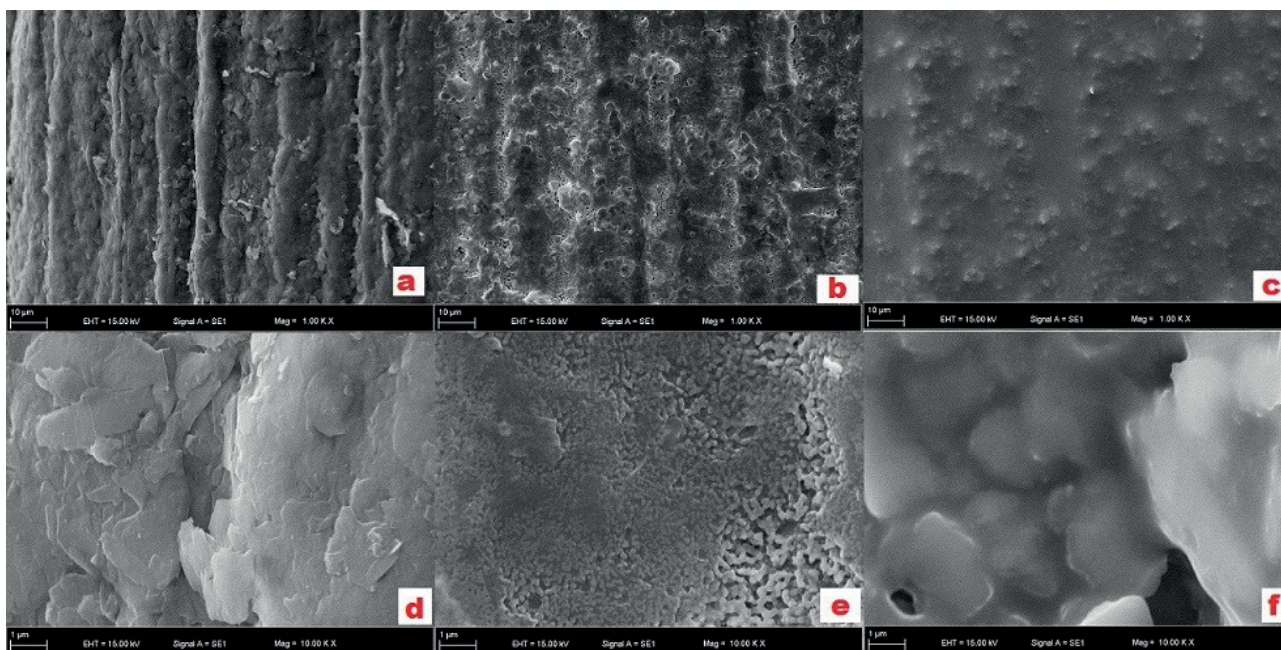**Figure 3**. SEM images of: a, d) bare PGE, b, e) Bi-modified PGE; c, f) IL/Bi-modified PGE (a, b, c: magnitude is $1000\times$; d, e, f: magnitude is $10,000\times$).

PGE also had a rough surface. Figures 3b and 3e show that the PGE was covered with Bi film as a black deposit and consisted of a porous three-dimensional structure. As can be seen in Figures 3c and 3f, IL/Bi/PGE presented a uniform structure and a cloudy surface due to the masking and adhesive effect of IL on the Bi/PGE.

