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Stripping Voltammetric Determination of Timolol Drug in Pharmaceuticals and Biological Fluids

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Abstract

A sensitive and reliable stripping voltammetric method was developed to determine timolol drug. This method is based on the adsorptive accumulation of the drug at a hanging mercury drop electrode (HMDE) and then a negative sweep was initiated, which yield a well defined cathodic peak at –850 mV versus (Ag/AgCl) silver reference electrode. To achieve high sensitivity, various experimental and instrumental variables were investigated such as supporting electrolyte, pH, accumulation time and potential, scan rate, frequency, pulse amplitude, convection rate and working electrode area. The monitored adsorptive current was directly proportional to the concentration of timolol and it shows a linear response in the range from 1×10^{-7} to 1.5×10^{-6} mol·l^{-1} of this drug (correlation coefficient = 0.998) and the detection limit (S/N = 3) is 1.26×10^{-9} mol·l^{-1} at an accumulation time of 30 sec. The developed adsorptive stripping voltammetry (AdSV) procedure shows a good reproducibility, the relative standard deviation RSD% (n = 8) at a concentration level of 1×10^{-6} mol·l^{-1} of timolol was 0.13%, whereas the method accuracy was indicated via the mean recovery of 110% ± 1.414. Possible interferences by several substances usually present in the pharmaceutical formulations have been also evaluated. The applicability of this approach was illustrated by the determination of the drug in pharmaceutical preparation and biological fluids such as serum and urine.

Keywords: Stripping Voltammetry, HMDE, Timolol, Urine, Serum

1. Introduction

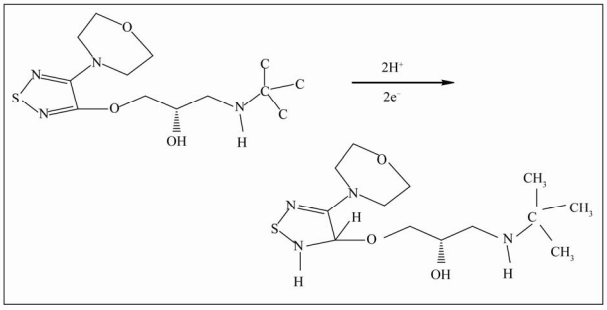
Of the most commonly used instrumental techniques, electroanalytical approach is the one of choice, and stripping voltammetric method has provoked particular interest because it is currently the most sensitive and widely used electrochemical technique. Its possibility of applications cover many fields ranging from environment, pharmaceutical and clinical to food and industrial samples. Many of the adsorptive stripping voltammetric (adsv) approach features such as sensitivity, selectivity, simplicity and versatility attributed to the combination of an effective preconcentartion step based on non-electrolytic adsorptive accumulation process with an advanced measurement procedures such as differential pulse (DP) or square wave (SW) [1-5]. Unlike conventional stripping approaches (anodic and cathodic stripping voltammetry), which are based on an electrolytic nature of preconcentration step, adsorptive stripping voltammetric approach in contrast is based on adsorptive accumulation of the analyte on the electrode at open circuit with no charge transferred. Consequently, for a wide range of surface-active organic and inorganic species, which cannot be preconcentrated electroanalytically, the adsorption approach allows these analytes to be interfacially accumulated on the electrode and hence analysed. There have been many reviews devoted to emphasize and illustrate the wide spectrum and scope of adsorptive stripping voltammetric applications and potentialities in the analysis of metal ions [6,7] organic analytes [8,9] and pharmaceutical drugs and biomedical compounds, such as, the anti-Inflammatory drug Lornoxicam, the antidepressant drug sulpiride and Josamycin, a Macrolide Antibiotic [10-14]. Timolol was the first beta (β) blocker to be used as an anti-glaucoma agent and to date remains as the standard because none of the newer β blockers were found to be more effective. Timolol maleate is the generic name for (*Ocumol* 0.5, *Timolo* 0.5%, 5 *mg/ml- Riyadh, Pharma-SA*), an eye drop (ophthalmic solution) indicated for the treatment of elevated intraocular pressure for those patients with primary open-angle glaucoma and ocular hypertension. Timolol maleate should be used

