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Application of *N*-4,4'-azodianiline(ferrocenyl Schiff base) for electrocatalytic determination of atenolol on modified carbon paste electrode

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Abstract

A carbon-paste electrode (CPE) chemically modified with the N-4,4'-azodianiline(ferrocenyl Schiff base) complex and multi wall carbon nanotubes (ADAFCNTE) was used as a highly sensitive electrochemical sensor for determination of trace amounts of atenolol. The oxidation peak potentials in cyclic voltammogram of ADAFCNTE occurred around 550 mV vs Ag/AgCl (at pH 6.0) while this peak potential at the carbon paste electrode appeared around 800 mV at the same scan rate of 10 mV s^{-1}. The kinetic parameters such as electron transfer coefficient, α, and rate constant for chemical reaction between atenolol and redox sites in modified electrode were 0.41 and 2.8×10^2 cm^3 mol^{-1} s^{-1}, respectively. The catalytic peak current was linearly dependent on atenolol concentration in the range of 0.1-57.0 µmol L^{-1} with a detection limit of 0.08 µmol L^{-1}. Finally, the sensor was examined as a selective, simple and precise new electrochemical sensor for the determination of atenolol in urine samples, with satisfactory results.

Keywords: Atenolol; *N*-4,4'-azodianiline ferrocene; multi wall carbon nanotubes; electrocataytic determination.

Introduction

The b-blocker drug atenolol was developed as a replacement for propranolol in the treatment of hypertension, angina, migraine headaches and cardiac arrhythmia [1,2]. β-blockers are exceptionally toxic and mostly have a narrow therapeutic range; i.e., the differences between the lowest therapeutic and the highest tolerable doses are small [3]. Hence, accurate methods for the measurement of atenolol are of great importance in pharmaceutical research. In recent years, ferrocene and its derivatives are of increasing importance as they regard biological applications [4]. Incorporation of ferrocene units into molecules of biological relevance introduces new useful features and properties of these compounds [5]. Because of their chemical stability and non-toxicity, ferrocenyl compounds are already applied as electroactive labels of biologically active compounds [6]. Other ferrocenyl derivatives display therapeutically useful properties that may open new avenues in the treatment of diseases like malaria [7] and cancer [8].

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Many analytical methods are available for quality control, stability testing, identification and clinical studies of atenolol. Gas chromatography (GC) [9,10], liquid chromatography (LC) with ultraviolet (UV) detector or mass spectrum (MS) have also been extensively used for the determination of atenolol [11,12] along with capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) [13,14]. These methods, however, are generally complicated and tedious. Electrochemistry has a well-defined role in drug analysis and various electroanalytical methods are being used from time to time for the purpose. Electrochemical techniques are suitable for determining drugs, even in samples containing complex matrix such as syrups, tablets, creams or biological fluids. Ferrocene compounds containing an azo moiety are of interest owing to their electrochemical properties and their ability to form oligomers and polymers [15]. Interestingly, no data have been reported so far on the application of electrocatalytic activity of azoferrocene molecules in sensors. In this study, we report the synthesis of *N*-4,4'-azodianiline(ferrocenyl Schiff base) where the unique physical and chemical properties of ferrocene moieties are combined with the unusual and interesting properties of azo group. Therefore, we have allocated the present study on Azoferrocene compound. Cyclic voltammetry (CV) study showed that the oxidation current of *N*-4,4'-azodianiline ferrocene (ADAF) was enhanced significantly in the presence of atenolol. The peak current of the electrochemical probes had a linear relationship with the concentration of atenolol. Also, the proposed method has been used for determination of atenolol in urine samples with satisfactory results. Table 1 presents the advantages and disadvantages of the proposed modified electrode for determination of atenolol as compared to other electrochemical methods reported in the literature.

