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# Voltammetric determination of atenolol at $C_{60}$-modified glassy carbon electrodes

Rajendra N. Goyal *, Sudhanshu P. Singh

*Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee, Uttaranchal 247 667, India*

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**Abstract**

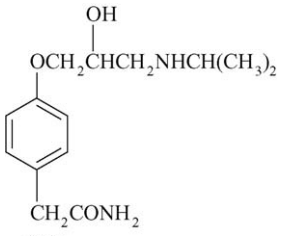
$C_{60}$-modified glassy carbon electrode has been found to exhibit excellent electrocatalytic activity towards atenolol oxidation for its voltammetric determination at physiological pH. Lowering of overpotential associated with atenolol oxidation indicates electrocatalytic nature of electrode. Determination of atenolol was carried out at pH 7.2 at modified electrode and a well-defined oxidation peak has been observed ~1040 mV versus Ag/AgCl electrode for atenolol oxidation. Calibration plot having good co-linearity with a correlation coefficient 0.997 was obtained in the concentration range of 0.25–1.5 mM atenolol and the sensitivity of the method has been found to be 8.58 μA $mM^{-1}$. The detection limit is found to be 0.16 mM. The method developed is applicable for atenolol determination in pharmaceutical preparations and urine samples. The modified electrode showed a good surface coverage (~85%) with $C_{60}$.


*Keywords:* Voltammetry; Atenolol; Electrocatalysis; Fullerene-$C_{60}$; Surface coverage

## 1. Introduction

Recent researches in chemistry, material science, physics and life science are focussed more towards their comparatively new common interface; i.e. nanotechnology and its applications. Current electroanalytical research is also attracting attention towards the use of nanomaterials for determination of variety of compounds. The discovery of fullerenes [1] and nanotubes [2,3] proved to be a landmark in nanomaterials research due to their well-known electrochemistry and electron accumulating properties [4]. Successful attempts have been made in the last few years to study electrochemical behaviour of fullerene films in aqueous solutions [5–9].

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 $\beta_1$-receptor blocking agent, which is of immense therapeutic use in the treatment of various cardiovascular disorders, such as angina pectoris, cardiac arrhythmia and hypertension [10]. Many analytical methods are available for quality control, stability testing, identification and clinical studies of atenolol. Gas chromatography (GC) with mass spectrometry or electron capture detector are in extensive use for the determination of atenolol [11,12]. High performance liquid chromatography (HPLC) has also been extensively used for the determination of atenolol [13]. Broderick et al. [14] quantified atenolol contents in tablets using water-in-oil microemulsion electrokinetic chromatography. Chromatographic 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. Most



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* Corresponding author. Tel.: +91 1332 285794; fax: +91 1332 273560.
*E-mail address:* rngcyfcy@iitr.ernet.in (R.N. Goyal).

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favourable property for modern electroanalytical methods is that excipients do not interfere. Hence, sample can be prepared simply by dissolution of pharmaceutical ingredient in suitable solvent. Alonso and co-workers [15] developed a high-performance liquid chromatographic method with electrochemical detection for the determination of β-blockers. Nikolelis et al. [16] described an electrochemical technique that is suitable for the rapid and sensitive screening of atenolol based on surface stabilized bilayer lipid membranes.

In this paper a simple and sensitive procedure to quantify atenolol in various samples using fullerene modified glassy carbon electrode is presented. The method described is simple and highly accurate provided a fresh electrode is prepared for each calibration to avoid any probability of surface contamination.

## 2. Experimental

### 2.1. Reagents and materials

$C_{60}$ was obtained from Aldrich, USA (purity 98%). Pure atenolol in powdered form was obtained as a gift sample from Fidalgo Laboratories Pvt. Ltd., Ludhiana. Atenolol containing tablets marketed by different medical companies were purchased from the local pharmacy. Phosphate buffer solutions ($\mu$ =1.0 M) were prepared according to the method of Christian and Purdy [17]. All other reagents used were of analytical grade. All solutions were prepared in double distilled water.

