Voltammetric Behavior of Telmisartan and Cathodic Adsorptive Stripping Voltammetric Method for Its Assay in Pharmaceutical Dosage Forms and Biological Fluids

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Abstract
Electrochemical behavior of Telmisartan and optimum conditions for its assay were investigated by using cyclic voltammetry and square-wave voltammetry. All studies were based on the quasi-reversible and adsorption-controlled electrochemical reduction signal of TS at about −1.50 V versus Ag/AgCl at pH 10.0 in Britton-Robinson buffer. The peak current was found to change linearly with concentration from 1.69 nM (0.87 μg/L) to 27.5 nM (14.15 μg/L). The limit of detection and the limit of quantification were found to be 1.05 nM (0.54 μg/L) and 3.49 nM (1.79 μg/L), respectively. The method was successfully applied to different samples with good recoveries at about 100 %.

Keywords: Telmisartan, Square-wave cathodic adsorptive stripping voltammetry, Pharmaceuticals, Spiked human serum, Hanging mercury drop electrode

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1. Introduction

Despite the considerable success of treatments, hypertension still remains one of the greatest public health problems all over the World. Telmisartan (TS), 4-((2-*n*-propyl-4-methyl-6-(1-methylbenzimidazolol-2-yl)-benzimidazol-1-yl)-methyl)-biphenyl-2 carboxylic acid, is an angiotensin II receptor antagonist (ARA II) widely used in the treatment of hypertension. Chemical structure of TS is given in Figure 1. Therapy with ARA II drugs offers a good quality of life for hypertensive patients due to the absence of side effects, specificity of their action and their once daily administration [1,2]. TS undergo minimal biotransformation in the liver to form telmisartan-1-*o*-acyl-glucuronide [3] its major inactive metabolite. Maximum plasma concentration occurs within about 3 h after its oral administration, giving plasma levels of 50 $\mu g L^{-1}$ for a 40 mg dose. Renal excretion is a minor elimination pathway for TS, hence the small amount of the dose excreted in urine (less than 1 %). The mean elimination half-life ($t_{1/2}$) is approximately 24 h, in contrast with the rapid absorption process of the drug. The rest of the parent compound (more than 98 %) is excreted in the faeces [4].

Several analytical techniques including high-performance liquid chromatography [5–8], liquid chromatographic–tandem mass spectrometric method [9], column-switching liquid chromatographic system with fluorescence detection [10], liquid chromatography–mass spec-

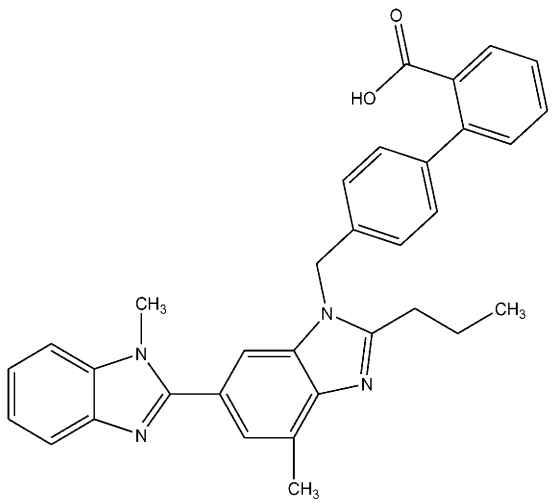

Fig. 1. Chemical structure of TS.

trometry method [11], automated solid phase extraction and liquid chromatography mass spectrometry [12], capillary electrophoresis [13–15], ratio derivative spectrophotometry [16], and immunoassay methods [17,18] have been devised for the determination of TS in pharmaceutical samples or biological fluids. All these reported methods require highly sophisticated instrumentation even they are sufficiently sensitive. TS is an electroactive molecule on different electrodes therefore there are three studies dealing with electrochemical determination of TS based on mercury [19,20] and carbon paste [21] electrodes. Reviewing the literature revealed that, up to the present time, there is no square-wave cathodic stripping voltammetric method using mercury electrode to assay of TS in pharmaceutical formulation and biological samples.

The voltammetric techniques, such as cyclic voltammetry, differential pulse voltammetry and square-wave voltammetry have been proved to be very sensitive for the determination of organic molecules including drugs and related molecules in pharmaceutical dosage forms and biological fluids [22–26]. These methods are faster, easier to be operated and cheaper than spectroscopic and chromatographic methods. The sensitivity increases when the stripping voltammetry is employed. Adsorptive stripping voltammetry has been shown to be an efficient electroanalytical technique for determination of sub-nanomolar level of a wide range of drugs having an adsorption-controlled electrode mechanism on working electrode. Its remarkable sensitivity is attributed to the combination of an effective accumulation step with an advanced measurement procedures that generates an extremely favorable signal to back ground ratio [27–29]. It usually involves a simple deposition step and most of the excipients used do not interfere in the subsequent determination of the drugs.