2.2. Electrochemical behavior and effect of surface modification on the response of CEF

The cyclic voltammograms of 10 μM CEF recorded at bare PGE (curve a), IL/PGE (curve b), Bi/PGE (curve c), and IL/Bi/PGE (curve d) in BR buffer solution of pH 4.0 in the potential range from 0.2 V to 1.2 V (vs. Ag/AgCl) are shown in Figure 4. For 10 μM solution of CEF, irreversible behavior with a sharp oxidation peak at around 0.9 V (vs. Ag/AgCl) was observed at the IL/Bi/PGE. In the case of bare PGE, IL/PGE (curve b), and Bi/PGE (curve c) the oxidation peak was weak and broad, located at around 0.9 V (vs. Ag/AgCl). The highest peak currents were obtained at the IL/Bi/PGE with respect to the other electrodes.
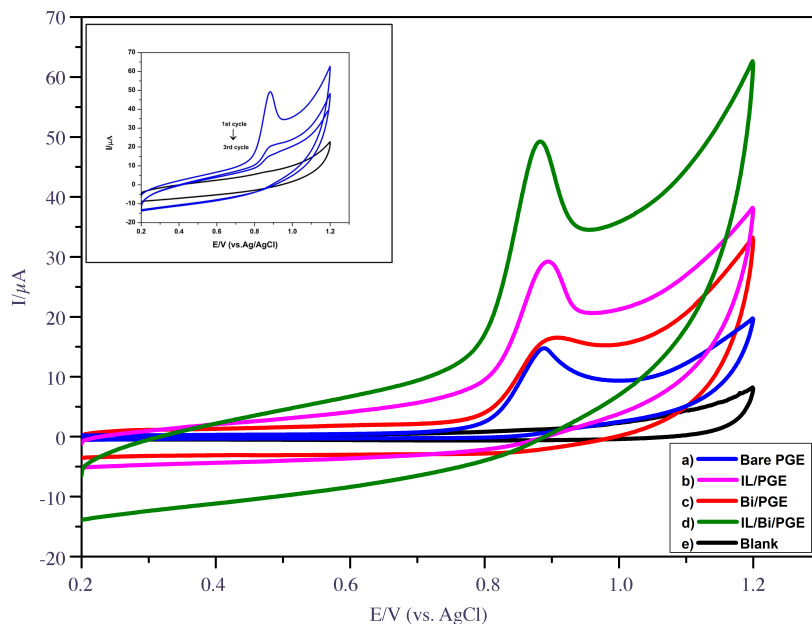

Figure 4. Cyclic voltammograms of 10 μM CFE at a- bare PGE, b- IL/PGE, c- Bi/PGE, d- IL/Bi/PGE, e- blank curve at PGE, at pH 4.0 BR, scan rate: 100mV s^{1}. Inset: repetitive cyclic voltammogram of 10 μM CFE at IL/Bi/PGE.

Bi/PGE provided high adsorption capacity and metallic conductivity and IL/PGE had ionic conductivity and electrocatalytic ability. When these features were combined, the IL/Bi composite exhibited a remarkable increment of CEF's anodic peak current. The enhancement in the peak current may be attributed to the presence of IL on Bi film, which provided a conductive and stable electrochemical surface for the CEF to be adsorbed. This approach caused an increment for the voltammetric signal of CEF. The anodic peak currents decreased after each successive scan, suggesting the possible consumption of adsorbed CEF molecules on the surface of the electrode (inset, Figure 4).

2.3. pH effect

To investigate the effect of pH on the anodic peak current of CEF at the IL/Bi/PGE, SWV was performed in the pH range from 2 to 8 (Figure 5).

Figure 5 indicates that the electrochemical oxidation of CEF was a pH-dependent process. The anodic peak current increased with increasing solution pH up to pH 4.0 and then decreased slightly. Hence, subsequent experiments were carried out in pH 4.0 BR buffer solution. The peak potential of the CEF shifted negatively
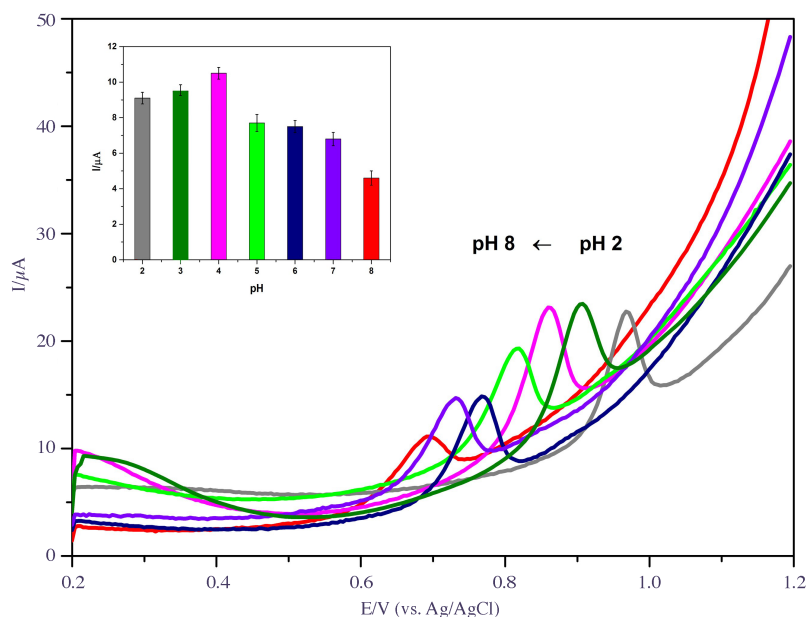
Figure 5. Effects of pH value on the voltammetric response of 0.1 μM CEF at IL/Bi/PGE by SWV in the range of 2 to 8. Conditions: pH 4.0; frequency 50 Hz; step amplitude 50 mV; pulse amplitude 4 mV (inset: effects of pH value on the oxidation peak current of CEF).