### 2.2. Instrumentation

The electrochemical experiments were performed with BAS (Bioanalytical Systems, West Lafayette, IN, USA) CV-50W Voltammetric analyzer and were carried out in a single-compartment three-electrode glass cell with a 3 mm diameter fullerene modified glassy carbon electrode as the working electrode, a platinum wire as counter electrode and Ag/AgCl electrode as reference (BAS; Model MF-2052 RB-5B) electrode. All experiments were carried out at an ambient temperature of $25 \pm 2\,^{\circ}C$.

### 2.3. Preparation of $C_{60}$-modified electrode

The surface of the glassy carbon electrode (GCE) was cleaned first by polishing with alumina using microcloth pads (BAS, USA) and then by using zinc oxide (Aldrich) until a mirror-like surface was obtained. The electrode was then dipped in 0.2 M $H_3PO_4$ solution followed by rinsing with distilled water and dried. $C_{60}$ solution (150 μM) was prepared by dissolving in dichloromethane. A known volume of the solution (40 μL) was adsorbed onto the surface of the clean and dried GCE using a microsyringe and dried in a stream of hot air. Electrode thus prepared was then pretreated in 1 M KOH in the potential range 0 to −1.5 V at 10 mV/s sweep rate. Finally, electrode surface was equilibrated by cycling potential in the range of 550 to −50 mV (versus Ag/AgCl) at a sweep rate of 20 mV/s for 20 min under nitrogen atmosphere in phosphate buffer solution of pH 7.2 [5]. The electrode was then ready to use.

### 2.4. Procedure

A stock solution of atenolol (4 mM) was prepared in doubly distilled water. Required amount of the stock solution was added to 1 ml of phosphate buffer solution ($\mu$ = 1.0 M, pH 7.2) and the total volume was made to 4.0 ml with double distilled water. The electrochemical measurements were then carried out. Differential pulse voltammetry employed had the following parameters: initial *E*: 0 mV, final *E*: 1200 mV, sweep rate: 20 mV/s, sensitivity: 100 μA/V, pulse amplitude: 50 mV, sample width: 20 ms, pulse width: 50 ms, pulse period: 200 ms.

## 3. Results and discussion

### 3.1. Determination of surface coverage

The fraction of the surface of GCE covered with $C_{60}$ was determined in order to determine efficacy of surface modification procedure. Chronocoulometric experiments were performed using 10 mM potassium ferrocyanide as redox substrate for the approximation of the surface area of GCE covered with $C_{60}$. Determination of surface coverage of GCE is based on the fact that for the surface modified electrodes, formation of diffusion layer begins in pores and diffusion towards whole geometrical surface starts after sometime; while for bare electrode, it occurs over the entire surface of the electrode right from the beginning [18,19]. Fullerenes have a football like geometry and for spherical nanoelectrodes, when the time scale is short, the diffusion layer is smaller than the electrode radius and the mass transport is dominated by semi-infinite linear diffusion. However, for long time scale, diffusion layer is larger than electrode radius and mass transport is dominated by spherical diffusion (Fig. 1). Since chronocoulometric experiments were carried out for very short time span; hence, for $C_{60}$-modified electrode diffusion layer forms only in pores while for bare GCE it forms over the whole surface.

The linear plots between charge and square root of time were obtained for both GCE and $C_{60}$-modified GCE and it was found that slopes of two plots were different (Fig. 2). Linear behaviour of plots is in accordance with integrated form of Cottrell's equation: $Q = (2nFAD_o{}^{1/2}\pi^{-1/2}C_o)\, t^{1/2}$ where $Q$ is the absolute value of charge, $n$ the number of electrons involved in reaction, $F$ the Faraday constant, $A$ the surface area of electrode, $D_o$ the diffusion coefficient of substrate, $C_o$ the bulk concentration and $t$ is the time. Surface area ratio of bare and $C_{60}$-modified GCE was

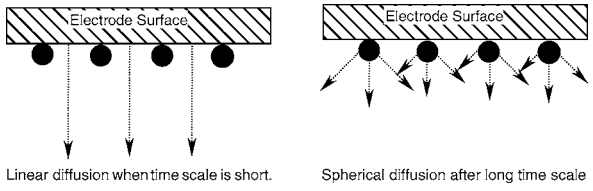


Fig. 1. Linear and spherical diffusion from $C_{60}$-modified glassy carbon electrode (where ● denotes fullerene).