Table 1. Comparison of figures of merit of the proposed method with recently published voltammetric methods for the determination of atenolol

Electrode	LOD (µM)	LDR (µM)		Interference	Ref.
Catalytic potential/mV	Sensitivity/µA µmol^{-1} L				
Indium tin oxide	0.13	0.5–1000.0	Amlodipine, Nifedipine, Propanolol hydrochloride	470 and 900	0.008	[16]
CPE	1.96	20.0–100.0	Not found	930	0.006	[17]
CPE	0.1	0.4-80.0	bisoprolol , bevantolol	600	0.676	[18]
surfacestabilized bilayer lipid membranes	15	20.0–200.0	Not found	600	0.011	[19]

C60-modified glassy carbon electrode	160	250-1500	Nifedipine, Propranolol	1040	0.009	[20]
Bare graphite-polyurethane composite electrode	3.16	4.0–100.0	Furosemide, propanolol	810	0.0001	[21]
	0.073	1.96-909	dopamine , propranolol	875	0.01	[22]
CPE	0.29	5.0–210.0	Not found	800	0.0109	[23]
CPE	2.1	8.0–205.0	Not reported	800	14.8	[24]
CPE	0.08	0.1-57.0	Ascorbic acid	550	0.281	This work

Experimental

Apparatus and reagents

All Voltammetric measurements were carried out using an electrochemical system comprising the Metrohm instrument (Herisau, Switzerland), Model 797 VA and a conventional three electrode cell assembly (Ag/AgCl electrode as reference electrode, a platinum wire as counter electrode and azodianilineferrocenyl carbon nanotube paste electrode (ADAFCNTE) as working electrode). The pH of the solutions was controlled by a Corning pH meter (model 146). The electrode feature was characterized by scanning electron microscopy (SEM) (Philips, XL30). All the chemicals were of analytical grade (Merck) and doubly distilled water was used throughout the experiments. A 1.0×10^{-3} mol.L^{-1} atenolol solution was prepared daily by 0.0068 g atenolol in 25 mL universal buffer solution, pH 10.0. Universal buffer (0.1 mol.L^{-1}) solutions with different pH (3-8) values were used.

Synthesis of N-4,4'-azodianiline(ferrocenyl Schiff base) complex

The Fe(II) complex, *N*-4,4'-azodianiline(ferrocenyl Schiff base), was prepared in the following way: ferrocene carbaldehyde (1 mmol, 0.214 g) and 4,4'-azodianiline (1 mmol,0.0212 g) were added to a porcelain mortar and ground using a porcelain pestle at 50-60 ºC for 3 h. The shiny red crystals of the *N*-4,4'-azodianiline(ferrocenyl Schiff base) complex were formed in a yield of 75%. Analytical calculation for $C_{23}H_{20}FeN_4$ (MW = 408.268): C, 67.66; H, 4.94; N, 13.73. Found: C, 67.85; H, 4.98; N, 13.82%. IR (KBr pellet, cm^{-1}): 1623(C=N) and 1582 (C=C). UV–Vis ($CHCl_3$, λ_{max}/nm ($\varepsilon/M^{-1}cm^{-1}$)): 1513 (80), 709 (89), 291 (67450), 267 (41551), 195 (84017). ^{1}H NMR ($CDCl_3$) CH=N (1H, s, 8.37 ppm), C_5H_5 (5H, s, 4.28 ppm), C_5H_4 (2H, t,4.90 ppm and 2H, t, 4.72 ppm).

