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Nanogold-modified Carbon Paste Electrode for the Determination of Atenolol in Pharmaceutical Formulations and Urine by Voltammetric Methods

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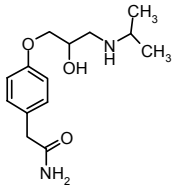
A gold nanoparticles modified carbon paste electrode (GN-CPE) has been used for the determination of atenolol (ATN) in drug formulations by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and chronocoulometric methods. The results revealed that the modified electrode shows an electrocatalytic activity toward the anodic oxidation of atenolol by a marked enhancement in the current response in buffered solution at pH 10.0. The anodic peak potential shifts by −80.0 mV when compared with the potential using bare carbon paste electrde. A linear analytical curve was observed in the range of 1.96×10^{-6} to 9.09×10^{-4} mol L^{-1}. The detection limit for this method is 7.3×10^{-8} mol L^{-1}. The method was then successfully applied to the determination of atenolol in tablets and human urine. The percent recoveries in urine ranged from 92.0 to 110.0%.

Key Words: Gold nanoparticles modified carbon paste electrode, Atenolol, Differential pulse voltammetry, Drug formulation, Urine

Introduction

Atenolol (ATN), designated chemically as 4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide (Scheme 1), is a member of the β-blocker family, and more specifically, a cardioselective β_1-adrenergic receptor-blocking agent. ATN is used therapeutically in the treatment of angina pectoris, hypertension, cardiac arrhythmia, myocardial infraction and migraine. β-blockers are exceptionally toxic and most have a narrow therapeutic range; i.e., the differences between the lowest therapeutic and the highest tolerable doses are small.[1-3] Hence, accurate methods for the measurement of ATN are of great importance in pharmaceutical research. Several methods have been reported for the determination of ATN, including UV spectrophotometry,[4,5] spectroflurometry[6] and capillary zone electrophoresis in plasma.[7] Gas chromatography (GC) with mass spectrometric or electron capture detection[3,8] and high performance liquid chromatography (HPLC)[9-11] have also been extensively used for the detection and determination of ATN. However, these methods require time-consuming sample extractions, expensive instrumentation and high-running costs.[12,13] In recent years, electrochemical determination of atenolol was performed using a C_{60}-modified glassy carbon (GC) electrode,[14,15] a nanogold-modified indium tin oxide electrode,[16] a graphite-polyurethane composite electrode[17,18] and a plastic membrane electrode,[19] with limits of detection of 0.16 mmol L^{-1},[14] 0.13 μmol L^{-1},[16] 3.16 μmol L^{-1},[17] and 1.0×10^{-7} mol L^{-1},[19] respectively. In this paper, we prepare a simple and selective gold nanoparticles modified carbon paste electrode and use it to quantify atenolol in a variety of samples. The advantages of carbon paste electrodes include the diverse range of paste modifications available and the convenience in handling.



Scheme 1. The molecular structure of ATN

Experimental

Reagents and solutions. All chemicals used were of analytical or pharmaceutical grade and solutions were prepared in deionized water. Carbon graphite powder, paraffin oil, $HAuCl_4$ and sodium citrate were supplied by MERCK. The pure form of ATN was supplied by local pharmaceutical company (Iran) and a stock solution of 0.01 mol L^{-1} ATN was prepared in Britton-Robinson buffer solution, pH 10.0.

Preparation of colloidal gold. Colloidal gold nanoparticles were prepared by adding 0.5 mL of 1% sodium citrate solution to 50 mL of a boiling solution of 0.01% $HAuCl_4$. The mixture was maintained at boiling point for 15 minutes, then stirred for another 15 minutes after removing the heating source. The method produced 24 nm-diameter colloidal gold nanoparticles.[20] The maximum UV-vis absorption of the colloidal gold was 520 nm. The solution was stored in a refrigerator in a dark-colored glass bottle.

