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Electrochemical determination of ceftriaxone using polyurethane-modified electrode containing caffeic acid and chitosan

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ABSTRACT

The aim of this work was to develop an electrochemical sensor based on caffeic acid and chitosan-containing polyurethane (CAC-PU) films modified platinum electrode for ceftriaxone (CFX) determination. For this aim, firstly the CAC-PU structures were synthesized from three different diisocyanates and these structures were characterized with spectroscopic, thermal and surface analysis techniques. Then, these CAC-PU structures were coated on bare Pt electrode surface for the preparation of CFX selective sensors. Throughout this study several analytical parameters, such as film thickness, electrolyte type, pH effect, and scan rate were investigated by using differential pulse voltammetry (DPV) method. Under the optimized conditions, the voltammetric test results showed best analytical performances for CFX detection with a linear range from 0.1 to 1.5 mM. Limit of detection (LOD) and correlation coefficient (R^2) were obtained 52 μ M and 0.9991 using CAC-PU modified electrode, respectively. Stability of the modified electrode was found to be 96.78% by using reproducibility experiments ($n = 10$). Applicable of the presented sensor was tested in two synthetic urine samples and recoveries values were obtained 98.7% and 110%, respectively. The prepared modified electrode exhibits high repeatability, fast response, and high sensitivity, which makes it suitable for the detection of CFX in urine samples.

ARTICLE HISTORY

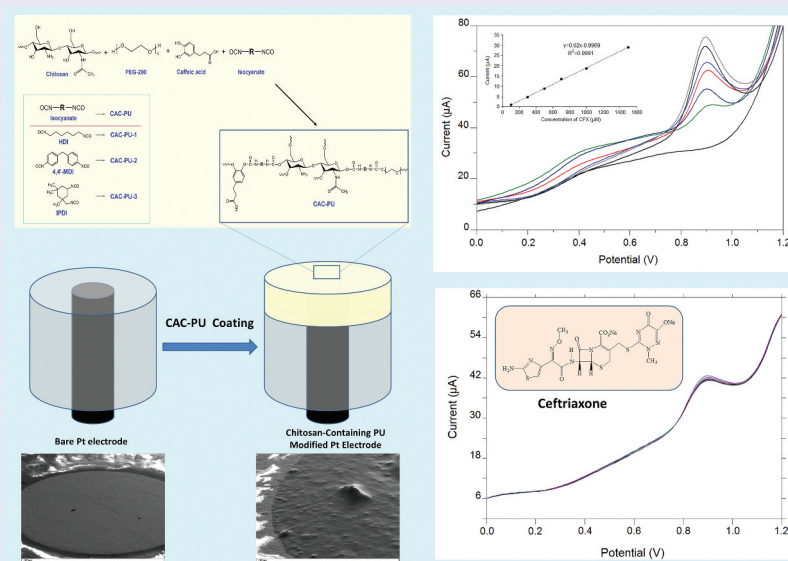
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KEYWORDS

Ceftriaxone; polyurethane membrane; electrode modification; sensor; voltammetry



1. Introduction

Ceftriaxone sodium (CFX) is an important antibiotic belonging to the class of cephalosporin (Figure 1). It is administered by parenteral route and has the ability to cross the blood-brain barrier.^[1] Cephalosporins are

widely used as anti-infective agents.^[2] Ceftriaxone sodium is a pharmaceutical widely used in the treating respiratory tract infection, urinary system infection and gonorrhea.^[3] Ceftriaxone is also used for the treatment of diseases, such as acute bacterial otitis, skin structural

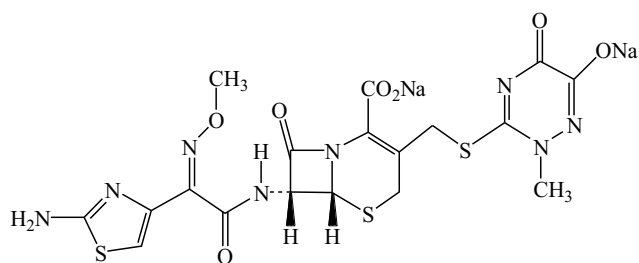


Figure 1. Structure of ceftriaxone sodium.

infections, pelvic inflammatory disease, bacterial septicemia, bone infections, and intra-abdominal infections.^[4] Besides, the wide range of applications of ceftriaxone in pharmaceutical and clinical procedures makes its detection and quantification important.

Various methods have been reported for the determination of ceftriaxone sodium-like chromatography, spectrophotometry, and capillary electrophoresis.^[5–13] Furthermore electrochemical methods have been used with different kind of type electrodes and modification layers.^[14] At present, chromatographic methods require many steps before measurements like sample pre-treatments, filtration all this makes these techniques time consuming and expensive. Modern electrochemical methods attract attention since they are cheaper, simpler, high sensitive, and don't require any pre-treatments if we compare them to the other analytical methods.^[15–20] Few works have elaborated on electrochemical detection of CFX. A group of researchers used a multiwall carbon nanotube modified glassy carbon electrode for the detection of CFX. Another group presented modified glassy carbon electrode with a polymeric film of Eriochrome black-T. The main goal of analytical methods is to achieve maximum efficiency and easiest experiments.^[21–23] This leads researchers to design sensors with simple methods of modification speed and low cost. According to this work, we prepared a thin film of polyurethane-chitosan composite for the electrode modification.

Polyurethane has suitable physical characteristics including high mechanical resistance and elasticity, high durability and water insolubility, porous surface for the adsorption of gas contaminants.^[24–27] Chitosan is a nontoxic, biodegradable polycationic polysaccharide and positively charged biopolymer, with antimicrobial properties derived from naturally abundant chitin. Due to the presence of carboxyl, phosphate and amino groups, the cellular membrane of the most microorganisms has a negative charge.^[28] The polyurethane/chitosan composite have been as nanofiber for air pollution control applications.^[29] Polyurethane structures have been successfully used as a membrane in the design

and modification of electrochemical sensors.^[30–33] In this area, the advantages, such as good surface adhesion, easy adjustment of the pore structure, chemical stability, mechanical strength and easy synthesis, have increased the use of polyurethanes in sensor modification.^[34–36]

CFX is a very important drug for medical applications and simple, selective, repetitive, and easy measurement techniques have been urgently desired to detection of ceftriaxone sodium in different physiological fluids.^[37,38] Thus, in the current study, a novel sensor has been developed based on a platinum electrode modified with CAC-PU films for the detection of CFX. The prepared modified electrodes can be used for the detection of CFX in biomedical application.

2. Experimental section

2.1. Reagent

Ferrocyanide ($K_4[Fe(CN)_6]$), potassium dihydrogen phosphate (KH_2PO_4), sodium monohydrogen phosphate (Na_2HPO_4), potassium nitrate (KNO_3), nitric acid (HNO_3), Sodium sulfate (Na_2SO_4), Ammonium chloride (NH_4Cl), dopamine (DA), ascorbic acid (AA) and uric acid (UA), Glucose (Glu), Lactose (Lac), Sucrose (Suc) and Calcium chloride dehydrate ($CaCl_2 \cdot 2H_2O$) were purchased from Sigma-Aldrich Chemical Company. Monomers and solvents used for the synthesis of polyurethane structures were also obtained from Sigma-Aldrich Chemical Company. Tris-HCl, sodium hydroxide (NaOH), phosphoric acid (H_3PO_4), boric acid (H_3BO_3) and sodium chloride (NaCl) sodium acetate ($CH_3COONa \cdot 3H_2O$), acetic acid (CH_3COOH), sodium perchlorate ($NaClO_4$) and potassium chloride (KCl) were purchased from Merck. All chemicals were supplied at the highest purity and used without any further purification. Aqueous solutions were prepared using ultrapure water.

