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Makale Başlığı / Title

The Ultra-sensitive method development using Nafion and multi-walled carbon nanotube coated glassy carbon electrode for atenolol determination

Atenolol tayini için Nafion ve çok duvarlı karbon nanotüp kaplı camı karbon elektrot kullanılarak ultra hassas yöntem geliştirilmesi

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10.0 were prepared from mixture solution of 0.04 M H_2BO_3 and 0.04 M CH_3COOH in deionized water. For each buffer solution, the pH of 50 mL mixture solution was adjusted to the desired value by adding 0.2 M NaOH, and then it was diluted to 100 mL with deionized water. Also, sulfuric acid (H_2SO_4) solutions were used as supporting electrolyte with different concentrations of 0.1-0.5 mol/L, throughout the study.

The supporting electrolyte was deaerated with argon for 30 minutes prior to all of the electrochemical measurements. Between consecutive additions, the voltammetric cell was also deaerated with argon for 30 s.

The effect of pH on the anodic peak current values resulting from the oxidation of ATN were investigated using cyclic voltammetry technique. After the required volume of ATN standard solution had been added to cell containing the supporting electrolyte which has different pH values in the range between 2.0 and 10.0 for each measurement, first of all, cyclic voltammograms of ATN were recorded under the same conditions.

In order to compare the obtained peak currents, both CV and DP voltammograms were also recorded at NGCE and MWCNT/NGCE in similar conditions.

The effective parameters for analytical performance, such as dynamic calibration range, limit of detection, limit of quantification repeatability, etc. were optimized by considering the peak current of ATN obtained from DP voltammograms recorded between 0.15 V and 0.30 V.

2.4 Atenolol Analysis in Tensinor Drug

The sample solution of Tensinor drug was carefully prepared. For this purpose, ten tablets were grounded to a fine powder and homogenized. A Tensinor tablet has a weight of 0.50 g (average of 10 tablets) and involves 50 mg ATN per tablet. Then, this obtained powder (0.50 g) was added into 100 mL water containing about 0.2 M HCl for dissolving. The obtained heterogenic tablet mixture solution was filtered and washed. After that, the obtained supernatant solution was diluted 200 mL water. The solutions of standard ATN and Tensinor tablet were stored in the dark refrigerated environment.

The content of ATN tried to be determined in Tensinor drug by being applied the voltammetric procedure under optimum experimental conditions. For this purpose, standard addition method was used. The standard addition is a useful method when the analyte present in a complicated matrix and no ideal blank is available in order to do that various solutions would be prepared with including the same unknown quantity, but different known quantities.

Under this condition, a known volume of standard solution was added into the voltammetric cell containing 5.0 mL BR buffer solution (pH 7.0) and DP voltammogram was recorded. Following this step, the known volumes of the standard ATN solution were consecutively added into the same voltammetric cell and then the voltammogram was recorded after each addition.

The standard addition calibration curve of the obtained current values from proposed method against concentrations of ATN standard solution added was plotted. Subsequently linear regression analysis was done to find out the concentration of analyte. Here the slope (m) and intercept (b) of the

calibration curve were made use of to measure the concentration.

3 Results and Discussion

The aim of the study, first type of working electrode was examined in detail by using CV and DPV techniques. Then, the resulting peak currents, which were obtained in the same supporting electrolyte (BR buffer solution containing, pH 7.0) at bare GCE, NGCE and carbon nanotubes modified GCE (MWCNT/NGCE) were compared with each other. Finally, the performance of voltammetric technique was also investigated for determination of ATN.

3.1 Cyclic Voltammetric Studies Using Different Working Electrode

To investigate the electrochemical behaviour of ATN, CV was recorded at MWCNT/NGCE, NGCE and bare GCE in the BR buffer solution (pH 7.0) by using cyclic voltammetry technique. The results obtained from these voltammograms (in the first scan) are given in Figure 1. When GCE was used as the working electrode, the peak current value of ATN was very low according to its concentration. When NGCE was used as the working electrode, the peak current value of ATN was found much higher than that of bare GCE. As shown in Figure 1, when MWCNT/NGCE was used as the working electrode, the peak current value of ATN was higher than that of bare GCE and NGCE.