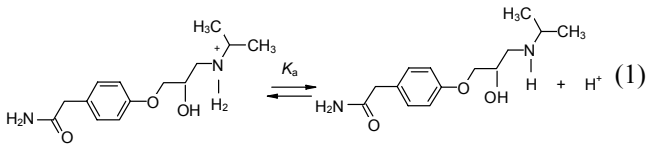
Fabrication of carbon paste electrodes (CPEs). The carbon paste electrode was prepared by thoroughly hand-mixing 0.50 g graphite powder with approximately 0.2 mL of paraffin oil. The colloidal gold nanoparticles modified CPE was prepared by thoroughly mixing 0.50 g graphite powder and 1.5 mL of colloidal gold nanoparticles solution (containing 0.075 mg Au) prior to adding paraffin oil. A portion of the paste was put into plastic syringe tubes with the inner diameter of 2.0 mm to form the GN-CPE. Electrical contact to the paste was established by inserting a copper wire into the plastic syringe tube.

Electrochemical measurement. Voltammetric measurements were carried out with a potentiostat/galvanostat EG&G (model

273A) and a conventional three-electrode system was adopted. The working electrode was the CPE described above while the auxiliary and reference electrodes were platinum wire and Ag/AgCl electrode, respectively. The electrochemical measurements were conducted in Britton-Robinson buffer solution. The cyclic voltammograms were recorded from 0.25 to 1.06 V using various scan rates. Differential pulse voltammetric (DPV) analysis was used for determination of atenolol in samples. All electrochemical measurements were done in an unstirred electrochemical cell at 25 ± 0.5 °C.

Results and Discussion

Voltammetric behavior of atenolol. Fig. 1 shows the voltammograms of ATN on the surface of bare CPE and GN-CPE in Britton-Robinson buffer solution of pH 10.0. At the bare electrode (c), ATN shows a relatively broad and weak oxidation peak at 955 mV. However, with the GN-CPE (b), the oxidation peak becomes well-defined and sharp, with a negative potential shift of 80.0 mV. The lower overpotential and the increase in current response are clear evidence of the catalytic effect of the modified electrode on the oxidation of ATN. The effect on the peak potential and current of varying the pH of the ATN test solution (1.0×10^{-4} mol L^{-1}) from 2.0 to 11.0 was investigated for the GN-CPE surface. A well-defined oxidation peak for ATN was obtained in a pH range 7.0 to 11.0. This electrochemical response for the ATN at pH values below 7.0 is blocked by the acid-base equilibrium of the amino group whose pK_a = 9.4,[21] as represented in Eq. (1).



(1)

The ATN oxidation response increases with pH, reaching a maximum at pH 10.0, and then decreases (Fig. 2A). The peak potential (E_p) shifts to more negative values with increasing pH of Britton-Robinson buffer solution. In the pH range of 7.0 to 10.0, there is a linear relationship between the two responses (Fig. 2B), as expressed by the Eq. (2). A slope of −75.8 mV pH^{-1} and a correlation coefficient of 0.9976 were observed.

$$E_p = 1564.3 - 75.8\ pH \quad (2)$$

The dependence of E_p on the pH indicates that the electrochemical reaction involves proton transfer. The decrease of overpotential with increasing pH shows the catalytic effect of GN-CPE on the anodic oxidation of ATN. The main process, which can be observed in both acidic and basic media, is probably due to the oxidation of secondary alcoholic group and it seems reasonable to assume that the oxidation occurring at secondary alcoholic group in a 2 e^-, 2 H^+ process gives corresponding ketone as reported in the literature.[16] To optimize the electrochemical response of ATN at the surface of gold nanoparticles modified CPE, the effect of varying DPV parameters including pulse height, pulse width, scan increment and scan

