MULTIWALLED CARBON NANOTUBE-CPE MODIFIED ELECTRODE AS A NANOSENSOR FOR ELECTROCHEMICAL DETERMINATION OF BETA-BLOCKER DRUG

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Abstract

The multiwalled carbon nanotubes (MWCNTs) modified carbon paste electrode (MWCNTs/CPE) was prepared and studied for a sensitive determination of β-blocker Atenolol (ATN). The nanocomposite electrode MWCNT/CPE exhibited excellent electrocatalytic activity towards ATN oxidation in 0.1 mol L^{-1} H_2SO_4 using cyclic voltammetry technique. The analytical performance of the modified sensor was evaluated using square wave voltammetry (SWV) and chronoamperometry (CA) in 0.1 mol L^{-1} sulphuric acid solution (H_2SO_4). Under optimal conditions, linear calibration curves were obtained for atenolol detection ranging from 66 to 909 μmol L^{-1} and 406 to 2475 μmol L^{-1} with detection limits of 8.0 and 5.7 μmol L^{-1} using CA and SWV techniques respectively. The modified nanocomposite MWCNTs/CPE sensor showed good sensitivity and good repeatability (RSD ≤ 1.1%) for ATN determination. This electrochemical nanosensor was successfully applied to determine atenolol in biological (human urine) and pharmaceutical (ATN tablet) samples. The obtained results proved that the developed method for atenolol determination might be successfully applied in routine laboratory practice.

Key words: *Atenolol; square wave voltammetry; CPE/MWCNT nanocomposite; cyclic voltammetry; pharmaceutical tablets.*

Përmbledhje

Është përgatitur dhe studiuar elektroda pastë karboni e modifikuar me nanotuba karboni shumë-shtresorë (MWCNTs/CPE) për një përcaktim të ndjeshëm të β-bllokuesit Atenolol (ATN). Elektroda nanokompozite

CPE/MWCNTs shfaqi aktivitet elektrokatalitik të shkëlqyer ndaj oksidimit të ATN në 0.1 mol L^{-1} H_2SO_4 duke aplikuar teknikën e voltametrisë ciklike. Performanca analitike e sensorit të modifikuar u vlerësua duke përdorur voltammetrinë me valë katrore dhe kronoamperometrinë në tretësirë elektroliti acid sulfurik 0.1 mol L^{-1} (H_2SO_4). Në kushte optimale, me teknikën CA dhe SWV u përftuan lakore kalibrimi lineare për përcaktimin e ATN, që varionë nga 66 në 909 µmol L^{-1} dhe 406 to 2475 µmol L^{-1} me kufijë zbulimi 8.0 dhe 5.7 µmol L^{-1} respektivisht. Sensori nanokompozit i modifikuar CPE/MWCNTs tregoi ndjeshmëri të mirë dhe përsëritshmëri të mirë (RSD ≤ 1.1%) për përcaktimin e ATN. Ky nanosensor elektrokimik u aplikua me sukses për përcaktimin e atenololit në mostrat bilogjike (urinës) dhe (farmaceutike) tableta ATN. Rezultatet e marra vërtetuan se metoda e zhvilluar për përcaktimin e atenololit mund të zbatohet me sukses në praktikën rutinë laboratorike.

Fjalë kyçe: *Atenolol; voltammetri me valë katrore; nanokompozit CPE/MWCNT; voltametri ciklike; tableta farmaceutike.*

Introduction

Hypertension is a growing disease of medical concern. Tremendous increase in the use of antihypertensive medications such as β-blockers points toward an increasing number of hypertension cases in last decade. Atenolol (I) is one of the most widely used β-blockers. It is a hydrophilic β1-receptor blocking agent, which is of immense therapeutic use in the treatment of various cardiovascular disorders, such as angina pectoris, cardiac arrhythmia and hypertension (Goyal *et al*., 2005, 2006). Many analytical methods are available for quality control, stability testing, identification and clinical studies of atenolol. Carbon-based nanomaterials have been being increasingly studied since the discovery of graphene, due to the possibility of formation of different nanomaterials of different sizes and crystalline structures.