Synthetic urine was prepared with a mixture of 6.25 g of urea, 0.40 g of KCl, 0.56 g of Na_2SO_4 , 0.25 g of NH_4Cl , 0.35 g of KH_2PO_4 , 0.28 g of $CaCl_2 \cdot 2H_2O$ and 0.73 g of NaCl by dissolving in deionized water.^[15] Ceftriaxone was purchased from IESEF®.

2.2. Apparatus

Polyurethane structures synthesized within the scope of the study were first characterized by using Fourier Transform Infrared Spectrometer (FTIR). The infrared spectrum of the polymer was recorded with Perkin Elmer spectrum two FTIR spectrometers in the range $4000\text{--}400\text{ cm}^{-1}$. The surface morphologies of the synthesized polyurethane-based surface coatings were

analyzed with the LEO EVO-40xVP scanning electron microscope. In addition, the surface structure of the modified electrodes and the chemical structure of the polymers on the surface are characterized by Energy Dispersive X-Ray Analysis (EDX). Röntech Xflash detector analyzer based on SEM (LEO EVO-40xVP) was used in these EDX analyzes. The thermal properties of the obtained polymer were determined by DTA and TGA thermograms. Schimadzu TGA-50 devices were used for thermal analysis. During TGA and DTA analyzes, 10 mg of polymer sample was heated at a heating rate of 10°C/min in the range of 20–700°C and in a constant air atmosphere. The surface morphologies of the modified electrodes were investigated by SEM (LEO Evo-40 VPX) and AFM (XE-100E; Park Systems Corp., Suwon, Korea) measurements.

For the electrochemical experiments, a traditional three-electrode cell system was used. A platinum wire was used as a counter electrode and platinum electrodes with active electrode diameter of 1.6 mm were used as working electrodes. The reference electrode used is a Ag/AgCl in saturated KCl solution (Ag/AgCl/(sat. KCl)). All electrochemical experiments were realized with a potentiostat controlled by computer with software Ivium soft for the data analysis. Differential pulse voltammetry was used for Ceftriaxone detection. Differential pulse voltammetry (DPV) were recorded from 0.0 V to 1.4 V with the amplitude of 100 mV in citrate buffer 0.1 M (pH = 7.0). All electrochemical measurements were carried out at room temperature conditions. All glassware and electrochemical cells were kept in Nitric acid (6.0 M) for one night to remove impurities.

2.3. Synthesis of the Polyurethane membranes

Within the scope of the study, three different polyurethane structures were synthesized with the solution polymerization technique for electrode modification. The chemical structures and synthesis reaction of the synthesized polyurethanes are given in Figure 2 schematically. In the synthesis of polyurethane structure; diisocyanate and -OH functional monomers (chitosan, PEG-200 and caffeic acid) were used equimolar. The -OH functional monomers mixture prepared from chitosan:PEG 200: caffeic acid molar ratios as 3:96:1. For the first polyurethane structure; hexamethylene diisocyanate (HDI), chitosan, PEG-200 and caffeic acid were used as monomers. For the synthesis of second polyurethane structure; isophorone diisocyanate (IPDI), chitosan, PEG-200 and caffeic acid were used. For the third polyurethane structure; 4,4'-methylenediphenyl diisocyanate (4,4'-MDI), chitosan, PEG-200 and caffeic acid were used as monomers. Three different polyurethane structures were carried out after 7–8 hours of reflux at 75–80°C in 15 mL solution medium (DMF) by taking equimolar from isocyanate and diol sources. At the end of the reaction period, the mixtures were cooled to room temperature and the polymer samples were removed from the solvent under vacuum. Chemical characterizations of the polyurethanes were made by FTIR analysis. The thermal properties of these polymers were determined by TGA and DTA measurements. In addition, the surface properties of the chitosan and caffeic acid based polyurethanes were determined by SEM and AFM techniques.

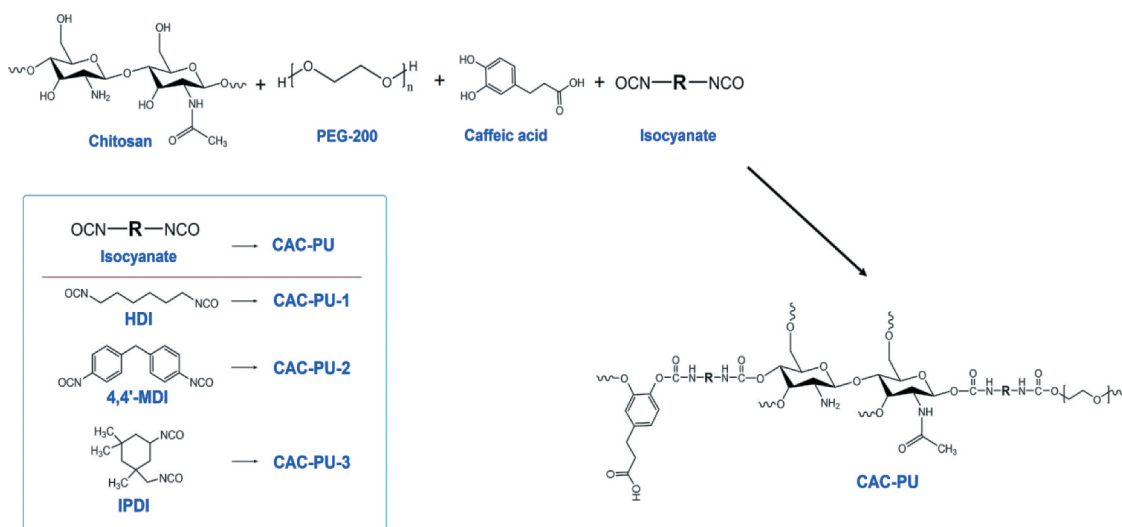


Figure 2. Synthesis of the caffeic acid (CA) and chitosan (C) based polyurethane membranes.

2.4. Electrode preparation

Platinum electrode surface was pretreated before the modification. Firstly, it was hand-polished with 10 μm alumina–water slurry using a polishing cloth. Secondly, the polished electrode was washed and sonicated in ultrapure water for 3 min and finally rinsed with ultrapure water in order to remove impurities on the electrode surface. Finally, 4 μL of the homogenized CAC-PU solution was dropped onto the platinum electrode surface. Then, CAC-PU solution coated electrode was dried at room temperature overnight. After the modification step, the surface of the modified electrode was washed with ultrapure water.

3. Results and discussion

In this study, in order to prepare CFX selective and sensitive electrodes, chitosan and caffeic acid based polyurethane structures that show affinity for CFX structure were synthesized. Synthesized polyurethane structures were examined using FTIR, thermal analysis techniques, and surface analysis methods. CFX selectivity and sensitivity were investigated by coating structurally defined polyurethane structures on platinum electrode surface with different film structures and film thicknesses. Structural and chemical diversity were achieved by using different diisocyanate structures as monomers in the synthesis of polyurethane structures prepared for platinum electrode modification. The polyurethane structure with high CFX selectivity, good film property and high structural stability was chosen as the CFX sensor for electrode surface modification. For the investigation of chemical structure of chitosan and caffeic

acid based polyurethanes and polymerization control were used primarily FTIR spectroscopy technique. In polyurethane synthesis reactions, FTIR spectra were taken during the reaction and the characteristic peak of the free isocyanate groups at a wavelength of 2265 cm^{-1} was observed. When this peak disappeared completely, the polymerization reaction was terminated. The disappearance of this peak indicates that no free isocyanate is left in the reaction mixture and the polymerization reaction is complete.

