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Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 761–766

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Short communication

Electrochemical behavior of thalidomide

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Received 24 November 2001; received in revised form 28 February 2002; accepted 23 March 2002

Keywords: Thalidomide; Cyclic voltammetry; Adsorption

1. Introduction

Thalidomide was introduced as a sedative in the 1950's and was later marketed to prevent nausea in pregnant women. It was withdrawn from the market in the early 1960's because of its ability to cause severe birth defects. In the 1990's, it was confirmed that thalidomide was very effective in alleviating erythema nodosum leprosum in leprosy patients. During the past several years there has been a resurgence of interest in thalidomide. Factors contributing to this renewed interest in thalidomide are the numerous treatment of actinic prurigo, chronic cutaneous lupus erythematosus, prurigo nodularis, cutaneous manifestations of graft-versus-host reaction, and aphthous ulcers in patients with AIDS. It also has inhibitive ability to tumor [1]. The determination of thalidomide by capillary electrophoresis (CE), capillary electrochromatography (CEC), high-performance liquid chromatography (HPLC), nano-HPLC, on-line HPLC-atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and solid-surface room-temperature phosphorimetry [2–13] has been reported, but its electrochemical behavior has never been investigated. The electrochemical investigation of thalidomide may provide us some information about its redox process, which is important for our understanding of its property as well as its metabolism in biological system. In the present paper the electrochemical behavior of thalidomide was studied. A sensitive analytical method is described and the electrode reaction mechanism is discussed.

2. Experimental

2.1. Instrumentation

Cyclic voltammetric and Chronocoulometry experiments were performed by using an EG&G PAR (Princeton Applied Research) Model 283 potentiostat/galvanostat controlled by an IBM microcomputer with EG&G PARC M270 software and with a PAR Model 303 Static Mercury Drop Electrode (SMDE). The electrode was used in a hanging mercury drop (HMDE) mode. The electrode surface area was 0.0171 cm^2, as determined by weighing a large number of mercury drops. CHI660A Electrochemical Station was also

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PII: S0731-7085(02)00126-7

used to record the CVs when a glassy carbon electrode (GCE)(d = 3mm) was used as working electrode. Before use in electrochemical experiments, GCE was polished with 0.06-μm alumina slurry and sonicated in fresh water. Electrochemical measurements were performed using a conventional electrochemical cell, with mercury drop electrode or glassy carbon electrode as working electrode, Ag/AgCl electrode as the reference electrode and platinum wire as the counter electrode.

Water was triply distilled from an all-quartz still. High purity nitrogen was used for deaeration. All the measurements were carried out at (approximately 25 °C) and the solutions were previously deaerated with high-purity nitrogen for at least 15 min.

2.2. Chemicals

The stock solutions of thalidomide were prepared by dissolving known amounts of thalidomide in a small amount of 1, 4-dioxane and being diluted to 50 ml using triply distilled water before measurements. Other reagents used were of analytical grade. The stock solution containing thalidomide was protected from light. All measurements were the average of at least three replicate measurements.

3. Results and discussion

The CV experiments on thealidomide show two electro-reduction peaks in the medium of 0.1M HCl solutions between 0.10 and − 0.80 V (Fig. 1). The properties of these two peaks will be discussed, separately as follows:

3.1. CV behavior of peak 1

3.1.1. Selection of experimental conditions

Various supporting electrolytes, such as H_2SO_4, HCl, HNO_3, $HClO_4$, HAc-NaOAc, Phosphate buffer solution were tested by cyclic voltammetric experiments, and HCl was found to be the best because the voltamogram of thalidomide was well defined and the sensitivity was reasonably high. Thus 0.1 mol/l HCl solution was chosen in all experiments. The experiments on the effect of preconcentration potential on the peak current, i_{p1}, showed that i_{p1} remained almost constant, over the range of preconcentration potentials 0.15–0.05 V. Thus, 0.1 V was chosen as the preconcentration potential.

As shown in Fig. 1, on HMDE the CV curve of thalidomide gives rise to two well-defined reduction peaks and one oxidation peak in 0.1 mol/l HCl solution. Furthermore, a strong adsorption process accompanies this faradic reaction. The first reduction process is reversible because a pair of reduction/reoxidation peaks at different scan rates are almost symmetrical, and the second reduction process is irreversible. The second reduction peak was applied to determination of thalidomide concentration, due to its high sensitivity. Under the optimized conditions, for a 100 mV/s scan rate, i_{p2} was linearly dependent on the