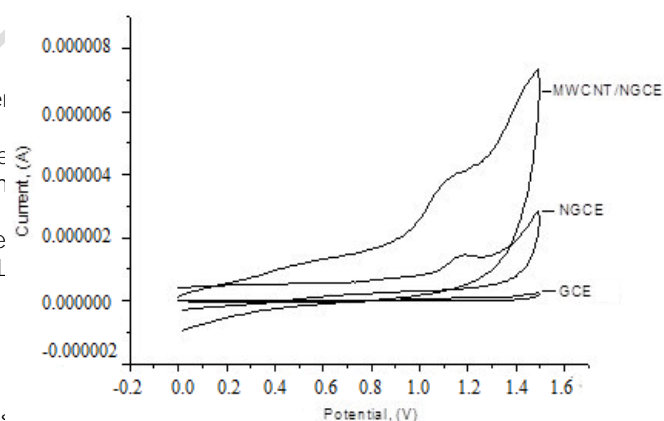


Figure 1. Cyclic voltammograms of measurement of ATN at GCE, NGCE, and MWCNT/NGCE in the presence of 10^{-2} mM ATN in BR buffer solution (pH 7.0)

As a result the highest peak current for ATN was obtained by using cyclic voltammetric technique at MWCNT/NGCE. In relation to this, studies on the investigation of cyclic and DP voltammetric behaviours of ATN were carried out only using MWCNT/NGCE. These studies including these optimization experiments were presented below.

3.2 Effect of pH and Supporting Electrolyte

Effect of pH was investigated by using cyclic voltammograms in BR buffer solutions (2.0 < pH < 10.0) at MWCNT/NGCE. The obtained cyclic voltammograms and results at a scan rate of 20 mV/s given in Figure 2. It is clear that the intensity was increased to a high value at pH 7 then the peak intensity decreases. Because the best result with respect to sensitivity accompanied with sharper response was

obtained with pH 7.0, it was selected for further experiments. Also, the effect of 10^{-2} mM ATN was evaluated using cyclic voltammetry (20 mV scan rate) employing MWCNT/NGCE. The effect of pH on peak potential and peak current of ATN was studied between 0.1-0.5 M sulfuric acid (H_2SO_4) (Figure 2.b).

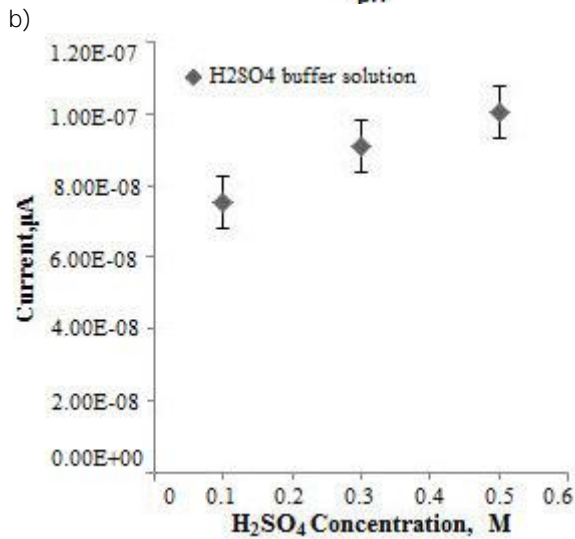
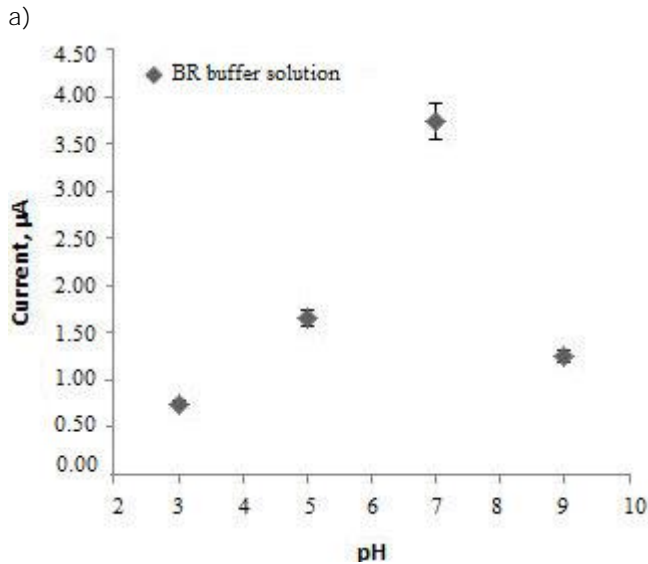