with care. It is important not to contaminate the solution, because this can cause infection and/or inflammation in the eye. If you are using more than one eye-drop medication, administration of the drugs should be spaced at least ten minutes apart to provide adequate time for absorption. Timolol is soluble in distilled water, its molecular weight is 316.421 g/mol and systematic IUPAC name is (S)-1-(tert-butylamino)-3-[(4-morpholin-4-yl-1, 2,5-thiadiazol-3-yl)oxy]propan-2-ol[15,16]. The chemical structure of this drug and the mechanism for the electrochemical reduction process of its compound are shown in **Scheme 1** (The mechanism proposed of electrochemical reduction for timolol drug). Timolol drug has been analysed in pharmaceutical formulations and biological samples by various analytical methods such as spectrophotometric [17-19], chromatographic [20,21], high performance liquid chromatography (HPLC) [22] and differential pulse voltammetric [23]. However, to the best of our knowledge, the square wave voltammetric behavior of timolol and thus its square-wave adsorptive stripping voltammetry (SW-adsv) have not been performed and reported so far. Consequently, the aim of this work was to develop more sensitive, reliable and simple SW-adsv procedure for the determination of timolol drug in biological media and pharmaceutical formulations.

2. Experimental

2.1. Apparatus

All adsorptive stripping measurements were carried out with 797 VA computrace (Metrohm, Switzerland) in connection with Dell computer and controlled by VA computrace 2.0 control software. Stripping voltammograms were obtained via a hp color laserjet CP1215 printer. A conventional three electrode system was used in the hanging mercury drop electrode (HMDE) mode. pH values were measured with Hanna instruments pH 211 (Romania made) pH meter. The labofuge 200 instrument , Heraeus sepatech (Germany) was used for centrifuging of biological fluids to suite for stripping analysis.



Scheme 1. The mechanism proposed of electrochemical reduction for timolol drug.

2.2. Reagents

All chemicals used were of analytical reagent grade and were used without further purification. Timolol stock solution of 1×10^{-2} mol·l^{-1} were prepared by dissolving the appropriate amount of this drug in distilled water in 10 ml volumetric flask. This stock solution was stored in the dark and under refrigeration in order to minimize decomposition. Standard solutions of this drug with lower concentrations were prepared daily by diluting the stock solution with distilled water. Britton-Robinson supporting buffer (pH ≈ 2, 0.04 M in each constituent) was prepared by dissolving 2.47 g of boric acid in 500 ml distilled water containing 2.3 ml of glacial acetic acid and then adding 2.7 ml of ortho-Phosphoric acid and diluting to 1 L with distilled water. In addition, phosphate supporting buffer (0.1 M NaH_2PO_4 and 0.1 M H_3PO_4) was prepared by dissolving 12 g of NaH_2PO_4 and 6.78 g of H_3PO_4 in 1000 ml distilled water. Acetate supporting buffer (0.02 M in each constituent) was prepared by dissolving 1.68 g of sodium acetate in 500 ml distilled water containing 1.12 ml of acetic acid and diluting to 1 L with distilled water. Finally, carbonate supporting buffer (0.1 M in each constituent) was prepared by dissolving 10.6 g of sodium carbonate and 8.4 g of sodium hydrogen carbonate in 1000 ml *distilled water.*

2.3. Procedure

The general procedure adopted for obtaining adsorptive stripping voltammograms was as follows: A 10 ml aliquot of B-R supporting buffer (unless otherwise stated) at the desired pH (e.g. 3.5) was pipetted into a clean and dry voltammetric cell and the required standard solution of timolol was added. The test solution was purged with nitrogen for 5 minutes initially, while the solution was stirred. The accumulation potential of –0.8 V vs. Ag/AgCl was applied to a new mercury drop while the solution was stirred for 30 seconds. Following the preconcentration period, the stripping was stopped and after 20 seconds had elapsed, cathodic scans were carried out over the range 0.0 to –1.2 V. All measurements were made at room temperature.