The main carbon nanomaterials are: fullerenes; carbon nanoparticles, also denominated quantum dots of graphene or quantum dots of carbon, that both have 0D structures, carbon nanotubes of 1D structure; graphene with 2D structure; and graphite with 3D structure (Goyal *et al*., 2005, 2006; Canevari *et al*., 2018, 2019). The development and application of modified carbon paste electrodes (CPE) have received considerable attention due to its low residual current, ease of manufacture, renewable surfaces, and low cost. However, the limit of detection of CPE is still limited for some compounds in trace analysis.

Therefore, some previous researches have modified the CPE for electrochemical determination of some compounds in low concentration.

Therefore, the simpler and faster methods for analyses of different receptor blocking agents are interesting in the quality of pharmaceutical formulations that contain them and also for therapeutic drug monitoring purpose (Fadillah *et al*., 2020; Broli *et al*., 2021). However, use of the functionalized carbon nanomaterials such as carbon nanotubes or graphene for voltammetric measurements is associated with the risk of obtaining hetero-geneous layers with different nanotubes orientations, which may cause the problem of low repeatability of obtained signals. Carbon nanotubes may also differ from each other considering the differences in its activation process or the various numbers of active centers or function groups on its surface, that also can affect working conditions. Voltammetry is considered an important electrochemical technique for electroanalytical chemistry because it has low cost, sensitivity, and precision, as well as accuracy, simplicity, and rapidity.

Voltammetric techniques, such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV), etc., have been proven to be very sensitive for the determination of organic molecules including drugs and related molecules in pharmaceutical dosage forms and biological fluids (Bargiel *et al*., 2021; Hoxha *et al*., 2024). Electroanalysis is a powerful analytical technique that is increasing in utility in the pharmaceutical industry (Ergi *et al*., 2024; Radi *et al*., 2004). The aim of this work was developing of a new, highly sensitive electrochemical method using carbon paste sensors modified with MWCNT-s for atenolol determination.

The nanostructures are effective in composite sensors for the determination of β-blocker (ATN) because these materials have the ability for fast electron transfer, good biocompatibility, ease of preparation which makes them very suitable for selective and sensitive determination of ATN. Analytical applications of the MWCNTs/CPE were demonstrated by estimating the ATN content in pharmaceutical formulations and biological samples (Sayed Mehdi Ghoreishi *et al*., 2015).

Materials and methods

Experimental

Reagents and chemicals

All chemicals and reagents utilized in the research were of analytical grade (Sigma and Merck). The standard stock solution of atenolol (ATN in powdered form; series 00336) was obtained by dissolving an appropriate weight of standard in 0.1 mol L^{-1} of H_2SO_4 solution and stored in the fridge (50 mL, 0.01 mol L^{-1}). Double-distilled water was used in the preparation of each solution. The supporting electrolyte was prepared using H_2SO_4 that was purchased from Merck (99% Merck). The synthetic graphite powder with a particle size of 90–71 μm was from Alfa Aesar (99.9% Alfa Aesar), and the paraffin oil (Olio di Vaselina) was from Zeta Farmaceutici. Multi-Walled Carbon Nanotubes (MWCNTs - OD: 10-20nm, Length: 0.5-2um-based) were used as nano modifiers for electrodic material.

Apparatus

All electrochemical measurements were conducted using a PalmSens4 (potentiostat–galvanostat) linked via Bluetooth to a personal computer and three-electrode system software. The MWCNTs/CPE sensor developed in this study as a working electrode, a platinum wire as the counter electrode and a silver-silver chloride Ag/AgCl/KCl (3M) as the reference electrode. The electrochemical analysis of ATN was carried out using cyclic voltammetry (CV) square wave voltammetry (SWV) and Chronoamperometry techniques. SW-voltammograms were obtained in a potential range of 1.0 to 1.7 V (versus SCE) for ATN, using an amplitude of 50 mV, frequency of 20 Hz and a scan rate of 100 mVs^{-1}. All voltammetric measurements were performed in an unstirred electrochemical cell containing 15 mL of 0.1 mol L^{-1} H_2SO_4 as the supporting electrolyte.