In this study we aimed to investigate the electrochemical reduction behaviors of TS on mercury electrode using voltammetric methods. Development of new validated square-wave adsorptive stripping voltammetric assay method with lower detection limit than given in electrochemical studies for direct determination of TS in different samples including pharmaceutical preparations and human serum was one of the other goals of present study.

2. Experimental

2.1. Apparatus

All voltammetric measurements such as cyclic voltammetry (CV), controlled potential coulometry (CPC), square-wave cathodic adsorptive stripping voltammetry (SWCAdSV) were carried out using a CH-instrument electrochemical analyzer (CHI 760). A three electrode cell system incorporating the hanging mercury drop electrode (HMDE BAS CGME 1108) as 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.

A three-electrode combination system for bulk electrolysis with mercury pool (55.4 cm^2) as working electrode, coiled platinum wire as an auxiliary electrode (BAS MW-1033) and Ag/AgCl reference electrode (BAS MF-2052 RE-5B) was used.

All pH measurements were made with Thermo Orion Model 720A pH ion meter by using combined Orion glass pH electrode (912600).

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

2.2. Reagents and Solutions

Standard TS (99.0 %, from Boehringer Ingelheim) was used to prepare the stock solution of TS by dissolution of precisely weighed amount of TS in 0.1 mol L^{-1} NaOH solution in order to have the TS concentration of 2.0×10^{-3} mol L^{-1}. Calibration solutions were prepared by diluting the stock solution with Britton–Robinson buffer (BR) and pH value of these solutions were adjusted by using 0.1 mol L^{-1} NaOH solutions.

All chemicals used in preparation of BR solution, 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 TS 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.

2.3. Preparation and Analysis of Samples

Pritor and Micardis tablets were used as pharmaceutical dosage form which contains 80 mg of TS and some amount of excipients per tablet. To prepare the solutions of tablets, initially the drug content of ten tablets was weighed, finely powdered and mixed separately for each brand. 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 which contained about 85 mL 0.3 mol L^{-1} NaOH solutions and completed to the mark with water. The contents of the flask were sonicated for 30 min to achieve complete dissolution of TS. After dissolution step, content of flask was centrifuged 30 min at 1500 rpm. 1.0 mL of sample from the clear supernatant liquor was withdrawn and quantitatively diluted to 250.0 mL with BR buffer. This solution was kept in refrigerator. 15, 35, 75, 90 and 110 μL from this tablet solution were transferred to electrochemical cell containing 10 mL of BR buffer, pH was adjusted to desired value and performed determination of TS in tablets by using calibration curve method.

Similarly, spiked human serum samples were analyzed. Serum samples, obtained from healthy individuals were

stored frozen until assay. After gentle thawing, 1.0 mL aliquot volumes of serum was added to electrochemical cell containing 9.0 mL of BR buffer and then 45 and 60 μL from tablet solution were transferred to this cell. After deaeration with argon measurements were performed to determine TS content of cell by using calibration curve method.

2.4. Voltammetric Procedure

In cyclic voltammetry, 10.0 mL of TS solution in BR was placed into the electrochemical cell for each time. The solution was deoxygenated with purified argon (99.99% purity) for 2 min before the first running and 30s between runnings. After deaeration, a hanging mercury drop was formed, and then the voltammograms were recorded by applying a negative-going scan from −1.05 V to −1.75 V.

3. Results and Discussion

3.1. Electrochemical Behavior of TS

The electrochemical behavior, diffusion and adsorption properties of TS were studied by using cyclic voltammetry, square-wave voltammetry, and controlled-potential electrolysis. In cyclic voltammetric studies a single well-defined reduction peak was observed at a potential of about −1.50 V at pH 10.0 (Figure 2). There is no peak when a blank BR solution was scanned at the same conditions, and peak intensity increases linearly with increasing concentration of TS, showing that this reduction peak is due to the reduction of TS molecules. As can be seen from Figure 2, there is also an anodic peak at reverse scan. Existence of this anodic peak depends on the concentration of TS.

