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Short communication

Rapid determination of telmisartan in pharmaceutical preparations and serum by linear sweep polarography

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Abstract

The polarographic behaviors of telmisartan (TE) are investigated in 0.8 mol/l $NH_3{\cdot}H_2O$–NH_4Cl (pH = 8.9) supporting electrolyte. The results demonstrated that the reduction peak is obtained at ca. −1.30 V, which corresponds to a catalytic hydrogen wave. Based on the catalytic hydrogen wave, a novel method has been developed for the determination of telmisartan by linear sweep polarography. Calibration curve is linear in the range 2.0×10^{-7} to 3.0×10^{-6} mol/l and the detection limit is 1.0×10^{-7} mol/l. The proposed method is applied to the rapid determination of the telmisartan in capsule forms and biological sample without pre-separation.

Keywords: Telmisartan; Catalytic hydrogen wave; Linear sweep polarography; Pharmaceutical analysis

1. Introduction

Telmisartan (TE), 4′-[(1,4′-dimethyl-2′-propyl[2,6′-bi-1H-benzimidazol]-1′-yl) methyl-[1,1′-biphenyl]-2-carboxylic acid, is a potent, long-lasting, nonpeptide antagonist of the angiotensin II type-1 (AT_1) receptor that is indicated for the treatment of essential hypertension. It selectively and insurmountably inhibits stimulation of the AT_1 receptor by angiotensin II without affecting other receptor systems involved in cardiovascular regulation. In clinical studies, TE shows comparable antihypertensive activity to members of other major antihypertensive classes, such as angiotensin-converting enzyme (ACE) inhibitors, beta-blockers and calcium antagonists. Experiments have confirmed the placebo-like safety and tolerability of TE in hypertensive patients [1]. Its chemical structure is shown in Scheme 1.

TE has become one of the most important advances in the treatment of hypertension despite its recent introduction in the market (1997). With the analytical techniques for the determination of TE, capillary zone electrophoretic (CZE) methods [2–4] and a micellar electrophoretic (MEKC) method [5] were described. However, these methods need expensive equipment and are time-consuming, and they exact rigid experimental conditions, such as the amount of reagent, reaction temperature, and reaction time. A simple and rapid method for detecting TE that requires no such

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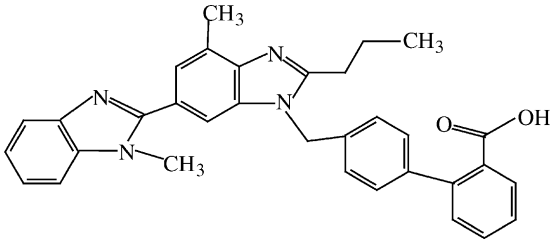

Scheme 1. Structural formula of TE.

procedures is, thus, highly desirable from a practical viewpoint.

Electrochemical methods have proved to be very sensitive for the determination of organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids. The advance in experimental electrochemical techniques in the field of analysis of drugs is due to their simplicity, low cost and relatively short analysis time as compared with the other techniques. However, since most drugs are less active electrochemically, little attention has been paid so far to the use of electrochemical detection methods. Since many nitrogen- and sulfur-containing organic compounds can cause catalytic hydrogen waves, it is interesting to study the catalytic hydrogen waves as detection methods of these organic compounds due to their high sensitivities. These waves have been applied to the determination of platinum-group metal ions [6–8], proteins and drugs [9]. With TE, owing to possessing nitrogen atoms, it is predicted that TE can produce a catalytic hydrogen wave. Surveying the literature revealed that nothing appears to have been published concerning the electrochemical behavior of TE in general or its catalytic hydrogen wave in particular. However, the usual catalytic hydrogen wave appears at a potential of ca. 100 mV less negative than that of the discharge of hydrogen ions in bulk buffer. The catalytic hydrogen wave is generally liable to bulk solution.