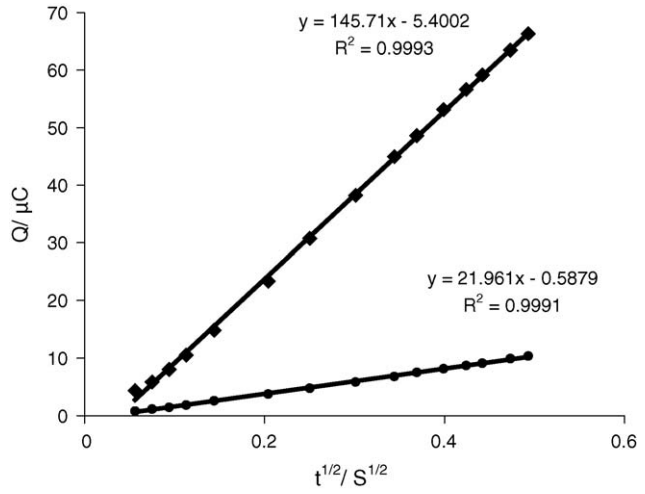


Fig. 2. $Q$ vs. $t^{1/2}$ plot at bare (♦ — ♦) and $C_{60}$-modified glassy carbon electrode (● — ●) obtained by performing chronocoulometric experiments on 10 mM potassium ferrocyanide.

determined directly from the ratio of slopes of two plots. Calculations indicate a good surface coverage of GCE with fullerene; $C_{60}$ covered ~85% of the electrode surface. This experiment indicated that surface modification procedure is quite effective.

### 3.2. Electrocatalytic oxidation of atenolol

Atenolol does not electrooxidise at bare glassy carbon electrode (GCE) in the working potential range 0–1200 mV. However, atenolol showed a well-defined oxidation peak ~1040 mV potential at $C_{60}$-modified GCE in differential pulse voltammetry. Oxidation peak of atenolol can be assigned to the oxidation of secondary alcoholic group, as is evidenced from literature [20]. The differential pulse voltammograms recorded at bare and $C_{60}$-modified GCE for atenolol electrooxidation at pH 7.2 are presented in Fig. 3(a). When CV of atenolol at 40 mV/s was recorded at modified electrode no oxidation peak was noticed (Fig. 3b). The appearance of oxidation peak in differential pulse voltammetry at $C_{60}$-modified electrode is an indication of catalytic nature of $C_{60}$-modified GCE for atenolol oxidation as it lowers the overpotential associated with atenolol oxidation and necessity of using differential pulse voltammetry. A well-defined peak for atenolol oxidation was obtained in the pH range 6.0–11.0 at $C_{60}$-modified GCE. Peak potential of oxidation peak was pH dependent and was found to shift towards less positive potential with increasing pH (Fig. 4). The relationship expressing linear dependence of $E_p$ on pH is

$$E_p[\text{pH } 6-11] = [1534 - 62.38\,\text{pH}]\,\text{mV versus Ag/AgCl} \quad (1)$$

