Electrochemical Study of Zolpidem at Glassy Carbon Electrode and Its Determination in a Tablet Dosage Form by Differential Pulse Voltammetry

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Received February 9, 2004; accepted June 1, 2004

The oxidative behaviour of, a hypnotic drug, zolpidem was studied at glassy carbon electrode in Britton–Robinson buffer over the pH range 2.0—11.0 using cyclic, linear sweep and differential pulse voltammetry. Oxidation of the drug was effected in a single irreversible, diffusion-controlled step. Using differential pulse voltammetry (DPV), the drug yielded a well-defined voltammetric response in Britton–Robinson buffer, pH 8.0 at +0.889 V (*vs.* Ag/AgCl) on glassy carbon electrode. This process could be used to determine zolpidem concentrations in the range 5.0×10^{-7} M to 1.0×10^{-5} M with a detection limit of 2.0×10^{-7} M. The method was applied, without any interference from the excipients, to the determination of the drug in a tablet dosage form.

Key words zolpidem; differential pulse voltammetry; glassy carbon electrode; electrochemical oxidation; pharmaceutical analysis

Zolpidem (Fig. 1), (*N*,*N*,6-trimethyl-2-(4-methylphenyl)-imidazole[1,2-*a*]pyridine-3-acetamide, is a non-benzodiazepine hypnotic drug with an imidazopyridine backbone, which acts in the brain principally at receptors of the ω_1-receptor subtype belonging to the γ-aminobutyric acid-(GABA)ergic system.[1)] Zolpidem is characterized by its fast onset of action and relatively short elimination half-life ranging between 1.4 and 4.5 h.[2)] For this reason bedtime administration carries a low risk of residual daytime sedative effects.[3,4)]

Many analytical methods have been published for the determination of zolpidem based on high-performance liquid chromatography (HPLC) combined with ultraviolet,[5)] fluorescence[6,7)] and with photodiode-array detection,[8)] gas chromatography-nitrogen phosphorus detection,[9)] gas chromatography-mass spectrometry,[10,11)] capillary electrophoresis with laser-induced fluorescence detection[12)] and radioimmunoassay.[13)]

However, to our knowledge no information about the electrochemical redox properties of zolpidem and its analytical application has appeared in the literature. The present study deals with the voltammetric oxidation behaviour of zolpidem on glassy carbon electrode and its determination by differential pulse voltammetry (DPV) in a tablet dosage form.

Experimental

Reagents Zolpidem hemitartrate was kindly supplied by Amriya for Pharmaceutical Industries (Alexandria, Egypt) and was used without prior purification. Stilnox® tablets were purchased from local pharmacies. Each tablet was labeled to contain 10 mg zolpidem hemitartrate. Methanol (Merck) was of HPLC grade; water was doubly distilled. All other chemicals were of analytical-reagent grade (Merck or Sigma) and were used as received.

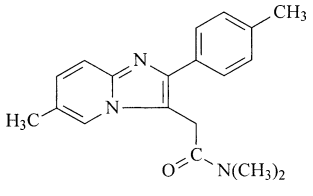

Fig. 1. Chemical Structure of Zolpidem

Zolpidem stock solutions were prepared daily by direct dissolution in methanol. Britton–Robinson buffers (0.04 M of each of acetic, *o*-phosphoric, and boric acids, adjusted to the required pH with 0.2 M sodium hydroxide solution) were used as supporting electrolytes.

Apparatus The voltammetric measurements were performed using a PC controlled AEW2 analytical electrochemical workstation with ECprog3 electrochemistry software (Sycopel, U.K.) connected to C-2 stand with a three-electrode configuration: a glassy carbon (Φ=3 mm) working electrode, an Ag/AgCl/3 M KCl reference electrode and a platinum wire counter electrode. OriginPro 7.0 software was used for the transformation of the initial signal. A CG 808 (Schott Geräte, Germany) digital pH meter with glass combination electrode served to carry out the pH measurements.

Procedure. Recommended Procedure Ten milliliters of the electrolyte solution were transferred into the voltammetric cell. After measurement of the blank solution, the appropriate amount of zolpidem solution is added and the anodic potential sweep was carried under different operational parameters. All measurements were carried out at room temperature, and peak heights were evaluated by means of the tangent method. The electrode must be rinsed with methanol and deionized water prior to each measurement.

Analysis of Tablets Ten tablets were weighed and powdered. Portion equivalent to a stock solution of a concentration about 1.0×10^{-3} M was accurately weighed, transferred into 10 ml volumetric flask and dissolved in methanol. The content was allowed to settle after stirring magnetically for 10 min. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting them with buffer solution in order to obtain a final solution of 5.0×10^{-6} M zolpidem. Each solution was transferred to a voltammetric cell and the differential pulse voltammogram was subsequently recorded following the optimized conditions. The content of the drug in tablet was determined referring to the calibration graph or regression equation.

