ELECTROCHEMICAL OXIDATION OF ATORVASTATIN AND ITS ADSORPTIVE STRIPPING DETERMINATION IN PHARMACEUTICAL DOSAGE FORMS AND BIOLOGICAL FLUIDS

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Electrochemical behavior of atorvastatin (AT) and optimum conditions to its quantitative determination were investigated using voltammetric methods. Some electrochemical parameters such as diffusion coefficient, surface coverage of adsorbed molecules, electron transfer coefficient, standard rate constant and number of electrons were calculated using the results of cyclic voltammetry. A tentative mechanism for the oxidation for AT has been suggested. The oxidation signal of AT molecule was used to develop fully validated, new, rapid, selective and simple square-wave anodic adsorptive stripping voltammetric (AdsSWV) and differential pulse anodic stripping voltammetric (AdsDPV) methods to direct determination of AT in pharmaceutical dosage forms and biological samples. For the AdsDPV and AdsSWV techniques, linear working ranges were found to be 1.0×10^{-7}–5.0×10^{-6} and 3.0×10^{-7}–5.0×10^{-6} mol l^{-1}, respectively. The detection limits obtained from AdsDPV and AdsSWV were calculated to be 6.55×10^{-8} and 1.53×10^{-7} mol l^{-1}, respectively. The methods were successfully applied to assay the drug in tablets, human blood serum and human urine.

Keywords: Atorvastatin; Cholesterol lowering agent; Anodic adsorptive stripping voltammetry; Pharmaceuticals; Human serum; Urine; Glassy carbon electrode.

Atorvastatin (AT), [*R*-(*R**,*R**)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid calcium salt, is an active substance of some drugs which are reducing cholesterol and triglyceride. This kind of substances, such as lovastatin, simivastatin, pravastatin, fluvastatin, cerivastatin, rosuvastatin and pitavastatin[1] is known as statins. These are competitive inhibitor for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase which catalysis the reduction of 3-hydroxy-3-methylglutaryl coenzyme A to

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mevalonate, and this step is the rate-limiting step in biosynthesis of cholesterol[1]. The chemical structure of atorvastatin is given in Fig. 1.

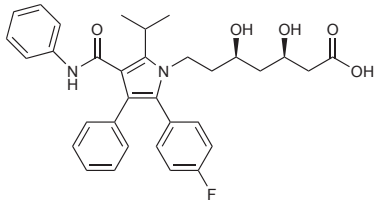


FIG. 1
Chemical structure of AT

Different analytical methods have been carried out the determination of AT in pharmaceutical samples or biological fluids including high-performance liquid chromatography[1–7], capillary electrophoresis techniques[8], spectrophotometry[9,10] and electrochemical methods[11–15]. Electrochemical stripping methods carried out by the deposition with the preconcentration of an analyte onto the working electrode surface are one of the most suitable methods for the determination of several pharmaceutical compounds. Moreover, these methods have the advantage of being relatively less expensive, highly sensitive and they have a low limit of detection. Therefore, electrochemical stripping methods are becoming more and more popular as alternative methods in pharmaceutical formulations and biological samples[16].

The electroanalytical determination of AT has been studied by voltammetric and polarographic methods[11–14]. However, there is no detailed electrode reaction mechanism for AT. For this reason, we aimed to investigate the detailed electrochemical oxidation behavior and electrode reaction mechanism of AT using voltammetric methods. Current study was also aimed to develop new validated square-wave adsorptive stripping and differential pulse adsorptive stripping voltammetric assay methods with lower detection limit than given in electrochemical studies for direct determination of AT in different samples including pharmaceutical preparations, human serum and human urine was one of the other aims of present study.

EXPERIMENTAL

Apparatus

All voltammetric measurements such as cyclic voltammetry (CV), square-wave anodic adsorptive stripping voltammetry (AdsSWV) and diffential pulse adsorptive stripping

voltammetry (AdsDPV) were carried out using a Bioanalytical Systems (BAS 100 B, USA) electrochemical analyzer. A three-electrode cell system incorporating glassy carbon electrode (MF-2012) as a working electrode, platinum wire as an auxiliary electrode (BAS MW-1034) and an Ag|AgCl reference electrode (MF-2052 RE-5B) were used in all experiments. IR spectra (ν, cm^{-1}) were recorded on a Mattson 1000 FTIR spectrometer in KBr disc.

Prior to each experiment, glassy carbon electrode, which had 0.071 cm^2 area, was polished successively in 1, 0.3 and 0.05 μm alumina slurries made of dry Buehler alumina on a smooth polishing cloth, rinsed with deionized water. Polished glassy carbon electrode was sonicated in methanol before each use.