3. Preliminary Observations

When the differential pulse polarographic behavior was investigated for timolol drug in acetate buffer at pH 3.5, a broad polarographic wave at E_p = –0.850 V was observed and this obtained polarographic wave, as shown in **Figure 1** (Differential Pulse Polarographic of 5×10^{-5} mol·l^{-1} Timolol in pH 3.5 Acetate buffer, scan rate 25 mV·s^{-1}), is probably due to the electrochemical reduction

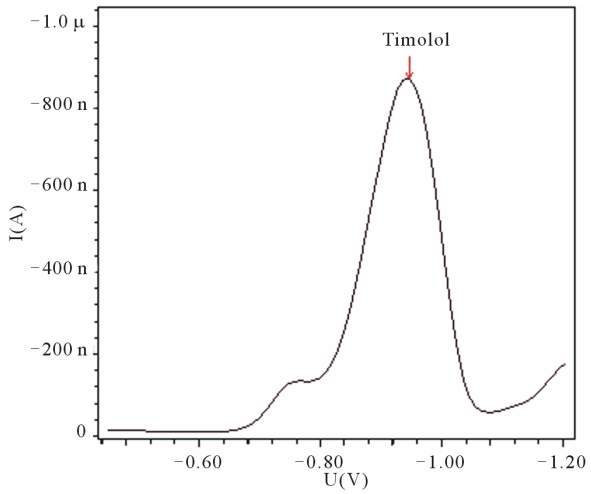


Figure 1. Differential Pulse Polarographic of 5 × 10^{-5} mol·l^{-1} Timolol in pH 3.5 Acetate buffer, scan rate 25 mV·s^{-1}.

of the double bond (–N=C) as shown in the previous scheme1, which including a proposed mechanism for the electrochemical reduction of this drug. This mechanism suggesting that the electrochemical reaction is an irreversible process, an assumption which was confirmed by cyclic voltammetric measurement at a scan rate of 50 mV^{-1} of timolol in acetate buffer (pH 3.5) as shown in **Figure 2** (Cyclic voltammogram of 5 × 10^{-5} mol·l^{-1} Timolol in pH 3.5 Acetate buffer, scan rate 50 mV·s^{-1}).

In order to obtain a voltammetric peak with better definition and higher sensitivity, a HMDE was used to study the adsorptive prosperities of timolol compound. The AdSV behavior of the drug was investigated in various supporting electrolytes at different pH values. This Drug compound yielded a well-developed and defined AdSV peak corresponding to the electroactive –N=C at peak potential of –0.85 V. A typical adsorptive stripping voltammogram for 5 × 10^{-7} mol·l^{-1} timolol in acetate buffer is shown in **Figure 3** (SW-AdSV voltammogram

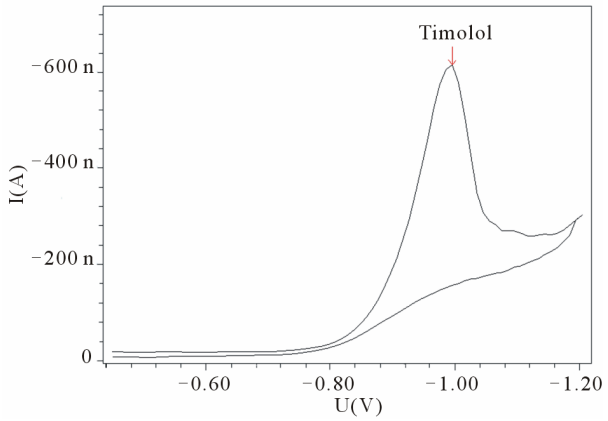


Figure 2. Cyclic voltammogram of 5 × 10^{-5} mol·l^{-1} Timolol in pH 3.5 Acetate buffer, scan rate 50 mV·s^{-1}.

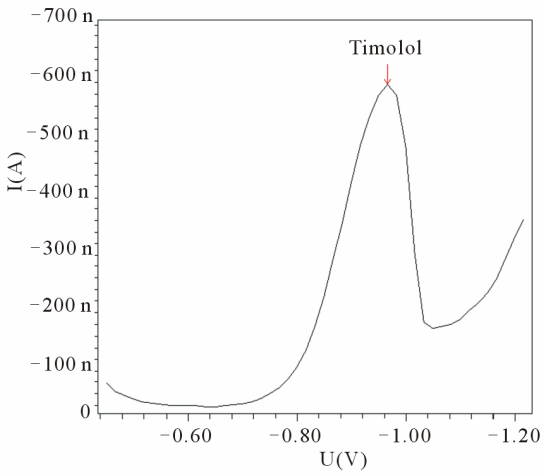


Figure 3. SW-AdSV voltammogram of 5 × 10^{-7} mol·l^{-1} Timolol in pH 3.5 Acetate buffer. Accumulation time 30 sec, accumulation potential –0.8 V and scan rate 250 mV·s^{-1}.

of 5 × 10^{-7} mol·l^{-1} Timolol in pH 3.5 Acetate buffer. Accumulation time 30 sec, accumulation potential –0.8 V and scan rate 250 mV·s^{-1}), which illustrates a well observed electrochemical peak indicating a strong and readily adsorption process at the surface of the working electrode.

