Cephalosporins are β-lactam antibiotics whose main core is formed by cephem derivative of 7-amino-cephalosporanic acid [1]. Due to their similar antimicrobial indications with penicillin’s, this group of antibiotics are widely used in treatment for penicillin resistive bacteria. Further, they have been prescribed safely for penicillin allergic patients. Cephalosporins show bactericide effect against various microbial organisms-bacteria and they have been classified into generations depending on their spectrum of activity. Specifically, ceftizoxime sodium (CFX), belonging to third generation of semi-synthetic cephalosporins, has broad-spectrum resistance against β-lactamases [2] (Fig. 1). The most important features of third-generation cephalosporins (except Cefoperazone) includes the increased efficiency to gram-negative bacteria, high stability against hydrolysis by β-lactamases and in particular, being free of serious side effects in patients. Furthermore, some of them are known to overcome the blood-brain barrier [3]. CFX shows high antibacterial effect against a broad range of gram positive and negative bacteria such as *Staphylococcus pneumoniae*, *Hae-

mophilus influenzae*, *Escherichia coli*, and *Neisseria gonorrhoeae* by penetrating into bacterial cell wall and consequently leading to effective destruction of bacteria [4]. CFX, with a relatively long elimination half-life (3–4 h), is administered through the oral route for the treatment of moderate bacterial infections [5]. It is active against putrefaction and clinically effective in eradicating bacteria causing urinary tract infections, acute pharyngitis, otitis media, tonsillitis, gonorrhea and pneumonia [3]. Given its pharmacological significance and extended use, quantitative analysis of CFX in biological fluids has great clinical value in order to reveal biochemical processes that occur in the drug metabolism and also in pharmaceutical formulations to assure drug quality.

Up to now, analysis of CFX in various matrixes, such as human and animals, biological materials, food, waters and pharmaceuticals have been studied by several chromatographic and spectrometric methods [6–10]. Although they provide very efficient analyses for the sensitive detection of CFX, these methods have some handicaps such as high cost instrumentation, long...
remarkably important surfaces owing to their excellent conductivity, high surface area and biocompatible properties [21]. To obtain highly electro-catalytic surfaces, AuNP’s combined with other nanomaterials have widely been used in various analyses and catalysis [22–25] applications.

Hence, in the present study, AuNPs combined with GO modified PGE was constructed for the stripping analysis of CFX. AuNP/GO modified PGE was considered as an attractive platform from the standpoint of easy use, cheapness and simple modification. Owing to considerable synergy between the AuNP and GO, anodic oxidation signal of CFX increased remarkably. The electrode surfaces were examined microscopically to prove the success of the modification. The experimental parameters such as adsorption time of graphene oxide, deposition time of gold nanoparticles, supporting electrolyte pH, pre-concentrating potential and time were optimized. Anodic stripping voltammetry (SWASV) measurements of CFX demonstrated good linear response with a low limit of detection (LOD) as 0.442 nM. The practical use of the sensor system was tested for commercial pharmaceutical tablets by standard addition method. The proposed sensor system has offered a low detection limit with respect to other studies in literature and also other parameters were comparable with the literature data.

MATERIAL AND METHODS

Reagents and Materials

Ceftizoxime sodium, gold (III) chloride trihydrate and graphene oxide (GO) were provided from Sigma Aldrich and Nanograf, respectively. 0.5 M of HAuCl₃ was prepared in 0.5 M of H₂SO₄. Britton-Robinson (B-R) buffer solution (0.04 M) was prepared by mixture of boric acid, acetic acid and phosphoric acid which was applied as a supporting electrolyte. Stock solution of CFX (10⁻² M) was prepared in double distilled water and stored at +4 ºC. More diluted solutions were prepared daily.