All pH measurements were made with Thermo Orion Model 720A pH ion meter having an Orion combined glass pH electrode (912600) calibrated with pH 4.13 (potassium hydrogen phthalate) and pH 8.20 (sodium bicarbonate) stock buffer solutions before measurements.

Double-distilled deionized water was supplied from Human Power I^+, Ultra Pure Water System. All the data were obtained at ambient temperature.

Reagents and Solutions

Standard sample of AT (99.0%, from Hifzissihha) was used to prepare the stock solution. Stock solution of AT was prepared by dissolution of precisely weighed amounts of atorvastatin calcium in ethanol in order to have the AT concentration of 2.295×10^{-3} mol l^{-1} (1.28 g l^{-1}). Calibration solutions were prepared by diluting the stock solution with Britton–Robinson buffer (BR) and pH values of these solutions were adjusted using 0.2 M NaOH solutions.

All chemicals used in preparation of BR buffer, such as phosphoric acid (Riedel), boric acid (Riedel), acetic acid (Merck) and sodium hydroxide (Merck) to adjust the pH of supporting electrolyte were of analytical reagent grade. Double-distilled deionized water was used in preparations of all the solutions.

All AT solutions were protected from light and were used within 24 h to avoid decomposition. However, electrochemical response of sample solutions recorded after preparation did not show any significant change in following studies.

Preparation and Analysis of Samples

Tarden tablets (from Abdi Ibrahim) were used as pharmaceutical dosage form which contains 40 mg of AT and some amount excipients per tablet. To prepare the solutions of tablets, initially the drug content of five tablets was weighed, finely powdered and mixed. The average mass per tablet was determined. A sample equivalent to one tablet was weighed and transferred into the calibrated flask of 250.0 ml volume and completed to the mark with ethanol. The contents of the flasks were sonicated for about 15 min to achieve complete dissolution of AT. After dissolution step, the content of the flask was centrifuged at 1500 rpm for 15 min. This solution was kept at refrigerator and given the name stock tablet solution. Sufficient volumes from stock tablet solutions were transferred to electrochemical cell containing 10.0 ml of BR buffer, pH was adjusted to desired value and performed determination of AT in tablets using direct calibration methods.

Similarly, spiked human serum and urine samples were analyzed. Serum and urine samples, obtained from healthy individuals, were stored frozen until assay. After gentle thawing, 1.0 ml of aliquot volumes of serum (or urine) was added to electrochemical cell containing

9.0 ml of BR buffer and then sufficient volumes from stock tablet solution were transferred to this cell. After deareation with argon, measurements were performed to determine AT content of cell using direct calibration methods.

Voltammetric Procedure

The pH value of the solution containing AT in appropriate concentration was adjusted with BR buffer. For the remove of the dissolved oxygen, argon was bubbled through a 5.0-ml portion of this solution for 2 min. After the deposition process under the optimized experimental conditions, differential pulse and square wave voltammograms were recorded by scanning in the anodic direction (from +0.70 to +1.40 V). The optimum parameters for the experiments performed by the two methods are AdsDPV: differential pulse amplitude 0.010 V, pulse width 15 ms, scan rate 0.020 V s^{-1} and AdsSWV: frequency 15 Hz, square wave amplitude 0.025 V, potential step 0.004 V, scan rate 0.060 V s^{-1}. The calibration graphs were plotted for AT as peak currents versus AT concentrations. Furthermore, voltammograms of the blank solutions were recorded to seek for a suitable working range.

RESULTS AND DISCUSSION

Electrochemical Behavior of AT

The electrochemical behavior, diffusion and adsorption properties of AT were studied using CV. As shown in Fig. 2, in cyclic voltammetric studies one well-defined oxidation peak was observed at a potential of about 1.0 V at pH 2.5 (100 mV s^{-1} vs Ag|AgCl electrode). There was no peak when a blank BR solution was scanned at the same conditions, and peak intensity increases linearly with increasing concentration of AT, concluded that this

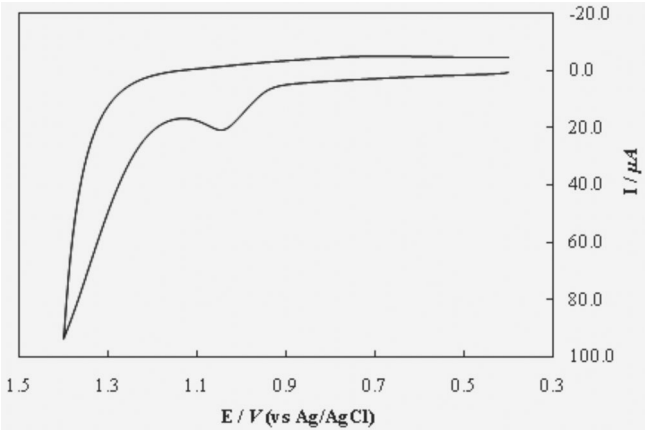


FIG. 2
Cyclic voltammogram of 3.41×10^{-4} M AT solution in BR buffer at pH 2.5 at scan rate of 0.10 V s^{-1}

anodic oxidation peaks are due to the oxidation of AT molecules. As can be seen from Fig. 2, no reduction peak was observed in the reverse scan.