The influences of the potential scan rate on cathodic peak current ($i_{p,c}$) and cathodic peak potential ($E_{p,c}$) were investigated for 3.85×10^{-5} mol L^{-1} TS in the 0.01–10 V s^{-1} range. The peak potential shifts to more negative values

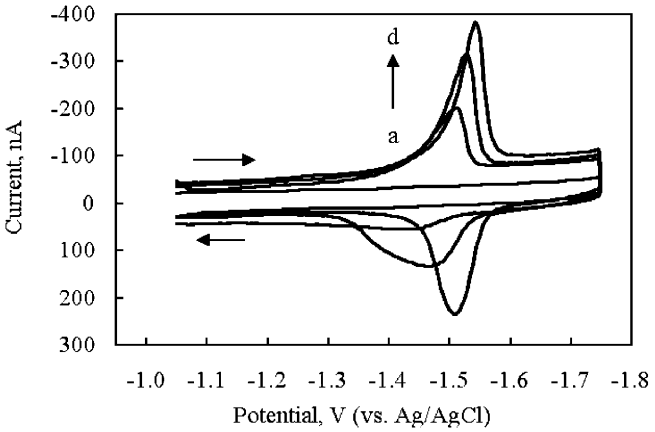

Fig. 2. Influence of TS concentration on peak current in cyclic voltammetry (a) blank BR, (b) 1.0×10^{-5}, (c) 2.8×10^{-5}, and (d) 4.6×10^{-5} mol L^{-1}; (scan rate: 0.10 V s^{-1}, pH: 10.0).

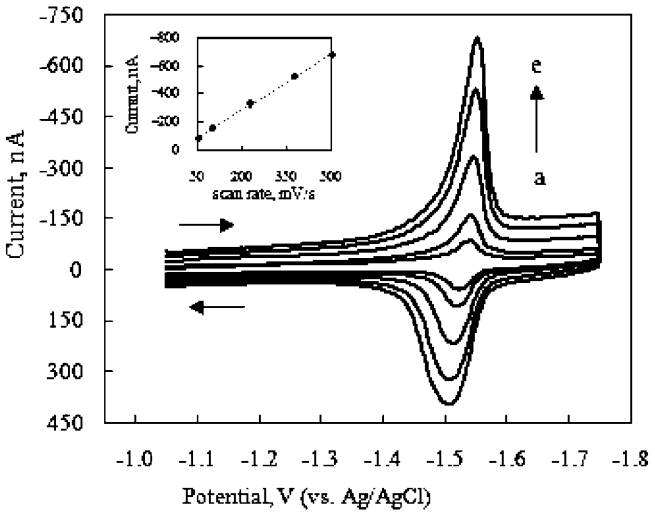

Fig. 3. Influences of scan rate on peak current and peak potential of 3.85×10^{-5} mol L^{-1} TS: (a) 0.050, (b) 0.100, (c) 0.225, (d) 0.375, and (e) 0.500 V s^{-1} (inset) plot of peak current versus scan rate at pH: 10.0.

with increasing scan rate (Figure 3). This behavior shows the irreversible character of electrode reaction, but as can be seen from Figures 2 and 3 there is also an anodic peak indicating the reversibility. In fact for an ideal reversible electrode reaction, peak potential does not effected by scan rate and ratio of anodic peak current ($i_{p,a}$) to cathodic peak current ($i_{p,c}$) is unity [22]. In present study, peak potential was affected by scan rate and peak current ratio is not unity. This ratio approaches to unity with increasing concentration of TS. At TS concentration higher than 2.0×10^{-4} mol L^{-1} this ratio is very close to unity. Oxidation peak is not recognized by M. Xu et al. [19] may be because of their TS concentration of about 1.0×10^{-6} mol L^{-1}. This behavior may be explained as follows: Reduction of TS molecule occurs on the surface of electrode meaning that adsorbed TS molecules are reduced. When the concentration gradient between the electrode surface and bulk solution is high enough, reduced molecules are removed from the surface and this lowers the current of oxidation peak. When the concentration of bulk solution is high enough to decrease the concentration gradient between the surface and bulk solution, slow removing process is established and the ratio of peak currents approach to unity.

Linear plots of peak current versus square-root of scanning rate ($i_{p,c}$ vs. $v^{1/2}$) should be obtained for diffusing electroactive species, whereas species adsorbed on the electrode surface should result in linear plots of $i_{p,c}$ versus v [22]. When the scan rate varied from 0.01 V s^{-1} to 0.500 V s^{-1} in 3.85×10^{-5} mol L^{-1} TS, a linear dependence of cathodic peak intensity $i_{p,c}$ (μA) upon the scan rate (V s^{-1}) was found as given equation $i_{p,c}(\mu A)=0.80\,v$ (μA s V^{-1}) + 0.01 (μA) with ($R^2=0.996$), confirmed an adsorption behavior.