Pharmaceutical Sample Preparation

Pharmaceutical samples such as atenolol (Atenolol SFDB was in the one film-coated tablet containing 100 mg ATN) were investigated to measure the atenolol content. Pharmaceutical tablets were obtained from a local pharmacy. For measurements, two atenolol tablets (100 mg) were ground into a very tiny powder using a mortar and pestle. Then, a weight precisely equal to one tablet was transmitted to a 50 mL calibrated flask and dissolved in sulfuric acid $molL^{-1}H_2SO_4$. After complete dissolving and homogenization, the solution was ready for analysis. As an unknown sample, a solution with a concentration of 3.0 μM was prepared from this solution in 0.1 mol L^{-1} H_2SO_4. The content of atenolol in the samples was evaluated by using the standard addition method and validated with the recovery parameter.

Urine Sample Preparation

The biological (Urine) sample was prepared by the dissolution of human urine with 5 mL of double distilled water. Then prepared urine samples 900 µL and 100 µL of ethanol absolute (*Sigma Aldrich form series 32221*) were transferred into the electrochemical cell containing 15 mL supporting electrolyte 0.1 mol L^{-1} H_2SO_4 and the corresponding voltammograms were recorded. Measurements were performed using square wave voltammetry and the standard addition method was used to validate the recovery parameter.

Preparation of bare and MWCNTs modified carbon paste electrodes

The ***CPE*** was prepared as follows: 1.00 g of carbon powder with particles size 90-70 µm was mixed with 300 µL of paraffin oil until to obtained a homogeneous carbon paste. The paste was packed into the plastic syringe 2 ml (insulin syringe) containing a copper wire as the external electric contact.

For preparation of the ***MWCNTs/CPE*** nanocomposite sensor, firstly was mixed 0.100 g MWCNTs with 1.00 g carbon powder with particle size 90-70 µm, optimised in the previous study and then 300 µL of paraffin oil was added to the mixture and mixed all component for 30 min until a uniformly robust paste was formed (Hoxha *et al*., 2024). In order to immobilize the nanomodifer material (MWCNT) into carbon paste, the composite electrodic materials were kept in a refrigerator at 4 ^{0} C for 24 h before measurement. The prepared paste was filled into the plastic syringe with an internal diameter of 8 mm and an outer diameter of 9.5 mm. The fresh surface was obtained by polishing the electrode on the glass surface until it showed a smooth appearance.

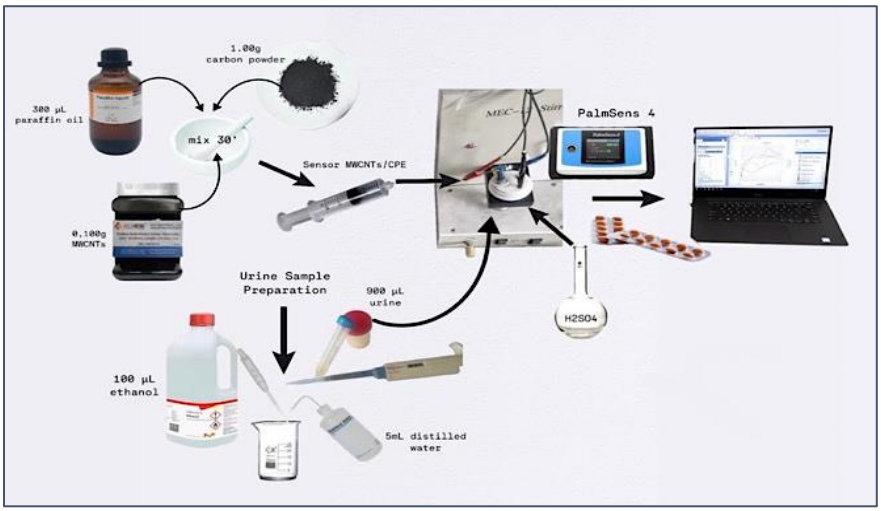


Figure 1: Schematic of preparation of nanomodified sensor and electrochemical measurements

Results and discussion

The electrochemical investigation of atenolol at nanocomposite electrode

Referring to our previous studies demonstrated that a carbon paste electrode (CPE) modified with natural ilmenite nanomaterials exhibits a good capability and high catalytic effect on the electro-oxidation of atenolol (ATN) (Broli *et a*l., 2021; Broli N *et al*., 2022). In the current work, the electrochemical behaviour of atenolol at the MWCNTs/CPE nanocomposite sensor was investigated using CV, SWV and CA techniques in 0.1 mol L^{-1} H_2SO_4 (pH 2) as the supporting electrolyte, with an amplitude of 50 mV/s and a frequency of 20 Hz. To study the effect of modifier MWCNTs incorporated into the CPE, the cyclic voltammograms of ATN in 0.1 molL^{-1} H_2SO_4 pH 2 were obtained on these electrodes and the corresponding voltammograms are shown in Figure 2.