The influence of the potential scan rate on anodic peak potential ($E_{p,a}$) and anodic peak current ($i_{p,a}$) at glassy carbon electrode were investigated for 6.65×10^{-5} M AT in the 0.01–1.0 V s^{-1} range. The peak potential shifts to more anodic values with increasing scan rate (Fig. 3). This behavior indicates the irreversible nature of oxidation process. When the scan rate varied from 0.01 to 1.0 V s^{-1} in 6.65×10^{-5} M solution of AT, a linear dependence of the anodic peak current $i_{p,c}$ (μA) upon the scan rate (in V s^{-1}) was found as given by equation $i_{p,a}$ (μA) = 1.318ν – 2.080 with R^2 = 0.9921, confirmed an adsorption behavior. Also a plot of logarithm of peak current (in A) versus logarithm of scan rate (in V s^{-1}) gave a straight line with a slope of nearly 0.60 for AT (inset in Fig. 3). Also the plot of peak current versus square root of scan rate was constructed and this graph is not linear even if the scan rate is extremely low or extremely high.

Furthermore, to investigate the electrode process for AT, CV voltammograms with 10 cycles are recorded. The oxidation peak current of AT indicated a spectacular decrease during the successive cyclic voltametric sweeps as seen in Fig. 4. The peak current decreased highly after the second scan and then the other scans remained unchanged.

These results confirm that the electrode process is controlled by adsorption under diffusion condition[11].

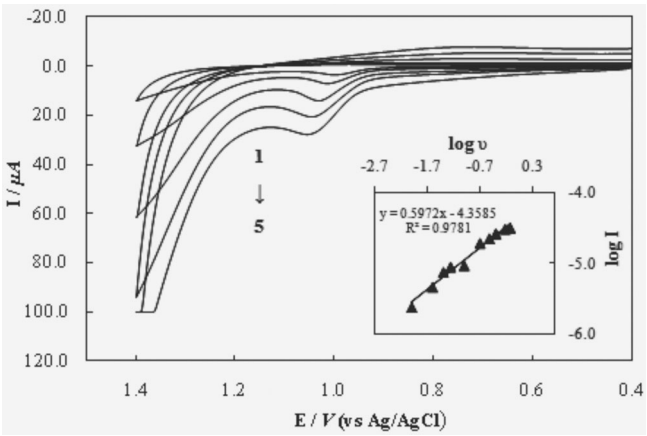


FIG. 3

Cyclic voltammograms of 3.41×10^{-4} M AT in BR buffer at pH 2.5 and at different scan rates (in V s^{-1}): 0.01 (*1*), 0.025 (*2*), 0.050 (*3*), 0.10 (*4*) and 0.20 (*5*). Inset: The plot of logarithm of peak current versus logarithm of scan rate (in V s^{-1})

The pH of a solution is a critical factor affecting both the rate and equilibrium state of the accumulation process and the rate of the electrode reaction. The pH values of solutions containing 3.76×10^{-5} M AT were adjusted in the range of 2.0–7.0 successive ΔpH being 0.5 unit before pH 4.0 and 1.0 unit after pH 4.0. Then CV voltammograms were recorded. The oxidation peak current for AT reaches the maximum value at pH close to 2.5 (Fig. 5), and this was selected as optimum value for quantitative analysis.

The pH is also one of the variables that commonly and strongly influence the electrochemical behavior of molecules. Before pH 4, potential remains pH-independent (Fig. 5). It indicates that the electroactive grouping respon-