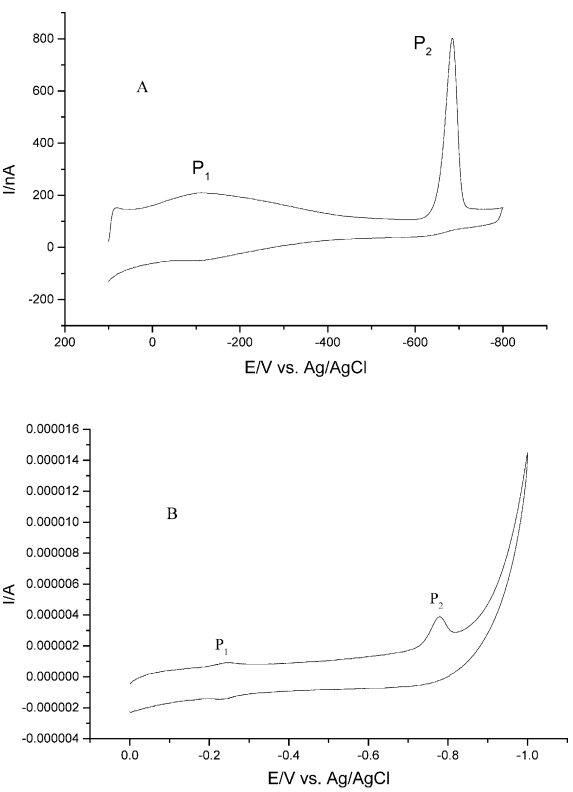

Fig. 1. (A) CVs of 1.0×10^{-5} mol/l thalidomide in 0.1M HCl solution. (Hg electrode), (B) CVs of 1.0×10^{-5} mol/l thalidomide in 0.1M HCl solution. (glassy carbon electrode)

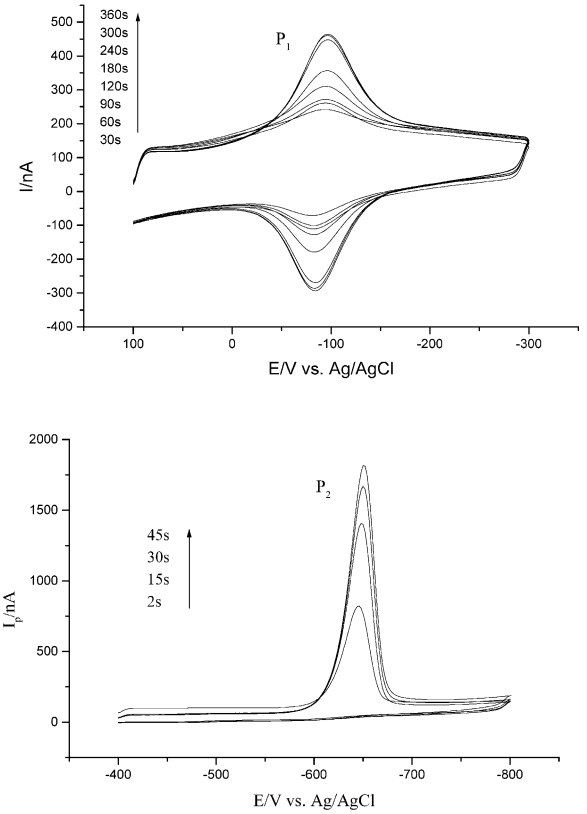

Fig. 2. Effect of accumulation time on voltammetric behavior of 1.0×10^{-5} mol/l thalidomide in 0.1M HCl solution. Scan rate: 0.1 V/s

thalidomide concentration over the range of 2.0×10^{-9}–2.0×10^{-6} mol/l, for the accumulation time of 30 s (correlation coefficient, $r = 0.9951$). The detection limit was 1.0×10^{-9} mol/l.

Fig. 1 depicts a CV behavior of thalidomide in 0.1mol/l HCl solution at glassy carbon electrode. Similarly, The CV scan shows two reduction peaks and one oxidation peak. Compared with the reduction of thalidomide on HMDE under similar conditions, the peak current was smaller, and the peak shape has changed slightly.

3.1.2. Effect of accumulation time of a mercury drop

In the case of adsorption, the peak current increases with prolonging accumulation time, owing to accumulation of the adsorptive species. Fig. 2 shows the CVs of thalidomide in different accumulation time. The peak current of thalidomide increased with increasing the accumulation time until the adsorptive equilibrium is approached. The electro-reduction and electro-oxidation peaks are symmetrical for the first pair of peaks and $E_{pc1} = E_{pa1}$. Furthermore, the peak current, i_{p1}, is linearly dependent on scan rate in the range of 0.05–0.5 V/s. These results indicated that the first electrode reaction of thalidomide is a reversible surface electrochemical reaction with both the reactant and product strongly adsorbed on the electrode surface [14].

The limiting value of the first reduction peak current for 1.0×10^{-5} or 5.0×10^{-5} mol/l thalidomide is approached with accumulation time of 240 or 180 s, respectively. This means that the adsorptive equilibrium is reached faster at higher concentration, which is also characteristic of adsorptive waves [15,16].