Figure 2.a) Results of cyclic voltammogram of 10^{-2} mM ATN in BR buffer solution at different pH values (2.0 < pH < 10.0) b) Results of cyclic voltammogram of 10^{-2} mM ATN in sulfuric acid (H_2SO_4) solution at different concentration values (0.1-0.5 M)

In this region, the increase of peak current and sulfuric acid concentration, and ease of oxidation was observed. Besides, peak potentials of ATN shifted to less positive values (+) as pH of the medium grew. The pH of the supporting electrolyte effects peak current and potential of ATN, which are showed involvement of proton in the oxidation reaction. The working environment, pH = 7.0 solution was selected for the most obvious, sharp peaks and the maximum current was observed at +1.2 V.

3.3 Effect of Scan Rate

The scan rate has an important effect on the peak currents. The effect of the scan rate on peak current was studied in the range of 10 mV/s to 100 mV/s of 10^{-2} mM ATN. The voltammogram was recorded in BR buffer solution (pH=7.0). As can be seen in Figure 3, the anodic peak of ATN grows with increasing scan rate. However, no cathodic peak of ATN was observed on the reverse scan.

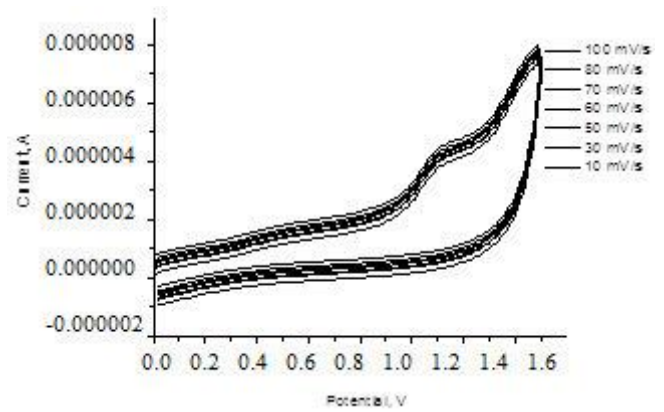


Figure 3. Cyclic voltammograms of 10^{-2} mM ATN at the different scan rates (10-100 mV/s) in BR buffer solution (pH=7.0)

The linear change of peak current values with the square root of the scan rate was also observed in BR buffer solutions (pH 7.0) in the range of 10-100 mV/s (Figure 4).

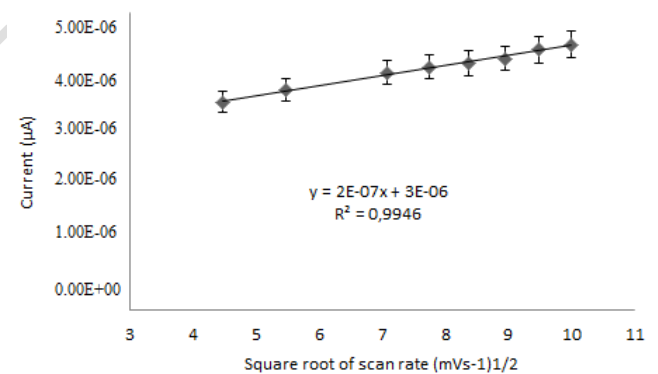


Figure 4. Linear plot of peak current values versus the square root of scan rate of 10^{-2} mM ATN pH = 7 BRT solution

The linear relationship between the peak current and the square root of scan rate, which has a regression coefficient of 0.9946, indicates that the oxidation reaction of ATN was also controlled by diffusion in the BR buffer solution (pH 7.0). In addition, the peak potential values shifted to more positive values with increasing scan rate.