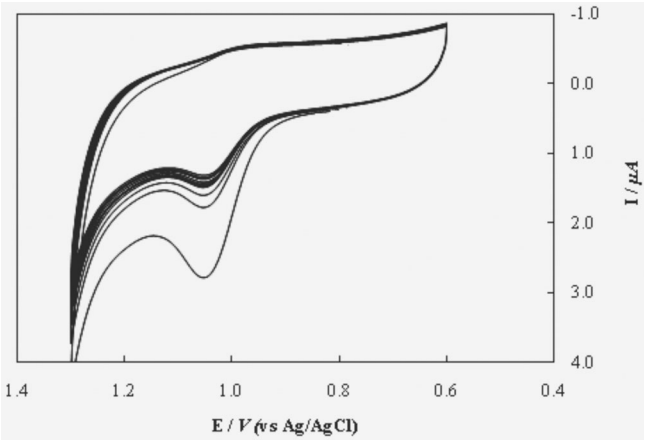


FIG. 4
Successive CV voltamograms of 3.0×10^{-5} M AT in BR buffer at scan rate of 0.10 V s^{-1}. Voltammogram includes 10 cycles

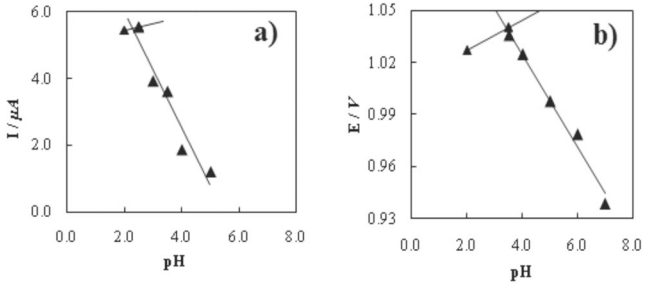


FIG. 5
Influence of pH on peak current (a) and peak potential (b) on CV voltammograms of 3.76×10^{-5} M AT

sible for the oxidation process is in acid–base equilibrium with pK_a about 4.0. The intersection of the curves is placed about pH 4.0, which is supposed to correspond to the pK_a value of atorvastatin[11].

In CV studies, peak potential changes linearly with the pH values (4.0–7.0) as given by equation E_p = 0.0257 pH – 1.13 with R^2 = 0.9838. The experimental value of the slope of the peak potential versus pH curves in CV studies was found to be 25.7 mV per unit pH value in given pH range. The slope is very close to the theoretical value of 30 mV per unit pH required for the assumed 2 e^-/H^+ process[11,16,19,20] of the electro oxidation of AT.

Based on literature, Eq. (*1*) was used in CV to find the number of protons in electrode mechanism[17].

$$E_p = E^0 + \frac{RT}{nF}\ln\frac{[Ox]}{[Red]} - \frac{\partial RT}{nF}\ln[H^+] \qquad (1)$$

In this equation ∂ is the number of proton participated in reaction mechanism and others are usual constants with their known values. Number of protons involved in reaction mechanism was found to be 1 from the slope value of the plot of E_p vs pH value.

To find out the number of electron(s), following relations proposed for adsorption process[16] were used in cyclic voltammetry

$$i_p = \frac{n^2F^2\Gamma A\nu}{4RT} \qquad (2)$$

and the relation

$$Q = nFA\Gamma \qquad (3)$$

where i_p is the peak current (A), Q is the charge (C) consumed by the surface process as calculated by the integration of the area under the peak, n is total number of electrons transferred in electrode reaction, Γ is the surface coverage of adsorbed substance (in mol cm^{-2}), A is the working glassy carbon electrode area (0.071 cm^2), F is the Faraday constant (96485 C mol^{-1}) and ν is the scanning rate (V s^{-1})[16,17]. By substitution the Γ term of Eq. (*3*) into Eq. (*2*), it is easy to get a new relation for n

$$n = \frac{4i_pRT}{FQ\nu}\,. \qquad (4)$$

In the scan rate range from 0.010 to 0.750 V s^{-1}, number of electron(s) transferred in electrode reaction (n) was calculated directly using an equation given above for each scan rate and using the slope of peak current versus scan rate. As a result of both methods, calculation and graph method, number of electrons in electrochemical step was found as 2.07 ± 0.38.

The surface coverage of adsorbed substance (Γ) was calculated from Eq. (*3*) and it was found as 7.27×10^{-11} mol cm^{-2} if $0.010 \leq \nu \leq 0.750$ V s^{-1}. So, from this value it is easy to say that each AT molecule at electrode surface occupies an area of 2.29 nm^2.

The following equation which expresses adsorption phenomena validated by Garrido[18] was used to calculate the diffusion coefficient of AT

$$i_p = 1.06 \times 10^6 n^2 ACvD^{1/2} t_p^{1/2}. \quad (5)$$

The mean of the diffusion coefficient calculated from this equation was obtained as 7.46×10^{-7} cm^2 s^{-1}.