Instruments

CV, EIS and SWASV were recorded by Gamry interface 1000 model potentiostat/galvanostat. AuNP/GO modified PGE served as a working electrode. Pencil leads were purchased from local store in Turkey (0.5 mm diameter, Tombow). An Ag/AgCl (3M KCl) electrode was used as the reference and a platinum wire as the counter electrode. The surface morphology of the developed sensor was characterized by Zeiss Evo 60 EP-SEM. Besides, the AuNP/GO modified graphite surfaces were characterized by Energy-dispersive X-ray spectroscopy (EDX, Tescan/Czech Republic).
Fabrication of AuNP/GO/PGE sensor

Firstly, 1 cm of pencil leads were immersed in graphene oxide (GO) solution for 30 minutes. Afterwards, the leads were soaked in double distilled water for 5 seconds to remove the excess of GO attached on the surface. The GO modified electrodes were allowed to dry for 5 min at room temperature. Potential controlled electrolysis was used at -0.3 V (vs. Ag/AgCl) for the electro-deposition of AuNPs on GO/PGE in 0.5 M HAuCl₄ solution (in 0.5 M H₂SO₄). The optimum electro-deposition time was found as 100 seconds.

Preparation of Drug Samples

Five tablets of Cefizox® (400 mg CFX per tablet) were weighed and pulverized in a mortar. Then, an average of 1 tablet weight was taken from this powder and dissolved in 100 mL double distilled water by ultrasonication for 1 hour. Upon centrifugation at 5000 rpm for 5 min, the supernatant was diluted in double distilled water.

Analytical Procedure

Electrochemical characterizations were performed by CV (between -0.2 V and 0.6 V (vs. Ag/AgCl)) and EIS (in the frequency range of 0.01–100,000 Hz) in redox probe solution (0.5 mM Fe(CN)₆³⁻/⁴⁻ prepared in 0.1 M KCl). The electrochemical determination of CFX was carried out in 10.0 mL cell and BR buffer (pH 2.5 as optimum pH) was used as the supporting electrolyte by CV at potential from 0.7 V to 1.1 V (vs. Ag/AgCl). Square wave anodic stripping voltammetry (SWASV) was recorded from 0.6 V to 1.0 V (vs. Ag/AgCl) after a pre-concentrating step of 300 seconds at 0.6 V (vs. Ag/AgCl) accumulation and deposition time, respectively. After this pre-concentration step, stirring was stopped and 15s was waited as a release time. The pulse amplitude 4 mV, pulse size 50 mV and frequency 50 Hz were used as parameters of SWASV. Before the all electrochemical measurements, supporting electrolyte was purged with the nitrogen gas for 5 minutes.

RESULTS AND DISCUSSION

Morphology and Electrochemical Characterization of AuNP/GO modified PGE

In order to reveal the gradual changes on the electrode after the modifications, surface morphology of bare and modified pencil graphite electrodes (PGEs) were investigated with scanning electron microscopy (SEM). Fig. 2.a showed that bare PGE surface displayed irregular graphite layers with highly rough structure, as expected. When graphene oxide (GO) was deposited onto PGE surface, it was observed that single or few-layers of GO nano-sheets with high amount of wrinkles due to GO sheets were entangled with each other (Fig. 2.b). The nanosized AuNPs which were deposited by potential control electrolysis, were in a form of spherical structure uniformly distributed over the bare electrode surface and over the GO layers, respectively (Fig. 2.c,d). The morphology of the AuNP/GO modified PGE showed more even structure.

As it was shown in Fig. 2.g-h, the main elements of GO including carbon, and oxygen were all observed in EDX spectrum as expected [26]. Also, the presence of gold which resulted from the AuNPs on the surface of GO/PGE was confirmed with EDX analysis (Fig. 2.h). The carbon and oxygen peaks were observed on the bare graphite surface in Fig. 2.e-f. These results clearly suggested that AuNP/GO were successfully coated on the graphite surface.