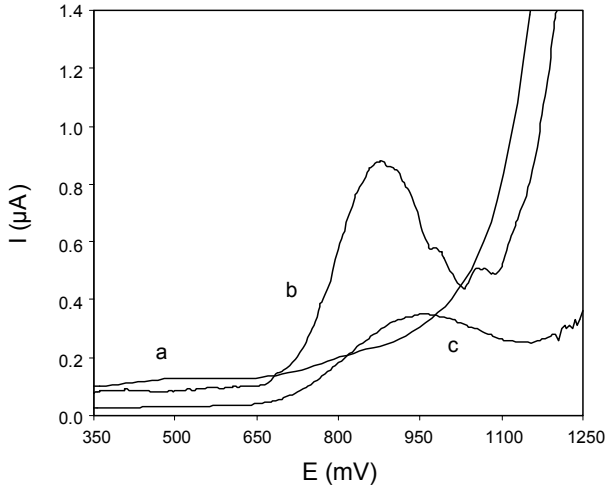


Figure 1. Differential pulse voltammograms of GN-CPE in Britton-Robinson buffer solution (pH 10.0) in (a) absence and (b) presence of 6.6×10^{-4} mol L^{-1} ATN. (c) As (b) for bare CPE.

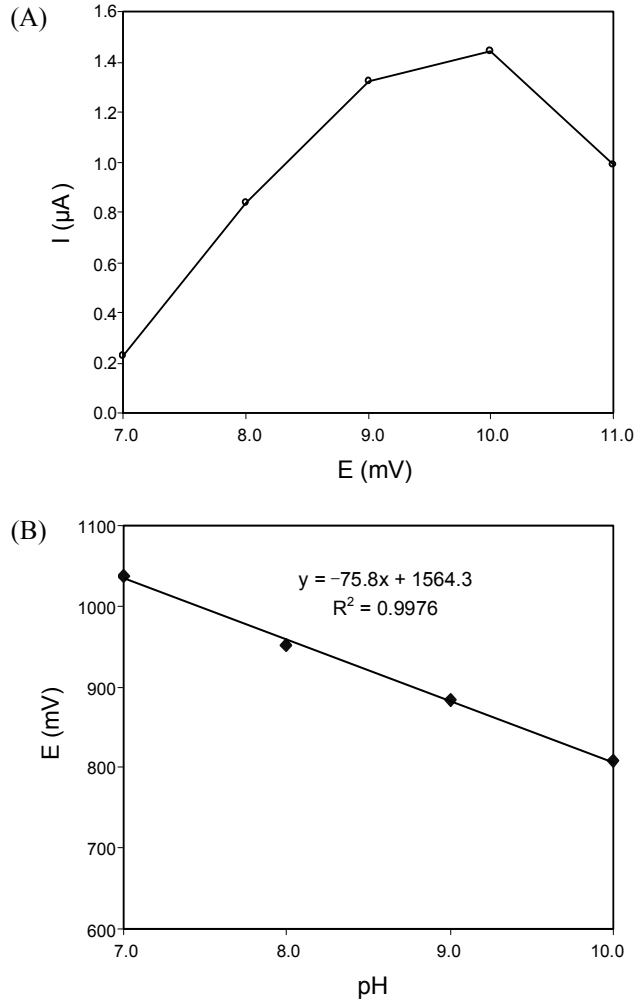


Figure 2. Effect of pH on (A) the peak current and (B) the peak potentials for the oxidation of 1.0×10^{-4} mol L^{-1} ATN in 0.2 mol L^{-1} Britton-Robinson buffer solution. DPV conditions: pulse height: 100 mV; pulse width: 50 ms; scan increment: 10 mV; scan rate: 15mV s^{-1}.