The equation related to the plot of logarithm of peak current $i_{p,c}$ (A) versus logarithm of scan rate (V s^{-1}) was

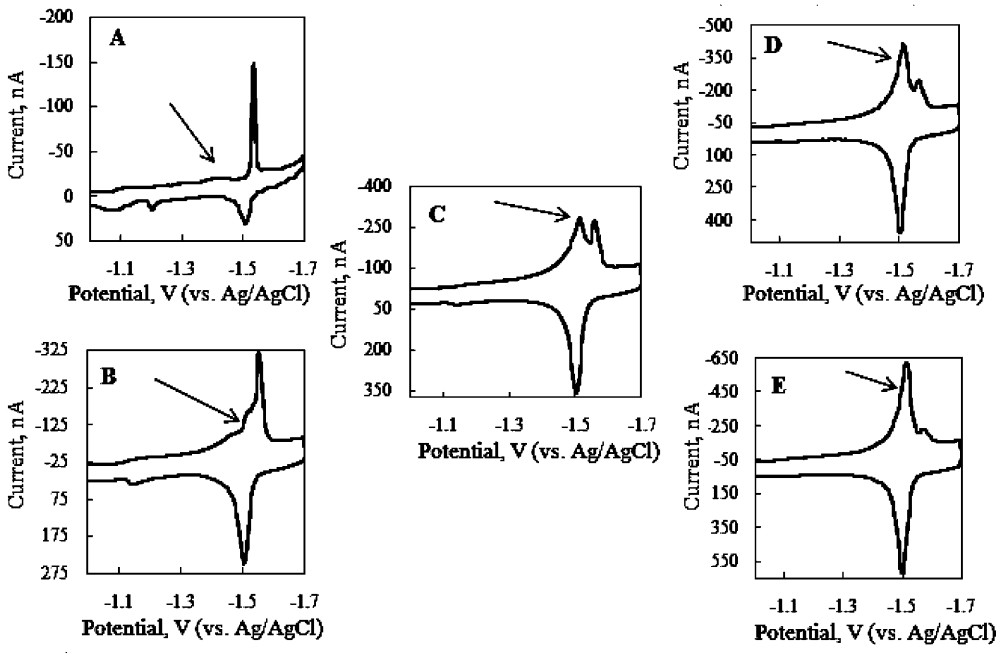

Fig. 4. Splitting of reduction peak with scan rate, concentration of TS is 2.0×10^{-4} M, BR buffer with pH of 10.0: (A) 0.025, (B) 0.175, (C) 0.250, (D) 0.325, and (E) 0.450 $V s^{-1}$.

found to be $\log(i_{p,c}) = 0.95 \log v - 5.96$ with ($R^2 = 0.999$). Slope of this curve (0.95 $\log i_p/\log v$) is very close to the theoretical value of 1.0 for adsorbed species. Moreover, cathodic peak was split with scan rate when TS concentration is higher than 1.0×10^{-4} mol L^{-1} (Figure 4). All these results show that reduction process is controlled by adsorption [22]. Furthermore anodic peak is wider than cathodic peak but areas of these peaks are nearly same. This behavior may show the different adsorption strength of reactant and product to the mercury surface. Some extra studies were carried out to control the adsorption phenomena according to literature [30,31]. As a result, it is found that the value of the ratio of cathodic peak current to concentration ($i_{p,c}/C$) decreases with increasing concentration; value of the ratio of cathodic peak current to multiplication of concentration and scan rate ($i_{p,c}/C\ v$) is nearly constant with increasing scan rate, and value of the ratio of cathodic peak current to multiplication of concentration and square root of scan rate ($i_{p,c}/C\ v^{1/2}$) increases with increasing scan rate. According to these observations, a quasireversible charge transfer mechanism that includes the adsorption of product and reactant with different strength to electrode surface may be proposed.

50.0 mL of 1.0×10^{-4} mol L^{-1} TS solution in BR was electrolyzed in the cell with mercury pool electrode having an area of 55.4 cm^2 by using controlled-potential electrolysis method. Solution was deoxygenated for 25 min before running electrolysis. The applied potential was hold constant at −1.70 V and the electrolysis was performed. The result of bulk electrolysis showed that no significant change in both peak current and peak potential was observed before and after electrolysis even it takes 5 hours. These results supported that deduction on the observed reduction wave as a catalytic adsorptive one [19,32,33].