Preparation of the modified electrode

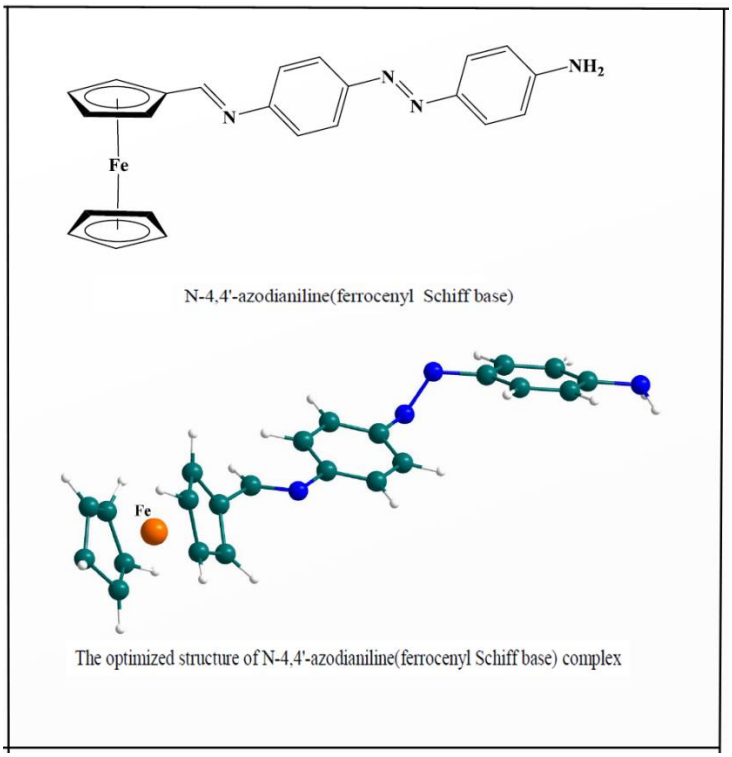
The mixture of 0.07 g multi wall CNTs (MWCNT), 0.01 g ADAF and 0.5 g

graphite powder were mixed together. Then diethyl ether was added and the mixture was mixed to get a uniform mixture. After evaporation of diethyl ether, 0.3 g paraffin oil was added and the mixture was mixed with mortar and pestle to obtain a uniformly wetted paste. The prepared paste was inserted into a glass tube (internal radius 2.2 mm). To prepare a blank solution, 5.0 mL of the buffer solution (universal buffer, 0.10 M, pH 6.0) was transferred into an electrochemical cell. The differential pulse voltammogram (DPV) was recorded with a pulse height of 50 mV and a pulse width of 5 mV and the potential scanned from +0.20 and +0.70 V vs. Ag/AgCl.

Preparation of real samples

Atenolol tablets (100 mg tablet, Pharmachemie Company, Iran) were administered to hypertensive patient and healthy volunteers. The urine samples taken from humans were analyzed after its centrifuge and dilution for 25 times. The standard addition method was used for the determination of the atenolol contents after dilution of the samples.

Atenolol (Sobhan Daru Company, Iran) labeled as containing 50 mg of atenolol per tablet was weighed and grounded to a homogeneous fine powder in a mortar. A portion equivalent to a stock solution of a concentration of about 0.01M was accurately weighed and transferred into a 100 mL calibrated flask and completed to the volume with doubly distilled water. The contents of the flask were sonicated for 15 min to affect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting them with the Universal buffer solution. Each solution was transferred to the voltammetric cell and analyzed by standard addition method.



Scheme 1. The structure of ADAF

Results and discussion

Characterization of electrode surface

Figure 1 shows SEM images of carbon paste electrode (CPE) and ADAFCNTE. As can be seen, Multiwall carbon nanotubes and azodianiline ferrocenyl were dispersed in the carbon paste and filled pores between graphite particles. It caused increasing active surface area of the electrode and electrooxidation current.

Figure 2 illustrates the Nyquist diagrams of CPE, CNTPE electrode, ADAF modified carbon paste electrode (ADAFPE) and ADAFCNTE in the presence of 1.0 $\times 10^{-3}$ mol L^{-1} $[Fe(CN)_6]^{3-/4-}$ (1:1) + 0.1 mol L^{-1} KCl. It can be seen that at the CPE, a semicircle with R_{ct} about 1.1 kΩ was obtained. However, the diameter of the high frequency semicircle was obviously reduced to 0.32 Ω by modification of the CPE with multi wall carbon nanotubes, suggesting that a significant acceleration for $[Fe(CN)_6]^{3-/4-}$ redox reaction occurred due to the presence of multi wall carbon nanotubes. Also, the R_{ct} of ADAFPE and ADAFCNTE obtained was about 2.5 and 0.57 kΩ, respectively. As expected, decrease of charge transfer resistance value for CPE spiked with multi wall carbon nanotubes is due to acting these multi wall carbon nanotubes as conductive shreds.