3.1. Characterization of the CAC-PU structures

FTIR spectra of polyurethane structures prepared from three different diisocyanate structures are given in Figure 3. In the FTIR spectra of three different polyurethane structures, the H-bond band originating from the OH groups and N-H structures in the polymer structure can be seen at $3000\text{--}3600\text{ cm}^{-1}$. In addition, we see C = O stress vibration at approximately 1670 cm^{-1} , C-N stress peak at 1450 cm^{-1} and N-H peaks at 1510 cm^{-1} caused by the urethane bond. The presence of these peaks proves that the urethane bond is formed and the polyurethane structure. In addition, we see C-O-C peaks around 1100 cm^{-1} and C-O peaks at 1060 cm^{-1} due to the chitosan structure. Aliphatic C-H stretching vibrations originating from aliphatic diisocyanate structures and chitosan groups are seen quite clearly at $2810\text{--}2980\text{ cm}^{-1}$. The C-H peak resulting from aromatic units in the caffeic acid structure is seen at approximately 950 cm^{-1} and 845 cm^{-1} . All these peaks prove that the desired caffeic acid and chitosan-based polyurethane structure is obtained.

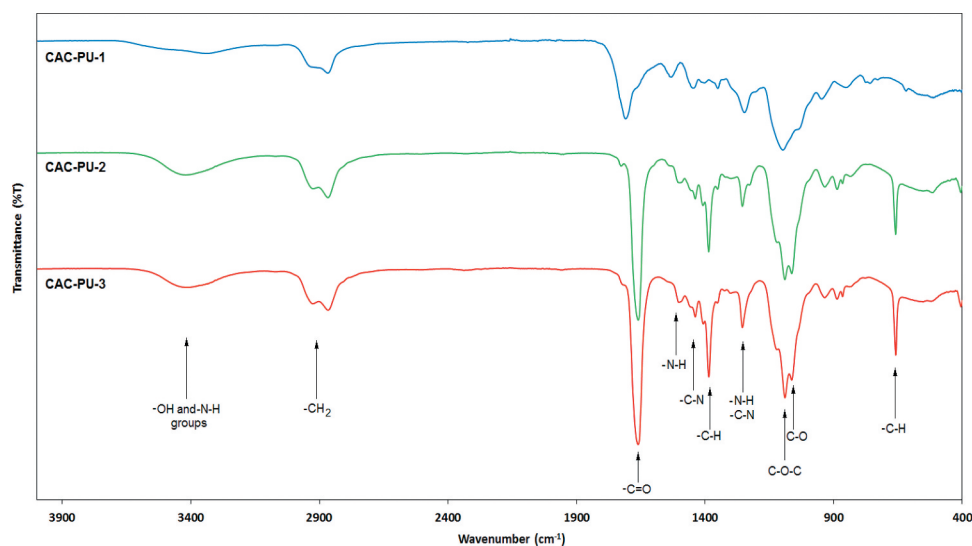


Figure 3. FTIR spectra of the CAC-PU structures.

In voltammetric sensor studies, an important parameter is the surface roughness and surface area of the electrode used. The increased surface area on the electrode surface increases the oxidation or reduction reaction of the analogue on the electrode surface, resulting in more sensitive, faster and more accurate measurement results. Within the scope of this study, CAC-PU structures were coated on the electrode surface in order to increase the area of the Pt electrode surface given in Figure 4(a-c) and to ensure the elimination of interferant molecules.

The surface images of these structures are given in Figure 4 comparatively at different magnifications. When these surface images are examined, it is seen that the uncoated Pt electrode surface in Figure 4(a-c)

has a fairly flat structure. This flat structure limits the surface area of the electrode. However, when the polyurethane structures given in Figure 4 are examined, their surface structures are quite homogeneous and fractal. There is no foreign phase or impurity in their structure. In addition, cracks and broken areas were not found in the surface structures. The surface of polyurethane structures is very rough compared with bare electrode surface. Therefore, the surface area of the CAC-PU modified electrodes is seen as high.

When the polyurethane film structures synthesized using three different diisocyanates are evaluated, a more ideal surface structure for electrode surface applications has been obtained due to the aliphatic structure in the polyurethane structure given in Figure 4(d-f) and the

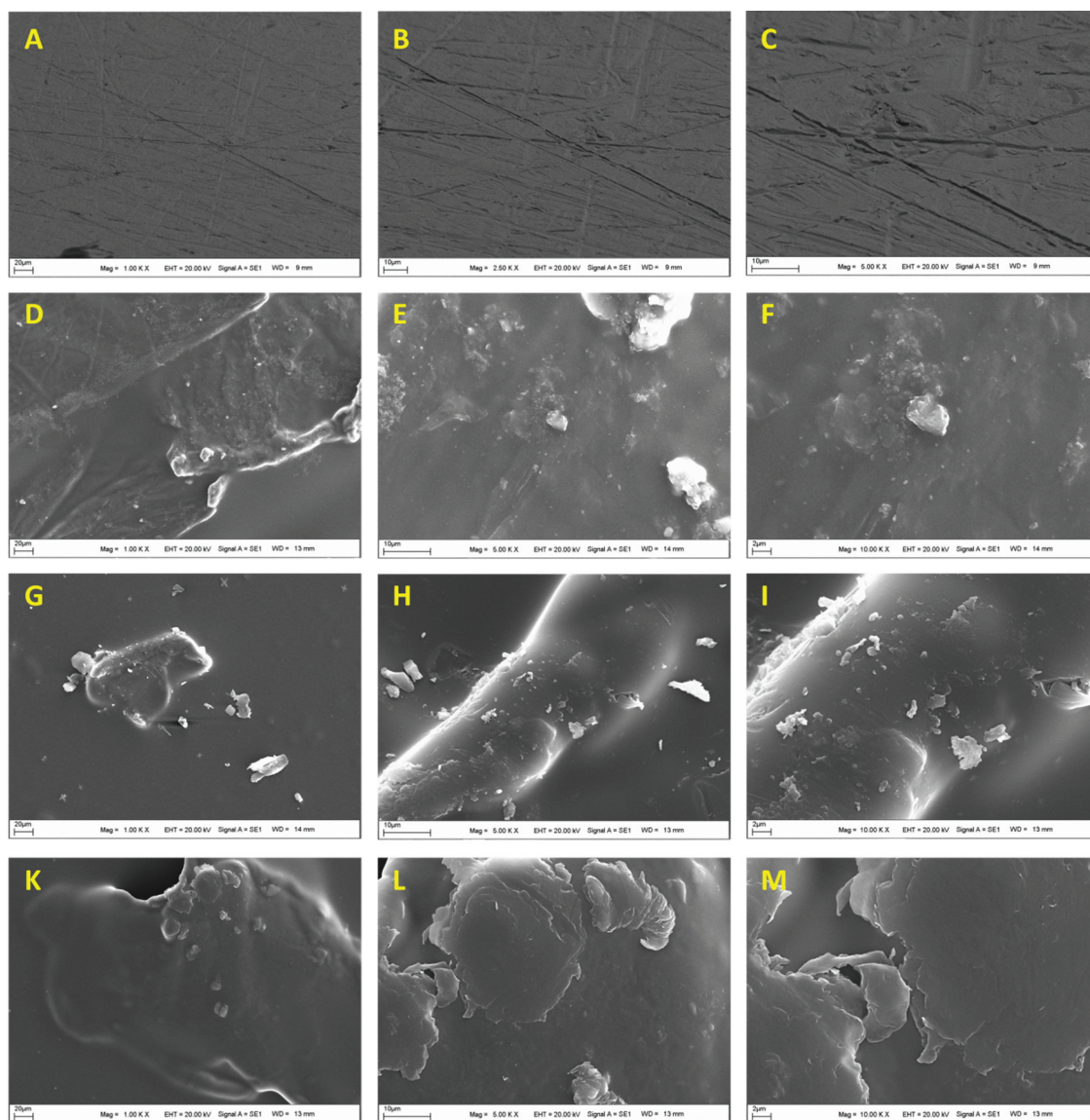


Figure 4. SEM images of the bare Pt electrode surface (A, B, C) and CAC-PU film structures (D, E, F: CAC-PU-1; G, H, I: CAC-PU-2 and K, L, M: CAC-PU-3).

chemistry of the hard segments similar to the soft segments. Large surface folds and anisotropic roughness are seen in the polyurethane surface structure given in Figure 4(g-i). In the SEM images of the polyurethane structure in Figure 4(k-m), deep layers and voids are seen on the surface. For these reasons, the polyurethane structure synthesized using HDI has shown a more suitable surface structure for electrode designs.