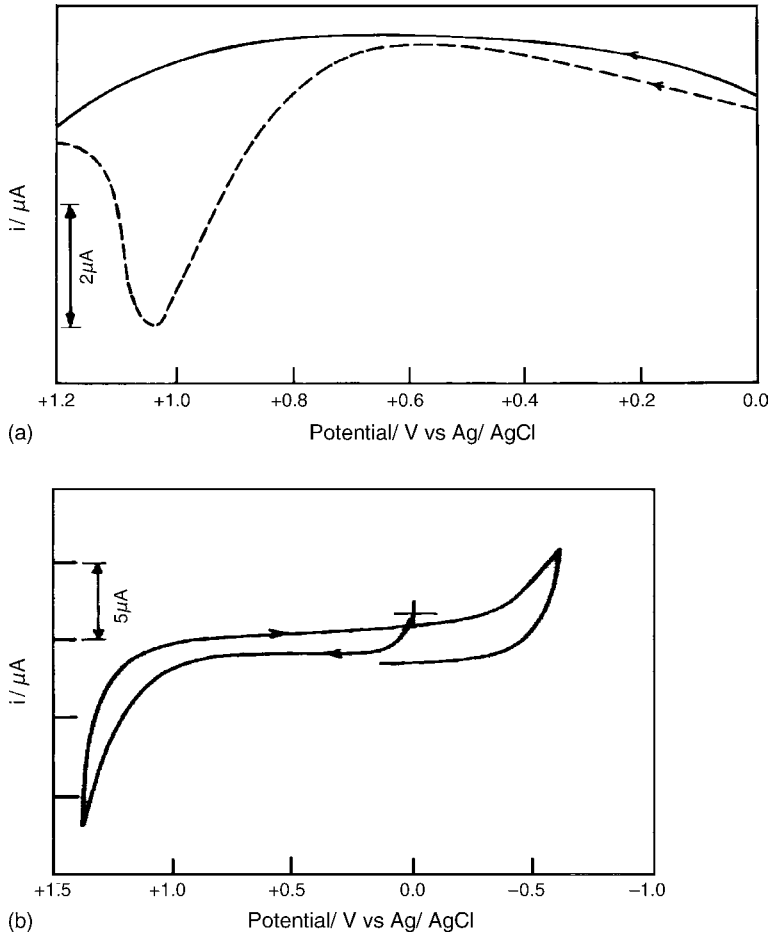


Fig. 3. (a) Observed differential pulse voltammograms of 0.5 mM atenolol at bare (—) and $C_{60}$-modified glassy carbon electrode (- - -) at pH 7.2. (b) Observed cyclic voltammogram of 0.5 mM atenolol at $C_{60}$-modified glassy carbon electrode at pH 7.2.

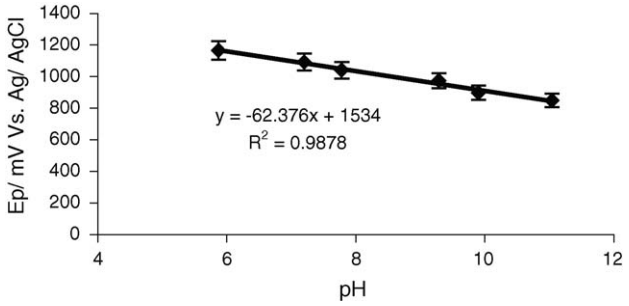


Fig. 4. Observed dependence of $E_p$ on pH for 0.5 mM atenolol at $C_{60}$-modified glassy carbon electrode.

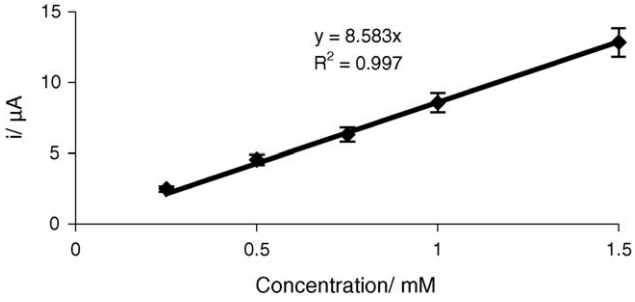


Fig. 5. Effect of concentration of atenolol on peak current at $C_{60}$-modified glassy carbon electrode at pH 7.2.

having correlation coefficient ~0.988. The slope of the $E_p$ versus pH plot was 62.4, which shows that equal number of protons and electrons are involved in the oxidation of atenolol. The peak current of the oxidation peak increased with the increase in concentration of atenolol. The $i_p$ versus concentration plot was linear in the concentration range 0.25–1.5 mM (Fig. 5). The linear relation expressing dependence of $i_p$ on concentration in the range 0.25–1.5 mM can be described as

$$i_p(\mu A) = 8.583C, \quad \text{where } C \text{ is in mM/L.} \tag{2}$$

having correlation coefficient ~0.997 and sensitivity 8.58 μA $mM^{-1}$. The detection limit of the electrode was found to be 0.16 mM at physiological pH. The values of the peak current function ($i_p \nu^{-1/2}$) remained practically constant with increase in sweep rate in the range 5–100 mV $s^{-1}$ (Fig. 6). This behaviour indicated that atenolol electrooxidation is