Results and Discussion

Cyclic Voltammetry The cyclic voltammetric study of zolpidem as a function of pH at glassy carbon electrode points out the appearance of a single irreversible oxidation peak in the pH range of 5.0—11.0, and the peak is no longer present at lower pH values. A typical cyclic voltammogram of 1.0×10^{-4} M zolpidem at glassy carbon electrode in Britton–Robinson buffer at pH 8.0 is shown in Fig. 2. Zolpidem has an anodic peak at 0.903 V. No peaks are observed in the cathodic branch indicating that the zolpidem oxidation is an irreversible process.

Cyclic voltammograms were then recorded at different potential scan rates between 20 and 500 mV s^{-1}. A positive

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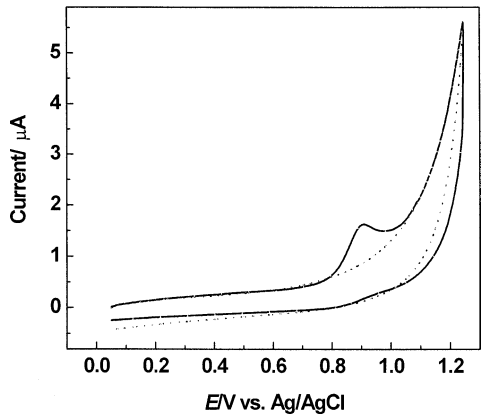

Fig. 2. Cyclic Voltammograms of 1.0×10^{-4} M Zolpidem Solution on Glassy Carbon Electrode in Britton–Robinson Buffer at pH 8.0

Scan rate, 100 mV s^{-1}. The dotted lines represent blank solution.

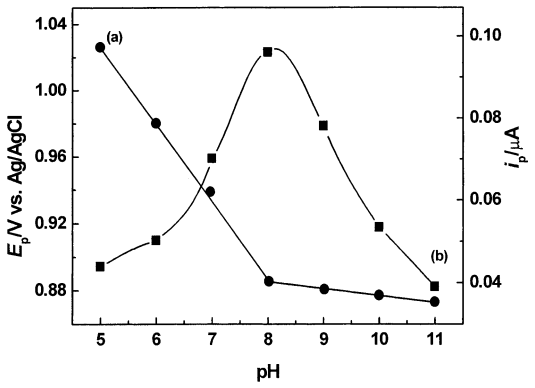

Fig. 3. Effect of pH on (a) Peak Potential and (b) Peak Current in Britton–Robinson Buffer Using Differential Pulse Voltammetry at Glassy Carbon Electrode

Zolpidem concentration, 3.0×10^{-6} M; scan rate, 10 mV s^{-1}; pulse amplitude, 50 mV; pulse width, 30 s.

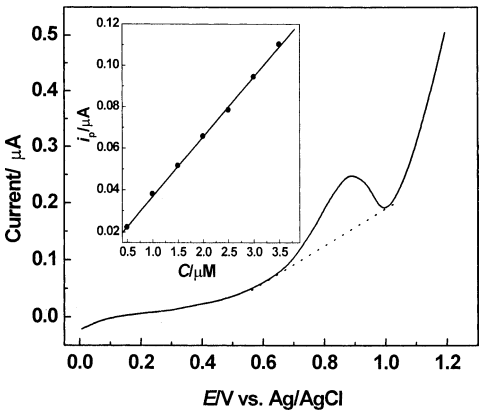

Fig. 4. Differential Pulse Voltammograms for 3.0×10^{-6} M Zolpidem in Britton–Robinson Buffer pH 8.0 at Glassy Carbon Electrode

Scan rate, 10 mV s^{-1}; pulse amplitude, 50 mV; pulse width, 30 ms. Inset is the calibration plot.

shift in the peak potential (E_p) was observed, which confirms the irreversibility of the process, with the simultaneous increase in peak current (i_p) when the scan rate (v) was increased. The linear relationship existing between peak current and the square root of the scan rate (correlation coefficient 0.999) gave a slope of 0.91, very close to the theoretical value of 1.0, which is expected for an ideal reaction of solution species,[14] so in this case the oxidation process is predominantly diffusion-controlled in the whole scan rate range studied.

In order to obtain information on the rate-determining step, the αn_a value (where α is the charge transfer coefficient and n_a is the number of electrons involved in the rate-determining step) was determined from a ($E_p - E_{p/2}$) value (where E_p is peak potential and $E_{p/2}$ is the half-peak potential) which is equal to $47.7/\alpha n_a$ mV for the totally irreversible diffusion controlled process.[15] The αn_a value obtained for 1.0×10^{-4} M zolpidem at pH=8.0 was 0.46. The number of protons transferred in the rate-determining step (p) calculated from the expression $\Delta E_p/\Delta\text{pH}=0.059p/\alpha n_a$ was found to be $\approx$1.0. The αn_a and p values are consistent with one electron-one proton transfer involved in the rate-determining step. Moreover, the current function ($i_p/v^{1/2}$) decreases with $v^{1/2}$, which is characteristic of a coupled chemical reaction following the electron transfer (EC mechanism).[16] This type of mechanism occurs quite frequently in organic compounds, which undergo electrochemical oxidation or reduction and produce a reaction species (radical or radical ions) that tend to dimerize.[16] It seems reasonable to conclude that the *N*-heterocyclic nitrogen of the imidazopyridine backbone on 1 e^-, 1 H^+ oxidation gives a free radical species, which is very unstable and combines readily with a similar species to form a dimer.