To find out the number of electron(s), following relations proposed for adsorption process [22] were used in cyclic voltammetry:

$$i_p = n^2\ F^2 \Gamma A v/4RT \tag{1}$$

and the relation

$$Q = nFA\Gamma \tag{2}$$

where i_p is the peak current (A), Q is the charge (C) 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 (mol cm^{-2}), A is the working mercury electrode area (0.0145 cm^2) and F is the Faraday constant (96485 C/mol) and v is the scanning rate ($V s^{-1}$) [22,34]. By substitution the Γ term of Equation 2 into Equation 1, it is easy to get a new relation for n:

$$n = 4i_pRT/FQv \tag{3}$$

In the scan rate range from 0.005 $V s^{-1}$ to 0.500 $V s^{-1}$ number of electron(s) transferred in electrode reaction (n) was calculated by directly using an equation given above for an each scan rate and by using the slope of peak current versus scan rate (according to Equation 3). As a result of calculation and graph method, number of

electrons was found between 1.90 and 2.17 electrons, so number of electron in electrochemical step is 2. Amount of adsorbed molecules depends on the time allowed (time interval from the formation of drop to the peak formation) and this parameter depends on the scan rate. Because of this approach, surface coverage was calculated for different scan rate interval. The surface coverage of adsorbed substance (Γ) was found as 2.2×10^{-11} mol cm^{-2} when $0.01\ \mathrm{V\,s^{-1}} \le v \le 0.075\ \mathrm{V\,s^{-1}}$ and 1.85×10^{-11} mol cm^{-2} for the scanning rate between 0.10 V s^{-1} and 0.50 V s^{-1}. At higher scan rates, shape of cyclic voltammograms is distorted. Thus it was not aimed to calculate the electrochemical parameters of TS at a scan rates higher than 0.50 V s^{-1}.

The following equation which expresses adsorption phenomena validated by Garrido [35] was used to calculate the diffusion coefficient of TS:

$$i_p = 1.06\times10^6\ n^2ACvD^{1/2}t_p^{1/2} \quad (4)$$

The mean of the diffusion coefficient calculated from this equation was obtained as 2.19×10^{-8} cm^2 s^{-1}.

By using data from frequency (f) studies in SWV and the Equation 5 given below, electron transfer coefficient (α) was calculated [34].

$$E_p = k + (RT/n\alpha F)\ \ln f \quad (5)$$

According to this equation, by using the slope value of the plot of E_p versus ln f and 2 for n, value of α was calculated as 0.59 and rate constant (k_s) was calculated according to equation given below [34].

$$\ln k_s = \alpha\ \ln(1-\alpha) + (1-\alpha)\ \ln\alpha - \ln(RT/nFv) - \alpha(1-\alpha)\ nF\Delta E_p/2.3RT \quad (6)$$

k_s values was found to be 2.15 s^{-1}.

3.2. Electroanalytical Determination of TS

Since TS has adsorption-controlled reduction mechanism on HMDE, adsorptive stripping technique was proposed for the determination of TS. These techniques 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 [22–29]. In the present study, initially instrumental parameters and experimental conditions such as type and the concentration of supporting electrolyte, pH, TS concentration, deposition time, and deposition potential were optimized for determination of TS.

The square-wave (SW) response markedly depends on the parameters of the excitement signal. In order to obtain a well-defined square-wave voltammetric peak shape, the optimum instrumental conditions such as frequency, f, scan increment, ΔE_i, and pulse-amplitude, ΔE_a, were studied for 1.0×10^{-8} mol L^{-1} TS in a BR buffer of pH 10.0 at a HMDE. The optimum instrumental conditions were found as $f=25$ Hz, $\Delta E_i=4$ mV and $\Delta E_a=15$ mV.

The peak responses for the studied drug were affected by the type of supporting electrolytes. Two different supporting electrolytes were examined including BR and NH_3/NH_4Cl buffers. The highest peak current and the best peak shape were obtained in the presence of BR buffer containing 0.04 mol L^{-1} for each component, although peak current increases with increasing buffer concentration in the range between 0.01 mol L^{-1} and 0.20 mol L^{-1}.

The pH of solution is a critical factor affecting both the rate and equilibrium state of the accumulation process and the rate of the electrode reaction. The influence of pH on the SWCAdSV responses was studied at HMDE between pH values from 6.5 to 12. At pH values lower than 6.5 peak potential may be in more negative region than hydrogen evaluation potential on HMDE because this, peak is not observable at lower pH values. In these studies it was evaluated that peak potential is not significantly changed by pH between pH values from 6.5 to 12 and there is a change in peak current with pH value (Figure 5A). In optimization of pH value, not only peak current was chosen as an important parameter, but also peak shape and peak symmetry were also chosen as another important parameters. As a result, 10.0 was selected as an optimum value for pH.

In stripping method the influence of deposition time on the peak current for 1.0×10^{-8} mol L^{-1} TS was examined at different deposition times over the range from 30 s to 240 s. In fact, optimum deposition time strongly depends on the concentration of molecule under investigation. The resulted peak current increased with the increase of the deposition time from 30 s to 150 s for 1.0×10^{-8} mol L^{-1} TS; then, begins to decrease by increasing deposition time (Figure 5B). According to this result, optimum deposition time was selected as 150 s.

