한국환경분석학회지 제15권 (제1호) 61~66, 2012
J. of the Korea Society for Environmental Analysis

Electrochemical Behavior of Udenafil on Glass Carbon Electrode

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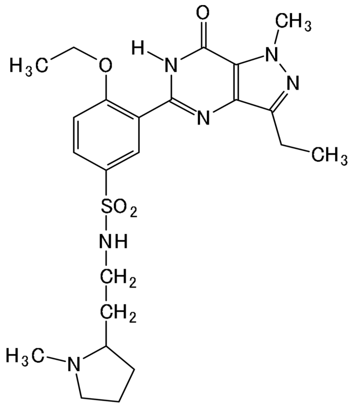
Received March 17, 2012/Accepted March 31, 2012

The voltammetric behavior of udenafil on glassy carbon electrode was studied using cyclic, linear sweep and square-wave (SW) techniques. Udenafil showed an irreversible oxidation behavior in aqueous buffer solutions containing 30% (v/v) acetonitrile over the pH range of 2 to 6. The oxidation pattern of 2-(2-aminoethyl)-1-methylpyrrolidine differs from that of udenafil. On the other hand, the oxidation of udenafil resembles those of the alkoxybenzene group and 6-hydroxypurine group of udenafil and 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]-7-pyramidinone (EMPP). From the linear relation between the peak current and the concentration of udenafil, square wave (SW) voltammetric method for its determination in udenafil tablets was developed. For analytical purpose, one well-defined diffusion controlled voltammetric peak was obtained in 0.2 M phosphate buffer at pH 2.0. The current-concentration plot was rectilinear over the range of 5×10^{-7} to 5×10^{-5} M with correlation coefficient of 0.998.

Key words: Udenafil, Oxidation mechanism, Drug analysis, Voltammetry

1. Introduction

Udenafil, which has recently developed by Research Laboratory of Dong-A Pharmaceutical Company (Yongin, South Korea) and is used to control erectile dysfunction, is a potent and selective inhibitor of phosphodiesterase type V (PDE- 5)1). Udenafil is designated chemically as 5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethylamidosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-yrazolo(4,3-D)pyrimidin-7-one and has the following structural formula (Fig. 1).



Udenafil, MW=516.66 g/mol

Fig. 1. Structure of udenafil.

Udenafil, Phosphodiesterase type 5 (PDE5) inhibitor, is vasoactive drug that has been developed for the treatment of erectile dysfunction2). PDE5 inhibitors act by blocking the degradation of cGMP, which is increased in the vascular smooth muscle cells in the endothelium by l-arginine in the presence of NO synthase, leading to relaxation of the vessels3). It was reported that zaprinast enhanced the endothelium-dependent and NO-mediated vasodilation in an intact lamb with experimental pulmonary hypertension4). Halcox et al. reported that sildenafil citrate, which was the first PDE-5 inhibitor

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approved for treating erectile dysfunction, dilated the epicardial coronary arteries, improved the endothelial dysfunction, and improved the physiologic coronary vasomotion in patients with coronary artery disease (CAD)5,6).

Udenal has been studied and determined by very few procedures: high-performance liquid chromatography-electrospray ionization mass spectrometry3), micellar electrokinetic capillary chromatography4) and sensitive liquid chromatography assay with ultraviolet detection7). The main problems encountered using such methods are either the need for derivatization or the need for time-consuming extraction procedures. Since these techniques have a slightly expensive instrumentation and high running costs, the use of simpler, faster and less expensive, but still sensitive, electrochemical techniques can be an alternative. Electroanalytical techniques have been used for the determination of a wide range of drug compounds8-11). Additionally, application of electroanalytical techniques includes the determination of electrode reaction mechanism. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity. There appears to be no analytical methods for the determination and oxidation mechanism of vardenal, in bulk form and pharmaceutical dosage forms.

The aim of this work was to study the electrochemical behavior and oxidation mechanism of udenal at glassy carbon electrode and the development of new voltammetric methods for the direct determination of udenal in raw material, pharmaceutical tablets.

2. Experimental

2.1. Apparatus and Chemicals

Voltammetric measurements were performed using CHI660A Potentiostat and the three-electrode system consisted of a glassy carbon working electrode, Ag/AgCl (3 M KCl) reference electrode, and a Pt auxiliary electrode. Before each measurement, the glassy carbon electrode was polished with aqueous slurry of 0.01 μm alumina powder on a damp polishing cloth until a mirror-like finish was obtained. The pH was measured using a pH/Ion Analyzer Model MA235 (METTLER - TOLEDO GmbH, made in Switzerland) with a pH combination electrode.