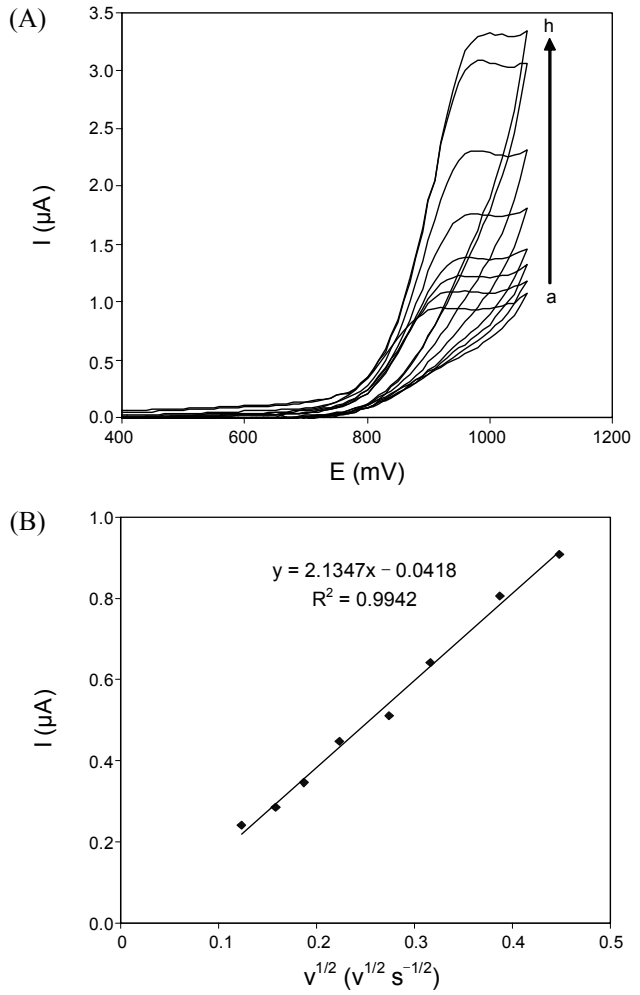


Figure 3. (A) Cyclic voltammograms of the GN-CPE in Britton-Robinson buffer solution (pH 10.0) at different scan rates (mV s^{-1}): (a) 15, (b) 25, (c) 35, (d) 50, (e) 75, (f) 100, (g) 150, (h) 200; (B) The relationship between the peak currents of ATN and the square root of the scan rates.

rate were investigated. The CV response using 6.7×10^{-4} mol L^{-1} ATN on GN-CPE was measured using different scan rates over the range of 15-200 mV s^{-1} (Fig. 3A). As the scan rates were increased, the anodic peak current also increased. The linear dependence of peak current on square root of scan rate (Fig. 3B), expressed by Eq. 3, reflects the diffusion-controlled nature of electrode reaction.[22]

$$i_p = -0.0418 + 2.1347\, \upsilon^{1/2} \quad (\upsilon \text{ in V s}^{-1},\ r = 0.9942) \qquad (3)$$

Chronocoulometric response of ATN. Chronocoulometry was used for calculating diffusion coefficient at gold nanoparticles modified electrodes. The chronocoulometric responses for various concentration of ATN at GN-CPE are shown in Fig. 4A. The plot of Q against $t^{1/2}$ for different concentrations of ATN is shown in Fig. 4B. The slopes of the resulting straight lines were plotted against the ATN concentration (Fig. 4C). According to the integrated Cottrell equation:[23]