pH Dependence Figure 3 shows the effect of pH on peak potential and peak current for 3.0×10^{-6} M zolpidem solution between pH 5.0—11.0 using differential pulse voltammetry (DPV). The anodic peak potential is shifted to less positive values by increasing the pH with slope of −46 mV/pH unit until pH 8.0. In the range from pH 8.0 to 11.0 the peak potential remains practically pH independent (Fig. 3a), which can be explained by change in protonation of the acid-base functions in the molecule. The effect of the solution pH on the peak enhancement is also shown in Fig. 3b. The best results with respect to signal enhancement accompanied by sharper response was obtained with Britton–Robinson buffer at pH 8.0. This supporting electrolyte was chosen for subsequent measurement experiments.

Analytical Application In order to develop a voltammetric methodology for determining the drug, we selected the differential pulse mode, since the peaks were sharper and better-defined at lower concentration of zolpidem than those obtained by linear sweep voltammetry, with a lower background current, resulting in improved resolution. The optimum instrumental conditions were chosen from a study of the variation of the peak current with pulse amplitude, pulse width and scan rate. The peak current increased with increasing pulse amplitude from 20 to 100, but the peak became less sharp and ill defined. However, the peak current decreased as the pulse width increased from 30 to 90 ms. The peak current increased linearly with the scan rate up to 20 mV. Thus, the best peak definition was recorded when using 50 mV pulse amplitude, 30 ms pulse width and 10 mV s^{-1} scan rate.

Using the optimum conditions described above, a calibration curve over a range of 5.0×10^{-7} M to 1.0×10^{-5} M was obtained, which fitted the equation i_p (μA)=0.0288+0.0081C (μM), with a correlation coefficient r=0.9993. Standard deviations for the intercept and slope of the calibration

curve were 0.0004 μA and 0.0009 μA μM^{-1}, respectively. Figure 4 reports a typical cyclic voltammogram with a well-defined peak at $E_p = +0.889$ V and shows as inset the calibration plot. The limit of detection (*LOD*) of the procedure was calculated to be 2.0×10^{-7} M, which were estimated as: $LOD = 3S_{y/x}/b$,[17] where $S_{y/x}$ is the standard deviation of *y*-residuals and *b* is the slope of the calibration plot. The reproducibility of the measurement was calculated from five independent runs of 5.0×10^{-6} M zolpidem solution. The relative standard deviations were calculated to be 0.45 and 1.51% for peak potential and peak current, respectively.

Interference Studies In order to investigate the analytical application of this method, the effect of the excipients present in the dosage form was examined by carrying out the determination of 5.0×10^{-6} M zolpidem in the presence of each of the different excipients at concentrations that can be found in the tablet dosage form. A deviation of more than 2% from the peak current of the solution containing no interfering additives was taken as a sign of interference. These studies showed that none of the excipients at the concentration level existing in the dosage form caused a positive or a negative error indicating that there were no serious interferences to the method.

Zolpidem Assay in Tablets The validity of the proposed voltammetric method was tested by determining zolpidem in Stilnox® tablets. Each tablet was labeled to contain: zolpidem hemitartrate (10.00 mg), lactose (90.40 mg), cellulose microcrystalline (12.10 mg), methylhydroxypropyl cellulose (8.10 mg), sodium carboxymethyl amidon (3.80 mg), magnesium stearate (1.20 mg), titanium dioxide (1.84 mg) and polyethylene glycol (0.56 mg). The results of the proposed voltammetric method were evaluated statistically as compared with a HPLC method[5] (Table 1). According to the results of *t*- and *F*-tests, the variances between the two methods were found to be insignificant at 95% probability level, indicating that no significant differences exist between the performances of the two methods regarding their accuracy and precision.

Table 1. Application of the Proposed Voltammetric Method to the Determination of Zolpidem in Stilnox® Tablets (10 mg/Tablet)

Item	DPV	HPLC method[5]
Mean (%)	98.66	99.96
S.D.	1.33	0.99
N	6	6
t-value	1.92 (2.23)	
F-value	0.554 (4.284)	

Figures in parentheses are the corresponding theoretical *t*- and *F*-values ($p = 0.05$).

Conclusions

Zolpidem is irreversibly oxidized at glassy carbon electrode. Application of the DPV method using glassy carbon electrode to pharmaceutical preparations is possible after a simple dilution step without interference from the ingredients of tablet matrix. The proposed DPV method is simple, inexpensive, selective and precise and does not require any complex pre-treatment except polishing the electrode surface.

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