The thermal stability of the obtained CAC-PU film structures was determined by TGA, DTA, and DSC analysis. The thermograms obtained in these thermal analyzes are comparatively given in Figure 5. In order

for the modified electrodes to be used in a wide temperature range and to be used in biomedical applications, the shelf temperature and storage temperature must be appropriate. In addition, it must be thermally resistant up to the sterilization temperature in order to be sterilized after use. For these reasons, the thermal stability of the developed CAC-PU based modified electrodes were determined by TGA and DTA analyzes. In addition, T_g temperatures of polymeric film structures used in electrode coating processes were determined by DSC thermograms. The results of TGA analyzes of the CAC-PU structures are given in Figure 5(a). In this

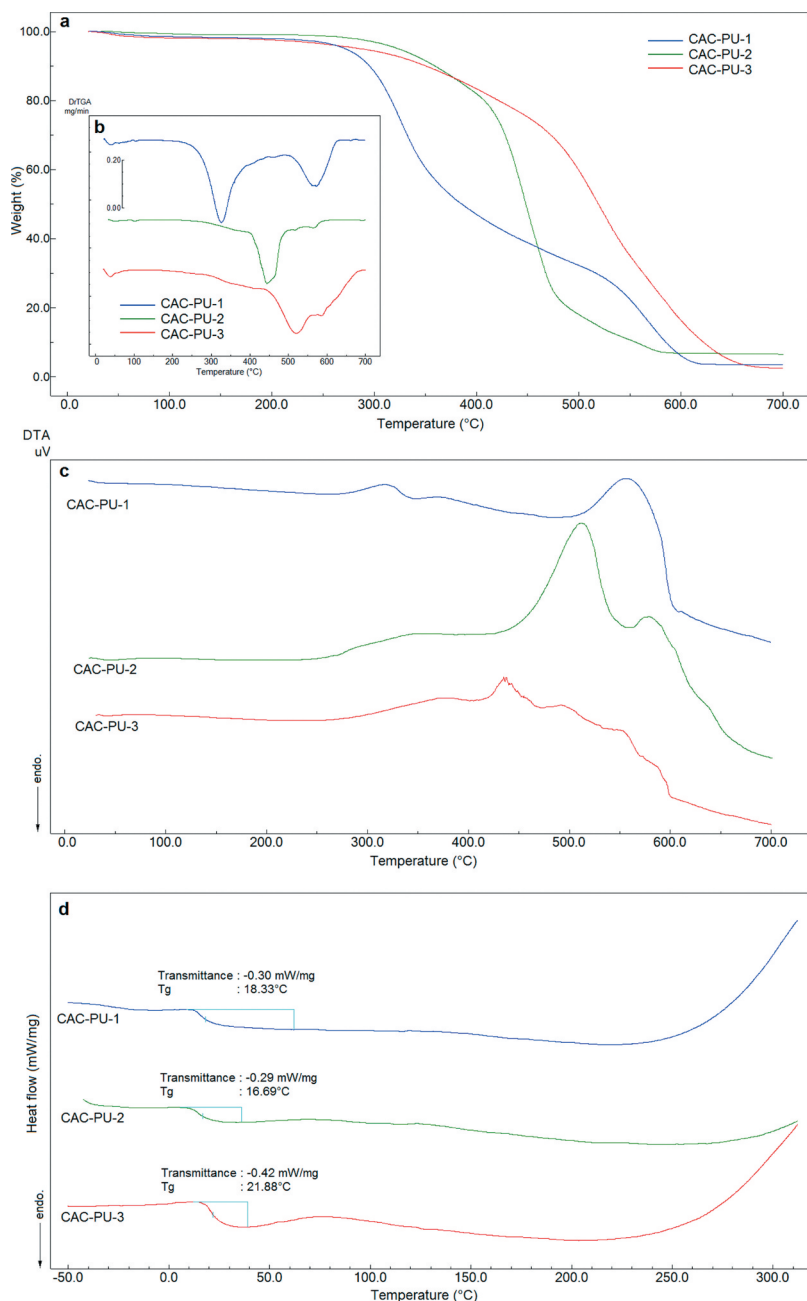


Figure 5. TGA (a), DTA (b) and DSC (c) thermogram of the CAC-PU structures.

way, three different mass losses are seen. The first loss of mass is the removal of free moisture in the structure. This mass loss occurs between 50°C and 120°C and is a mass loss of approximately 3%. The second mass loss occurs between 250°C and 450°C. This mass loss is approximately 80% mass loss. It is caused by the degradation of soft segments and cross-link points in the structure. The third mass loss is between 450°C and 600°C. This loss of mass is caused by the degradation of hard segments and isocyanate residues. It is approximately 15%. The resulting PU structure is thermally stable up to approximately 220°C due to its cross-linking and can be easily used in many biomedical applications.

Derivative TGA plots are given in Figure 5(b) so that these mass loss values can be seen more clearly. According to these data, it is seen that the thermal stability of CAC-PU-2 structure is higher than other polyurethane structures. However, in all three polyurethane structures, the decomposition initial temperature is above 220°C. Therefore, it has the thermal stability required for electrode modification and sterilization in three polyurethane structures. DTA thermograms of the synthesized chitosan-based polyurethane structures are given in Figure 5(c). When the DTA thermogram of the synthesized polyurethane is examined, it is seen that it is compatible with TGA. DTA thermogram shows three exothermic bands up to 650°C. The first band lasts up to 150°C and is caused by the removal of moisture from the structure. It is a dehydration band. The second band starts at 250°C and lasts up to 450°C and shows the degradation of the soft segments. These are cross-linking points, peg groups and CH₂ groups in aliphatic units structure. The third exotherm band is between 450°C and 650°C, caused by the degradation of rigid segment structures.

In the DSC thermogram of the synthesized PU structures (Figure 5(d)), a distinct T_g transition was seen. T_g transition values were determined as 18.33°C, 16.69°C, and 21.88°C for CAC-PU-1, CAC-PU-2, and CAC-PU-3 structures, respectively. According to these values, T_g transitions of chitosan and caffeic acid-based polyurethane structures are very close. This result shows that the crosslinking ratio is achieved at the desired rate in all polymer structures and that the processability and applicability of the polymers to the electrode surface are suitable.

3.2. Characterization of the modified Pt electrode

Within the scope of the study, the structural characterization of electrodes modified with CAP-PU structures was primarily performed by SEM analysis. SEM image of the bare (A) and CAC-PU-1 modified

electrode (B, C, and D) surfaces are given in Figure 6. It is seen that the surface of the bare electrode is quite flat and smooth. Therefore, the electrode active surface area is very low. However, the modified electrode surface is quite rough due to the coated CAP-PU structures. This rough structure increases the surface area of the electrode. In addition, no cracking and any impurities were observed in the coatings made on the modified electrode surface.

Surface and morphological features of synthesized PU structures were determined in more detail by AFM examinations. AFM results are given in Figure 7. These images show that the surfaces of all three electrodes are very rough. Especially, it contains small micro-hillocks with CAC-PU-3 surface. In the CAC-PU-2 structure, there are wide elevations in certain regions. In AFM images of the CAC-PU-1 structure, it is seen that the film is well spread over the surface and the surface roughness increases significantly after the modification. As a result, the AFM images in Figure 7 show that the modification on the electrode surface was achieved successfully.

In order to electrochemical detection of CFX, firstly, the ceftriaxone and blank responses of the bare Pt electrode were determined. These results are given in Figure 8. According to Figure 8, CFX response was seen at about 0.9 V on the bare electrode surface.