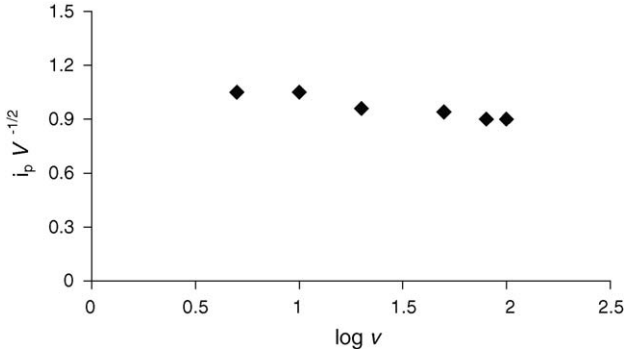


Fig. 6. Dependence of peak current function on sweep rate for 0.5 mM atenolol at $C_{60}$-modified glassy carbon electrode.

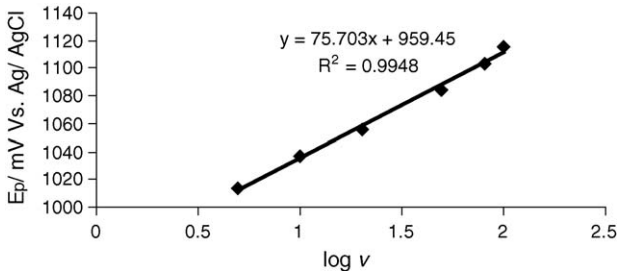


Fig. 7. Dependence of $E_p$ on log $\nu$ for 0.5 mM atenolol at pH 7.2.

free from adsorption complications. The linear dependence of oxidative current on square root of potential scan rate is an indication of diffusion-controlled nature of the electrode reaction [21]. The $E_p$ of the oxidation peak was also dependent on sweep rate and shifted to more positive potentials with increasing sweep rate. The plot of $E_p$ versus log $\nu$ was linear having a correlation coefficient of 0.995 (Fig. 7). The relation between $E_p$ and $\nu$ can be expressed by the equation

$$E_p(\text{mV}) = 75.70 \log\nu + 959.45 \tag{3}$$

### 3.3. Effect of interferents

Propranolol, metoprolol, amlodipine and nifedipine are some other commonly used drugs for the treatment of hypertension. Hence, it was considered necessary to determine their interference in atenolol determination. Response of $C_{60}$-modified electrode towards atenolol oxidation was observed in the presence of propranolol, metoprolol, amlodipine and nifedipine drugs by recording differential pulse voltammograms for mixture of atenolol (0.25 mM) and different concentrations of each interferent in the range 0.25–1.0 mM. It was found that propranolol and nifedipine interferes even when their concentration is 0.25 mM, i.e., when they are at same concentration as that of atenolol. However, amlodipine and metoprolol do not affect the peak current of atenolol even upto four-fold excess, i.e., 1.0 mM. Oxidation peak potential of atenolol remained almost constant and the change in current response in the presence of interferents was within ±0.180 μA as compared to their absence (Table 1) in all such cases where interference is less. Hence, atenolol can be safely detected in the presence of these interferents provided their amount is in the range shown in Table 1. The oxidation peak of atenolol gets distorted if these interferents are at much

Table 1
Effect of interferents on the differential pulse voltammetric response of 0.25 mM atenolol at the $C_{60}$-modified GCE

| Interferents | Concentration (mM) | Current (μA) | Signal change[a] (μA) |
|---|---|---|---|
| Atenolol (no interferent) | 0.25 | 2.500 | – |
| Amlodipine | 1.0 | 2.679 | +0.179 |
| Metoprolol | 1.0 | 2.658 | +0.158 |
| Nifedipine | 0.25 | 0.623 | −1.877 |
| Propranolol | 0.25 | 11.650 | +9.150 |

[a] Average of at least three runs.