and showed linearity with increasing pH in the range of 2–8, obeying the following equation:

$$E_{pa}\left(V\right) = -0.05\,pH + 1.062 \quad R^2 = 0.998 \tag{1}$$

The slope obtained from the equation was 50 mV pH^{-1}, which is characteristic of the electrooxidation process and close to the theoretical value of 59 mV pH^{-1}.[36] This behavior demonstrated that electrons and protons participated in the same ratio in the oxidation process of CEF.

2.4. Deposition time of bismuth and adsorption time of IL effect

The effect of deposition time of bismuth was optimized. After this step, the adsorption time of IL was studied and optimized. The obtained results are given in Figure 6. The bismuth deposition was carried out at –0.3 V (vs. Ag/AgCl) with constant potential electrolysis. It was observed that the oxidation peak current of CEF was increased until the deposition time of 10 s with respect to the bare electrode surface. There was a slight decrease in the peak current after this point (Figure 6A). This may be due to the formation of a thicker film layer with higher deposition times, which may lead to less conductive and stable Bi film by reducing the electron transfer between the electrolyte and electrode surface.[37] After this step, the physical adsorption time of IL was studied. Ten-second bismuth-deposited electrode surfaces were left for adsorption of IL at different times. The results proved that the best response was obtained with the electrode left for adsorption for 15 min (Figure 6B). Up to 15 min of IL adsorption time, the oxidation peak current of CEF increased, and then it slightly decreased, suggesting that the binding of CEF and IL had reached saturation.
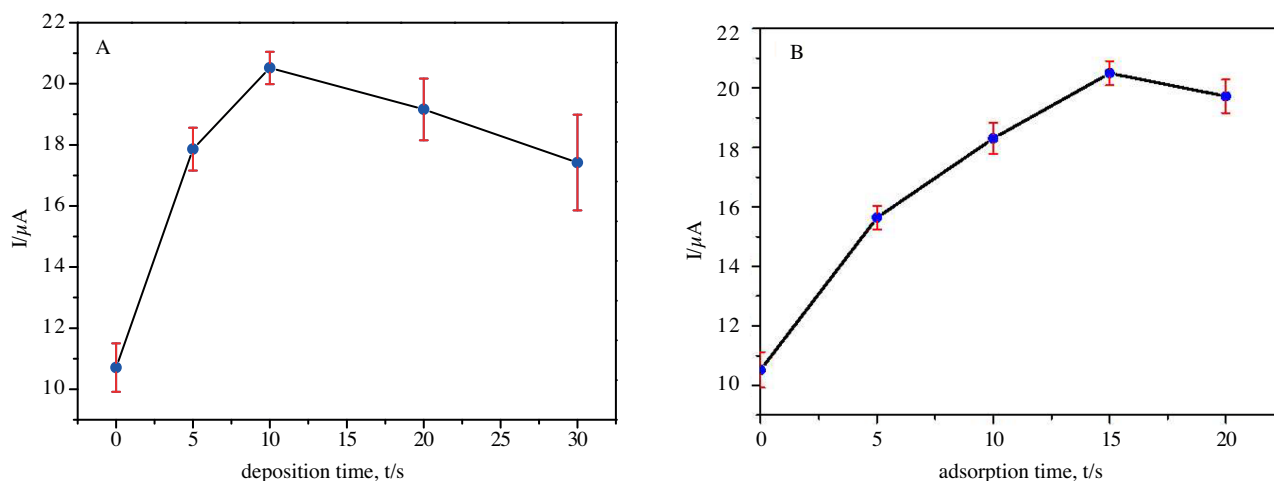
Figure 6. A) Effect of the deposition time of Bi and B) adsorption time of IL on the oxidation peak current of 0.4 μM CEF with SWV.