3.1. Parameters Affecting the Adsorptive Stripping Response

3.1.1. Effect of Supporting Electrolyte and pH

The nature and acidity of the supporting buffer are some of the most important factors which strongly influence the stability of the analyte and its cathodic reduction and adsorption processes. Among the various investigated buffers (B-R, acetate, carbonate and phosphate) the best voltammetric signal in terms of sensitivity (peak height) and resolution (peak shape) have been secured using acetate buffer. In addition, when the AdSV peak current was measured as a function of pH over 2-6 range, the stripping voltammetric signal increased steadily over the acidic region and the peak current reached its maximum value at pH 3.5 which was selected as optimal value for subsequent studies. It is noteworthy that when more than 3.5 acetate supporting electrolyte was used, timolol was barely detectable and nearly no stripping voltammetric signal was observed. The variation of AdSV peak current with pH, obtained for 1 × 10^{-6} mol·l^{-1} Timolol drug concentration accumulated for 30 sec is exhibited in **Figure 4** (Effect of pH on AdSV peak current of 1 × 10^{-6} M Timolol at Acetate buffer).

3.1.2. Effect of Accumulation Time and Potential

Preconcentration of the analyzed drug on the surface of the working electrode (HMDE) is one of the essential

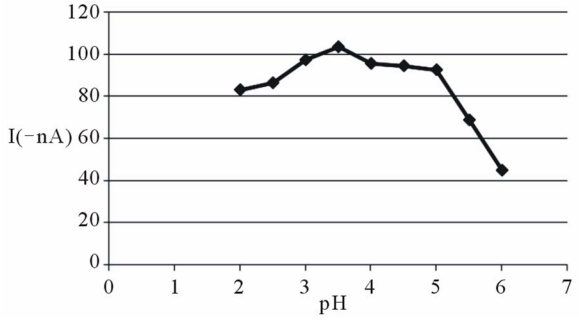


Figure 4. Effect of pH on AdSV peak current of 1 × 10^{-6} M Timolol at Acetate buffer.

conditions for highly sensitive determinations. Variation of the accumulation time over 0 - 150 sec period for 1 × 10^{-6} mol·l^{-1} timolol drug solution at a preconcentration potential of 0.0 V, showed a gradual enhancement for the monitored peak current over the range 0 - 30 sec. The dependence of peak current on accumulation time is presented in **Figure 5** (Effect of accumulation time on AdSV peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer). The proportional relationship was nearly ob- served up to 30 sec and then it became virtually curved and leveled off owing to the saturation of the hanging mercury drop by the analyte. For further experiments an accumulation time of 30 sec was selected as optimal because it provided relatively high peak current with adequate practical time. The variation of accumulation time did not produce significant shifts in peak potential value.

In addition, as can be seen from **Figure 6** (Effect of accumulation potential on AdSV peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer and accumulation time 30 sec), when the influence of accumulation potential on the monitored electrochemical response was examined over the –0.8 to +0.8 V range at 30 sec preconcentration time, the peak current was directly reached its maximum value at E_p = –0.8 V then it decreased sharply after potential –0.8V. Thus, E_{acc}= 0.8 V will be adopted as optimum operational value for the following works as it ensured the highest AdSV signal.

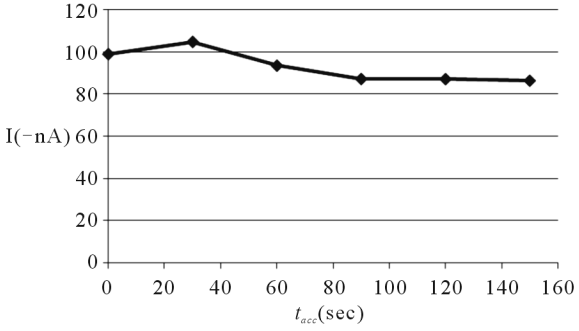


Figure 5. Effect of accumulation time on AdSV peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer.