3.1.3. Effect of scan rate

According to the Randles–Ševick equation in a linear diffusion-controlled process, $I_p \propto v^{1/2}$; for the adsorptive process $I_p \propto v$. The peak currents of thalidomide are plotted against the scan rate. The peak currents I_p increase linearly with increasing scan rate v; the I_p versus $v^{1/2}$ curve shows an upward incline. This points out to the adsorptive nature of the peak. The plots of $\log I_p$ against $\log v$ for 1.0×10^{-5} mol/l thalidomide are straight lines with slopes of 0.99. These calculated results agree with the theoretical value of 1.0, indicating the adsorptive nature of the reduction species [14,16,17].

3.1.4. Relationship between current function and scan rate

The relationship between the current function and the scan rate may be simply indicated by the curve of $I_p/v^{1/2}$ versus scan rate, which decides the various electrode process [16]. If an electrode is diffusion controlled, the current function will be independent of scan rate. In the case of adsorption, I_p versus $v^{1/2}$ increases with increasing scan rate. Fig. 3 shows that the electrode process is adsorption controlled.

For a reversible adsorption peak [18]:

$$W_{1/2} = \frac{3.52\,RT}{nF}(\text{mV}) \quad (1)$$

where $W_{1/2}$ is the width of the peak at mid-height. For the first reduction peak, $W_{1/2}$ is 60 mV. Thus, the estimated number of electron transferred during the electrode reaction (n) is 2. The width of the peak is larger than that theoretically predicted, which might be caused by the deviation of the adsorption from the Langmuir isotherm.

3.1.5. The effect of pH value

The influence of pH value on the reduction peak potential was examined. The first reduction peak potential changed to more negative linearly with the increase of pH value, indicating that protons take part in the reduction reaction. Based on our experimental data, a linear regression equation $E' = -62.2$ pH-47.7 (E', taken as the average of E_{pc} and E_{pa}(mV); pH 1.0–5.6; correlation coefficient, $r = 0.9962$) was obtained, which suggested that the uptake of electrons is accompanied by an equal number of protons.

3.1.6. Determination of diffusion coefficient

The chronocoulometric method is suitable for determination of the diffusion coefficent of a reactant, according to the equation [19]:

$$Q = \frac{2n\text{FAC}(Dt)^{1/2}}{\pi^{1/2}} + Q_{dl} + Q_{ads} \quad (2)$$

Q_{dl} is double-layer charge (integration of charging current), Q_{ads} the faradaic component given to the reduction of adsorbed species. The plot of Q versus $t^{1/2}$ is shown in Fig. 4. From the slope, the diffusion coefficient was determined, $D = 2.48 \times 10^{-6}\text{cm}^2/\text{s}$.

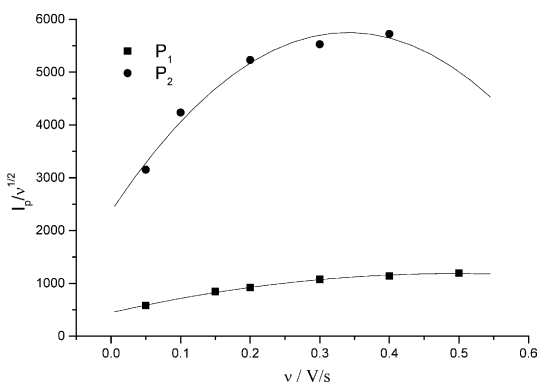

Fig. 3. Relationship between current function and scan rate.

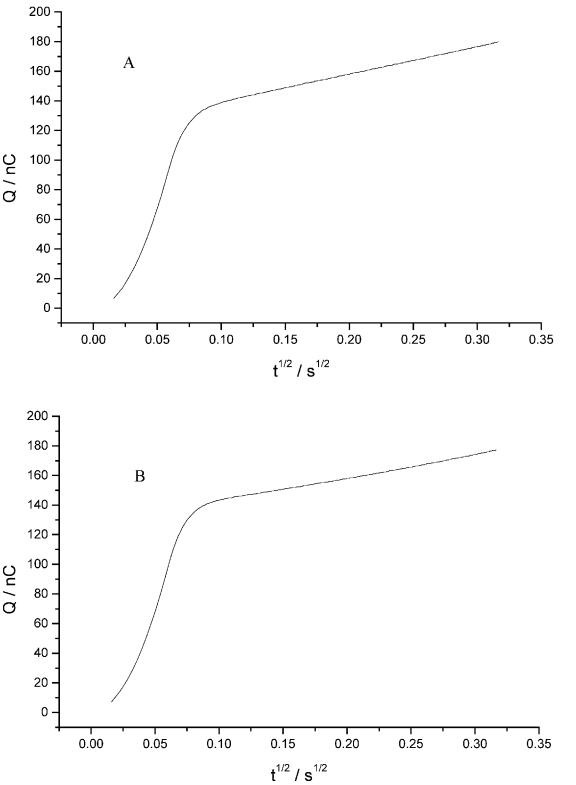

Fig. 4. Chronocoulometric dependence of charge on square root of time. 1.0×10^{-5} mol/l thalidomide in 0.1 m HCl solution. Step time: 500 ms, potential step: 0.10 V to -0.30 V (A), -0.40 V to -0.80 V (B).