Experimental variables

To obtain the best conditions for the modified electrode, we optimized the ratio of MWCNTs and ADAF in ADAFCNTE. Therefore, the mixture of 0.07 g carbon nanotubes (MWCNT), 0.01 g ADAF and 0.5 g graphite powder were mixed together. Then diethyl ether was added and after evaporation of diethyl ether, 0.3 g paraffin oil was added and the mixture was mixed with mortar and pestle to obtain a uniformly wetted paste.

It is well known that the electrochemical behavior of atenolol is dependent on the pH value of the aqueous solution [16], whereas the electrochemical properties of *N*-4,4'-azodianiline(ferrocenyl Schiff base) is pH independent. The electrochemical behavior of 50 μmol.L^{-1} of atenolol solutions in universal buffer atdifferent pH values (3.0 < pH <8.0) was studied using the ADAFCNTE and differential pulse voltammetry. The results showed that maximum signal was obtained in pH 6.0.

Electrochemical behavior of ADAFCNTE

The CV of ADAFCNTE exhibited an anodic peak with E_{pa} =0.55 V and cathodic peak with E_{pc}= 0.45 V. The peak separation potential, $\Delta E_p=(E_{pa}-E_{pc})$ = 100.0 mV, was more than 59/n mV excepted for a reversible system which indicates that the redox couple in ADAF shows quasi-reversible in an aqueous medium. In addition, its anodic and cathodic peak potentials does not show any shift in various buffered solutions. Therefore, the electrochemical behavior of the $ADAF/ADAF^+$ redox couple is independent of the pH.

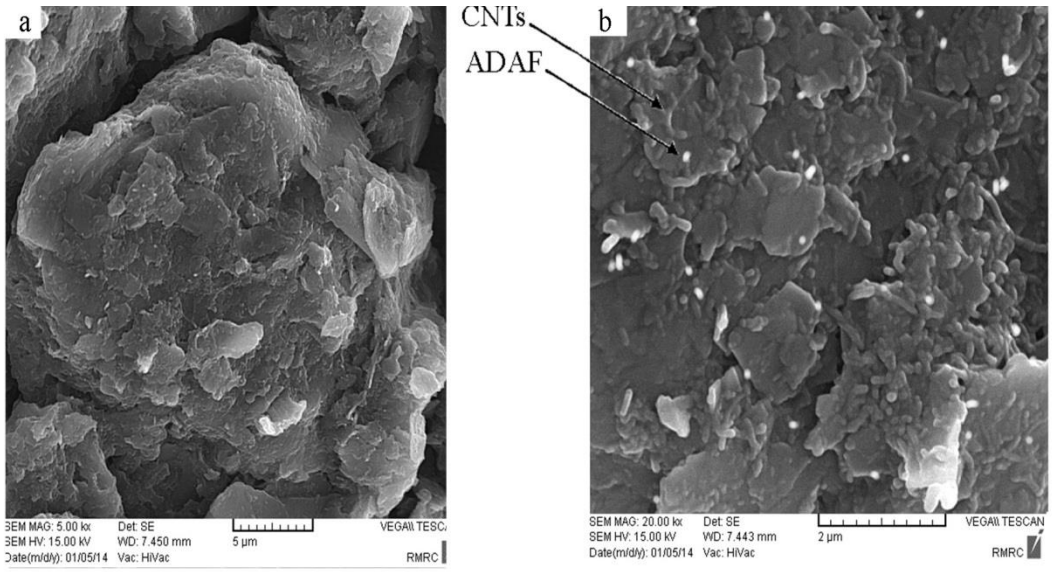


Figure 1. SEM image of a) carbon nanotube paste electrode and b) azodianilineferrocenyl carbon nanotube paste electrode