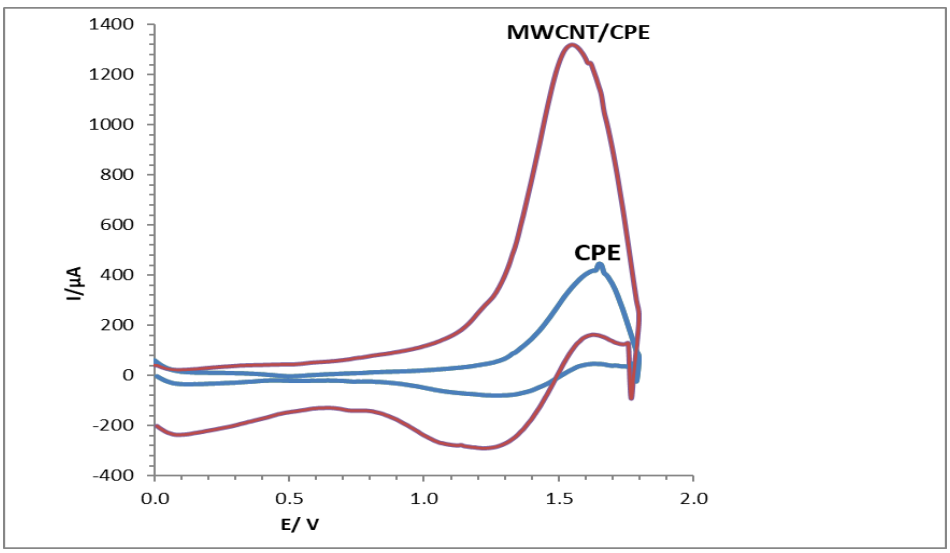
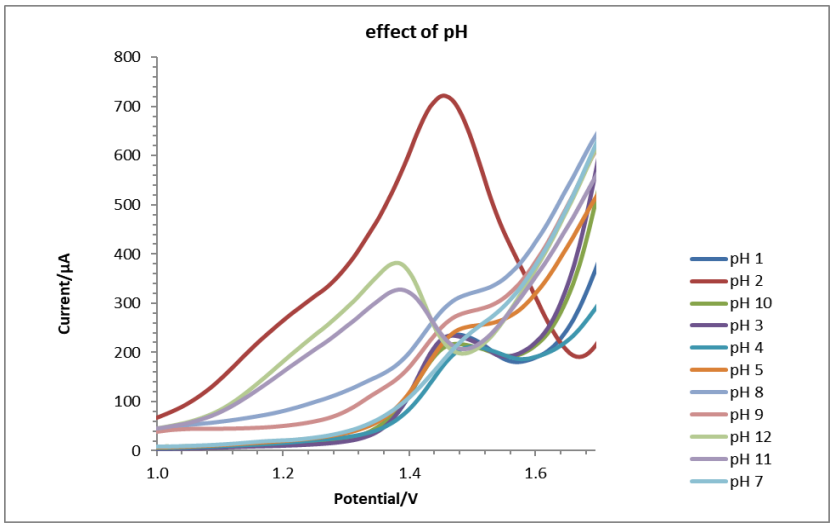


Figure 2. The Cyclic voltammograms of ATN (506 µmol L^{-1}) at the bare CPE and the modified MWCNTs/CPE sensor in 0.1 mol/L^{-1} H_2SO_4 at υ= 100 mV s^{-1}

As shown in Figure 2, an oxidation peak of atenolol appeared at the potential of +1.55 V. In comparison to the bare CPE electrode, the modified MWCNT/CPE sensor presents a well-defined irreversible oxidation peak with a higher current signal (for atenolol concentration 506 μmol L^{-1}). From these results, it was concluded that the high electrocatalytic effect and low resistance for atenolol oxidation were observed at MWCNTs/CPE because of the faster electron transfer capability of the nanomodifier immobilized into the CPE electrode. This reduction of resistance can be due to the high conductivity of MWCNT on the electrode surface (Sayed Mehdi Ghoreishi *et al*., 2015).