The influence of the deposition potential (from 0.0 V to −1.15 V) on the SWCAdSV signal was studied for 1.0×10^{-8} mol L^{-1} TS solution. Variation of the cathodic peak current ($i_{p,c}$) values versus deposition potential for 1.0×10^{-8} mol L^{-1} TS is shown in Fig. 5C. The dependence of cathodic peak current on the deposition potential showed a decrease at more negative potentials after −0.45 V. The maximum peak current in the deposition step was observed for the deposition potential of −0.45 V in SWCAdSV method.

To establish the linearity range (working concentration range) of TS in SWCAdSV different standard solutions were used ranged from 2.65×10^{-10} mol L^{-1} to 5.00×10^{-8} mol L^{-1}. 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 reduction peak current changes linearly with TS concentration, in

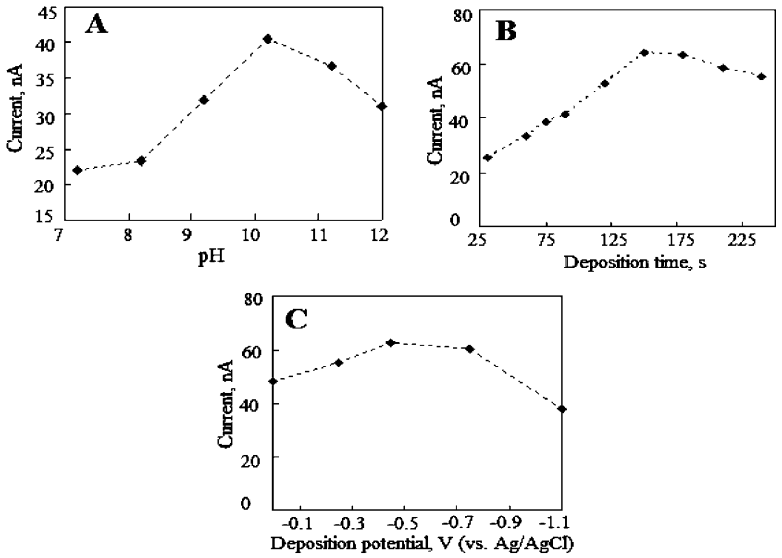

Fig. 5. A) Influence of pH on reduction peak current of 1.0×10^{-8} mol L^{-1} TS in SWCAdSV (deposition time: 150 s, deposition potential:−0.45 V). B) Effect of deposition time on peak current of 1.0×10^{-8} mol L^{-1} TS at pH 10.0 in SWCAdSV (deposition potential:−0.45 V) C) Effect of deposition potential on peak current of 1.0×10^{-8} mol L^{-1} TS at pH 10.0 in SWCAdSV (deposition time: 150 s).

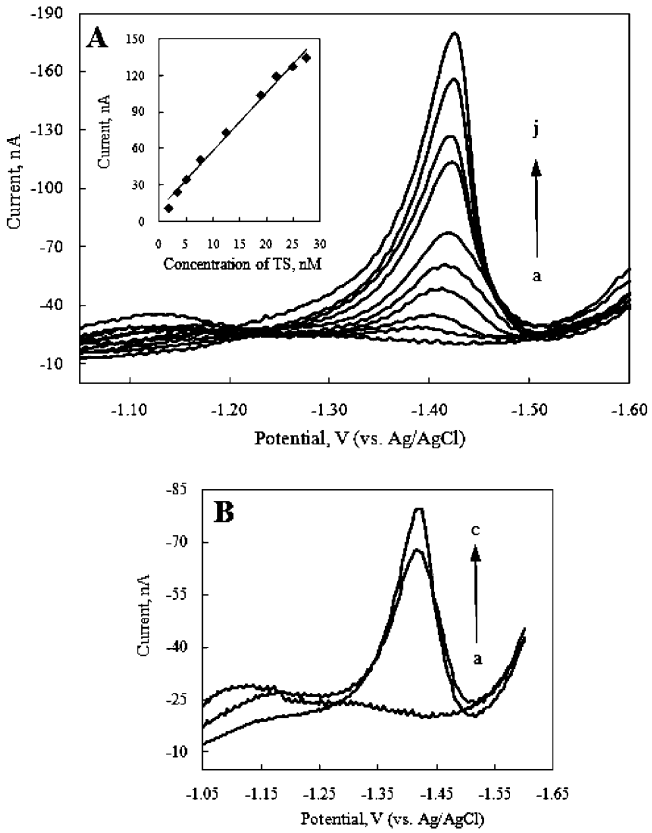

Fig. 6. A) SWCAdS voltammograms of TS at different concentrations: (a) blank BR, (b) 1.69 (c) 3.36, (d) 5.02, (e) 7.65, (f) 12.50, (g) 19.0, (h) 21.9, (i) 25.0 and (j) 27.5 nM. Inset in A: Calibration curve for corresponding solutions of TS. B) SWCAdS voltammograms of TS solutions spiked into the mixture of BR and human serum (a) 1.0 mL human serum + 9.0 mL BR, (b) a + 45 μL of tablet solution of Micardis sample (c) a + 60 μL tablet solution of Pritor sample.