Using data from CV studies and the Eq. (*6*) given below, electron transfer coefficient (α) was calculated[16].

$$E_p = k + \frac{RT}{n\alpha F} \ln \nu \quad (6)$$

According to this equation using the slope value of the plot of E_p vs ln ν and 2 for n, value of α was calculated as 0.58 and rate constant (k_s) was calculated according to equation given below[17]

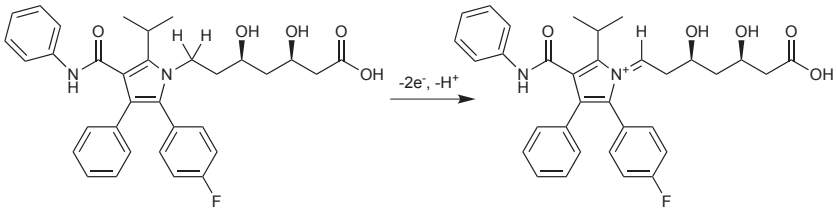
$$\ln k_s = \alpha \ln(1-\alpha) + (1-\alpha)\ln\alpha - \ln\frac{RT}{nF\nu} - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT} \quad (7)$$

and k_s value was found to be 1.73 s^{-1}.

Proposed Mechanism

Results of voltammetric studies given above and also bulk electrolysis carried out at 1.15 V were evaluated to propose a tentative electrode reaction mechanism for AT. The solutions of AT before and after bulk electrolysis were analyzed by IR spectrometry. The peak at 1625 cm^{-1} shows that at the end of the reaction there is a product which called iminium cation. Our results confirm that, the electroactive center corresponding to the anodic

peak was pyrrole ring as it can be observed from the same group member to give in 2 e^- and H^+ oxidation process. On the basis of all experimental results, it can be concluded that pyrrole ring of AT is oxidized to corresponding iminium cation as given in Scheme 1.



SCHEME 1

Proposed mechanisms and a similar type of mechanism are also given for the oxidation of pyrrole ring in literature[11,20–24].

Electroanalytical Determination of AT

The anodic peak is obviously suitable for use as the bases of a quantitative analytical technique for the determination of AT, because it is well resolved and adsorption controlled. This peak is also adequate for the precise and accurate measurement of current. Hence, all subsequent work was based on the measurement of the current at this peak potential.

Because of the adsorptive character of AT on glassy carbon electrode, the electrochemical assay of AT was established on adsorptive methods. These methods are effective and rapid electroanalytical techniques. Especially adsorptive stripping analysis greatly enhances the scope of stripping measurements toward numerous low amounts of organic compounds. Short adsorption times (1–5 min) result in a very effective interfacial accumulation[16]. In the present study, initially instrumental parameters and experimental conditions such as type and the concentration of supporting electrolyte, pH, AT concentration, deposition time and deposition potential were optimized for determination of AT. Two alternative techniques, AdsDPV and AdsSWV were employed.

The peak responses for the studied drug were affected by the type of supporting electrolytes. Two different supporting electrolytes were examined including BR buffer and 0.1 M H_2SO_4 solution. The highest peak current and the best peak shape were obtained in the presence of BR buffer contain-

ing 0.045 mol l^{-1} of each component, although peak current increases with increasing buffer concentration in the range from 0.01 to 0.20 mol l^{-1}.

To optimize the sensitivity and selectivity of the procedure, the effects of deposition potential and deposition time on the peak current were investigated. AdsDPV and AdsSWV voltammograms were recorded after deposition at various potentials with an increments of 0.10 V between 0.00 and 1.0 V. The graphs indicate that the peak currents for AT increase in the range from 0.00 to 0.40 V, then suddenly decrease in the range from 0.50 to 1.0 V. As a result, the optimum deposition potential was selected as 0.50 V for AdsDPV and 0.45 V for AdsSWV (Fig. 6).

For the choosing of optimum deposition time for each method, deposition was carried out in the range of 0–100 s (with increments of 15 s) and then stripping voltammograms were recorded by Adds and AdsSWV. Variations of peak currents with the deposition time for each method are shown in Fig. 7. The graphs indicate that peak current increases up till 50 s and

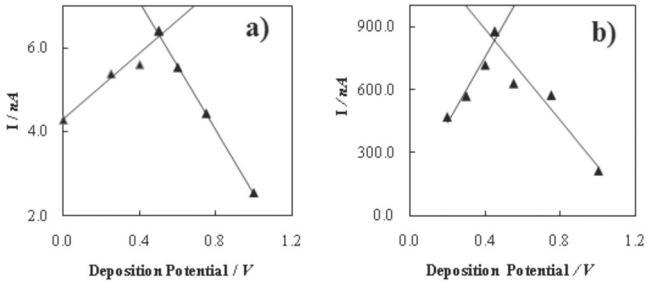


FIG. 6
Effect of deposition potential on peak current of 1.0×10^{-7} M AT at pH 2.5 in AdsDPV (a) and AdsSWV (b), deposition time 30 s