Udenafil (mol. Mass = 516.657 g/mol) was obtained as a gift from Research Laboratory of Dong-A Pharmaceutical Company (Yongin, South Korea) and Zydena® tablets containing 100 mg udenafil was also obtained from Dong-A Pharmaceutical Company. 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]-7-pyramidinone (EMPP, CAS No. 139756-21-1) and 2-(2-aminoethyl)-1-methylpyrrolidine (CAS No. 51387-90-7) were supplied by Sigma-Aldrich Chemical Company. Other chemicals, all of analytical-reagent grade (Sigma-Aldrich) were also used.

The stock solution of udenafil (2×10^{-3} M) was prepared in selected supporting electrolyte. The 0.2 M phosphate and acetate buffers for pH 2~6 were used as the supporting electrolytes. These buffer solutions were prepared daily before use. All solutions were kept in the dark in a refrigerator and used within several hours to avoid hydrolysis. Udenafil solutions were stable and their concentration did not change with time. All measurements were carried out at ambient temperature of the laboratory.

2.2. Tablet Assay Procedure

Ten tablets were weighed accurately and finely powdered. A portion of the powder, equivalent to 2×10^{-3} M udenafil, was transferred to a 100 ml volumetric flask and completed to volume with selected supporting electrolyte and sonicated for 15 min. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with selected supporting electrolyte. The nominal content of the tablet amounts were calculated from the corresponding regression equations of previously plotted calibration curve. To study the accuracy of the proposed method and check the possible interferences from common excipients, recovery studies were carried out after the addition of known amounts the pure udenafil to various pre-analyzed formulation of udenafil. The recovery of udenafil was calculated using the corresponding regression equations of previously plotted calibration curve.

3. Results and Discussion

3.1. Electrochemical Oxidation Behavior of Udenafil

The electrochemical behavior of Udenafil (Zydena®) was investigated over various pH range (2~6) at the glassy carbon electrode in buffered media using cyclic voltammetry (CV) and linear sweep voltammetry (LSV). Square wave (SW) voltammetric technique was developed for the quantitative determination of udenafil.

Cyclic voltammograms for udenafil on a glassy carbon electrode are shown in Fig. 2. We used the supporting electrolyte containing 30% acetonitile to maintain the solubility of udenafil. The cyclic voltammetric behavior of 2×10^{-3} M udenafil yielded one well-defined wave (E_{p2}) in strongly acidic solution (<pH 2.5). At 2.5< pH<6, this one peak was split into two waves. No cathodic peak was observed in this potential window (0.0 V to 1.8 V vs. Ag/AgCl reference electrode).

Both peaks (E_{p1} and E_{p2}) are shifted to less positive potentials by increasing the pH from 2 to 6 (Fig. 3). The first peak potential (E_{p1}) of udenafil continues to shift to less positive potentials, with a slope of about 62 V/pH, by increasing the pH from 3.5 to 7.0. The second peak potential (E_{p2}) of udenafil continues to shift to less positive potentials, with a slope of about 12 mV/pH, by increasing the pH from 2.0 to 4.0 and 93 mV/pH, by increasing the pH from 4.0 to 7.0.

Scan rate studies were carried out to assess whether the processes on glassy carbon electrode were under diffusion or adsorption control. When the scan rate was varied from 5 to 1000 mV/s in 2×10^{-3} M solution of