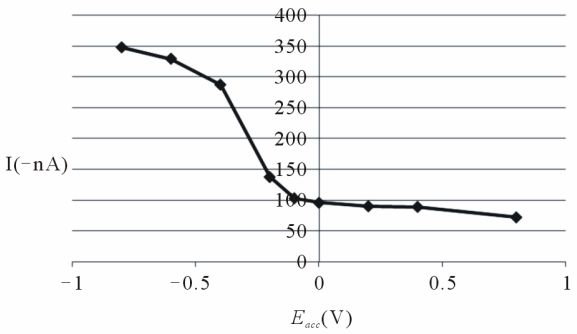


Figure 6. Effect of accumulation potential on AdSV peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer and accumulation time 30 sec.

3.1.3. Effect of Scan Rat

The cathodic peak current of timolol drug was found to be forthwith proportional to the scan rate, particularly at low scan rate values, a phenomenon characterized for adsorbed materials [24]. When the stripping voltammetric peak current of 1 × 10^{-6} mol·l^{-1} timolol drug in pH 3.5 acetate buffer was measured over the range 10 - 300 mV/s, it was found that peak height was observed in the scan rate 250 mV/s as it was shown in **Figure 7** (Effect of scan rate on AdSV peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer, accumulation time 30 sec and accumulation potential –0.8 V). However, after this maximum value the peak current started to decrease directly with faster scan rate. Accordingly, 250 mV/s scan rate value was adopted as optimum condition for further investigations.

3.1.4. Effect of Pulse Amplitude and Frequency

In addition, the impact of varying the excitation wave pulse amplitude on the voltammetric current intensity was also evaluated. The effect of this operating variable was studied over the rang 10 - 120 mV (see **Figure 8**: Effect of pulse amplitude on the peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer, accumulation time 30 sec, accumulation potential –0.8 V and 250 mV/s scan

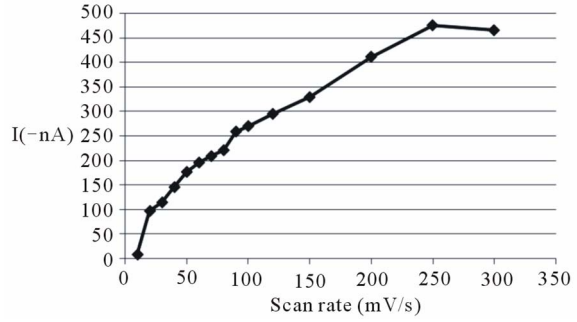


Figure 7. Effect of scan rate on AdSV peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer, accumulation time 30 sec and accumulation potential -0.8 V.

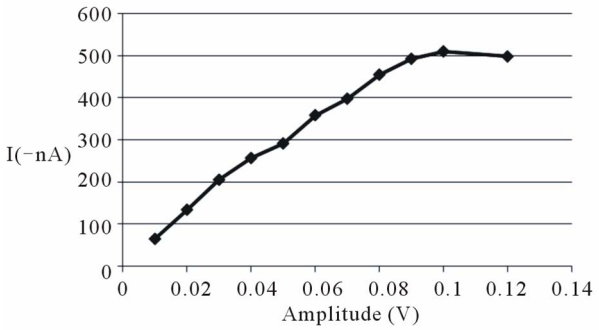


Figure 8. Effect of pulse amplitude on the peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer, accumulation time 30 sec, accumulation potential –0.8 V and 250 mV/s scan rate.

rate) and it was concluded that in order to assure maximum peak current, 100 mV pulse amplitude was the ideal choice for this operational parameter. Moreover, varying the value of square wave frequency also plays an important role for the measured signal of square wave- adsorptive stripping voltammetric (SW-AdSV) approach. When the voltammetric peak current of 1 × 10^{-6} mol·l^{-1} timolol drug in acetate buffer pH 3.5 was measured over the range 10 - 50 Hz, as in **Figure 9** (Effect of frequency on the peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer, accumulation time 30 sec, accumulation potential –0.8 V, Scan rate 250 mV/s and pulse amplitude 0.10 V), it was found that peak height was observed at a low frequency 10 - 15Hz only, after this value (15Hz) the peak current started to decrease with increasing frequency. Accordingly, for farther work 15 Hz square wave frequency value was adopted.