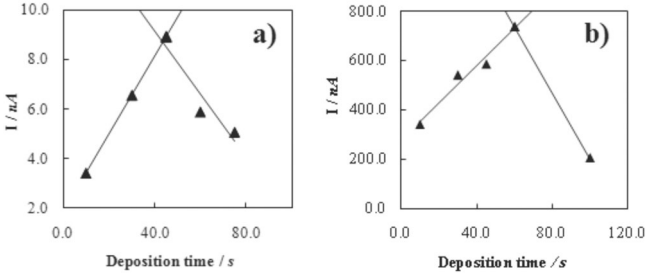


FIG. 7
Effect of deposition time on peak current of 1.0×10^{-7} M AT at pH 2.5 in AdsDPV (a) and AdsSWV (b)

then suddenly decreases in the range of 50–100 s for AdsDPV. A similar trial on the AdsSWV method was indicated. As a result of these experiments, deposition times of 45 and 60 s are suitable for AdsDPV and AdsSWV, respectively.

The another oxidation peak at more positive potential was observed. This peak was not given any attention anyway, as we concentrated on the first anodic oxidation. The second anodic peak is obviously suitable for use as the bases of a quantitative analytical technique for the determination of AT, because it is well resolved and adsorption controlled. This peak is also adequate for the precise and accurate measurement of current. Hence, all subsequent work was based on the measurement of current at this peak potential.

To establish the linearity range (working concentration range) of AT in different standard solutions for both methods, concentrations ranging from 8.0×10^{-8} to 3.0×10^{-5} mol l^{-1} were used. For each concentration, five reproducible measurements were taken and mean of these measurements was used to plot the calibration curve. Result of concentration studies showed that an average peak current of oxidation peak is changed linearly with AT concentration in the range from 1.0×10^{-7} mol l^{-1} (0.056 mg l^{-1}) to 5.0×10^{-6} mol l^{-1} (2.8 mg l^{-1}) for AdsDPV and 3.0×10^{-7} mol l^{-1} (0.168 mg l^{-1}) to 5.0×10^{-6} mol l^{-1} (2.8 mg l^{-1}) for AdsSWV. The calibration curves can be seen in the insets of Figs 8 and 9.

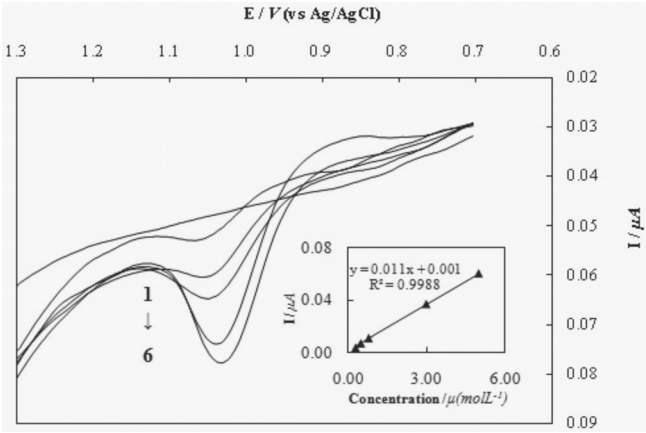


FIG. 8
Voltamograms of AT at different concentrations in AdsDPV (in μmol l^{-1}): base line (*1*), 0.3 (*2*), 0.5 (*3*), 0.8 (*4*), 3 (*5*) and 5 (*6*). Inset: The calibration curve

The characteristics of the calibration plots were summarized in Table III.

Application of Method to Dosage Form and Biological Samples

In order to evaluate the adequacy of the proposed method, AT was determined by quantifying commercial pharmaceutical tablets of Tarden (labeled as 40 mg AT per tablet). No pretreatment such as time-consuming extraction or evaporation step was required for sample preparation. The proposed AdsDPV and AdsSWV methods were applied to the direct determination of AT in pharmaceutical dosage forms using standart addition calibration method. The results of analysis found using proposed method for pharmaceutical preparations are given in Table I, for spiked human urine and for spiked human serum are given in Table II. The accuracy of the proposed method was determined by its recovery values.

It can be seen from Tables I and II that average recovery values are in good agreement with the RSD values less than 10%, which is good evidence of validity of method. Thus, the precision is very satisfying for the analysis of biological samples as well as bulk formulation. These results indicate that the content of AT in the pharmaceuticals and biological fluids can be safely determined using proposed voltammetric method without interference from other substances in the samples. The proposed method can be applied to pharmaceuticals, human serum and human urine after a simple dilution step with direct measurements.