3.4 Analytical Characteristics and Validation Parameters

A calibration study was carried out over the range of 10^{-8} to 10^{-2} mM ATN concentration to establish a reliable analytical response in the determination of ATN, under optimized conditions using a MWCNT/NGCE. These DP experiments were carried out at the scan rate of 20 mV/s in BR buffer solution (pH 7.0) (Figure 5). The obtained peak currents were plotted against concentrations of ATN. The linear segment was observed in the range of 10^{-8} to 10^{-4} mM.

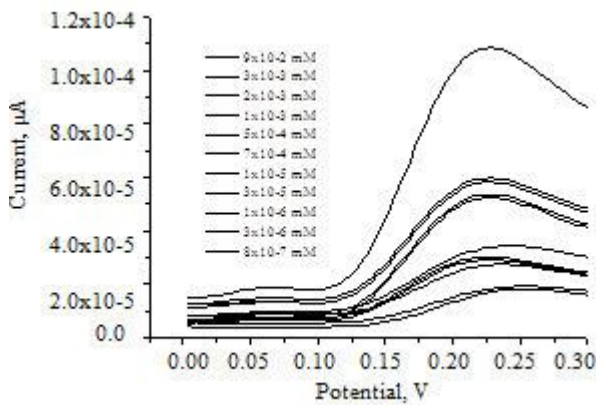


Figure 5. Dependence of the DP voltammograms with ATN at concentrations of 9x10⁻² mM, 3x10⁻³ mM, 2x10⁻³ mM, 1x10⁻³ mM, 5x10⁻⁴ mM, 7x10⁻⁴ mM, 1x10⁻⁵ mM, 3x10⁻⁵ mM, 1x10⁻⁶ mM, 3x10⁻⁶ mM, and 3x10⁻⁷ mM.

The LOD and limit of quantification (LOQ) were calculated as 5 mmol L⁻¹ and 10 mmol L⁻¹ ATN, respectively, using the equation $LOD = 3.3/s$ and $LOQ = 10/s$, where m is the slope of the first calibration plot and s is the standard error of the estimate of the calibration curve (Eq. 2)

$$s_{y/x} = \frac{1}{N-2} \sqrt{\frac{\sum_{i=1}^N (y_i - \hat{y}_i)^2}{N-2}} \quad (2)$$

where y_i is measured signal, \hat{y}_i concentration of measured for this signal and N is the number of points in the calibration graph.

3.5 Real Sample Analysis

Sample solutions of Tensinor Drug (in the tablet form) were prepared with ten tablets. The voltammograms were recorded under optimum working conditions (BR buffer solution pH 7.0) by applying standard addition method. The standard addition graph was plotted against the calculated concentration of standard ATN in the cell (Figure 7)

Table 1: Comparison of the proposed with another method

Method	Analytical range	LOD	LOQ	Ref
Bare graphite/polyurethane composite electrode	4			[24]
C60modified glassy carbon electrode	0.25-1.5 mM	0.16 mM	0.53 mM	[21]
MOR-modified CPE	0.4	0.1		[25]
Nanogold modified indium tin oxide (ITO)	1.0			[26]
MWCNT/NGCE	5x10 ⁻⁵ - 2 mM			This work