Then, most suitable film structure for electrode modification and CFX determination was determined. For this purpose; CAC-PU-1, CAC-PU-2 and CAC-PU-3 structures were coated on the Pt electrode surface with same thickness and the resulting modified electrodes were used for determination of the CFX with DPV technique. Comparing the CFX responses of modified electrodes using these polyurethane structure, it was found that the best results were obtained with the electrode structure prepared using CAC-PU-1 (Figure 9). Therefore, the CAC-PU-1 modified Pt electrode was chosen as the optimum electrode for CFX determination. Analytical optimizations and further sensor studies for CFX determination were performed using a CAC-PU-1 modified electrode.

The bare platinum and modified electrode were characterized with the electrochemical method cyclic voltammetry (CV) (scan rate of 100 mV/s) in 6 mM of ferrocyanide containing 1.0 M KNO₃. Figure 10 shows the cyclic voltammograms of the bare and the modified electrode.

Oxidation and reduction peaks of the ferrocyanide are clearly detected before and after the modification of the electrode. After the modification of the platinum electrode surface with the polyurethane-chitosan film a large decrease of peak current is observed. These

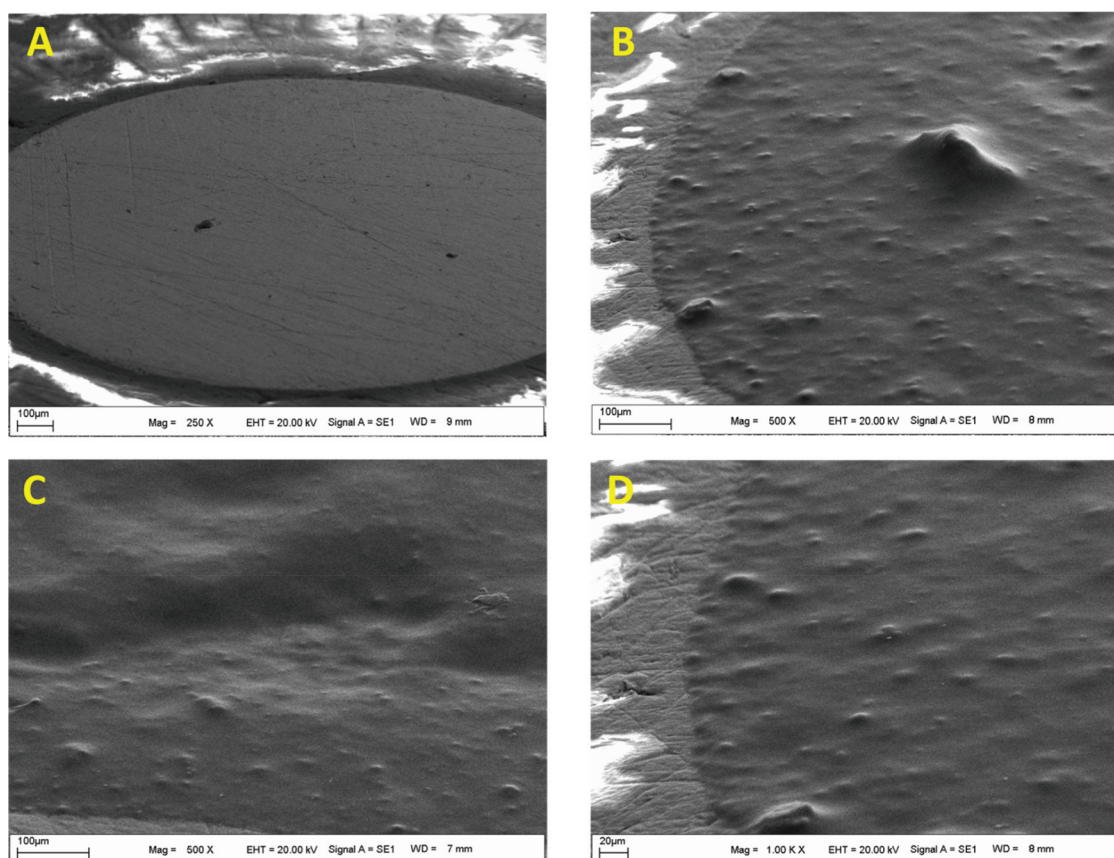


Figure 6. SEM images of the bare (A) and CAC-PU-1 modified electrode (B, C and D) surfaces.

differences in the electrochemical behavior show that the polymeric films adhere well to the surface of the electrode.

3.3. Optimization of the thickness of the CAC-PU-1 film

In order to check the influence of the film thickness on the electrochemical response of the modified electrode, different volumes of the polymeric mixture were dropped onto the electrodes. Those electrodes were studied by DPV in buffer solution (0.1 M) at pH 7.0.

2, 3, 4, 5, and 6 μL of the mixture were dropped and studied separately. Experimental results are summarized in the [Figure 11](#). The response of the modified platinum electrode in the presence of ceftriaxone increased as the film thickness increased from 3 to 4 μL and then decreased. It could be assigned to the electron transfer

due to the blocking effect of polyurethane. 4 μL was chosen for the electrode modification for all the experiments.

3.4. Electrolyte type and pH optimization

Analytical parameters were optimized using the DPV technique to develop in order to obtain the best response for CFX detection. Voltammograms were optimized in the potential range of 0.0 V to +1.4 V for all experiments. For the determination of the electrolyte-type effect on the CFX detection, the response of 600 μM of CFX was studied in different solutions at pH 7.0 of buffer: citrate buffer (CB), tris buffer (TrisB), Brinton Robinson buffer (BR), Acetate buffer (AB) and PBS. Results are gathered in the [Figure 12\(a\)](#). It was observed that highest peak current for CFX was obtained in CB. Thus, CB was chosen for all the incoming experiments.

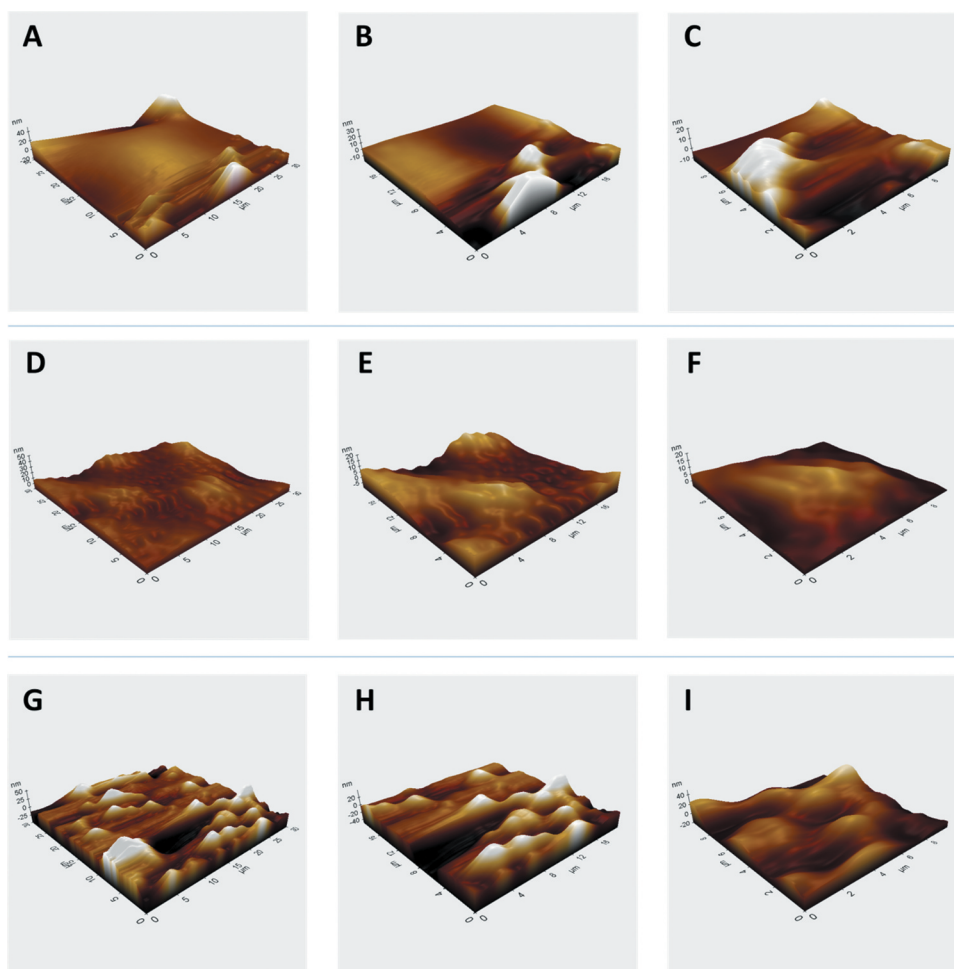


Figure 7. AFM images of the CAC-PU structures. (A, B, C: CAC-PU-1; D, E, F: CAC-PU-2 and G, H, I: CAC-PU-3).