3.1.5. Effect of Instrumental Parameters

The monitored adsorptive stripping voltammetry (AdSV) peak height could be further maximized by optimizing other experimental factors that can affect the adsorption process of the formed drug. The influence of both the surface size of the mercury drop working electrode and electrode convection rate was also evaluated. An increase in the surface of the working electrode (over 0.15 - 0.60 mm^2) yielded, as expected, a linear enhancement in the analytical signal and did not affect the value of the stripping voltammetric potential. In addition, an increase in the stirring rate (raising it from 0.0 to 3000 rpm) yielded, a linear enhancement in the analytical signal from 0.0 to 2000 rpm, after that it is decreased and did not affect the value of the stripping voltammetric potential. Thus, for optimal sensitivity, 0.60 mm^2 drop size and 2000 rpm stirring speed were chosen for subsequent practical works (see **Figures 10**: Effect of electrode area on the peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer, accumulation time 30 sec, accumulation potential –0.8 V, Scan rate 250 mV/s, pulse amplitude 0.10 V and frequency 15 Hz, **Figure 11**: Effect of convection rate on the peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer, accumulation time 30 sec, accumulation potential –0.8 V, Scan rate 250 mV/s, pulse amplitude 0.10 V, frequency 15 Hz and drop size 0.6 mm^2).

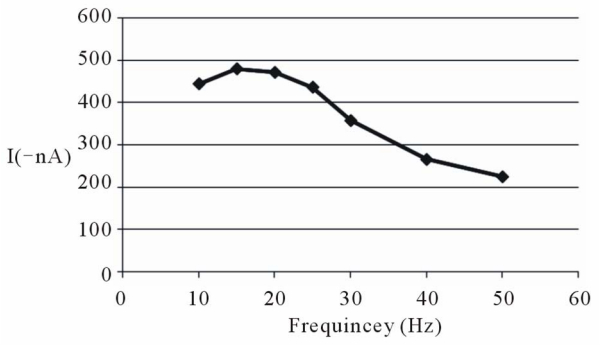


Figure 9. Effect of frequency on the peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer, accumulation time 30 sec, accumulation potential –0.8 V, Scan rate 250 mV/s and pulse amplitude 0.10 V.

3.2. Analytical Performance (Method Validation)

Once the most ideal and suitable chemical conditions and instrumental parameters for the adsorptive determination were established, a calibration plot for the analyzed drug was recorded to estimate the analytical characteristics of the developed method.

3.2.1. Calibration Graph

Under the optimum conditions a very good linear correlation was obtained between the monitored voltammetric peak current and timolol concentration in the range 1 × 10^{-7} - 1.5 × 10^{-6} mol·l^{-1}, is constant in all measurements, (see **Figure 12**: SW-AdSV voltammogram for Timolol in acetate buffer, pH = 3.5, T_{acc} = 30 sec, E_{acc} = –0.80 V.

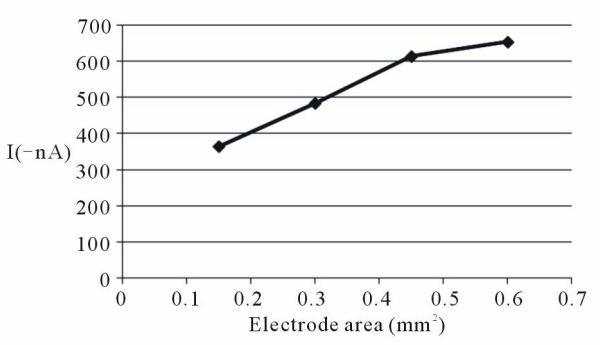


Figure 10. Effect of electrode area on the peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer, accumulation time 30 sec, accumulation potential –0.8 V, Scan rate 250 mV/s, pulse amplitude 0.10V and frequency 15 Hz.

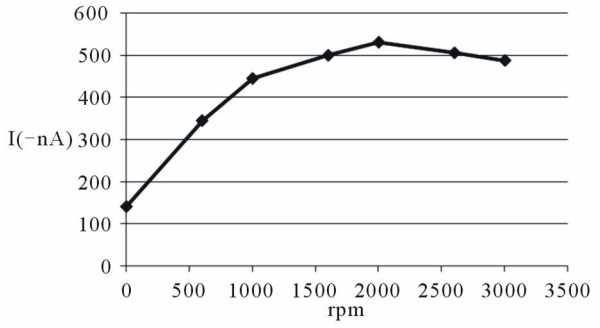


Figure 11. Effect of convection rate on the peak current of 1 × 10^{-6} M Timolol in pH 3.5 Acetate buffer, accumulation time 30 sec, accumulation potential –0.8 V, Scan rate 250 mV/s, pulse amplitude 0.10V, frequency 15Hz and drop size 0.6 mm^2.