2.5. Scan rate effect

In order to investigate the kinetic parameters, a scan rate study was performed at a fixed concentration of 10 μM CEF (Figure S1). The anodic peak currents increased linearly with scan rate in the range of 10–400 mV s^{-1} as expressed in Eq. (2).

$$I\ (\mu A) = 0.197\,\vartheta\ \left(mVs^{-1}\right) + 4.571 \quad R^2 = 0.991 \tag{2}$$

This result indicated the adsorption controlled the nature of the electrode process. A plot of the logarithm of peak current (log I$_p$) versus the logarithm of scan rate (log υ) was linear and the slope of 0.792 (Eq. (3)), which is close to the theoretical value of 1.0, was attributed to the adsorption-controlled electrode process as follows:

$$\log I\ (\mu A) = 0.792 \log \vartheta\ \left(mVs^{-1}\right) - 0.164 \quad R^2 = 0.987 \tag{3}$$

Also, as shown in Figure S1, anodic peak potential E$_p$ was shifted to more positive potentials as the value of the scan rate increased. The logarithm of the scan rate (log υ) and peak potential (E$_p$) were linearly changed, which indicated that the oxidation of CEF was adsorption-controlled and totally irreversible. The obtained equation was:

$$E_p\ (V) = 0.0408 \log \vartheta\,(V.s^{-1}) + 0.8055 \quad R^2 = 0.987 \tag{4}$$

To calculate the number of electrons (n), transfer coefficient (α), and electron transfer rate constant (k$_s$) during oxidation of CEF, the following formula, which is based on Laviron's equation for an adsorption-controlled and irreversible reaction[38], was used:

$$E_{pa} = E^{0\prime} + \left(\frac{2.303RT}{\alpha nF}\right) \log \left(\frac{RTk_s}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \log \upsilon \tag{5}$$

Here α = transfer coefficient, k$_s$ = standard heterogeneous reaction rate constant, n = number of electrons transferred, υ = scan rate, and E$^{0\prime}$ = formal redox potential. The slope of log υ versus E$_p$ was found to be

0.0408. Using this value, αn was calculated as 1.425. In order to find α, the following equation was used:

$$E_{p/2} - E_p = 1.857RT/\alpha F \tag{6}$$

The electron transfer coefficient (α) was determined as 0.634 for Bi/IL/PGE. Then n was calculated to be 2.25, which indicated that the number of electrons transferred in the oxidation of CEF was equal to 2 on the Bi/IL/PGE. This result was consistent with previous literature.[12,13,15] A single voltammetric oxidation peak was observed due to possible oxidation of the electroactive 2-aminothiazole group in the side chain on C_7 in the CEF structure.[13] According to the mechanism suggested, free radicals are produced and combine to form a hydrozo compound by dimerization and further a 2 e-, 2 H$^+$ oxidation process of the generated dimer takes place by formation of an azo compound as the final product (Figure S2). Thus, a total of four electrons per two molecules of CEF give an overall two-electron process per molecule.

The proposed mechanism has also been suggested in earlier studies in the literature.[13,39,40,41] The value of k_s, was found as 7.56 s^{-1} using the intercept of Eq. (5), indicating a relatively fast electrode reaction process, and this value was greater than previous values reported for CEF in the literature.[15]