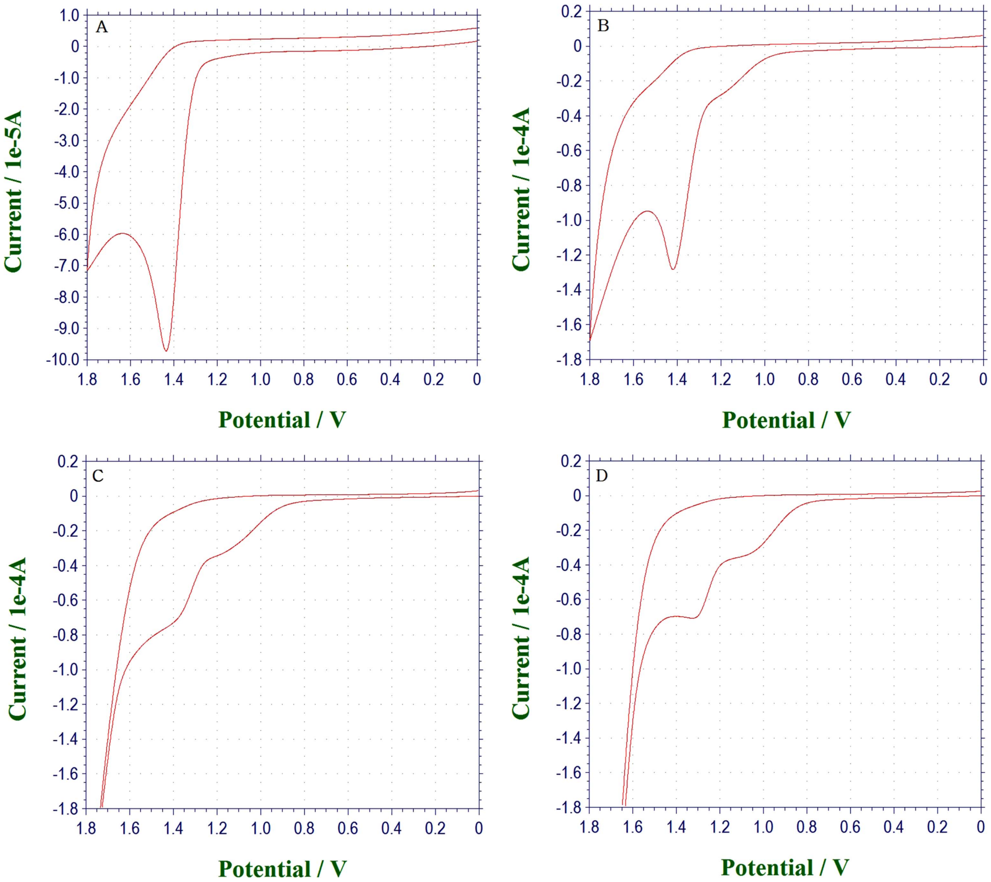

Fig. 2. Cyclic voltammograms of 0.2 mM solution of udenafil using a glassy carbon electrode. Current-voltage curves recorded from 0.0 V (vs. Ag/AgCl electrode) to +1.8 V and back to 0.0 V. Scan rate: 100 mV/s. In phosphate buffers of (A) pH 2.38, (B) pH 3.76, (C) pH 4.68 and (D) pH 5.60 containing 30% MeCN.

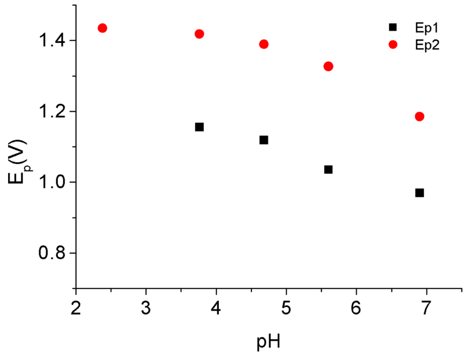

Fig. 3. Dependence of peak potentials (E_{p1} and E_{p2}) obtained in a solution of 2×10^{-3} M udenafil on pH.

udenafil, a linear dependence of the peak intensity i_p (μA) upon the square root of the scan rate ($v^{1/2}$) (mV/s) was found, demonstrating a diffusional behavior (Fig. 4). When the scan rate was varied from 5 to 1000 mV/s in 2×10^{-3} M solution of udenafil, a linear dependence of the peak intensity i_p (μA) upon the square root of the scan rate ($v^{1/2}$) (mV/s) was found, demonstrating a diffusional behavior. A plot of logarithm of peak current versus logarithm of scan rate gave a straight line with a slope of 0.441 very close to the theoretical value of 0.5, which is expressed for an ideal reaction for the diffusion-controlled electrode process (Fig. 5)[12].

To identify the oxidation process of udenafil, the study on some selected model compound were realized by cyclic voltammetry at the glassy carbon electrode (Fig. 6). The first model compound (B), 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo [4,3-d]-7-pyramidinone (EMPP), is oxidized over most of pH range, producing one well-defined, main irreversible oxidation peak by cyclic voltammetry in pH 2~6 ranges. The peak potentials (E_p) is shifted with increasing pH to less positive potentials similarly as E_{p2} of udenafil and became pH-dependent at pH lower than about 7. So, we expect that the monoprotonated form of 1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]-7-pyramidinone is oxidized at pH < 7. The second model compound (C), 2-(2-aminoethyl)-1-methylpyrrolidine, is oxidized over most of pH 2~6 ranges producing a very weak and broad wave by cyclic voltammetry. This wave