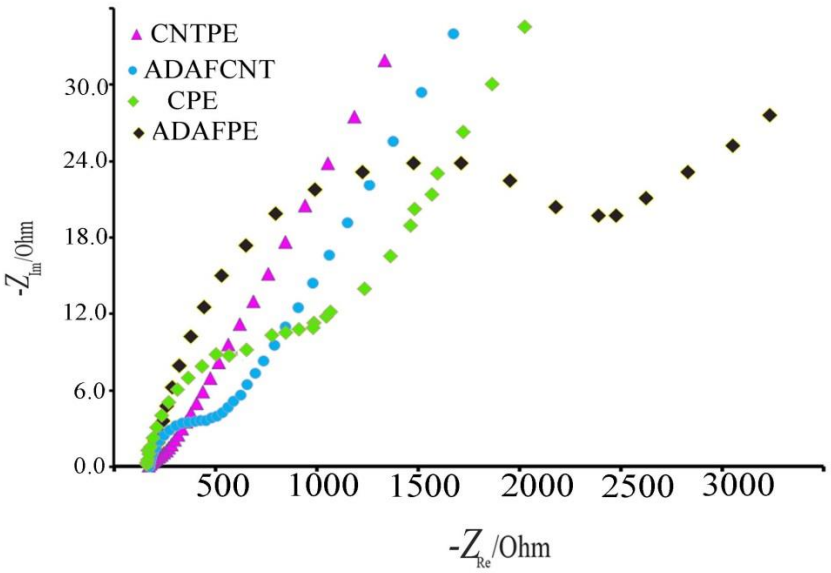
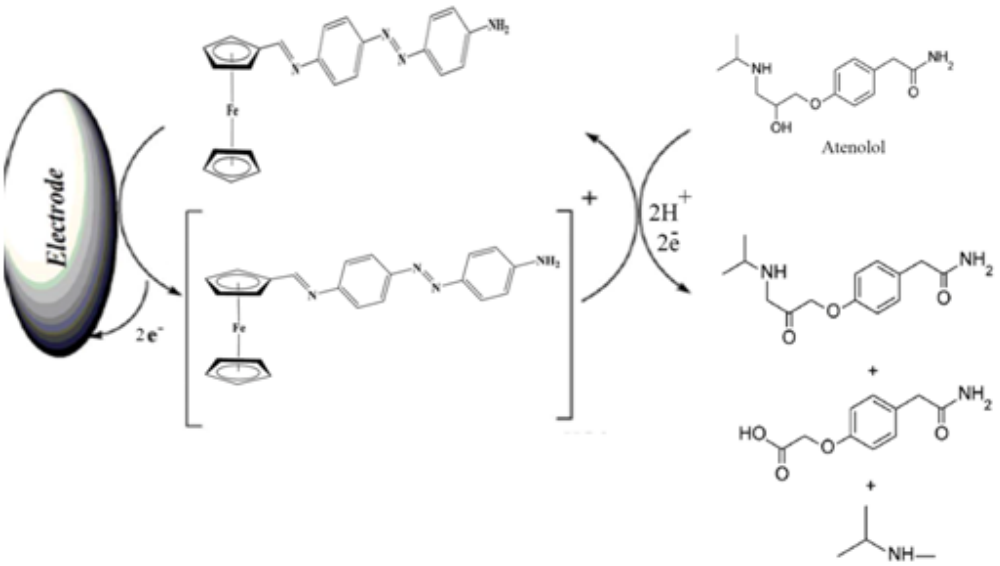


Figure 2. Nyquist plots for the Faradaic impedance measurements of a 1.0 mM solution of 1:1 $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ performed on bare carbon paste (CPE), carbon nanotubes carbon paste electrode (CNTPE), *N*-4,4'-azodianiline(ferrocenyl Schiff base) carbon paste electrode (ADAFPE) and ADAF as a mediator in carbon nanotube paste electrode (ADAFCNTE) Bias is 0.50 V with E_{ac} = 5 mV and frequency range of 10 kHz to 1 Hz.

Electrochemical behavior of atenolol on ADAFCNTE

The voltammetric behavior of ADAFCNTE is shown in Figure 3. Cyclic voltammogram of CPE in atenolol solution had a peak current in 800 mV in the pH 6.0. Cyclic voltammogram of ADAFCNTE in the

absence of atenolol had a well-defined electrooxidation peak current at 0.55 V (Figure 3-c). In the presence of atenolol in solution, anodic peak current was increased and cathodic peak current was decreased (Figure 3-d). This behavior is typical of that expected for electrocatalysis at chemically modified electrodes. Adding MWCNT to the modified electrode caused to increase peak current (Figure 3-e). Based on the results obtained on the oxidation of atenolol on the surface of ADAFCNTE, scheme 2 shows the mechanism of the electrocatalytic oxidation of atenolol [24].