the range from 1.69×10^{-9} mol L^{-1} (0.87 μg L^{-1}) to 2.75×10^{-8} mol L^{-1} (14.15 μg L^{-1}) (Figure 6A).

The characteristics of the calibration plots were summarized in Table 1.

3.3. Application of Method to Dosage Form and Biological Samples

In order to evaluate the adequacy of the proposed method, TS was determined by quantifying commercial pharmaceutical tablets of Pritor and Micardis (labeled as 80 mg TS per tablet). No pretreatment such as time-consuming extraction or evaporation step was required for sample preparation. The proposed SWCAdSV method was applied to the direct determination of TS in pharmaceutical dosage forms and biological sample. In this study only human serum is studied as a biological sample because less 1% of taken TS is excreted in urine [4]. The

Table 1. Regression data of the calibration curve for assay of TS by SWCAdSV.

Calibration parameters	Value
Linearity range (mol L^{-1})	1.69×10^{-9}–2.75×10^{-8}
Slope of calibration curve, ALmol^{-1} (m)	4.75
Intercept (A)	7.81×10^{-9}
SD (standard deviation) of calibration (A)	5.18×10^{-9}
SD of slope, ALmol^{-1}	0.17
SD of intercept, A , (s)	1.66×10^{-9}
Limit of detection (*LOD*) (mol L^{-1})	1.05×10^{-9}
Limit of quantification (*LOQ*) (mol L^{-1})	3.49×10^{-9}
Regression coefficient, R^2	0.9909
Repeatability of peak current (*RSD*,%)	2.78
Repeatability of peak potential (*RSD*,%)	0.34

Table 2. Analytical results of proposed method for tablets.

Sample	Nominal value per tablet (mg)	Found values per tablet (mg)	Recovery value [a] (%)	*RSD* [b] (%)
Micardis	80	81.5, 80.6, 82.6, 81.5, 80.8	101.8 ± 1.2	0.97
Pritor	80	80.2, 80.2, 80.1, 80.1, 80.2	100.2 ± 0.1	0.11

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

Table 3. Recovery results of proposed method for spiked serum samples (solutions of tablets were spiked)

Sample	Spiked amount (ng)	Found values (ng)	Recovery value [a] (%)	*RSD* [b] (%)
Micardis Tablet in Serum I	55	56.4, 56.3, 56.5, 56.4, 56.3	102.5 ± 0.2	0.16
Micardis Tablet in Serum II	75	76.1, 76.7, 77.0, 77.3, 76.1	102.2 ± 0.9	0.67
Pritor Tablet in Serum I	55	55.3, 54.9, 55.0, 55.1, 55.2	100.2 ± 0.3	0.24
Pritor Tablet in Serum II	75	74.8, 75.1, 75.3, 75.1, 74.9	100.0 ± 0.3	0.26

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

Table 4. Recovery results of proposed method for spiked serum samples (solution of standard TS was spiked).

Sample	Spiked amount (ng)	Found values (ng)	Recovery value [a] (%)	*RSD* [b] (%)
Standard in Serum I	75	75.0, 75.7, 75.1, 74.7, 75.5	100.3 ± 0.7	0.53
Standard in Serum II	85	85.2, 84.9, 85.3, 84.7, 84.7	99.9 ± 0.4	0.30

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

results of analysis found by using proposed method for pharmaceutical preparations were given in Table 2 and results of spiked human serum were given in Figure 6C, Table 3 and Table 4. The accuracy of the proposed method was determined by its recovery values.

It can be seen from these tables that average recovery values are in good agreement with the *RSD* values less than 1.0 %, which is good evidence of validity of method. Thus, the precision is very satisfactory for the analysis of serum samples as well as bulk formulation. These results indicate that the content of TS in the pharmaceuticals and biological sample can be safely determined by using proposed voltammetric method without interference from other substances in the samples. The proposed method can be applied to pharmaceuticals and human serum after a simple dilution step with direct measurements.

3.4. Method Validation

The elements required for method validation are: linearity range, limits of detection and quantitation, accuracy, reproducibility, stability, selectivity and robustness [36].