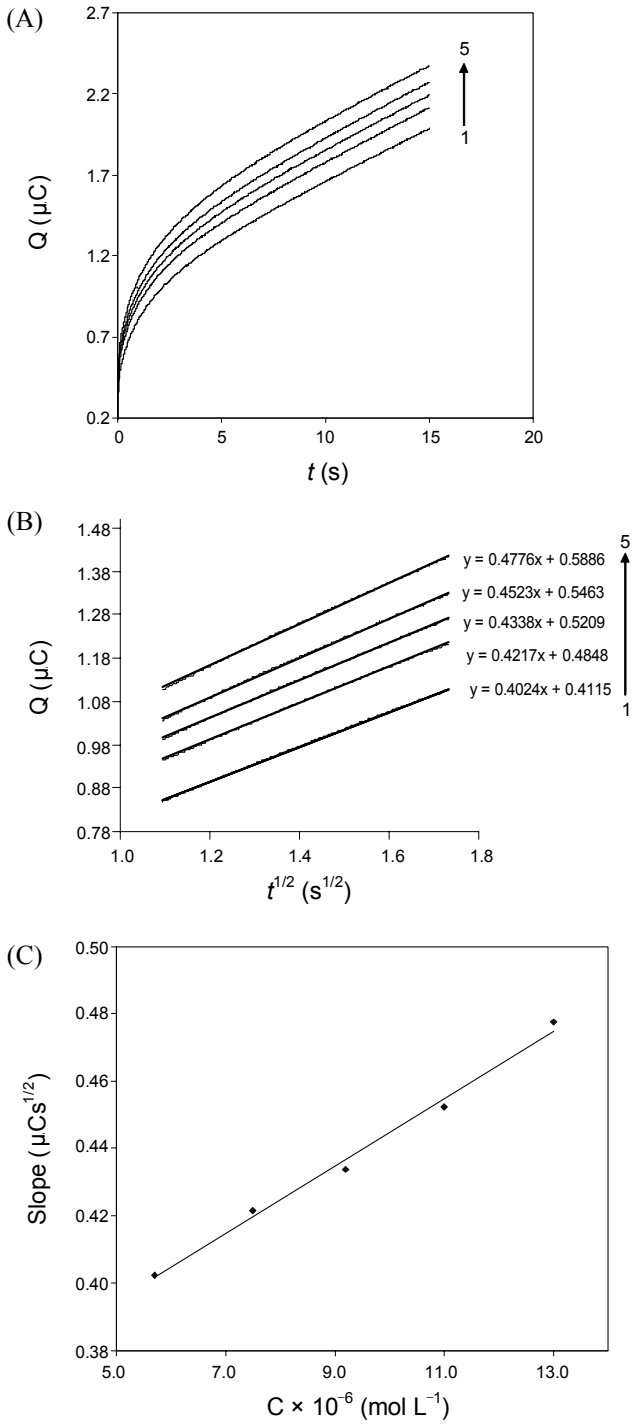


Figure 4. (A) Chronocoulomograms obtained with GN-CPE in Britton-Robinson buffer solution (pH 10.0) for different concentration of ATN. The numbers 1-5 correspond to 5.7×10^{-6}, 7.5×10^{-6}, 9.2×10^{-6}, 1.1×10^{-5} and 1.3×10^{-5} mol L^{-1} of ATN; (B) Plots of the charge (Q) *vs* square root of the time ($t^{1/2}$) obtained from Chronocoulomograms 1-5; (C) Plot of the slope of straight lines against the ATN concentration.

$$Q = \frac{(2nFAD^{1/2}Ct^{1/2})}{\pi^{1/2}} \qquad (4)$$

the apparent diffusion coefficient (D) of ATN was calculated to be about 2.1×10^{-6} $cm^2 s^{-1}$.

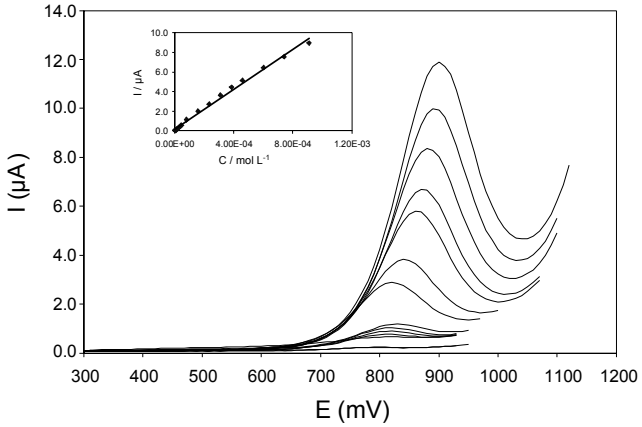


Figure 5. Differential pulse voltammograms of GN-CPE in Britton-Robinson buffer solution (pH 10.0) containing different concentrations of ATN (from inner to outer): 1.96, 3.47, 15.7, 23.4, 31.0, 38.5, 45.8, 157, 234, 385, 458, 602, 741 and 909 μ mol L^{-1}. The insert shows the plot of the peak current as a function of ATN concentration in the range of 1.96×10^{-6} to 9.09×10^{-4} mol L^{-1}.