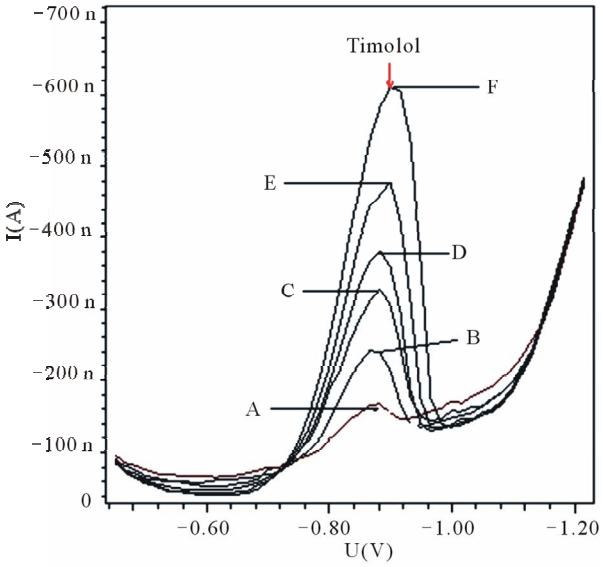


Figure 12. SW-AdSV voltammogram for Timolol in acetate buffer, pH = 3.5, T_{acc} =30 sec, E_{acc} = –0.80 V. Drug Conc.:- (A = 1 × 10^{-7} M, B = 3 × 10^{-7} M, C = 5 × 10^{-7} M, D = 7 × 10^{-7} M, E = 1 × 10^{-6} M, F = 1.5 × 10^{-6} M). Drug Conc.:- (A = 1 × 10^{-7} M, B = 3 × 10^{-7} M, C = 5 × 10^{-7} M, D = 7 × 10^{-7} M, E = 1 × 10^{-6} M, F = 1.5 × 10^{-6} M).

Least-square treatment of the calibration graph yielded the following regression equation:

$$i_p\ (\text{nA}) = 53.1 + 3.1 \times 10^8\ \text{C (mol l}^{-1}) \quad r = 0.998,\ n = 6.$$

where i_p is the adsorptive stripping peak current, C is the analysed drug concentration and r is the correlation coefficient.

3.2.2. Detection Limit

The lowest detectable concentration of this drug was 1.26 × 10^{-9} mol·l^{-1}(0.4 ppb), which was estimated based on the signal-to-noise ratio (S/N = 3) and this obtained analytical sensitivity is very promising since it was achieved after employing very short accumulation time (30 s) comparing to that reported for the previous voltammetric determination of timolol using differential pulse voltammetry (DPV) technique which required 2.5 ppb detection limit [23].

3.2.3. Reproducibility

The high sensitivity of adsorptive voltammetry is accompanied by very good reproducibility. This analytical performance was evaluated from eight repeated measurements of electrochemical signal of 1 × 10^{-6} mol·l^{-1} timolol drug solution. The precision of the electrochemical developed method in terms of the relative standard deviation (RSD%) was 0.13%.

3.2.4. Accuracy

The accuracy of the proposed method was checked by calculating the recovery of known amount of timolol (5 × 10^{-7} mol·l^{-1}) solution added to acetate buffer solution and analysed via the optimized stripping voltammetric procedure. The value of the recovery obtained by the standard addition method was 110% ± 1.414.

3.2.5. Stability

Under the optimum conditions, the stability of 1 × 10^{-6} mol·l^{-1} timolol solution was evaluated by monitoring the changes in the height of adsorptive stripping voltammetric (AdSV) peak over a period of 90 min. The electroanalytical signal was gradually constant with time. The acidic media (pH 3.5) of the acetate electrolyte solution probably initiated a slow degradation process for the drug.