Results of linearity range studies were given in early section and also shown in Figure 6A. The regression equation was obtained as $i_{p,c}$ (A) $= 4.75 C_{TS}$ (mol L^{-1}) $+ 7.81 \times 10^{-9}$. From the values of correlation coefficient ($R^2 = 0.9909$), peak current linearly increase with increasing concentration of TS at given concentration range. This result supported the validity of the SWCAdSV method for the assay of TS.

Limit of detection (*LOD*) and limit of quantitation (*LOQ*) values were calculated using the relations: $LOD = 3\ s/m$ and $LOQ = 10\ s/m$ [37] (s is the standard deviation of intercept of calibration curve and m is the slope of the related calibration curve). *LOD* and *LOQ* values were found 1.05×10^{-9} mol L^{-1} (0.54 μg L^{-1}) and 3.49×10^{-9} mol L^{-1} (1.79 μg L^{-1}) respectively. In all other electrochemical studies [19–21] and almost all chromatographic studies [5–12], relatively high concentration of TS was studied and limit of detection value was found to be higher than current study. Both *LOD* and *LOQ* values confirmed the sensitivity of the proposed methods.

The accuracy of measurements by means of the described procedure was checked by calculating the recovery of a known concentration of TS following proposed method at optimum instrumental and experimental conditions. Recovery values range between 100.2 % and 101.8 % for tablet analysis, found between 100.0 % and 102.5 % for spiked tablet preparations into serum samples and found between 99.9 % and 100.3 % for spiked standard into serum samples (Tables 2–4). From these recovery values it is concluded that proposed method is highly accurate.

The high sensitivity of an analytical method is usually accompanied by very good reproducibility. This analytical performance was evaluated from five repeated measurements of electrochemical signal of different TS solutions following the proposed method. The precision of the proposed procedure is excellent because the relative standard deviation of recovery values ranges between 0.11 % and 0.97 % for all measurement includes tablets and serum samples (Tables 2–4).

The stability of TS in a BR buffer of pH 10.0 was evaluated under the optimal procedural conditions by moni-

toring the changes in both the cathodic peak potential and the cathodic peak current of standard TS solution and repeatabilities of peak current and peak potentials were investigated by calculating the relative standard deviations of ten serial measurements of the same solution. Relative standard deviations of peak current and peak potential were found to be 2.78 % and 0.34 % respectively (Table 1). As a result, there is no significant change in both peak potential and peak current which confirms the stability of TS over the time period of measurements. According to these results TS 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 or tablet solutions of TS, voltammetric base line of biological medium was measured by applying the same procedures as applied to calibration studies with standard samples. In such applications no extra voltammetric signal in studying potential window observed indicates that there is no significant interferences of various inorganic cations, anions and some organic substances found in pharmaceutical preparations (tablets) and biological medium (human serum). In some cases, TS is used as combined with hydrochlorothiazide; a drug active molecule of diuretic class. A series of electrochemical measurement were carried out for hydrochlorothiazide at the same conditions. These studies show that hydrochlorothiazide is electrochemically inactive on HMDE but electrochemically active on carbon electrode. It could be said that the proposed method would be used to determine TS in pharmaceuticals and biological fluids containing hydrochlorothiazide.

The robustness [38] of the measurements by means of the described SWCAdSV procedure to assay of TS was examined by studying the effect of small variation of some important procedural conditions such as pH value, deposition potential, deposition time and room temperatures of different days. Small changes (± 1 %) in such conditions do not affect the recovery of procedure.

4. Conclusions

In this study electrochemical reduction behavior of TS was studied on HMDE. Reversibility of electrode reaction was recognized for the first time. Electrochemical behaviors of pharmaceutical compounds may have valuable findings in either understanding the mechanism of their action in living organisms or determining their concentration in living organisms at various times after intake.

Proposed and validated method provides a sensitive, fast, cost-effective, high-throughput and simple approach to the determination of TS in tablet dosage forms and spiked human serum samples. As applied to serum 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 separation steps. Also proposed method has lower detection limit than other electrochemical methods given in literature.

Table 5. Statistical analysis of results obtained by the proposed method and the method given in [16] for TS at 95 % confidence level.

Method	Mean recoveries (%)	*RSD* (%)	*N*
Proposed	100.89	1.07	8
Literature	100.23	1.82	6
F-Test significance	2.82	*F* (tabulated) = 6.39	
t-Test significance	1.45	*t* (tabulated) = 2.23	

The results of *t*- and *F*-tests, the variances between two methods were found to be insignificant at 95 % confidence level indicating that no significant differences exist between the performances of the two methods regarding their accuracy, precision and recoveries (Table 5). As a result proposed method might be alternatives to the HPLC techniques.

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