Effect of pH

The influence of pH on the response of MWCNTs/CPE toward ATN was examined. The oxidation of ATN at MWCNTs/CPE is significantly affected by conditions such as pH or ionic strength during ion exchange reactions. To test the effect of pH on the oxidation peak current of atenolol, solutions with different pH were prepared from H_2SO_4 solution (0.1 mol/L^{-1}) as supporting electrolytes by the addition of NaOH. The square wave voltammograms were registered in 909 μmol L^{-1} of ATN, at the surface of the nanocomposite MWCNTs/CPE electrode. Figure 3 shows the effect of electrolyte pH on the ATN oxidation peak current in the range of 2.0 to 11.



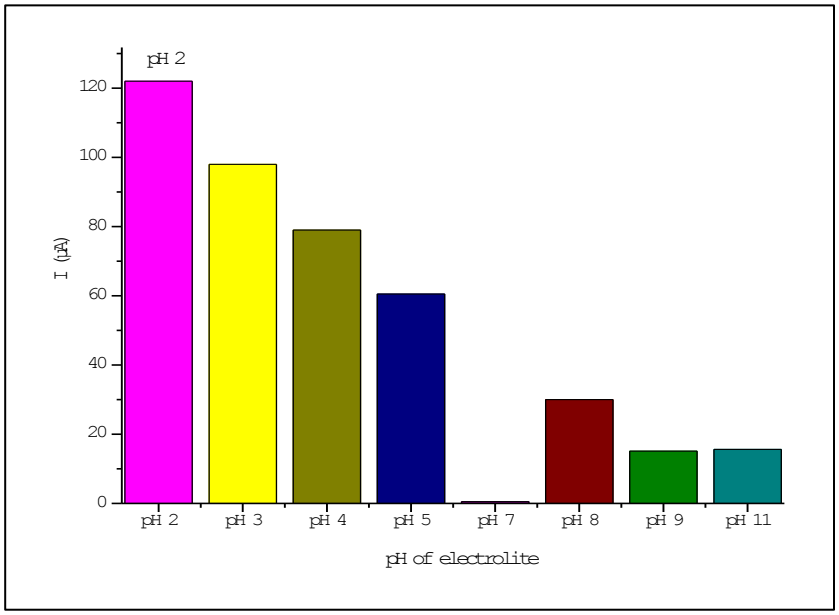


Figure 3. SW-voltammograms registered at MWCNT/CPE, in diffident pH value of supported electrolyte. B) The effect of electrolyte solution pH on the oxidation peak current of ATN (909 μmol/L^{-1})

The graph clearly demonstrates that pH significantly impacts the oxidation process, as evidenced by changes in the voltammogram shape, oxidation potential, and intensity of current. The maximum anodic peak current was recorded at an acidic pH of 2.0 using a 0.1 molL^{-1} H_2SO_4 solution. Due to the highest value of the peak current at pH=2.0, it was chosen as the best pH for the analytical applications. This observation aligns with findings from our previous studies on ATN oxidation (Canevari *et al*., 2018; Hoxha *et al*., 2024; Broli N *et al.,* 2022). In this process, ATN selectively penetrates the paste due to straightforward electrostatic attraction, as it exists in cationic form at pH 2.0.

Square Wave Voltammetry Analysis of Atenolol

The square wave voltammetry (SWV) was used for the determination of atenolol at the MWCNT/CPE sensor due to its high sensitivity compared with the bare one. The SW-voltammograms registered in H_2SO_4 0.1 molL^{-1} pH 2 in different concentrations of atenolol were illustrated in Figure 4 (pulse

amplitude 50 mV and frequency 20 Hz). The linear range was found to be from 406 µmol L^{-1} to 2475.5 µmol L^{-1}.

The linear equation was I(µA) =102.79+0.1556 C atenolol (µmol $L^{-1)}$ with a correlation coefficient of 0.9999. The detection limit, defined as 3Sb/m (Sb is the blank standard deviation for 4 replicates blank determination and m is the slope of the calibration graph), was computed equal to 5.7 µmol L^{-1}. This low detection limit indicated that the developed procedure is suitable for the measurement of ATN concentration in different environments having a low content of this drug.