Table 2
A comparison of observed and reported atenolol concentration in different tablets

| Tablet name (Company name) | Reference concentration (mM) | Observed concentration (mM) | Error (%) |
|---|---|---|---|
| Tenopress (Fidalgo Lab. Pvt. Ltd., Ludhiana) | 0.700 | 0.670 | −4.2 |
| Tenolol (Ipca Lab. Ltd., Mumbai) | 1.050 | 1.049 | −0.09 |
| Atecard (Dabur Pharm. Ltd., N. Delhi) | 0.350 | 0.350 | 0.0 |
| Zibloc (FDC Ltd., Andhra Pradesh) | 0.470 | 0.447 | −4.89 |

higher concentrations (>2.0 mM) as compared to atenolol concentration.

### 3.4. Analysis of commercial tablets

To optimize the method for pharmaceutical industry various atenolol containing tablets were examined for estimating atenolol content present in them. Solutions obtained by dissolving atenolol tablets were subsequently diluted so that atenolol concentration falls in the range of calibration plot. Differential pulse voltammograms were then recorded under exactly identical conditions that were employed while recording differential pulse voltammograms for plotting calibration plot. It was found that atenolol concentration determined for various tablets using this method are in good agreement with the reported values. The values of experimentally determined atenolol and reported atenolol amounts in various tablets are tabulated in Table 2.

### 3.5. Recovery test

Recovery test of atenolol was carried out by spiking of atenolol in highly diluted urine samples. The urine sample was spiked with different amount of atenolols. The results obtained are listed in Table 3. Recoveries have been found to lie in the range 99.6–105.3% with a relative standard deviation of 5.1%.

### 3.6. Stability of the $C_{60}$-modified GCE

The electrode stability of $C_{60}$-modified electrode is not of prime importance in these studies as the electrode can be prepared quickly. However, $C_{60}$-modified GCE showed variation in response after 3 days of its preparation and thus it is recommended that it should not be used for a longer time and a new electrode is to be prepared after 3 days. Experimental results indicated that current responses deviated intraday by 0.40% and interday by 1.54% for first 3 days. Thereafter peak current values started decreasing and increase in peak potential was also observed. The response time of the electrode was very fast and all measurements were carried out easily and quickly.

Table 3
Recovery data observed for spiked atenolol in highly diluted urine sample

| Spiked (mmol $l^{-1}$) | Detected (mmol $l^{-1}$) | Recovery (%) |
|---|---|---|
| 0.25 | 0.249 | 99.6 |
| 0.30 | 0.313 | 104.3 |
| 0.40 | 0.415 | 103.7 |
| 0.50 | 0.495 | 101.0 |
| 0.60 | 0.601 | 101.0 |
| 0.75 | 0.710 | 105.3 |

## 4. Conclusion

The surface modification of the electrode alters its characteristics in such a way that its performance improves manifold. Fullerene modified electrode show electrocatalytic nature and this behaviour is attributed to higher surface activity of fullerene because of the presence of more surface atoms in comparison to regular size material [22].

Some analytes react very slowly at the surface of electrode. Electrocatalytic nature of the electrodes is useful in such cases as catalyzed electrode reactions occur at lesser potential, significantly improving detection limit and selectivity of modified electrode [23]. The electroanalytical method described here employed electrocatalytic nature of $C_{60}$-modified electrode. Modified electrode showed a well-defined peak at ∼1040 mV for atenolol while it was totally absent at bare GCE. The 85% coverage of GCE indicates effectiveness of surface modification procedure. Moreover, excipients do not interfere with atenolol in this method. Hence, the method eliminates the need for derivitization prior to analysis. The differential pulse voltammetric method can be effectively used for the determination of atenolol at $C_{60}$-modified GCE because of its electrocatalytic nature and not at bare GCE. The method developed also showed good ability to quantify drug contents in tablets with reliable accuracy and can also be used to determine unmetabo-lised drug in urine samples.

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