2.6. Validation of the proposed method

Under optimal conditions, the proposed sensor system exhibited good response in the concentration interval from 0.005 to 0.5 μM (Figure 7). Beyond 0.5 μM, the anodic peak current of CEF changed slightly because the IL/Bi/PGE surface reached saturation and so the upper limit of the linear range was determined as 0.5 μM.
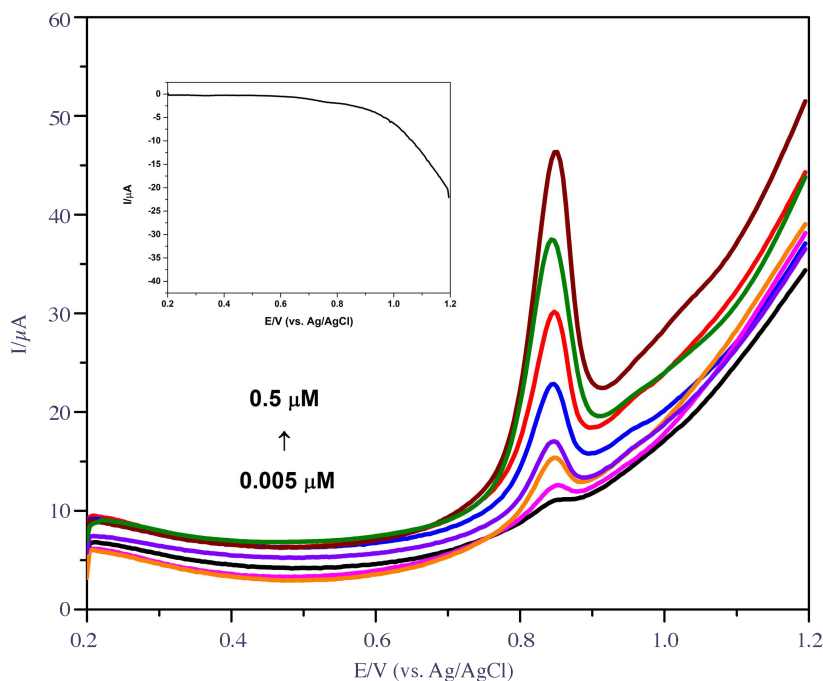

Figure 7. SWV curves of CEF at IL/Bi-modified PGE in 0.04 M BR containing 0.005-0.5 μM. Conditions: pH 4.0; frequency 50 Hz; step amplitude 50 mV; pulse amplitude 4 mV (inset: blank voltammogram).

The obtained linearity between these values is given by the equation below:

$$I\ (\mu A) = 64.37\,C\ (\mu M) + 2.582 \quad R^2 = 0.996 \tag{7}$$

Using the formula 3 Sb/m, LOD was calculated as 0.22 nM and 10 Sb/m (LOQ) was found as 0.73 nM (m: slope of the calibration curve, Sb: standard deviation of the analytical signals for measurements of blank, n = 3). The analytical performance data of the developed sensor system are summarized in Table 1.[42]

Table 1. Analytical data obtained for CEF using IL/Bi/PGE.

Parameter	Value
Linear range (μmol L^{-1})	0.005–0.5
Correlation coefficient (R^2)	0.996
Slope (μA mol^{-1})	64.37
Standard deviation of slope (μA L mol^{-1})	1.65
Intercept (μA)	2.58
Standard deviation of intercept (μA)	0.36
Detection limit (nmol L^{-1})	0.22
Quantification limit (nmol L^{-1})	0.73
Repeatability of peak current (intraday)^{a,b}	3.67
Repeatability of peak current (interday)^{a,b}	3.62
Application	Tablets
^{a} Relative standard deviation (%).	
^{b} n = 6.	

This LOD value showed that this new sensor system is superior to all electrochemical detection results of CEF studies in the literature (Table 2). It can be said that surface modification provides a lower detection limit according to the results obtained with other solid electrodes (GCE and HMDE) in Table 2. Thanks to the developed surface, it was possible to carry out analysis at sufficiently low concentrations without applying stripping analysis such as ASDP. As a result, our system offers a more practical, quick response and detects low concentrations in comparison to other sensor studies in the literature.[3,6,10−15,43−45]

In order to evaluate the reproducibility of the IL/Bi-modified PGE, six surfaces were prepared in the same way independently. Under optimum experimental conditions 0.1 μM CEF's anodic peak currents were compared with interday and intraday results by performing replicate measurements. The relative standard deviation (RSD) values were found as 3.62% (n = 6) and 3.67% (for 6 days), which revealed that fabrication of these modified surfaces showed good reproducibility with high precision. Moreover, the IL/Bi-based single-use sensor offered significant advantages such as low cost and fast, reliable, effortless analysis by overcoming surface passivation.