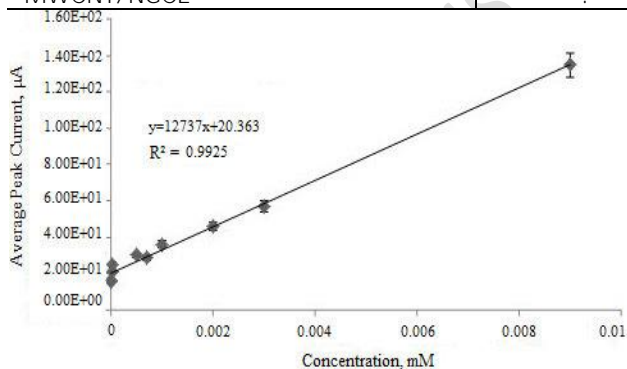


Figure 6. Calibration curves ($i = f(C)$) for determination of ATN at MWCNT/NGCE. Data were extracted from Figure 5. Equation is as follows:

$$i = 12737 \text{ ATN (M)} + 20.363 \quad (1)$$

where i is the peak current and C is concentration of ATN.

In order to evaluate the limit of detection (LOD), we fitted a linear regression line $i = 12737 \text{ ATN (M)} + 20.363$ to the data in Figure 6, and applied the widely-used criterion for LOD [23].

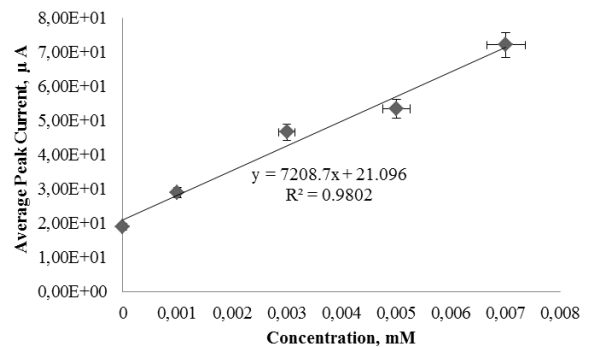


Figure 7. The standard addition graph for determination of ATN in Tensinor tablet

The data were in well concordance with the content marked in the label of Tensinor (Table 2).

Table 2: Determining atenolol in pharmaceutical preparation using MWCNT/NGCE electrode

Sample	Stated content (mg/tablet)	Detected content (mg/tablet)	Relative standard deviation (R.S.D.) (%) (n=3)
Tensinor	50	49.21	1.79

3.6 Effect of Excipients

The outcomes of the use of some often used ingredients of the pharmaceutical preparations were studied for possible analytical applications of this method. The effects of these excipients on the differential pulse voltammetric response were carried out by testing sample solutions with a fixed concentration (10⁻² mM) containing with different excess quantities of each excipient in the same experiment environment.

Table 3: Influence of potential excipients on the voltammetric response of 10⁻² mM ATN

Excipients (1 mM) (2 mM)	Potential (V)	Change (%)
ATN	0.224	0
Sucrose+ ATN	0.229	2.2
Maltose+ ATN	0.231	3.1
Sorbitol+ ATN	0.227	1.3

The experimental results (Table 3) showed that excess of sucrose, maltose and sorbitol did not affect the voltammetric signal of ATN. Thus, the procedures were able to determine the ATN in the existence of excipients, and this was understood that can be considered as specific.

4 Conclusion

In this study, it was aimed to investigate voltammetric behavior of ATN and develop a method for the determination of ATN, especially using MWCNT/NGCE. For this aim, it was carried out an investigation on bare GCE, NGCE and MWCNT/NGCE in BR buffer solutions using both the cyclic and DP modes. The effective parameters such as pH of supporting electrolyte solution, scan rate of potential and type of working electrode were determined.

The proposed voltammetric procedure was successfully applied to the direct determination of ATN in Tensinor. A very low cost and simple DP voltammetric procedure was developed for the determination of ATN. It was found that the voltammetric methods reported by Priscila et al. (2007) have lower LOD value than the proposed method. This low LOD value arises from the use of a controlled mercury drop electrode. However, the use of mercury has been increasingly abolished from analytical methodology due to its toxicity in recent years. The linear calibration range of the proposed method has comparability with or is better than mentioned study (Table 1). Main advantage is that the excipients do not intervene and separation is not necessary. Moreover, the present method is acceptable for pharmacokinetic studies.

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