The aim of this work is to develop a simple and sensitive catalytic hydrogen wave method for the determination of TE. The experiments show that the catalytic hydrogen wave of TE appears at ca. −1.30 V, which was far from the discharge of hydrogen ions in the solution used in this work. Accordingly, the proposed method is not interfered by bulk solution. The method is applied to the determination of TE in pharmaceutical preparations and biological sample such as human serum.

2. Experimental

2.1. Reagents

A TE stock standard solution (1.0×10^{-3} mol/l) was prepared by dissolving 0.1286 g of TE (Boehringer Ingelheim, Ingelheim, Germany) in 25 ml 0.1 mol/l NaOH and diluting to 250 ml with water. The standard solution was stored in the dark under refrigeration. Working standard solutions were prepared by appropriate dilution of the stock standard solution with water. $NH_3{\cdot}H_2O$–NH_4Cl buffers (pH = 8.9) were prepared by mixing 2.0 mol/l of NH_4Cl solution with 2.0 mol/l of $NH_3{\cdot}H_2O$ solutions in appropriate volume ratio. All chemicals were of analytical-reagent grade or better, and were used without further purification. Twice distilled water was used throughout the experiments.

2.2. Apparatus

Single sweep polarograms were recorded by a model JP-303 polarographic analyzer (Chengdu Instrument Factory, China). A three-electrode set-up was equipped with a dropping mercury working electrode, a platinum-wire counter electrode and a saturated calomel reference electrode. The drop time was 7 s. The potential scan rate was 0.25 V/s. Cyclic voltammograms were recorded on a model CHI660 electrochemical workstation (CH Instruments, USA) coupled with a model 303A static mercury drop electrode system (EG&G PARC, USA), including a hanging mercury drop working electrode, a platinum-wire auxiliary electrode and a saturated calomel reference electrode. The workstation was controlled by CHI660 software and operated under Windows 98 environment.

Unless otherwise stated, potentials were referred to the potential of the saturated calomel electrode.

2.3. Procedure

A certain amount of standard or sample solution of TE, 4.0 ml $NH_3{\cdot}H_2O$–NH_4Cl buffers (pH = 8.9) were successively pipetted into 10 ml volumetric flask, and the mixture was diluted to mark with water. The prepared solution was transferred into a polarographic cell. The linear potential scan was cathodically

performed from −1.10 to −1.50 V without deaeration. The second-order derivative peak current of the reduction wave at about −1.30 V was recorded versus the concentration of TE. The TE content in the samples was obtained by using the calibration curve method.

3. Results and discussion

3.1. Effect of supporting electrolyte

TE was practically insoluble in water and in the pH range of 3–9, sparingly soluble in strong acid (except insoluble in hydrochloric acid), and soluble in strong base. Therefore, solutions were prepared in 0.01 mol/l NaOH. The effects of the nature of the supporting electrolyte on polarographic reduction of TE were studied in various supporting electrolytes in the range of pH 4.0–11.0. Some supporting electrolytes such as HAc–NaAc (pH 4.0–5.6), Britton–Robinson (pH 4.0–10.0), $Na_2B_4O_7$–KH_2PO_4 (pH 6.0–9.0), KH_2PO_4–Na_2HPO_4 (pH 5.8–8.0) and $NH_3{\cdot}H_2O$–NH_4Cl (pH 8.0–11.0) buffers were examined. TE yielded a stable and well-defined reduction wave in supporting electrolytes such as KH_2PO_4–Na_2HPO_4, $Na_2B_4O_7$–KH_2PO_4 and $NH_3{\cdot}H_2O$–NH_4Cl buffers. However, best performance was obtained in $NH_3{\cdot}H_2O$–NH_4Cl buffer. Therefore, this work selected the $NH_3{\cdot}H_2O$–NH_4Cl buffer as supporting electrolyte.