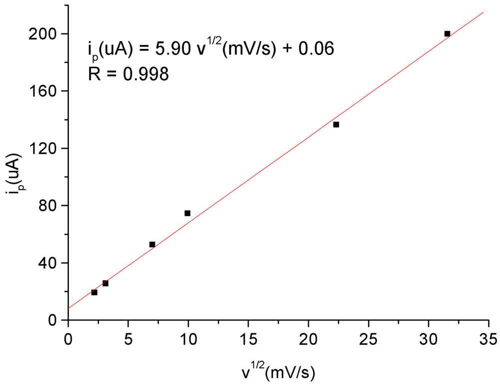

Fig. 4. A plot of peak current (i_p) versus square root of scan rate ($v^{1/2}$) for 2 mM udenafil in 0.2 M phosphate buffer at pH 2.38.

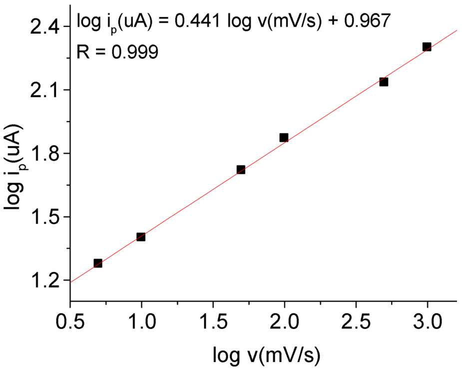

Fig. 5. A plot of logarithm of peak current (log i_p) versus logarithm of scan rate (log v) for 2 mM udenafil in 0.2 M phosphate buffer at pH 2.38.

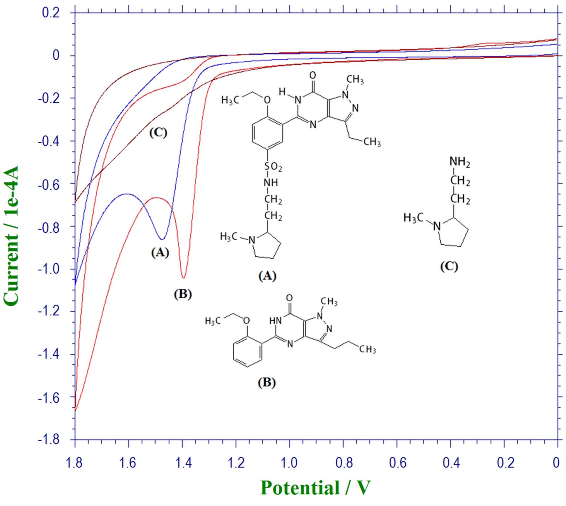

Fig. 6. TCyclic voltammograms of (A) udenafil, (B) 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]-7-pyramidinone and (C) 2-(2-aminoethyl)-1-methylpyrrolidine in 0.2 M phosphate buffer containing 30% acetonitile at pH 2.50. scan rate 100 mV/s

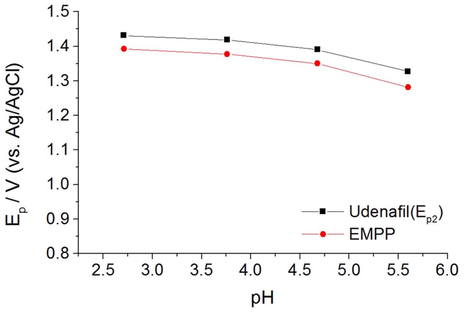

Fig. 7. TThe peak potentials versus pH for 2 mM udenafil and EMPP in 0.2 M phosphate buffer containing 30% acetonitile.

is also shifted with increasing pH to less positive potentials. However the peak potentials of 2-(2-aminoethyl)-1-methylpyrrolidine could not be determined due to broad as shown at Fig. 6. Both peaks (E_{p2} of udenafil and E_p of EMPP) are shifted to less positive potentials by increasing the pH from 2 to 6 (Fig. 7). The E_{p2} of udenafil and E_p of EMPP continue to shift to less positive potentials, with a slope of about 12 mV/pH, by increasing the pH from 2.0 to 4.0 and about 93 mV/pH, by increasing the pH from 4.0 to 7.0.

The anodic oxidation behavior of udenafil is also comparable to tamsulosin, which was reported[10)]. Our results for the second peak potential (E_{p2}) of udenafil revealed a good agreement with the redox mechanism postulated for tamsulosin including alkoxybenzene group. Also, the anodic oxidation behavior of udenafil is also comparable to ganciclovir, 6-hydroxypurine and pentoxifylline, which were reported[13-15)]. Our results revealed a good agreement with the redox mechanism postulated for ganciclovir, 6-hydroxypurine and pentoxifylline. They have a 6-hydroxypurine group. Therefore we suggest that oxidation process of udenafil may be occurring on the alkoxybenzene group and 6-hydroxypurine group of udenafil and 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]-7-pyramidinone (EMPP).