2.7. Analytical applications

The proposed method was successfully employed with pharmaceutical tablets in order to establish the content of CEF with a standard addition method. Recovery values were calculated for these samples and obtained results are given in Table 3. The recovery values were found in the range of 97%–103% for CEF tablets, which indicated that there was no considerable interfering effect of excipients on the voltammetric response of CEF. Therefore, it was proved that the IL/Bi-modified PGE can be used for the detection of CEF as an alternative in pharmaceutical analysis with high accuracy.

Table 2. Comparison of this work with literature data.

Electrode	Method	LOD (μM)	LR (μM)	Ref.
HMDE	DPP	0.046	0.06–12.0	[11]
GCE	SWV	0.064	6.0–200.0	[13]
GNPs/MWCPE	SWV	0.003	0.01–200	[12]
NiFe$_2$O$_4$-MWCNTs/GCE	CV	0.09	0.1–600	[14]
Pt-W/MWCNTs/GCE	Amperometry	0.005	0.01–3.2	[43]
AgDs/MIP/MWCNTs/GCE	ASDP	0.001	0.01–600	[15]
-	Spectrophotometric	2.87	4.4–88.4	[6]
	Spectrophotometric	132.6	176.8–4442	[3]
	Spectrophotometric	0.3877	5.525–77.35	[44]
	LC-MS	0.1105	0.1105–17.68	[10]
	RP-HPLC-UV	0.00221	0.00884–11.05	[45]
IL/Bi/PGE	SWV	0.00022	0.005–0.5	This work

HMDE: Hanging mercury drop electrode, DPP: differential pulse polarography, GCE: glassy carbon electrode, GNPs: gold nanoparticles, MIP: molecularly imprinted, AgDs: silver nanoparticle, MWCNTs: multiwalled carbon nanotubes, LC-MS: liquid chromatography-mass spectroscopy, RP-HPLC-UV: reverse phase-high pressure liquid chromatography-ultraviolet.

Table 3. Determination of CEF in tablet samples by standard addition method (n = 3).

Sample	Added (M)	Found (M)	RSD %	Recovery (%)
Cefixime tablet	2.64×10^{-8}	$2.54 (\pm 0.11) \times 10^{-8}$	1.62	97.0
	6.00×10^{-8}	$5.70 (\pm 0.31) \times 10^{-8}$	2.19	95.0
	15.0×10^{-8}	$15.9 (\pm 0.75) \times 10^{-8}$	1.89	103

2.8. Interferences

Some organic and inorganic compounds that can be found in urine were examined for the interference study. A 1000-fold excess of tartaric acid, K^+, Na^+, SO_4^{2-}, NO_3^{2-}, and CO_3^{2-}; a 500-fold excess of glucose, Ca^{2+}, and Mg^{2+}; a 200-fold excess of glycine, ascorbic acid, urea, and uric acid; and a 20-fold excess of histidine did not have interference effects on the anodic peak current of CEF (relative error less than $\pm 5\%$). These results showed the high selectivity of the developed method for the electrochemical determination of CEF in real samples.

In conclusion, an IL/Bi-modified PGE was used for the first time as a working electrode for the electrochemical detection of CEF. This modified surface remarkably enhanced the CEF's anodic peak current owing to the high electron transfer rate, surface area, and adsorption capacity. LOD was found as 0.22 nM, which is lower than the reported values in the literature (Table 1). The standard heterogeneous rate constant (k_s) was found to be 7.56 s^{-1}, which indicates a relatively fast electrode reaction process. The practical use of the developed system was established by performing the direct analysis of real samples, commercial drug samples, without any separation process. The obtained recovery values indicated that the proposed method is quite reliable and can

be effectively used for analysis of CEF in real samples with high accuracy. Due to the high analytical performance, this novel sensor system is an excellent alternative with respect to expensive, time-consuming methods used with pharmaceutical samples. This low-cost and single-use sensor platform has promising features for various electrochemical applications.

3. Experimental

3.1. Chemicals

CEF, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, 1-butyl-3-methylimidazolium hexafluorophosphate, absolute ethanol, HCl, and $Bi(NO_3)_2$ were purchased from Sigma Aldrich. The 1 mM CEF was prepared in methanol as a standard stock solution and stored at 4 °C in a refrigerator. More diluted solutions such as 0.1 mM and 0.01 mM were prepared daily. The 0.04 M Britton–Robinson (BR) buffer solution was prepared from a mixture of glacial acetic acid, phosphoric acid (85%), and boric acid. HCl and NaOH were used for adjusting the pH of the supporting electrolyte.