3.2. Effect of pH value

The effect of pH value in the range of pH 8.0–11.0 on both peak current and peak potential of the reduction wave of TE was examined in the $NH_3{\cdot}H_2O$–NH_4Cl buffer. It showed that the peak current i_p decreased dramatically upon increasing pH values from 9.1 to 9.8, and remained nearly unchanged in the range of 8.0–9.1 and 9.8–11, respectively (Fig. 1, curve a). On the other hand, the peak potential shifted obviously with pH values changing. The peak potential shifted towards positive direction with pH values from 8.0 to 8.9, while shifted towards negative direction from 8.9 to 11.0 (Fig. 1, curve b) according to the equation: $-E_p/\text{mV} = 46.2\ \text{pH} + 906.0$ ($r = 0.9893$, $n = 7$). Therefore, the $NH_3{\cdot}H_2O$–NH_4Cl buffer of pH 8.9 was selected.

3.3. Effect of buffer concentration

The effect of the total concentration of $NH_3{\cdot}H_2O$–NH_4Cl (pH = 8.9) buffer was tested over the

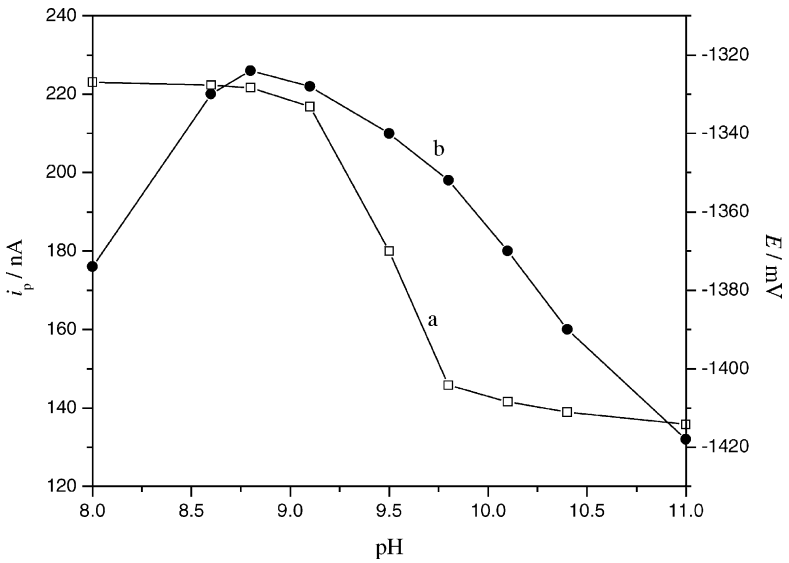

Fig. 1. Effect of pH values on peak current (curve a) and peak potential (curve b) of 1.0×10^{-6} mol/l TE in 0.8 mol/l $NH_3{\cdot}H_2O$–NH_4Cl supporting electrolyte.

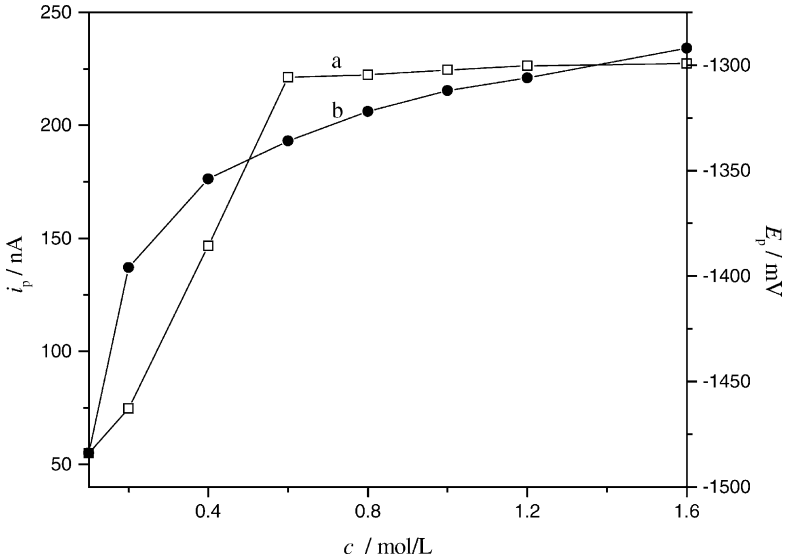

Fig. 2. Effect of total concentration of $NH_3{\cdot}H_2O$–NH_4Cl (pH = 8.9) on peak current (curve a) and peak potential (curve b) of 1.0×10^{-6} mol/l TE.