Scheme 2. Oxidation of atenolol at the surface of ADAFCNTE

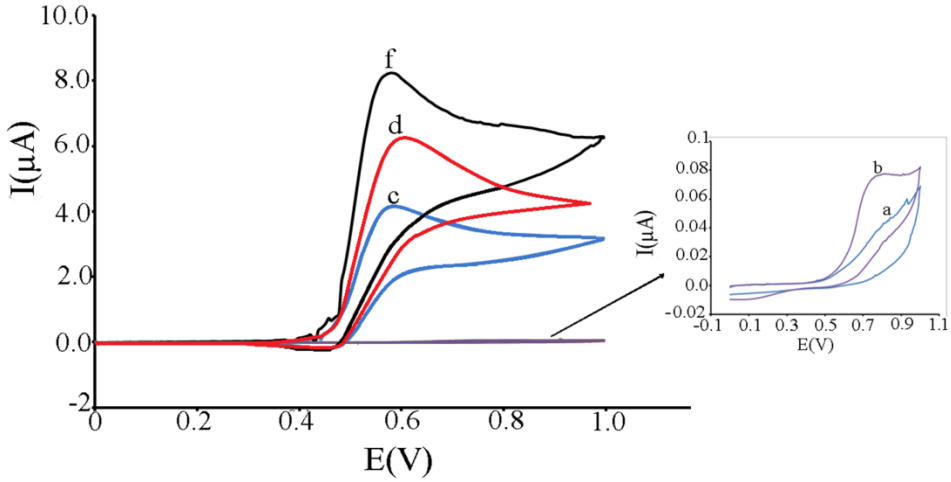


Figure 3. CVs of CPE in 0.05 mol L^{-1} universal buffer (pH 6.0) at a scan rate of 10 mV s^{-1} in the absence (a) and in the presence (b) of 50.0 μ mol L^{-1} atenolol. (c) as (a) and (d) as (b) for ADAFPE.(f) as (b) for ADAFCNTE.

The catalytic rate constant, k_h, (cm^3 mol^{-1} s^{-1}) for the chemical reaction between atenolol and redox sites in ADAFCNTE, k_h, can be evaluated by chronoamperometry according to the method described in [25]:

$$I_C/I_L = \pi^{1/2}(k_h C_b\, t)^{1/2}$$

where, I_C is the catalytic current of ADAFCNTE in the presence of atenolol, I_L is the limited current in the absence of atenolol, C_b is the bulk concentration of atenolol and t is time elapsed (s). From the slope of I_C/I_L versus $t^{1/2}$ plot, the value of k_h can be simply calculated as 2.8×10^2 cm^3 mol^{-1} s^{-1}. To study the rate determining step, a Tafel plot was developed for ADAFCNTE using the data derived from the current—voltage curve. The slope of the Tafel plot is equal to 2.3RT/ n(1−α)F V $decade^{-1}$. Using this data gives n(1-α)=0.69. If assuming n = 1, then α = 0.41. Calibration curve, which obtained from differential pulse voltammetry, exhibited a linear dynamic range of 0.1–57.0.0 µmol L^{-1} for atenolol with the regression equation of $I_p(\mu A)=(0.2806\pm0.0030)C_{atenolol}$ + (29.33±0.90) (R^2=09914, n=5), where $C_{atenolol}$ is concentration in µmol L^{-1}. The detection limit (defined as $3s_b/m$ where s_b is the standard deviation of the blank signal (n=10) and m is the slope of the calibration curve) was found to be 0.08 µmol L^{-1}.