Figure 2. The scanning electron micrographs of a) Bare PGE, b) GO modified PGE, c) AuNP modified PGE, d) AuNP/GO modified PGE (Scale bar: 1 µm) EDX mapping of e) PGE (The colors: Blue:C; Yellow:O) and g) AuNP/GO/PGE (scale bar: 100 µm. The colors: Red:O; Blue:C; Yellow: Au) and corresponding elemental compositions of f) PGE and h) AuNP/GO/PGE.
To investigate the electrochemical behaviour of developed disposable nanosensor platform, cyclic voltammetry and impedance spectroscopy were employed in redox probe solution. Fig. 3.A depicts the decrease in anodic/cathodic peak currents of redox pairs at GO/PGE compared to bare PGE (Fig. 3.A.b and 2.A.a, respectively). Besides, peak-to-peak separation increased due to the negatively charged carboxyl groups of GO. When the medium pH is high enough, carboxyl groups in the GO structure are negatively charged and repel the negatively charged Fe(CN)$_6^{3-/4-}$ redox pair from the surface with electrostatic effects. Hence, the presence of GO on PGE impeded the electron transfer and reduced the peak currents [27]. When AuNP electrodeposition was performed onto GO film, AuNP/GO/PGE showed a well-defined CV, with approximately equal and reversible peaks (Fig. 3.A.c). The highest peak current values indicated a faster electron-transfer than the GO modified PGE. This was realized by the catalytic effect of AuNPs and increased electrode surface area, accordingly. The corresponding results were summarized in Table 1.

To support this behavior of AuNP/GO/PGE, EIS method was utilized and obtained Nyquist diagrams were presented in Fig. 3.B. The charge transfer resistance (Rct) values were calculated from the obtained spectrum using Randles circuit at the electrode/solution interface (Fig. 3.B inset). The Rct values of bare and GO/PGE were calculated as 260 Ω and 440 Ω, respectively. The increased Rct can be explained by the restriction of electron transfer due to GO modification. The resistance of AuNP/GO modified electrode at the electrode/solution interface decreased, due to increased charge transfer, so, AuNP/GO/PGE had the lowest Rct (43 Ω) as expected. These results proved that modification of PGE surface was performed successfully.

The influence of modification of PGE on the electrochemical behavior CFX

The voltammetric response of CFX was examined at bare and modified surfaces (Fig. 4). All the CVs revealed an oxidation peak at about 0.9 V (vs. Ag/AgCl) and no reduction signal was recorded in the reverse scan due to irreversible nature of the electrode process for cephalosporins [28].

As previously reported, the oxidation of amino group at aminothiazole substituent to imino radical was responsible for the electrode process of this group of antibiotics [12] (Fig. 5).

**Table 1.** Anodic/cathodic peak currents (Ipa/Ipc) and peak potentials (Epa/Epc) at bare, GO modified and AuNP/GO modified PGEs.

| Electrode     | Ipa (μA) | Ipc (μA) | Epa (V) | Epc (V) | ΔEp (V) |
|---------------|----------|----------|---------|---------|---------|
| PGE           | 336.0    | 323.0    | 0.306   | 0.173   | 0.133   |
| GO/PGE        | 288.2    | 268.8    | 0.343   | 0.166   | 0.177   |
| AuNP/GO/PGE   | 461.7    | 468.6    | 0.292   | 0.175   | 0.117   |

**Figure 3.** a) CVs showing a) bare, b) GO/PGE and c) AuNP/GO/PGE in 5 mM Fe(CN)$_6^{3-/4-}$/0.1 M KCl at 50 mVs$^{-1}$. B) Nyquist diagrams of a) bare, b) GO/PGE and c) AuNP/GO/PGE in 5 mM Fe(CN)$_6^{3-/4-}$/0.1 M KCl. (E: 0.2 V, Frequency: 100000-0.1 Hz). Inset: equivalent circuit for EIS spectrum. Rs: the electrolyte resistance, Cdl: double-layer capacitance, Rct: the electron transfer resistance, Zw: the Warburg impedance.

**Figure 4.** CVs for 50 µM of CFX in pH 3.0 B-R buffer solution at a) bare, b) GO modified, c) AuNP modified, d) AuNP/GO modified PGE. e) CV for AuNP/GO/PGE in the absence of CFX. (Scan rate: 100 mVs$^{-1}$ vs. Ag/AgCl).

**Figure 5.** Electrochemical oxidation reactions of CFX.
As previously reported, the oxidation of amino group at aminothiazole substituent to imino radical was responsible for the electrode process of this group of antibiotics [12] (Fig. 5).