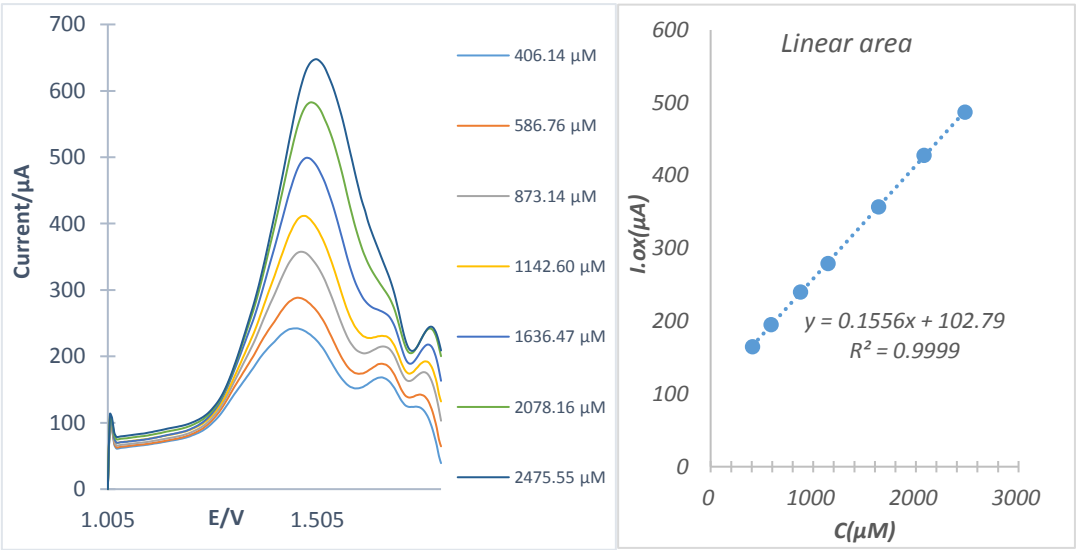


Figure 4. SWVs obtained at MWCNTs/CPE in 0.1 molL^{-1} H_2SO_4 (pH=2) containing different concentrations of atenolol (from 406.1 to 2078.2 µmol L^{-1}). Inset: Plots of electrocatalytic peak current as a function of atenolol concentration.

The relative standard deviations (RSD) for sequential determinations of 92 and 141 µmol L^{-1} ATN were obtained at 1.1% and 0.21% (n=4), respectively, indicating suitable precision of the determination by the constructed modified electrode MWCNTs/CPE.

Chronoamperometric analysis of atenolol

The electrochemical oxidation of ATN at the surface of the MWCNTs/CPE was also investigated using chronoamperometry (CA). As the oxidation peak of ATN appears at +1.5 V (pH 2), a more positive potential (+1.6 V) was

selected to obtain chronoamperometric responses. This potential will ensure the complete oxidation of the analyte (ATN).

The amperometric response of the proposed nanosensor MWCNTs/CPE at the constant potential of +1.6 V was used as an analytical signal to construct a calibration graph for atenolol in the concentration range of 66.2 µmol L^{-1} to 909.1 µmol L^{-1}. Figure 5 shows the Chronoamperograms obtained at the MWCNTs/CPE sensor, in H_2SO_4 0.1 molL^{-1} pH 2, with different ATN concentrations at experimental times (t=600 s).