3.2. Apparatus

An Interface 1000 Potentiostat/Galvanostat/ZRA model (Gamry Instruments, Warminster, PA, USA) was used for the electrochemical characterization of the surface and determination of the CEF with a three-electrode cell system. An IL/Bi-modified PGE was used as a working electrode. The other members of the electrode cell system were Ag/AgCl (3 M KCl), a platinum wire as a reference, and an auxiliary electrode, respectively. PGEs were Tombow (Tokyo, Japan) leads (0.5 mm in diameter, HB), which were purchased from a local bookstore. The connection between the potentiostat and pencil leads was provided with a Rotring pencil (Hamburg, Germany), which was prepared by soldering a copper wire to the pencil to provide the electrical contact. Electrochemical measurements were performed in a 10-mL cell into which 1 cm of modified lead was dipped. To show the effect of SWV variables on the voltammetric signal of CEF the frequency, pulse size, and amplitude were changed in different ranges. It was seen that the most suitable frequency value was 50 Hz from the measurements taken at 10-Hz increments in the frequency range of 20–60 Hz. The oxidation peak current decreased at higher frequencies. The pulse amplitude was examined at increments of 1 mV in the range of 1–10 mV. The highest peak current and the best reproducibility was achieved at 4 mV and it was determined as the optimum value. The pulse amplitude was examined with an increase of 10 mV in the range of 20–60 mV and the highest peak current was read at 50 mV. As a result, the parameters we have given in this article are optimum values. Pulse amplitude of 50 mV, pulse size of 4 mV, and frequency of 50 Hz were used for SWV as optimum parameters. SEM figures were obtained with a Zeiss Evo 60 EP-SEM (Cambridge, UK).

3.3. Preparation of IL/Bi/PGE electrode

The concentration of Bi(III) was examined at a range of 0.001–0.02 M in HCl solution. The oxidation peak current of CEF increased up to 0.01 M, but after this point it decreased. It was determined that when the Bi(III) concentration was over 0.01 M, the film blocked the electron transfer between the surface and analyte. Therefore, 0.01 M Bi(III) concentration was chosen as the optimum value. The oxidation peak current of Bi(III) was observed at around –0.2 V (vs. Ag/AgCl). The effect of the deposition potential was examined at 0, –0.1, –0.25, –0.3, and –0.4 V (vs. Ag/AgCl). The sensor response greatly reduced at more negative and positive potentials than –0.30 V (vs. Ag/AgCl). Therefore, Bi(III) deposition was carried out –0.30 V (vs. Ag/AgCl) in further experiments.

Bi(NO$_3$)$_2$ (0.01 M, optimum concentration) in 1.0 M HCl was transferred into the voltammetric cell and then 1 cm of PGE was immersed into this solution and ex situ bismuth film was deposited at –0.3 V (optimum deposition potential) (vs. Ag/AgCl/ 3 M KCl) by constant potential electrolysis for 10 s as the optimum deposition time. These electrodes were located vertically and then dried for 5 min at room temperature. Next, Bi/PGEs were immersed into 200 μL of IL (10%, in N,N-DMF) solution and kept 15 min (optimum adsorption time) for physical adsorption. Finally, these surfaces were dried at room temperature for 20 min. This modified surface was called IL/Bi-modified PGE (IL/Bi/PGE). The modified sensors are single-use type and are practical to use because surface polishing or treatment procedures are not needed for these electrodes before the experiments.

3.4. Preparation of drug sample

Five tablets of Suprax (each tablet contains 400 mg CEF) were weighed and powdered in a mortar. The powder equivalent of one average tablet was weighed, transferred into a 100-mL volumetric flask, and dissolved in methanol. This mixture was ultrasonicated for 1 h and then centrifuged at 5000 rpm for 5 min. Finally, the obtained clear supernatant was diluted in methanol and used for the standard addition method.