The recorded CVs for 50 µM of CFX in pH 3.0 B-R buffer showed that maximum peak current intensity was provided by GO/AuNP/PGE surface. The oxidation peak current was 3.5 times higher and the peak potential appeared at more negative potentials with respect to bare PGE. This can be ascribed to larger effective surface area and improved catalytic activity of the modified sensor surface. Thus, it was concluded that AuNP/GO modified PGE sensor system was suitable for sensitive CFX detection.

In order to understand the effect of several experimental parameters, optimization studies were performed as below.

**Effect of Scan Rate**

The effect of scan rate was examined in the range of 10-400 mVs⁻¹ at AuNP/GO/PGE (Fig. 6). The plot of oxidation peak current vs square root of scan rate showed excellent linearity. This linearity was given as follows (Eq.1.)

\[
I(\mu A) = 5.085g^{0.5} + 12.28 \quad R^2 = 0.990
\]  

This behavior demonstrated that the electro-oxidation reaction of CFX was a typical diffusion controlled electrocatalytic process at AuNP/GO/PGE [11].

**Effect of Electrolyte pH**

The voltammetric behavior of CFX on the sensor was studied in BR buffer solutions of different pH values since the electrochemical characteristics is strongly dependent on the pH of the medium. Highest peak current was observed with the pH value of 2.5 and decreased up to pH value of 5.0 (Fig. 7). Thus, optimum pH value was determined as 2.5.

**Effect of AuNP and GO deposition time**

After it was clearly demonstrated that the anodic oxidation of CFX increased when the modifying agents were combined together, AuNP/GO/PGE was employed in order to obtain improved electrochemical signal of the analyte. The dependence of response enhancement on GO and AuNP deposition time was examined. The physical adsorption time of GO was investigated between 15-90 minutes and the maximum oxidation peak current was obtained at 30 min (Fig. 8.a). After this adsorption time, peak current decreased which might have been due to electron transfer restriction with the increase of GO layer thickness. So, the optimum adsorption time of GO was used as 30 min.

After determining the optimization of physical adsorption time of GO, the bulk electrolysis time of AuNP onto GO/PGE was studied in the range of 25-300 s as deposition times (Fig. 8.b). The maximum peak current was obtained with deposition time of 100 s which promoted the electron transfer between the CFX and the electrode, and higher deposition times induced hinderance of the transfer. Therefore, 100 s of AuNP electrodeposition time was applied for further studies.

**Effect of Pre-concentration Step**

The pre-concentration of stripping analysis has two main steps; namely deposition potential and deposition time of the analyte. The deposition potential was changed at fixed concentration of CFX in the range of 0.0 to 0.7 V (vs. Ag/AgCl) at AuNP/GO modified PGE and stripping voltammograms were recorded. As shown in Fig. 9.a, according to the plot for applied deposition potential vs. the oxidation current of CFX, the measured peak current increased from 0.0 V to 0.6 V (vs. Ag/AgCl), and a roll-off rate was observed on the peak current after this value indicating that at more cathodic potentials which are close to oxidation potential of the analyte, CFX molecules are
not strongly adsorbed on the electrode surface. Therefore, the optimum deposition potential was determined as 0.6 V (vs. Ag/AgCl).

To identify the optimum deposition time of CFX, different durations (60–420 s) at the optimum deposition potential were examined (Fig. 9b). Up to 300 s, the peak currents increased and further increase in accumulation time yielded to almost stable response. This result proved that the sensor surface was saturated at 300 s deposition time. So, the optimum deposition time was selected as 300 s.

Analytical performance of the AuNP/GO/PGE

Since stripping analysis provides more sensitive measurements, it has frequently been applied for various drug determination studies [29–31]. Hence, under the optimized conditions, SWASV was performed for the quantitative detection of CFX (Fig. 10). The well-defined oxidation peak currents with respect to the increasing CFX concentrations were monitored and the measured peak currents increased linearly with CFX concentrations in the range of 0.02-2.0 µM. The corresponding calibration graph was illustrated in Fig. 10 (inset).