0.1–1.6 mol/l range. The response increased gradually upon increasing the $NH_3{\cdot}H_2O$–NH_4Cl concentration from 0.1 to 0.6 mol/l, and then reached a current plateau until 1.6 mol/l, while the peak potential E_p shifted positively from −1484 to −1292 mV (Fig. 2). The total concentration of $NH_3{\cdot}H_2O$–NH_4Cl (pH = 8.9) used in this experiment was 0.8 mol/l.

According to the above studies, the optimal supporting electrolyte was 0.8 mol/l $NH_3{\cdot}H_2O$–NH_4Cl (pH = 8.9) solution.

3.4. Calibration curve, detection limit and precision

Since a relatively large background current existed at the negative potential region that recorded the polarogram, the detection of trace amounts of TE was hindered. It was known that the derivative technique could efficiently suppress the background current. The second-order derivative polarogram, therefore, was recorded for the determination of TE. In the optimal supporting electrolyte chosen in this work, a stable and well-defined reduction peak with peak potential −1.30 V was obtained as shown in Fig. 3 and the second-order derivative peak current i''_p of the reduction peak was linearly proportional to the TE concentration in the range of 2.0×10^{-7} to 3.0×10^{-6} mol/l. The linear regression equation was i''_p (nA/s^2) $= -18.1 + 2.4 \times 10^8 C$ (mol/l) with a regression coefficient $r = 0.9997$ ($n = 7$). The detection limit of 1.0×10^{-7} mol/l TE was calculated based on an S/N = 3. The precision was determined from 11 independent determinations of 1.0×10^{-6} mol/l TE. The percentage relative error was ca. 1.4% and the percentage relative standard deviation (R.S.D. (%)) was 1.8%.

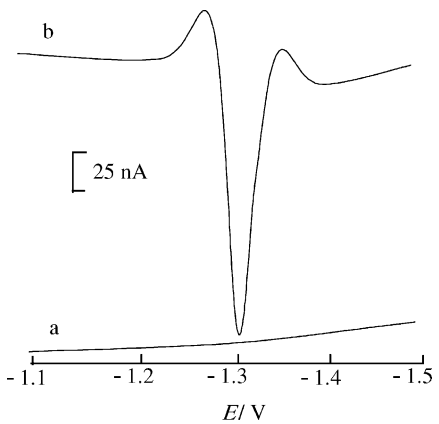

Fig. 3. 2nd-order derivative polarograms in 0.80 mol/l $NH_3{\cdot}H_2O$–NH_4Cl (pH = 8.9) supporting electrolyte in the absence (curve a) and the presence (curve b) of 1.0×10^{-6} mol/l TE.

3.5. Interferences

The possible interferences of various inorganic cations, anions and some organic substances were investigated by adding these substances to the optimal supporting electrolyte containing 1.0×10^{-6} mol/l TE. The experiments showed that a 500-fold of Ca^{2+}, Mg^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, Cl^{-}, Br^{-}, NO_3^{-}, CO_3^{2-}, 250-fold of uric acid, amylum, glucose, carbamide, ascorbic acid, phenylformic acid, oxalic acid, glutamic acid, tyrosine, 100-fold of Zn^{2+}, Fe^{2+}, Mn^{2+}, $S_2O_3^{2-}$, cystine, serine, valine, arginine, 50-fold of Fe^{3+}, Co^{2+}, lysine did not interfere with the determination of TE.

3.6. Sample analysis

3.6.1. Determination of TE in capsules

All the powder in 10 hard capsules was emptied and mixed and further produced. Portion of the obtained powder was accurately weighed and transferred into a 50 ml volumetric flask and dissolved in 0.1 mol/l NaOH. The contents of the flask were sonicated for 10 min to insure complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the supernatant liquor. The sample solution obtained above was applied to the polarographic determination of TE. The results were summarized in Table 1.