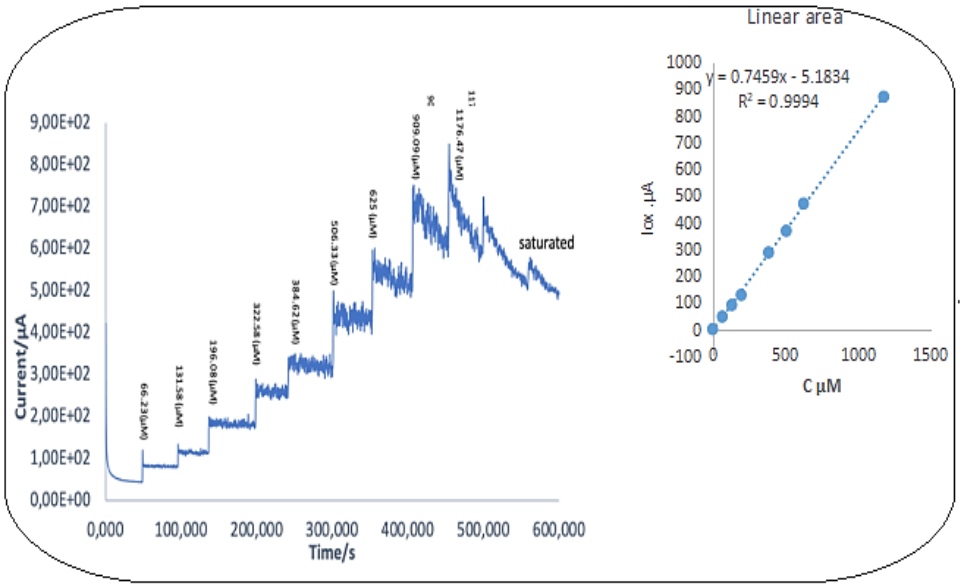


Figure 5. CA-s obtained at MWCNTs/CPE in 0.1 M H_2SO_4 0.1 molL^{-1} (pH=2.0) containing various concentrations of atenolol (from 66. to 1176µM) Inset: Plot of the anodic current as a function of ATN concentration.

As can be seen, the modified sensor showed a linear behaviour in the concentration range of 66.2 µmol L^{-1} to 909.1 µmol L^{-1}. The regression equation was: $I(\mu A) = 07459\ (\mu mol\ L^{-1\ ATN}) + 5.1834$ with $R^2 = 0.9994$. The limit of detection (LOD) and quantification (LOQ) based on 3sb/b and 10sb/b criteria were found to be 8 µmol L^{-1} and µmol L^{-1} µM, respectively. The reproducibility of the MWCNTs/CPE sensor for detecting 66.2 µmol L^{-1} ATN was assessed, with the results expressed as the relative standard deviation

percentage (RSD%). The obtained RSD% was 1%, highlighting the satisfactory precision of the proposed MWCNTs/CPE nanocomposite sensor.

Real Sample Analysis

The practical usage of the MWCNTs/CPE electrode was evaluated in real samples like tablets, and urine by using the additional standard method with the SWV technique. The samples were prepared according to the procedure from points 2.2 and 2.2.1 Before successive addition, the measurement procedure was applied to the blank matrix to ensure the primary matrix solution itself contains no ATN. Table 1 gives the results of ATN determinations in various real samples.

Table 1. Determination of ATN in real samples.

Sample	Added (μmol L^{-1})	Founded (±SD) (μmol L^{-1})	Recovery (%)	RSD (%)
Tablet	0.0	3.05±0.26	101.6	8.52
	65	68.9±4.94	106.0	7.16
	130	128.1±2.44	98.5	1.88
Urine	0.0	ND		
	123	135.0±4.3	109.7	3.49
	244	257.6±3.3	105.6	1.35

ND, not detected; SD, standard deviation for 3 independent measurements

The obtained data from tablet analysis were in good agreement with the producer declaration and the calculated recovery parameter in the range from 98.5 to 106% was good. Also, the recovery values for the spiked urine sample were achieved to be between 105.6% and 109.7%. These results indicate that the proposed method employing the developed MWCNTs/CPE nanosensor is suitable for practical applications.

Conclusions

In this work, the MWCNTs nano modifier incorporated into carbon paste electrode CPE was studied for sensitive detection of atenolol. The electrochemical performance of the nanosensor based on the MWCNTs modifier was evaluated, emphasizing its high electrical conductivity, large surface area, and excellent absorption properties. The MWCNTs/CPE nanocomposite sensor demonstrated enhanced atenolol detection capabilities, including a wide linear range, low detection limit, high average percent recovery, and reliable precision.

The modified sensor with low cost and easy preparation features provides selective and precise measurement of ATN in pharmaceutical tablets and urine samples. As ATN is commonly used in treating various heart conditions, the proposed method is expected to be effective for ATN analysis offering valuable applications in clinical and pharmaceutical industries.

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