3.2. Analytical application

We selected the SW voltammetric method, which is effective and rapid technique with well established advantages, including good discrimination against

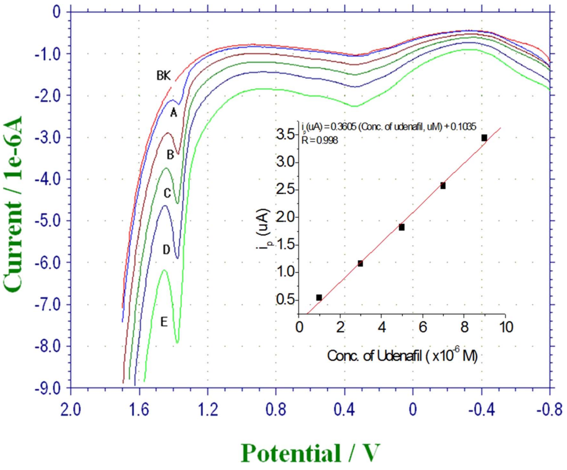

Fig. 8. TSquare wave voltammograms obtained for various concentration of udenafil, BK; blank, A; 1×10^{-6} M, B; 3×10^{-6} M, C; 5×10^{-6}, D; 7×10^{-6} M and E; 9×10^{-6} M, in supporting electrolyte containing of 0.2 M phosphate buffer at pH 2.0. Curves at -0.8 to 1.8 V.

background currents and low detection limit. The best result for SW voltammetric technique was obtained with 0.2 M phosphate buffer at pH 2.0. Because udenafil in 0.2 M phosphate buffer of pH 2.0 has the solubility of 0.0274 g/mL, 0.2 M phosphate buffer at pH 2.0 as supporting electrolyte was chosen. The SW voltammograms obtained at various concentrations of udenafil were shown in Fig. 8. The good linear correlation between the peak current and concentration (R=0.998) were obtained from SW voltammograms in supporting electrolyte containing of 0.2 M phosphate buffer at pH 2.0 for concentration between 5×10^{-7} – 5×10^{-5} M. are summarized in Table 1. The limit of detection (LOD) was obtained using equation LOD = 3 s/m and the limit of quantitation (LOQ) using LOQ = 10 s/m. Where s is the standard deviation of the peak currents (three runs) of sample and m is the slope of the related calibration curve[16)]. The characteristics of the calibration curve and the LOD and LOQ values are shown in Table 1.

On the basis of above results, square wave voltammetric technique applied to the direct determination of udenafil in tablets using the related calibration curve without the special pretreatment. The accuracy of the proposed method was determined by its recovery. Recovery studies were carried out after the addition of

Table 1. Regression data of calibration curve for determination of udenafil by SW voltammetry

	SWV technique
Measured potential (V)	1.38
Linear range (M)	5×10^{-7} - 5×10^{-5} M
Slope (μA/M)	3.61×10^{-5}
Intercept (μA)	0.1035
Correlation coefficient	0.998
LOD (M)	9.1×10^{-8}
LOQ (M)	3.0×10^{-7}

Table 2. Assay results of udenafil tablets (Zydena®)

	SWV technique
Labeled claim (mg)	100.0
Amount found (mg) *	99.4
RSD %	0.87
Added (mg)	20.0
Found (mg) *	19.8
Recovery %	99.0

*Each value is the mean of five experiments.

known amounts the pure udenafil to various pre-analyzed formulation of udenafil. According to the results, excipients presented in tablet do not interfere. Square wave voltammetric technique can be successfully applied to the determination of udenafil in tablets (Table 2).

4. Conclusion

The electrochemical behavior of udenafil on glassy carbon electrode was studied. Cyclic voltammetric behavior of udenafil is irreversibly oxidized at high positive potentials. We suggested that the oxidation process of udenafil may be occurring on the alkoxybenzene group and 6-hydroxypurine group. SW voltammetry is effective and rapid electroanalytical technique with well-established advantages, including good discrimination against background current and low detection limits. Square wave voltammetric technique applied to the direct determination of udenafil in tablets using the related calibration curve without the special pretreatment and the excipients presented in tablet do not interfere with analysis.

Acknowlegements

This work was supported by Hanseo University Research Grants in 2009.

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