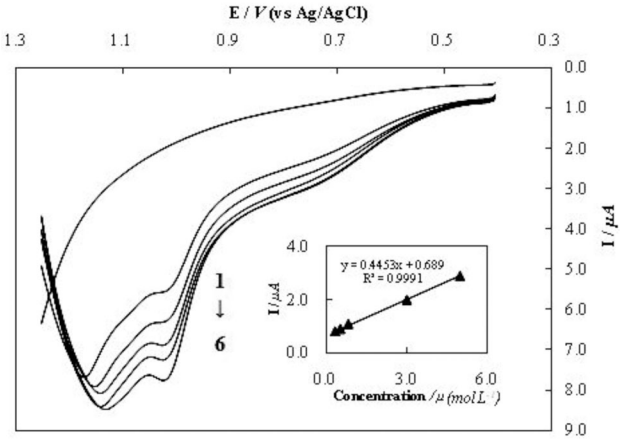


FIG. 9

Voltamograms of AT at different concentrations in AdsSWV (in μmol l^{-1}): base line (*1*), 0.3 (*2*), 0.5 (*3*), 0.8 (*4*), 3 (*5*) and 5 (*6*). Inset: The calibration curve

TABLE I
Results of AT amounts in Tarden tablets determined using proposed AdsDPV and AdsSWV methods

Parameter	AdsDPV	AdsSWV
Nominal value per table, mg	40.00	40.00
Amount found[a], mg	39.02 (±2.49)	39.24 (±0.93)
Recovery value[b], %	97.53 (±4.95)	98.10 (±1.85)
RSD[c], %	6.23	2.33
F-test	$F_{experimental} = 1.95$	
	$F_{critical} = 9.28$ (95% confidence level)	

[a] Each value of the mean 4 experiments. [b] Results of recovery values are given as mean ± $ts/\sqrt{n}$ (at 95% confidence level). [c] RSD is relative standard deviation.

TABLE II
Results of AT amounts in human urine and human serum spiked tablet AT determined using proposed AdsDPV and AdsSWV methods

Parameter	AdsDPV		AdsSWV	
	Human urine	Human serum	Human urine	Human serum
Reference concentration, mol l^{-1}	5.0×10^{-7}	5.0×10^{-7}	5.0×10^{-7}	5.0×10^{-7}
	8.0×10^{-7}	8.0×10^{-7}	8.0×10^{-7}	8.0×10^{-7}
	1.0×10^{-6}	1.0×10^{-6}	1.0×10^{-6}	1.0×10^{-6}
	3.0×10^{-6}	3.0×10^{-6}	3.0×10^{-6}	3.0×10^{-6}
Found concentration, mol l^{-1}	5.05×10^{-7}	5.02×10^{-7}	5.07×10^{-7}	4.91×10^{-7}
	8.15×10^{-7}	7.88×10^{-7}	8.01×10^{-7}	7.99×10^{-7}
	9.43×10^{-6}	9.86×10^{-6}	9.97×10^{-7}	1.00×10^{-6}
	3.04×10^{-6}	3.01×10^{-6}	2.99×10^{-6}	2.95×10^{-6}
Recovery value[a], %	99.57 (±2.83)	99.46 (±0.79)	100.29 (±0.60)	99.17 (±0.77)
RSD[b], %	3.57	1.00	0.75	0.97

[a] Results of recovery values are given as mean ± $ts/\sqrt{n}$ (at 95% confidence level). [b] RSD is relative standard deviation.

Method Validation

Validation of an analytical method is the process by which it is established that the performance characteristics of the method meets the requirements for the intended analytical applications. The elements required for method validation are linearity range, limits of detection and quantitation, accuracy, repeatability, stability, selectivity and robustness[25].

To establish the working concentration range (linearity range) of AT in AdsDPV and AdsSWV, eleven different standard solutions with concentrations ranging from 8.0×10^{-8} to 3.0×10^{-5} mol l^{-1} were used. A good linearity is evident from values of correlation coefficient (R^2) of 0.999 for AdsDPV and AdsSWV (Figs 8 and 9). The characteristics of the calibration plots are summarized in Table III.