3.3. Interference Studies

In order to evaluate the selectivity of the developed AdSV procedure, the influence of various interferences was examined. Considerable interference can be caused by co-existing surface-active compounds capable of competing with the analyte of interest for the adsorption site on the electrode surface, resulting in decreased or increased peak height. The competitive co-adsorption interference was evaluated in the presence of various substance usually occur in the pharmaceutical eye-drops and formulations. For these investigations, the interfering species were added at different concentrations (one, 5-fold and 50-fold) higher than the concentration of timolol (1 × 10^{-6} mol·l^{-1}). The addition of starch at these concentration levels caused the adsorptive stripping voltammetric (AdSV) peak current to decrease by about 3%, 4% and 6%, respectively, of its original peak current. Also the addition of 50-fold of sucrose in the test drug solution (1 × 10^{-6} mol·l^{-1}), caused the stripping voltammetric peak current to decrease by about 8%. Apparently,

these inhibition effects were caused by the working electrode surface blockage due to adsorption of interferences. In contrast, the addition of 50-fold of lactose in the drug solution, caused the square wave adsorptive stripping voltammetry (SW-AdSV) response of the drug to increase by about 16%.

3.4. Practical Applications

The reliability of the proposed adsorptive stripping voltammetry (AdSV) method for the determination of timolol drug was investigated by assaying this drug in some real samples. Following the developed electroanalytical procedure described above, timolol drug was analysed in pharmaceutical formulation. The timolol content of commercially available eye drop (Ocumol 0.5, Timolol 0.5%, 5 mg/ml) was determination directly by the square wave adsorptive stripping voltammetry (SW-AdSV) method after the required dissolving steps. Four aliquots of the dissolved sample were diluted to the required concentration level and measured via the standard additions approach. For these studies, results obtained gave a recovery mean 80% with standard deviation of ± 2.94%. As can be seen from **Table 1** (Analysis of Timolol drug in its commercial eye drop).

In addition, the applicability of the stripping voltammetric procedure for the analysis of timolol drug in biological samples was also evaluated by estimating its recoveries from spiked human urine and serum samples. A simple and fast pretreatment (clean-up) procedure, which is in fact a slight modification of the sample preparation method develop for the determination of some antagonist drugs [25] was used. By adding a small amount of 5% $ZnSO_4 \cdot 7H_2O$ solution, NaOH and methanol to the serum sample and centrifuging the mixture, most of the interfering substances (mainly proteins) were simply removed and eliminated by precipitation. As can be extracted from **Table 2** (Analytical results for timolol drug recoveries from urine and serum samples), this adsorptive stripping voltammetric (AdSV) method (after appropriate dilution) allowed the determination of timolol drug in urine and serum samples with mean recoveries 87% ± 1.83 and 107.5% ± kmi 1.3, respectively.

Table 1. Analysis of Timolol drug in its commercial eye drop.

	Found (mg)	Recovery %
Labeled	3.85	77
Content	3.90	78
5 mg/ml	4.10	82
Timolol	4.15	83
	Mean	80
	Standard Deviation	±2.94

Table 2. Analytical results for timolol drug recoveries from urine and serum samples.

Recovery%:	Spiked Urine	Spiked Serum
	86	108
	88	107
Added Timolol	85	109
5.0×10^{-7} mol·l^{-1}	89	106
Means	87	107.5
Standard Deviations	**±1.83**	**±1.3**

4. Conclusions

Voltammetric methods have proved to be very sensitive for the determination of organic molecules, including drugs and related molecules in pharmaceutical dosage forms. These techniques have several advantages, including that they are quick and reproducible, present low limits of detection and quantification and have relatively low cost compared with the more traditional techniques. Moreover, they provide a better discrimination against background currents.

In this study, a new, simple, selective, accurate and precise SW-AdSV method developed for the determination of Timolol in pharmaceutical formulation. In this study, all experimental and instrumental parameters were optimized, as their values strongly affect the sensitivity of the voltammetry. The peak current was investigated using differential pulse polarography and cyclic voltammetry. The electrochemical reduction of Timolol is an irreversible process controlled by adsorption under the conditions described in this work. The proposed method with the optimized parameters demonstrated a good linear relationship between the peak current and the Timolol concentration for a wide range of concentration. The applicability of the proposed procedure was tested using a commercial pharmaceutical formulation of Timolol. This drug was quantified in the pharmaceutical preparation and biological fluids such as serum and urine, and no pretreatment or time - consuming extraction was required prior to the analysis. The results are in good agreement with the labeled values. Accuracy and selectivity of the developed method were demonstrated by recovery studies. Reproducibility, stability, and interferences studies of this proposed method suggest that this method could be used in quality control analysis, clinical laboratories, and pharmacokinetic studies.

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