To study the accuracy of the proposed method, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. Each recovery was calculated by comparing the results obtained before and after the addition. The results were shown in Table 2.

3.6.2. Determination of TE in spiked serum samples

Serum samples, obtained from healthy individuals (from the Hospital of Northwest University, Xi'an), were stored frozen until assay. After gentle thawing, an aliquot volume of sample was fortified with TE dissolved in ethanol to achieve appropriate concentration. The solution was centrifuged for 15 min at 4000 rpm to remove the precipitated serum proteins and the supernatant was taken carefully and the supernatant was taken carefully. Appropriate volume of supernatant liquor was transferred in the 10 ml volumetric flask. The sample solution obtained above was applied to the polarographic determination of TE. The results were summarized in Table 2.

Table 2
Recovery results in samples ($n = 7$)

Samples	Added (mol/l)	Found (mol/l)	Mean recovery (%)	R.S.D. (%)
Capsule	6.00×10^{-7}	6.02×10^{-7}	100.3	2.2
	1.00×10^{-6}	1.01×10^{-6}	101.0	2.1
	2.00×10^{-6}	1.96×10^{-6}	98.0	2.5
Serum	6.00×10^{-7}	6.06×10^{-7}	98.3	4.6
	1.00×10^{-6}	9.78×10^{-7}	97.8	3.6
	2.00×10^{-6}	1.88×10^{-6}	94.0	4.5

As shown in the Tables 1 and 2, the proposed method had a good accuracy and precision.

3.7. Discussion on reaction mechanism

In order to understand the electrochemical process occurring on the mercury electrode repetitive cyclic voltammetry of the system was investigated on a hanging mercury drop working electrode. Typical cyclic voltammetric curves were shown in Fig. 4. TE gave a cathodic peak at about −1.30 V in 0.80 mol/l $NH_3{\cdot}H_2O$–NH_4Cl (pH = 8.9) supporting electrolyte. No peak was observed on the anodic branch, indicating that the reduction was totally irreversible. The reduction peak in the first scan after an accumulation time of 10 s was much higher than that in the second one, indicating that the reduction peak at ca. −1.30 V was an adsorption peak. Furthermore, in the presence of a low concentration of neutral surfactant (gelatin

Table 1
Analytical results of capsules

Samples	Label value (mg/tablet)	Determined value (mg/tablet)	Mean value ($t_{0.05,6}$) (mg/tablet)	R.S.D. (%)
A	40	39.5, 39.7, 39.2, 40.3, 39.8, 40.4, 40.1	39.9 ± 0.4	1.1
B	80	79.2, 79.6, 79.7, 79.5, 80.1, 79.7, 80.3	79.7 ± 0.3	0.5

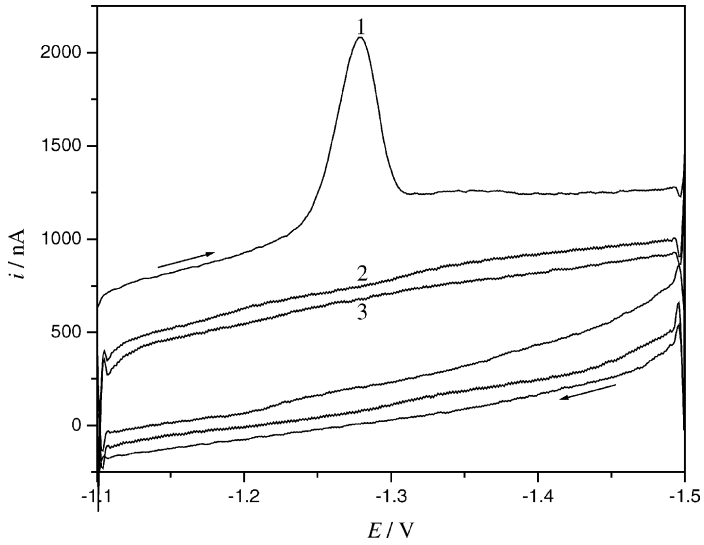

Fig. 4. Repetitive cyclic voltammograms: 0.80 mol/l $NH_3{\cdot}H_2O$–NH_4Cl (pH = 8.9); 1.0×10^{-6} mol/l TE. Accumulation for 10 s at −1.10 V; scan rate 100 mV/s; initial potential −1.10 V.