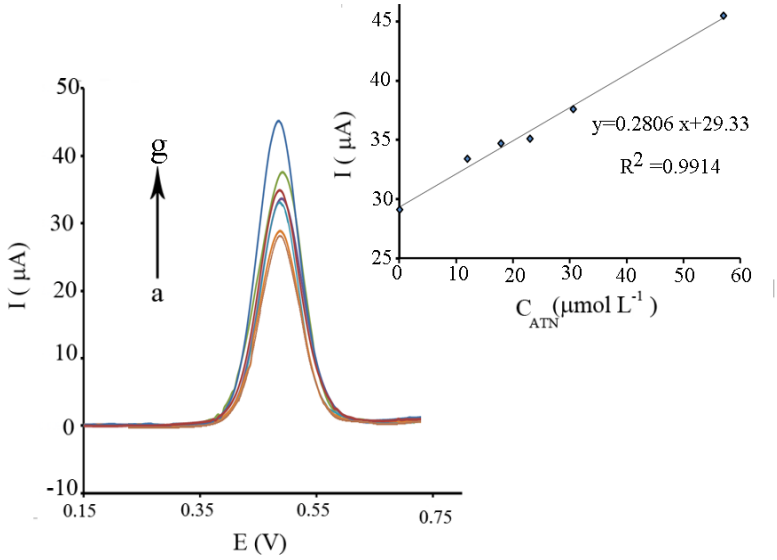


Figure 4. Differential pulse voltammograms at the ADAFCNTE for: a) 0.0; b) 0.1 ; c) 12.0; d)17.9; e) 23.0; f) 30.6 and g) 57.0 µM atenolol in phosphate buffer solution (pH 6.0). Insert) Plot of electrocatalytic peak current for DPV vs. atenolol concentration

Interferences

The effect of various potential interferences on the signal of atenolol was studied under the optimum conditions with 50µmol L^{-1} atenolol at pH 6.0. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than ±5% for the determination of atenolol. The results showed that 1000 fold of Na^+, Br^-, F^-, Mg^{2+}, Ca^{2+}, K^+, $NO_3^-$and NO_2^-, 600 fold of SO_4^{2-} and glucose, 500 fold of fructose, sucrose, lactose, urea and aspartic acid and 200 fold of citrate did not effect on the signal of atenolol. The result shows that only ascorbic acid could be interference for the

determination of atenolol using this modified electrode. The interference of ascorbic acid can be minimized by using ascorbic oxidase enzyme, which exhibits high selectivity to oxidation of ascorbic acid, if necessary.

Application

To evaluate the analytical applicability of the proposed sensor, determination of atenolol was done in the urine samples by the standard addition method. The accuracy of the method was examined by obtaining a recovery of experiment that varying from 93.4% to 105.1%. This proved that this procedure was not affected by the sample matrix. The results (Table 2) clearly confirm the capability of the ADAFCNTE in the voltammetric determination of atenolol with high selectivity, accuracy and good reproducibility.

Table 2. Determination of atenolol in real samples

Sample	Atenolol added(µM)	Expected value (µM)	Proposed method (µM)*	Standard method (µM) [26]	Recovery %
	-	1.0	1.03 ± 0.3	0.98 ± 0.1	105.1
Tablet[a]	10.0	11.0	11.2±1.1	-	102.0
	15.0	26.0	25.7±0.9	-	98.9
	-	20.0	18.4 ± 0.9	19.7 ± 1.2	93.4
Tablet[b]	5.0	25.0	23.3±1.6	-	94.3
	10.0	35.0	33.8±2.1	-	97.4
	-		10.8±1.3	11.4±0.8	94.7
Urine[c]	10.0	-	22.5±2.1	21.6±1.2	104.2
	-	-	22.4±3.1	23.3±1.7	96.1
Urine[d]	7.0		30.3±1.7	29.7±2.7	102.0

[a]50 mg tablet, Sobhan daru Company, Iran.
[b]100 mg tablet, Pharmachemie Company, Iran.
[c]Sampling after 3.0 h of administration of atenolol tablet from female heart patient and [d] healthy female.
* Average of three replicated measurements.
± Standard deviation

Conclusion

A new derivative of ferrocene (ADAF) was synthesized and used for modification of CPE. The electrochemical behavior of atenolol on the modified electrode was studied. ADAF had an excellent electrocatalytic effect on the oxidation

of atenolol. Electrochemical approaches were used for determination transfer coefficient. The proposed method has good linear dynamic range and it was applied for determination of atenolol in urine samples.