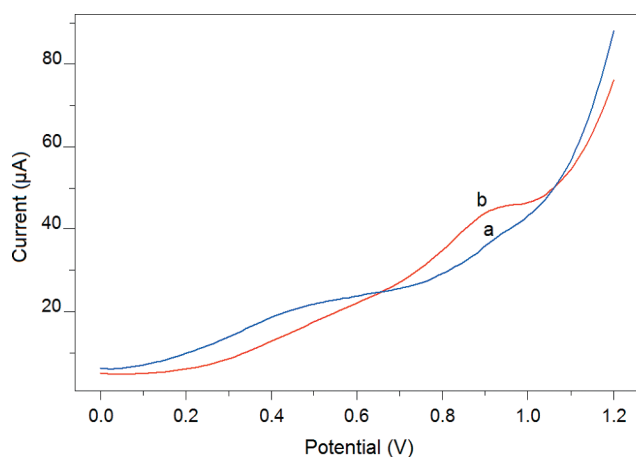


Figure 8. A: DPV voltammograms of blank and b: DPV voltammograms of the bare Pt for 1200 μM CFX in 0.1 M citrate buffer (pH 7.00)

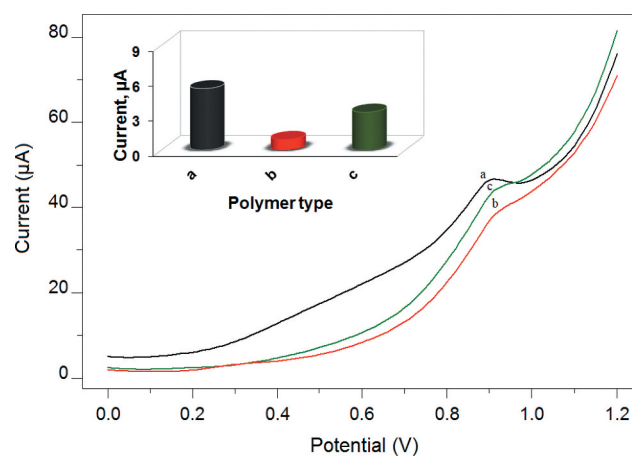


Figure 9. For the 600 μM CFX, DPV response of the CAC-PU-1 (a), CAC-PU-2 (b) and CAC-PU-3 (c) coated Pt electrode with same thickness in 0.1 M citrate buffer (pH 7.00).

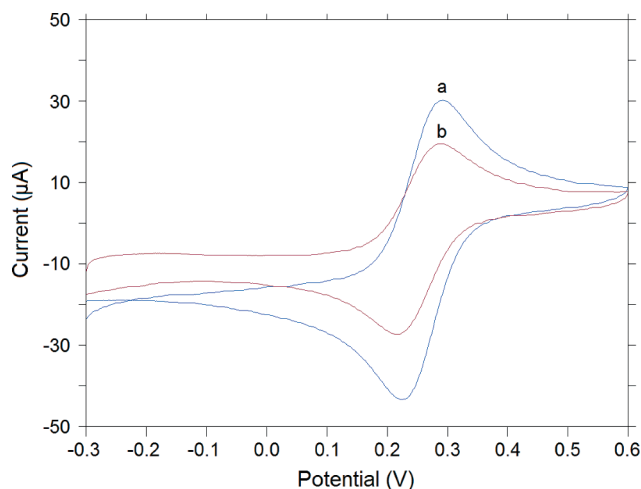


Figure 10. Cyclic voltammograms of the bare Pt (a) and CAC-PU-1 modified electrodes (b) in ferrocyanide (6 mM) and KNO_3 (1.0 M) at a scan rate of 100 mV/s vs. Ag/AgCl/ (sat. KCl).

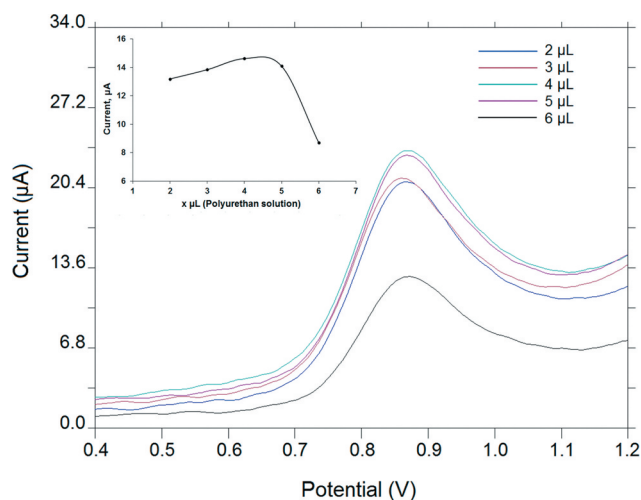


Figure 11. For 600 μM CFX, DPV responses of modified electrode obtained by dropping 2 μL, 3 μL, 4 μL, 5 μL and 6 μL of CAC-PU-1 solution, in 0.1 M citrate buffer pH 7.0.

The electrolyte pH was also investigated using DPV method and the modified electrode in 0.1 M CB from pH 2.0 to 8.0 (Figure 12(b)). A maximum peak current value was obtained for pH 7.0. Thus, the optimal pH value was chosen as 7.0.

3.5. Electrochemical parameter optimization

The influence of DPV parameter such as the scan rate and amplitude were studied in the presence of 600 μM of CFX. Results are presented in the Figure 13(a,b). It was clearly notified that parameter could strongly affect the response of the electrode toward the CFX detection. As can be seen, the anodic peak current varies according to

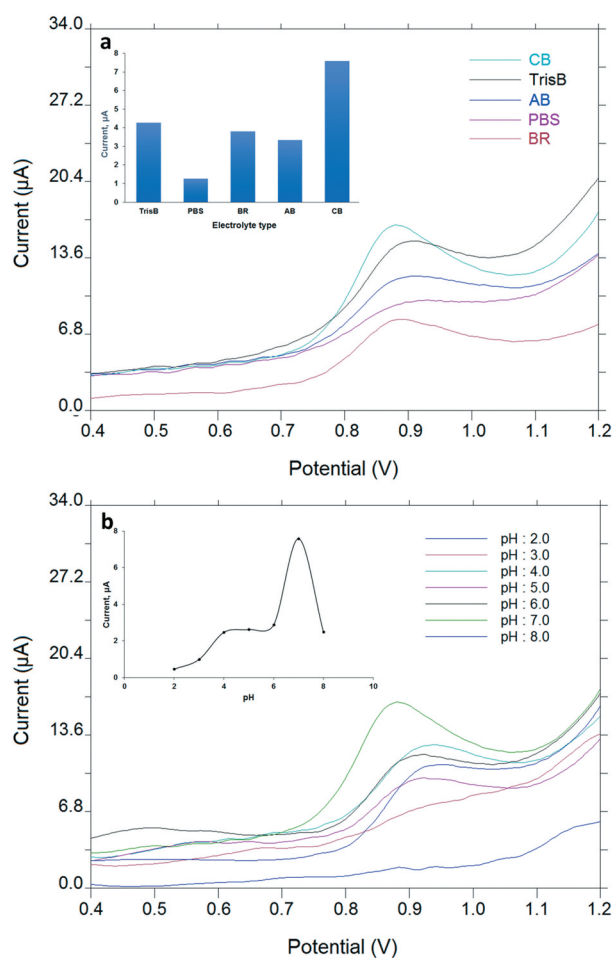


Figure 12. Electrolyte type (a) and pH (b) effect at the CAC-PU-1 modified electrode in the presence of 600 μM CFX.

the variation of these parameters. The optimal scanning rate and amplitude were chosen as 60 mV/s and 100 mV, respectively.