3.5. Analytical procedure

The electrochemical characterization of bare, ex situ bismuth film (Bi)-modified and ionic liquid (IL)/Bi-modified PGEs was performed by CV and EIS. K$_3$Fe(CN)$_6$/K$_4$Fe(CN)$_6$ (5 mM; 0.1 M KCl) was used as a redox agent for cyclic voltammetry measurements at 100 mV s^{-1} scan rate over the range of –0.20 to 0.60 V (vs. Ag/AgCl/ 3 M KCl). Open-circuit value of 0.2 V and frequency range of 10^5 to 10^{-1} Hz with amplitude of 5 mV were used as the EIS measurement parameters and measurements were obtained in the redox probe solution. The supporting electrolyte (pH 4.0, 0.04 M BR) was transferred to the 10-mL voltammetric cell. CV was used for the electrochemical behavior of CEF and the SWV method was used for the electrochemical detection of CEF by scanning the potential from 0.20 to 1.20 V (vs. Ag/AgCl/ 3 M KCl). Pulse amplitude of 50 mV, pulse size of 4 mV, and frequency of 50 Hz were applied for SWV.

Acknowledgment

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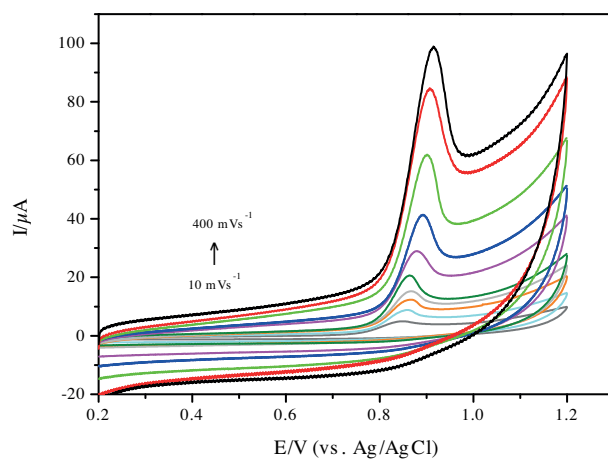
Figure S1. Cyclic voltammograms of 10 μM CEF at IL/Bi-film/PGE with different scan rates. Curves from bottom to top are obtained at 10, 20, 30, 40, 60, 80,150, 200, 300, and 400 mV s1, respectively. Conditions: pH 4.0 BR; Ei: 0.2 V; Ef: 1.2 V.

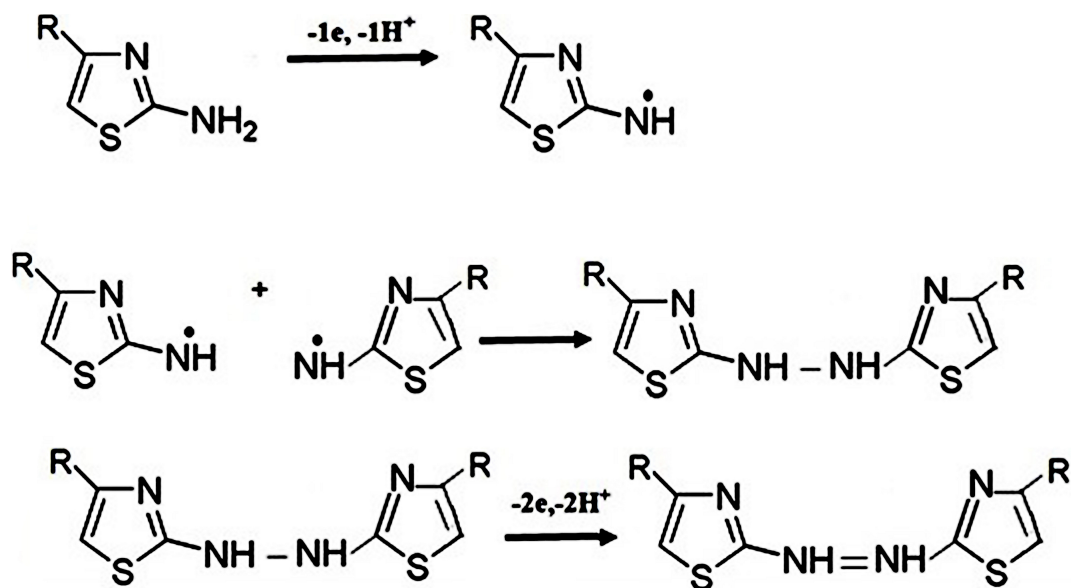**Figure S2.** Suggested oxidation mechanism of CEF.