3.2. CV behavior of peak 2

The electroreduction peaks, between -0.40 and -0.80 V, are revealed in the cyclic voltammograms (Fig. 1) for thalidomide in 0.1mol/l solution where $E_{p2} = -0.66$ V.

The longer the accumulation time, the larger the i_{p2} values before the adsorptive equilibrium are reached (Fig. 2). Furthermore, the peak current is linearly dependent on the scan rate. The peak potential E_p becomes more negative with increasing the scan rate. These mean that the electrochemical reactions are adsorption-controlled irreversible electrode reaction.

As the system studied belongs to those where reactant is strongly adsorbed, and the electrode reaction is irreversible, we may use Laviron's equation [14,20,21]:

$$E_p = E' + \frac{RT}{\alpha nF}\ln\frac{RTk_s}{\alpha nF} - \frac{RT}{\alpha nF}\ln \upsilon \quad (3)$$

where α is the transfer coefficient, k_s the standard rate constant of the surface reaction, and K' the formal potential. According to Eq. (3), the plot of E_p versus lnv should be linear. From its slope, the αn value can be determined, and from the intercept, the k_s can be calculated, if the value of K' is known. It has been shown that if υ approaches zero [20], both the cathodic and anodic peaks tend to be reversible. The curves in the plot of E_p versus υ are linear at the small scan rate. Therefore, the value of E' in Eq. (3) can be determined from the ordinate intercept of the E_p versus υ plot by extrapolating the line to $\upsilon = 0$. A set of cyclic voltammograms for 1.0×10^{-5} mol/l thalidomide in 0.1M HCl at different scan rate were recorded. The $\alpha n = 1.840$ values could be obtained from the slopes of the E_p versus $\ln \upsilon$ plots. The E′ value were -0.641 V, as determined from the intercepts of the E_p versus υ plot. Then, $k_s = 3.57\ s^{-1}$ value could be calculated from the intercepts of the E_p versus lnv plot.

Besides the diffusion coefficient, also the number of electrons transferred can be determined by chronocoulometry. Fig. 4 presents a plot of Q versus $t^{1/2}$ for the thalidomide. Curve A in Fig. 4 is plotted for the potential step 0.1– – 0.3 V. The slope of the curve is $1.85 \times 10^{-7} C/s^{1/2}$. Ccurve B in Fig. 4 is plotted for the potential step -0.3– -0.8 V. Its slope is $1.55 \times 10^{-7}\ C/s^{1/2}$. Assuming that the diffusion coefficients of thalidomide and the products of the first electroreduction are the same, number of electrons, $n = 2$, of the second electroreduction can calculated. Furthermore, the influence of pH value on the second reduction peak potential was also examined. A linear regressive equation was obtained ($E_p = -54.1$pH -648.2; E_p (mV); pH 1.0 to 5.6; correlation coefficient, $r = 0.9937$), indicating that the uptake of electrons is accompanied by an equal number of protons.

According to [22], thalidomide undergoes spontaneous hydrolysis to twelve distinct products, each of which can be hydroxylated to several additional structures. Thus over 100 different metabolic products can be postulated, indicating that thalidomide was unstable in solution. That is the reason why the first pair of reduction/reoxidation peaks could not be observed after the placement of the stock solutions of thalidomide for nearly 5 days, that is also the reason for the electrode reaction mechanism is too complex to be explained clearly, although the two reduction reaction of thalidomide were two electron transfer process separately. To sum up, investigation on the electrochemical behavior of thalidomide in an aqueous solution not only enriches the study of its property, but also gives us more information on its metabolism.

4. Conclusion

Electrochemical behavior of thalidomide has been studied on the hanging mercury drop electrode. It can be concluded that the first reduction reaction of thalidomide is a two-electron reversible adsorptive process, and the second reduction reaction of thalidomide is a two-electron irreversible adsorptive process. The second adsorptive peak of thalidomide is sensitive, and can be applied to the determination of thalidomide. The investigation is important for better understanding the characteristics of thalidomide molecule.

Acknowledgements

The support of the National Natural Science Foundation of China (No. 20105001) is gratefully acknowledged.

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