3.6. Analytical Performances of the Modified Electrode for Ceftriaxone Detection

The development of sensing polymeric materials to enhance sensitivity and selectivity of the platinum electrode is reported in this part. DPV was used to investigate the relationship between current peaks and analyte concentrations (CFX). The polyurethane/chitosan modified platinum electrode was used. Figure 14 shows the voltammograms obtained for CFX quantification using the modified electrode. The peak current of the modified electrodes has a linear behavior with the CFX concentrations variation. As shown in Figure 14 (inside), a linear dependence of the response current is obtained ($R^2 = 0.9991$) in the concentration range of 100 μM to

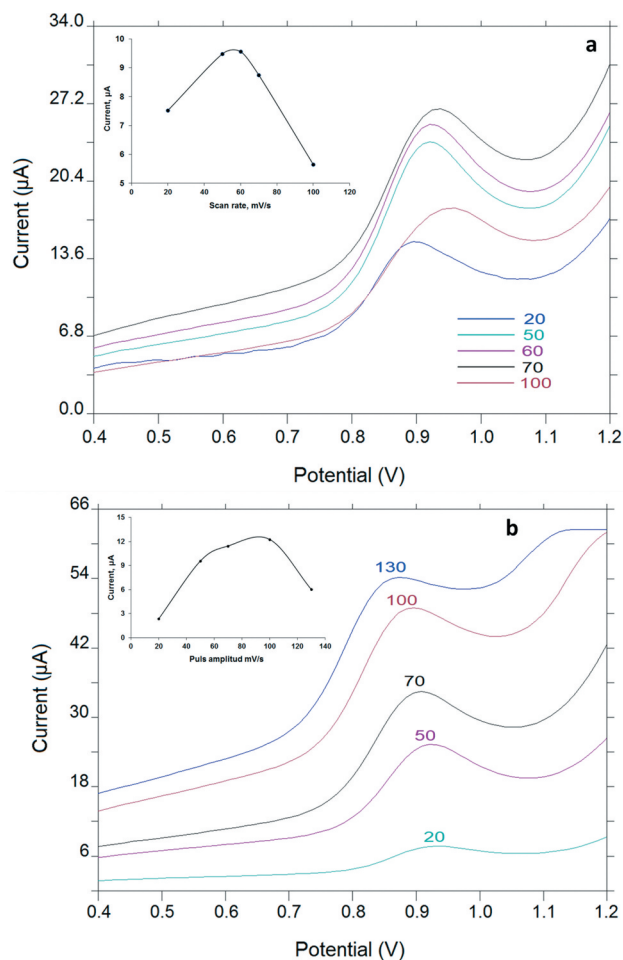


Figure 13. (a) DPV responses of the CAC-PU-1 modified electrode at different scan rate for 600 μM CFX in 0.1 M citrate buffer pH 7.0. (b) The effect of the amplitude values on the DPV responses of the CAC-PU-1 modified electrode for 600 μM CFX in 0.1 M citrate buffer pH 7.0.

1500 μM . A low limit of detection was obtained 52 μM . According to these results, our modified electrode provided a good sensitivity (Figure 14).

In this study, bare Pt electrode surface was modified with the CAC-PU-1. This polyurethane coating on the surface increased the accumulation of CFX molecules on the electrode surface and also eliminated the interfering species. To demonstrate this feature of the prepared modified electrode, DPV responses of the bare electrode and the modified electrode were compared in the presence of the electroactive (uric acid (UA), ascorbic acid (AA) and dopamine (DA)) and nonelectroactive interferents (lactose (Lac), sucrose (Suc) and glucose (Glu)). The bare Pt electrode and modified electrode responses are given in Figure 15. According to

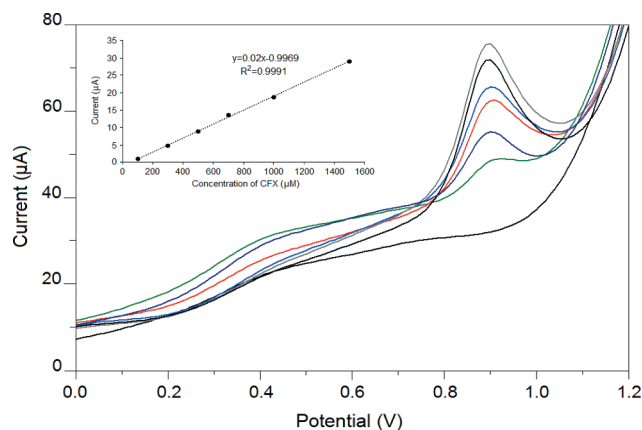


Figure 14. DPV voltammograms in the presence of different concentration (100, 300, 500, 700, 1000 and 1500 μM) of CFX in 0.1 M CB (pH = 7.0) (Amplitude 100 mV and scan rate 60 mV/s) **Inside:** Relationship between the anodic peak current and concentration of CFX.

these results, the bare Pt electrode gives a significant response for 150 μM UA (Figure 15(a)), 1000 μM AA (Figure 15(b)) and 30 μM DA (Figure 15(c)). However, CAC-PU-1 modified electrode is not affected by these interference species and does not respond to these species due to the inhibition of polyurethane membrane structure. Furthermore, prepared modified electrode is not affected by non-electroactive interferant such as 2 mM Glu, 2 mM Suc, and 2 mM Lac (Figure 15(d)). All these results show that the prepared CAC-PU-1 modified electrode is more selective for CFX than the bare electrode.

Time-based measurement was also performed to investigate the effect of interferences on the CFX responses of the prepared CAC-PU-1 modified electrode. In this study, measurements were made with prepared modified electrode in CB buffer (pH 7.0) with successive addition of 1200 μM CFX, 1 mM Glu, 1 mM Lac, 1 mM Suc, 100 μM DA, 100 μM UA, 100 μM AA, and 1200 μM CFX. The resulting current-time graph is given in Figure 16. In this way, it was observed that interfering molecules did not affect the CFX responses of the prepared CAC-PU-1 modified electrode. In particular, very close peak current results were obtained without interference (first CFX addition) and in the presence of interferant species (last CFX addition). These results show that the prepared electrode is sensitive and