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References

[1] J.E.F. Reynolds (Ed.), Martindale, 30th ed., Pharmaceutical Press, London, **1993**, 629.
[2] H. Winkler, W. Ried, B. Lemmer, *J. Chromatogr. Biomed. Appl*., **1982**, *228*, 223-234.
[3] H. Siren, M. Saarinen, S. Hainari, P.Lukkari, M.L. Riekkola, *J. Chromatogr. A*, **1993**, *632*, 215-227.
[4] N. Metzler-Nolte, M. Salmain, John Wiley & Sons, London, **2008**, 499.
[5] M.F.R. Fouda, M.M. Abd-Elzaher, R.A. Abdelsamaia, A.A. Labib, *Appl. Organomet. Chem*., **2007**, *21*, 613-625.
[6] M. Salmain, Wiley-VCH: Weinheim **2006**, 181.
[7] F. Dubar, J. Khalife, J. Brocard, D. Dive, C. Biot, *Molecules*, **2008**, *13*, 2900-2907.
[8] A. Vessieres, S. Top, W. Beck, E. Hillard, G. Jaouen, *Dalton Trans*., **2006**, *28*, 529-541.
[9] J. Paster, L. Mejstr´ıkov´, J. Zoulov´, K. Macek, J. Kvetina, *J. Pharm. Biomed. Anal*., **2007**, *44*, 674–679.
[10] M. Caban, P. Stepnowski, M. wiatkowski, N. Migowska, J. Kumirska, *J. Chromatogr., A*, **2011**, *1218*, 8110–8122.
[11] S. Dwivedi, N. Upadhaya, A. Uday, P. Sanjay, *Int. J. Drug Dev. Res*., **2011**, *1*, 134–137.
[12] D. Kong, S. Li, X. Zhang, J. Gu, M. Liua, Y. Menga, Y. Fua, X.J. La, G. Xue, L. Zhang, Q. Wang, *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci*., **2010**, *878*, 2989–2996.
[13] A. Van Eeckhaut, M.R. Detaevernier, Y. Michotte, *J. Pharm. Biomed. Anal.*, **2004**, *36*, 799–805.
[14] M. Spanakis, I. Niopas, *Journal of Chromatographic Science*, **2013**, *51*, 128–132.
[15] M. Kurihara, M. Kurosawa, T. Matsuda and H. Nishihara, *Synthetic Metals*, **1999**, *102*, 1517-1518.
[16] R.N. Goyal, V.K. Gupta, M. Oyama, N. Bachheti, *Electrochem. Commun*., **2006**, *8*, 65-70.
[17] R.H. Patil, R.N. Hegde, S.T. Nandibewoor, *Ind. Eng. Chem. Res*., **2009**, *48*, 10206-10210.
[18] M. Arvand, M. Vaziri, M. Vejdani, *Mater. Sci. Eng* C, **2010**, 30, 709-714.
[19] D.P. Nikolelis, S.S.E. Petropoulou, M.V. Mitrokotsa, *Bioelectrochemistry*, **2002**, *58*, 107-112.
[20] R.N. Goyal, S.P. Singh, *Talanta*, **2006**, *69*, 932-939.
[21] P. Cervini, L.A. Ramos, E.T.G. Cavalheiro, *Talanta*, **2007**, *72*, 206-209.
[22] M. Behpour, E. Honarmand, S.M. Ghoreishi, *Bull. Korean Chem. Soc*., **2010**, *31*, 845-849.
[23] A. Khoobi, S.M. Ghoreishi, S. Masoum, M. Behpour, *Bioelectrochemistry*, **2013**, BIOJEC-06673.
[24] M. Hasanzadeh, M.H. Pournaghi-Azar, N.Shadjou, A. Jouyban, *RSC Adv*., **2014**, *4*, 4710-4717.
[25] M. Mazloum-Ardakani, H. Beitollahi, B. Ganjipour, H. Naeimi, M. Nejati, *Bioelectrochemistry*, **2009**, *75*, 1-8.
[26] C. Vetuschi, G. Ragno, *Int. J. Pharm*., **1990**, *65*, 177-181.