Table 1. Relative standard deviation for different concentrations of ATN

Concentration of ATN (mol L^{-1})	RSD (%)	*N*
5.0×10^{-5}	0.27	8
1.0×10^{-4}	2.10	8
4.8×10^{-4}	3.10	9

Table 2. Application of the proposed method to the determination of ATN in spiked human urine

Added (mol L^{-1})	Found (mol L^{-1})	Recovery (%)
0.0	Not detected	-
4.0×10^{-5}	4.4×10^{-5}	110.0
1.0×10^{-4}	1.0×10^{-4}	100.0
1.9×10^{-4}	1.8×10^{-4}	92.0
3.0×10^{-4}	2.9×10^{-4}	98.0

Table 3. Determination of ATN in four pharmaceutical formulations using the GN-CPE

Sample	Stated content (mg/tablet)	Detected content (mg/tablet)
Atenolol (Daroupakhsh)	50	54.5
Atenolol (Sobhan Darou .Co)	100	87.4
Atenolol (Pharmachemie)	100	95.6
Atenolol (Tolidaru)	100	110.2

Calibration plot. The differential pulse voltammetry for ATN in the range of 4.00×10^{-8} to 1.94×10^{-3} mol L^{-1} was recorded. The DPV voltammograms show a linear relation between peak current and concentration of ATN in the range of 1.96×10^{-6} to 9.09×10^{-4} mol L^{-1} (Fig. 5), which can be represented by the following equation:

$$i_p\ (\mu A) = 0.1426 + 10227\ c\ (\text{mol } L^{-1}) \quad (5)$$

The observed correlation coefficient was 0.994 and the detection limit (3 S_b/m) was 7.3×10^{-8} mol L^{-1}, which is lower than the values of 1.6×10^{-4} mol L^{-1} of C_{60}-modified GC electrode,[14] 3.16×10^{-6} mol L^{-1} of graphite-polyurethane composite electrode[17] and 1.0×10^{-7} mol L^{-1} of plastic membrane electrode.[19] The relative standard deviations for different concentrations of ATN are summarized in Table 1. The results show excellent day-to-day reproducibility. The stability of the GN-CPE was investigated by measuring the oxidation response of 5.0×10^{-5} mol L^{-1} ATN over a period of 7 days. The RSD of 4.0% demonstrates that the GN-CPE exhibits excellent reproducibility over time.

Interference study. The effect of the anti-hypertensive drug (propranolol), and the effect of acetaminophen, ascorbic acid, dopamine, and other foreign substances on the determination of 5.0×10^{-5} mol L^{-1} ATN was investigated. The tolerance limit was determined as the maximum concentration of the material that caused a ± 5.0% relative error in the determination of ATN. The tolerated concentrations were 2.5×10^{-2} mol L^{-1} for NH_4Cl, 5.0×10^{-3} mol L^{-1} for $Ca(NO_3)_2{\cdot}2H_2O$, 2.5×10^{-3} mol L^{-1} for $NaNO_3$, and 1.0×10^{-3} mol L^{-1} for ascorbic acid, acetaminophen, uric acid and KNO_3, respectively. Also, dopamine and propranolol showed interference on the determination of ATN. The results revealed that the proposed method had good selectivity for ATN.

Recovery test of atenolol in urine. The high sensitivity of the method allows the determination of ATN in spiked human urine. The recovery of ATN was measured by spiking ATN into highly diluted urine samples. The differential pulse voltammograms were recorded after the urine sample was spiked with various amounts of ATN within the working concentration range. Recoveries were found to lie in the range from 92.0 to 110.0%. The results are listed in Table 2.

Analysis of commercial tablets. To investigate the real-life applicability of the GN-CPE, the concentrations of ATN in four commercial tablets were examined. The tablets were grounded to powder, dissolved in water, filtered with sinter glass and diluted so that the concentration of ATN was in the working range. A standard addition method was applied to measure accuracy. The values of experimentally determined ATN were compared to the reported ATN amounts in various tablets and the results are summarized in Table 3.

Conclusion

This work describes a simple and sensitive method for the determination of ATN. The gold nanoparticles modified CP electrode shows excellent electrocatalytic activity toward the oxidation of atenolol. An increase in the current response and a decrease in the anodic overpotential are obtained compared to the electrochemical response of a bare CPE. High sensitivity, low detection limit, easy regeneration of the electrode surface, low cost, simple method of preparation and excellent stability and reproducibility make the GN-CPE system useful for the determination of ATN in pharmaceutical dosage forms and urine.

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