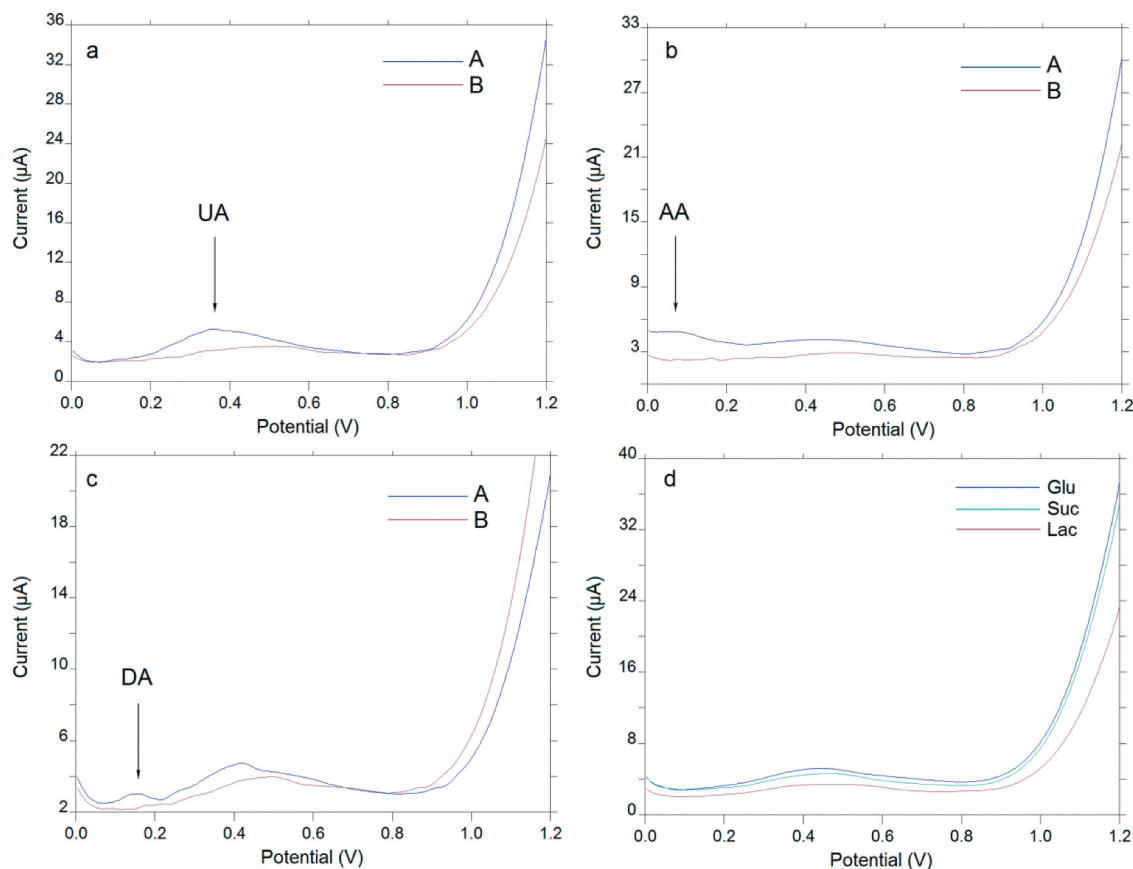


Figure 15. (a) DPV curves obtained for 150 μM UA in 0.1 M CB (pH 7.0) with bare Pt electrode (A) and the CAC-PU-1 modified electrode (B), (b) DPV curves obtained for 1000 μM AA in 0.1 M CB (pH 7.0) with bare Pt electrode (A) and the CAC-PU-1 modified electrode (B), (c) DPV curves obtained for 30 μM DA in 0.1 M CB (pH 7.0) with bare Pt electrode (A) and the CAC-PU-1 modified electrode (B), (d) DPV curves obtained for 2 mM Glu, 2 mM Lac and 2 mM Suc in 0.1 M CB (pH 7.0) with the CAC-PU-1 modified electrode.

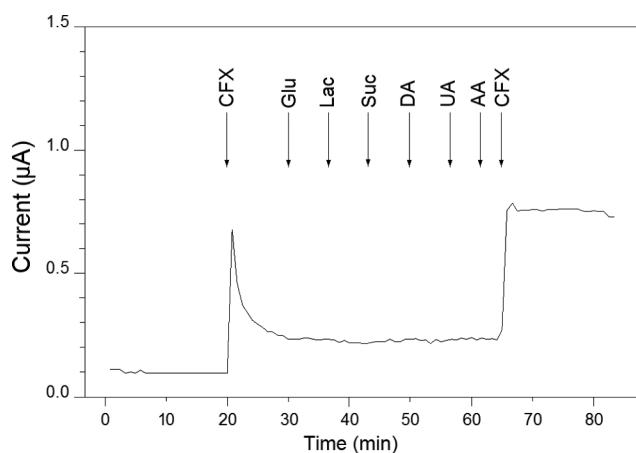


Figure 16. Current-time graph for the prepared CAC-PU-1 modified electrode (in CB buffer (pH 7.0) with successive addition of 1200 μM CFX, 1 mM Glu, 1 mM Lac, 1 mM Suc, 100 μM DA, 100 μM UA, 100 μM AA, and 1200 μM CFX).

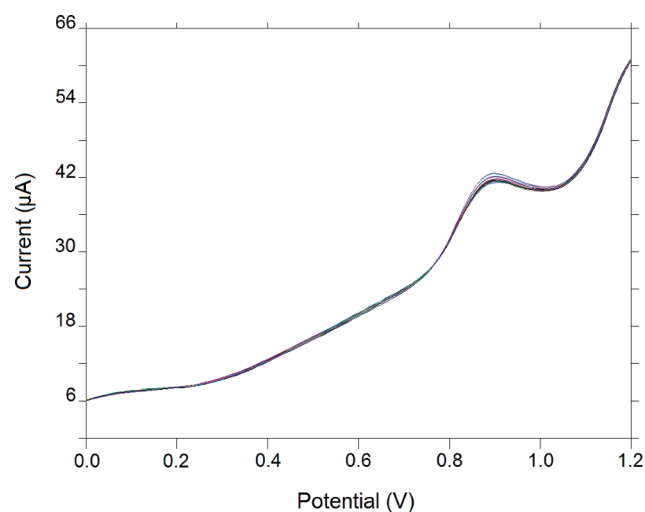


Figure 17. The repeatability of CAC-PU-1 modified Pt electrode for 500 μM CFX in 0.1 M CB (pH = 7) ($n = 10$).

Table 1. Determination of CFX in real synthetic urine samples by DPV.

Samples	Added concentration (μM)	Found Concentration (μM)	Recovery %
1	500	493.5	98.7
2	700	773.0	110

selective. However, in each addition for CFX, a current response was observed in approximately 45 second. This result shows that the detection time of the modified electrode prepared is quite small.

3.7. Repeatability Results of Modified Electrode

To test the repeatability of the prepared CAC-PU-1 modifies Pt electrodes, DPVs were obtained of 500 μM CFX in 0.1 M CB at pH 7.0 on the CAC-PU-1 modifies Pt electrode at the same electrode on the same day. For repeatability measurement of modified electrode, the obtained DPV voltammograms with 10 measurements using the CAC-PU-1 modifies Pt electrode are given in Figure 17. According to these voltammograms, the stability of the present CFX measurement method is very good. The stability of the CAC-PU-1 modified electrode for the 10 repeated measurements was 96.78%. A very small decrease of current sensitivity with a relative standard deviation (R.S.D.) of about 3.22% was observed which can be attributed to the good stability of the CAC-PU-1 modified electrode.

3.8. Determination of CFX in real synthetic urine samples with modified electrode

The elaborated procedure was applied to CFX determination in synthetic urine samples. Different CFX concentration was spiked to the samples and the results are presented in Table 1. Results indicate that very good recoveries (98.7% and 110%) were achieved for spiked urine samples. The reproducibility of the DPV responses obtained after 10 measurements using the same modified Pt electrode shows that the stability of the sensor prepared for the CFX assay is good.

4. Conclusion

Ceftriaxone (CFX) is an antibiotic molecule of the cephalosporin group. It is a pharmaceutical widely used in the treating respiratory tract infection, urinary system infection and many infections. Therefore, in this study, a suitable biosensor for the electrochemical detection of CFX was developed by modifying the Pt electrode surface

with chitosan and caffeic acid-based polyurethane membrane structures. The developed modified electrode exhibited good electrochemical performances with a wide detection range of 0.1 mM – 1.5 mM and a detection limit of 0.052 mM. The prepared polyurethane modified electrode was showed good storage stability and reproducibility. In addition, the proposed sensor was successfully used for the determination of CFX in synthetic urine samples and the obtained results showed acceptable recovery amounts. The polyurethane membrane modified electrode was satisfactory used for sensitive and selective determination of CFX in synthetic urine samples. According to this research, polyurethane/chitosan composite modified electrode can be suitable sensing platforms for the CFX detection due to its high sensitivity, high selectivity and good reproducibility.

Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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