or Triton X-100), the peak current at ca. −1.30 V was strongly depressed; on adding a cationic surfactant and an anionic surfactant, the peak current remained essentially unchanged, which indicates the characteristics of neutral adsorption. This could be ascribed to that the benzimidazole group carried a positive charge and the carboxyl group carried a negative charge in TE, and as a net amount of charge TE did not carry charge in the buffer of pH 8.9 used in the work.

From the structure of TE as shown in Scheme 1, the molecule possessed a carbonyl group and two benzimidazole groups. The pK_a value (4.45 ± 0.09) of TE at an ionic strength of 0.5 mol/l was calculated by spectrofluorimetry [10]. The pK_a value obtained for this compound corresponded to the acid–base equilibrium in which the carboxylic group was involved. This pK_a value implied that the carboxyl groups in TE molecules changed into carboxylate anions. However, the peak current and peak potential of the reduction wave of TE at pH 8.8 possessed the same inflexion point. This was related to nitrogen atoms of the TE because the nitrogen atom in benzimidazole group of TE molecule could combine proton. Generally speaking, one thought that the carboxyl group was electroinactive because the reduction wave of the carbonyl group C=O on carboxyl group could only be observed at ca. −2.0 V in organic solvent as media. The discharge of hydrogen ion in bulk solution overlapped the reduction wave of the carbonyl group, which made it impossible to observe the latter in the aqueous solution. On the other hand, according to literature [11], benzimidazole group was not reducible in aqueous solution. Accordingly, it was deduced that the reduction wave of TE at ca. −1.30 V in the optimal supporting electrolyte should be the result of the reduction of the proton rather than that of the other groups, which was a catalytic hydrogen wave. The ability of some organic compounds to bring about catalytic hydrogen evolution seemed to be due to possessing an unshared pair of electrons, to which a proton could be added. Owing to the ability of nitrogen atom combining hydrogen ion higher than that of oxygen atom, nitrogen atom of TE combined hydrogen ion, yielding onium compounds capable of participating in an electrochemical reaction on the cathode. This was consistent with the change of the peak current and peak potential of the reduction wave of TE with pH values.

In order to further confirm the deduction on identity of the reduction peak, some experiments were performed. The controlled-potential electrolysis was conducted with mercury working electrode with an area of approximately 7 cm^2, a platinum-wire auxiliary electrode and a saturated calomel reference

electrode. The potential was controlled at −1.40 V and the electrolysis was performed for 5 h in 0.80 mol/l $NH_3{\cdot}H_2O$–NH_4Cl (pH = 8.9) supporting electrolyte after deaeration. The experiment showed that no obvious current change was observed before and after electrolysis. In addition, as shown in Sections 3.2 and 3.3 the peak current decreased with pH values increasing and the peak current increased with the buffer concentration increasing. What is more, the peak current decreased with the increase of temperature and at 70 °C the peak current disappeared nearly, indicated that the reduction peak was controlled by a surface reaction [12]. These results supported the deduction on the observed reduction wave as a catalytic hydrogen wave.

According to the theory of catalytic hydrogen wave of organism [13], the production of the catalytic hydrogen wave of TE involved following steps. The TE combined with the hydrogen ion to form the protonated form of TEH^+. Then, the TEH^+ was polarographically reduced to produce an uncharged particle THE^* in the nature of a free radical. The uncharged particle THE^* entered subsequently into a bi-molecular interaction, resulting in the evolution of hydrogen molecule. The total scheme could be described as follows:

$$TE + H_3O^+ \Leftrightarrow TEH^+ + H_2O$$

$$TEH^+ + e^- \rightarrow TEH^*$$

$$2TEH^* \rightarrow 2TE + H_2 \uparrow$$

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