The obtained linear equation was (Eq.2),

\[ I(\mu A) = 45.21C(\mu M) + 3.412 \quad R^2=0.9985 . \] (2)

As a result, based on 3σ/m equation (σ is standard deviation of the analytical signal for the blank solution (n=3), m is the calibration curve slope), the LOD and LOQ were determined as 0.442 nM and 1.32 nM, respectively. When compared with other reported electrochemical studies about CFX (Table 2), the proposed sensing system had compatible results.

Furthermore, the intra/inter-day reproducibility of the AuNP/GO modified surface was examined by SWASV. To estimate reproducibility of the electrode, six AuNP/GO/PGE were fabricated independently and the RSD values for these electrodes were 2.43% (n=3) and 2.26% (for 6 days). The developed AuNP/GO/PGE showed good reproducible results. The interference study results showed...
that 250 fold concentration of ascorbic acid, 150 fold concentration of glucose and 150 fold concentration of lidocaine had no significant influence on the measured 0.5 µM CFX peak current (RSD values less than 5%).

Real Sample Analysis

The commercial CFX tablet was used to show the practical application of AuNP/GO modified PGE. The tablet samples were analysed by standard addition method and the recovery values of different concentrations of spiked CFX were given in Table 3.

The recovery and precision values confirmed that the adjuvants of the drug did not show important interference on the voltammetric response of CFX. These results clearly proved that this sensor system can be used in real samples for the detection of CFX with high accuracy.

CONCLUSION

A simple and an effective disposable sensor system based on AuNP/GO was developed for the electrochemical detection of CFX for the first time. When PGE surface was coated with AuNP/GO, the oxidation peak of CFX improved remarkably due to a synergistic effect. The optimum conditions were examined for the detection of CFX by SWASV. The described ultra-sensitive detection strategy had good sensing performance and exhibited comparable results with the literature. To demonstrate the success of the practical application, the developed sensor system was applied to the commercial CFX tablet and the obtained recovery values showed that the proposed system can be used in real matrices with high accuracy. The disposable modified sensor offers favourable features for the electrochemical detection of CFX in terms of high sensitivity, rapidness, simplicity and cost-effectiveness.

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GCE: Glassy carbon electrode, AgNPs-ND-G: silver nanoparticles Decorated Nano Diamond, SDS: sodium dodecylsulphate, CPE: carbon paste electrode, MIP/Ag@AuNPs/ILs: molecularly imprinted polymer based silver@gold nanoparticles/ionic liquid, HGPNs/rGO: Hollow gold nanoparticles/reduced graphene oxide, AdDPV: adsorptive differential pulse voltammetry.

| Working electrode type | Method            | LOD (M)         | Linear Range (M) | Reference |
|------------------------|-------------------|-----------------|------------------|-----------|
| AgNPs-ND-G/GCE         | LSV               | 6.0x10^-9       | 2.0x10^-8-8.7x10^-8 | [12]      |
| Fullerene/GCE          | SWV               | 6.6x10^-10      | 2.96x10^-6-6.25x10^-6 | [11]      |
| Poly(o-anisidine)/SDS/Ni/CPE | chronoamperometry | 8.0x10^-5       | 1.0x10^-5-2.0x10^-5 | [13]      |
| MIP/Ag@AuNPs/ILs/GCE   | DPV               | 2.0x10^-12      | 1.0x10^-9-1.0x10^-11 | [32]      |
| HGPNs/rGO/PGE          | AdDPV             | 3.5x10^-13      | 1.0x10^-12-1.0x10^-11 | [33]      |
| AuNP/GO/PGE            | SWASV             | 4.42x10^-10     | 2.0x10^-8-2.0x10^-6 | This work |

Table 2. List of electrochemical sensors of CFX.

| Sample               | Added (µM) | Detected (µM) | RSD (%) | Recovery (%) |
|----------------------|------------|---------------|---------|--------------|
| Tablet               | 0.0264     | 0.0261        | 1.60    | 101          |
|                      | 0.0600     | 0.0581        | 1.39    | 97.0         |
|                      | 0.0750     | 0.0742        | 1.29    | 99.0         |

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

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