Limit of detection (LOD) and limit of quantitation (LOQ) values were calculated using the relations LOD = 3 s m^{-1} and LOQ = 10 s m^{-1} (ref.[26]). The abbreviation of *m* is the slope of the related calibration curve and *s* is the standard deviation of working concentration values of AT which is selected as 3.0×10^{-7} and 5.0×10^{-7} for AdsDPV and AdsSWV, respectively. At these concentrations, five voltammograms were recorded for each method and then the standard deviation is calculated for each method. After that, LOD and LOQ values were found as 6.55×10^{-8} mol l^{-1} (0.036 mg l^{-1}) and 2.18×10^{-7} mol l^{-1} (0.122 mg l^{-1}) for AdsDPV, 1.53×10^{-7} mol l^{-1} (0.085 mg l^{-1}) and 5.16×10^{-7} mol l^{-1} (0.288 mg l^{-1}) for AdsSWV, respectively.

TABLE III
Regression data of the calibration curve for assay of AT by AdsDPV and AdsSWV

Calibration parameter	AdsDPV	AdsSWV
Linearity range, mol l^{-1}	1.0×10^{-7}–5.0×10^{-6}	3.0×10^{-7}–5.0×10^{-6}
Slope of calibration curve, A l mol^{-1}	0.0110	0.4450
Intercept, A	1.0×10^{-9}	6.89×10^{-7}
SD of slope	0.000188	0.0088
SD of intercept	0.5×10^{-9}	2.33×10^{-10}
Limit of detection (LOD), mol l^{-1}	6.55×10^{-8}	1.53×10^{-7}
Limit of quantification (LOQ), mol l^{-1}	2.18×10^{-7}	5.16×10^{-7}
Regression coefficient, R^2	0.999	0.999
Repeatability of peak current, RSD%	3.29	2.73
Repeatability of peak potential, RSD%	0.46	0.41

The accuracy of measurements by means of the described procedure were checked calculating the recovery of a known concentration of AT following proposed methods at optimum instrumental and experimental conditions. For AdsDPV, the recovery values of AT are ranging between 91.37 and 106.0% for tablet analysis, found between 94.27 and 101.81% for urine analysis and between 98.53 and 100.38% for serum analysis. For AdsSWV between 95.80 and 100.75% for tablet analysis, found between 99.70 and 101.39% for urine analysis and between 98.27% and 100.11% for serum analysis (see Tables I and II). From these recovery values it is concluded that proposed method is highly accurate.

This analytical performance was evaluated from five repeated measurements of electrochemical signal of different AT solutions following the proposed methods. The accuracy of the proposed methods is excellent because the relative standard deviation of recovery values ranges between 0.75 and 6.23% for all measurement including tablets, urine and serum samples (see Tables I and II).

F-test is applied in order to compare precision of two proposed methods. According to the test, the value of experimental *F* is lower than the critical one. These results show that there is no significant precision difference between the proposed AdsDPV and AdsSWV methods.

The stability of AT in a BR buffer of pH 2.5 was evaluated under the optimal procedural conditions by monitoring the changes in both the anodic peak potential and the anodic peak current of standard AT solution and repeatabilities of peak current and peak potential were found to be as 3.29 and 0.46% for AdsDPV, 2.73 and 0.41% for AdsSWV, respectively (Table III). As a result, there is no significant change in peak potential and peak current confirmed the stability of AT over the time period of measurements. Also these results show that the precision of the proposed methods is excellent. Furthermore, AT solution was found to be stable at least 2 months when kept in refrigerator.

During an application of proposed method to biological samples and tablets, before adding a standard solution of AT, voltammetric base line of biological medium was measured by applying the same procedures applied to calibration studies with standard samples. In such applications, no extra voltammetric signal in studied potential window indicates that there is no significant interference of various inorganic cations, anions and some organic substances found in pharmaceutical preparations (tablets) and biological mediums (human urine and human serum). These results show that oxidation peak is specific for AT and this peak can be used selectively to determine the AT in biological fluids.

The robustness[27] of the measurements by means of the described AdsSWV and AdsDPV procedures to assay of AT was examined by studying the effect of small variation of some important procedural conditions such as pH value, accumulation potential, accumulation time and room temperature of different days. Small changes (±1%) in such conditions do not affect the recovery of procedure.

CONCLUSION

In this study, the electrochemical oxidation behavior of AT was studied on glassy carbon electrode. Electrochemical behavior of pharmaceutical compounds may have valuable findings in either understanding the mechanism of their action or determining their concentration in living organisms at various times after intake.

Developed methods provide a sensitive, fast, cost-effective, high-throughput and simple approach to the determination of AT in tablet dosage forms, spiked human serum and spiked human urine samples. As applied to serum and urine samples, the proposed method offers the advantage that no prior extraction procedure is required. Furthermore, the proposed methods have distinct advantages over other existing methods regarding sensitivity, time-consuming, lower detectability and no excipients as interfering with the analysis, avoiding a separation step. The proposed methods might be alternatives to the HPLC techniques.

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