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Square wave voltammetric determination of valproic acid in pharmaceutical Preparations

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Abstract: The electrochemical behavior of valproic acid (VAL) was investigated using square wave voltammetry, cyclic voltammetry, and sampled direct current polarography and a new square wave voltammetric method was developed for determination of VAL in pharmaceutical preparations. VAL showed two reduction peaks at about –0.2 V and –0.8 V vs. Ag/AgCl 3 M KCl with a hanging mercury drop electrode in 0.05 M pH 3.3 Britton–Robinson (BR) buffer. These peaks were named peak I and peak II, respectively. The types of limiting current of both peaks were determined as diffusion controlled based on cyclic voltammetry studies. A linear calibration graph was obtained in the range 1.46 $\times$ 10^{-4}–1.0 $\times$ 10^{-3} M. The limit of detection (LOD) and limit of quantification (LOQ) were 1.09 $\times$ 10^{-4} M (21.05 μg/mL) and 1.10 $\times$ 10^{-4} M (144.20 μg/mL), respectively. Recovery studies for the accuracy of the method were performed by adding known amounts of VAL and it was found to be 109.67 $\pm$ 4.85%. The proposed method was successfully applied to pharmaceutical products on the market.

Key words: Square wave voltammetry, drug determination, valproic acid, valproate analysis, carboxylic acid electrochemical reduction

1. Introduction

Valproic acid (VAL) is small molecular weight, branched, short-chain fatty acid (Figure 1). It is derived from valeric acid and naturally produced by the plant *Valeriana officinalis*.[1] VAL is the most commonly used anticonvulsant agent in the world and the drug of choice for the treatment of all types of epilepsy.[2] Determination of VAL is necessary not only for the control of dosage forms but also for monitoring the level of the drug in biological fluids to establish pharmacotherapy. Over 90% of VAL is bound to plasma proteins (mainly albumin)[3] and the unbound concentration has been shown to be a better predictor of therapeutic response than the total concentration.[4]

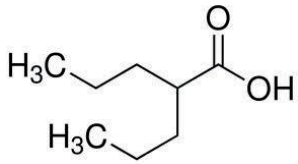

Figure 1. Chemical structure of VAL.

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As a weak organic acid, VAL and its conjugate base valproate are influenced by the pH of the medium. It is well known that the importance of pH regulation for the viability of cells is widely recognized. The cells of the central nervous system (CNS) do not differ from those of other tissues. Indeed, many of the mechanisms responsible for long-term "housekeeping" of hydrogen ions are similar. The study of pH in the CNS is also distinguished by the occurrence of rapid increases or decreases in H^+ that arise from electrical activity. The mechanisms generating and regulating these pH changes are of considerable neurobiological interest.[5] Moreover, the mechanism of action of VAL is still unknown[6] but there are different suggestions such as effect on GABA signaling, sodium channels, and the glutamatergic system, methylation, and inhibition of histone deacetylases.[7] There is a need to reveal the action mechanism of VAL clearly and to monitor it. For this purpose analytical techniques have been used.

Numerous methodologies have been described for determining VAL such as gas chromatography (GC) coupled with mass spectrometry (MS),[2,8–10] GC-flame ionization detector (FID),[11,12] liquid chromatography (LC)-MS,[13–15] high performance liquid chromatography (HPLC)-MS,[16,17] HPLC-fluorescence methods,[1,18] HPLC- electrochemical detection (ECD),[19] ultra-performance liquid chromatography (UPLC)- MS,[20] and capillary electrophoresis (CE) with a contactless conductivity detector.[21,22] Some biosensor studies were also performed to determine VAL.[23,24] All these methods are chromatography-based and they are time consuming and include cumbersome multiple-step procedures for sample preparation such as solvent extraction, evaporation, and derivatization. Only biosensors can be separated from other techniques due to based theory. However, preparation of a biosensor is also a procedure that takes time and needs experience to provide good results.

Electrochemical techniques are powerful and versatile analytical techniques that offer high sensitivity, accuracy, and precision as well as a large linear dynamic range, with relatively low cost instrumentation. Voltammetry is a current-voltage technique; the recording of current vs. potential is termed a voltammogram. Electrochemical measurements are two-dimensional techniques. The qualitative properties can be determined by using methods investigating by thermodynamic or kinetic control, beside the current related to quantitative properties controlled either by mass transport process or reaction rates. Thus, compounds can be selectively detected by electrochemical methods. This selectivity depends on the accessible potential range, the number of compounds that are active in this range, and the half-width of the single signals.[25] Due to similarity in the electrochemical and biological reactions, it can be assumed that the oxidation/reduction mechanisms taking place at the electrode and in the body share similar principles. Biologically important molecules can be investigated electroanalytically by voltammetry in order to determine the molecule in different ways. The redox properties of drugs can give researchers insight into their metabolic fate in in vivo redox processes or pharmacological activity. Furthermore, the electroanalytical techniques have been shown to be excellent for the determination of pharmaceutical compounds in different matrices.[26]

Electrochemical methods can be an alternative choice for VAL determination because there is still need for a simple, reliable, precise, and cheap method for VAL monitoring. Biosensors opened up the possibility of investigating VAL by electrochemical methods. To the best of our knowledge, the electrochemical behavior of VAL has not been published. This study has revealed the electrochemical behavior of VAL by using square wave voltammetry (SqW) on a hanging mercury drop electrode (HMDE). As mentioned previously, the action mechanism of VAL is still unknown, and so it is hoped that the proposed electrochemical mechanism in relation with pH can contribute to enlightening the action of mechanism of VAL on CNS.

2. Results and discussion

2.1. Mechanism studies

2.1.1. Effect of supporting electrolyte

The electrochemical behavior of VAL was studied in various supporting electrolytes over a wide pH range (2.0–10.0) at a mercury multimode electrode using cyclic, linear sweep voltammetry and square wave voltammetry. VAL did not give any peak in salted electrolytes like KCl or NaCl. For this reason, pH buffers were investigated to determine VAL. In general, VAL gave two cathodic peaks in acidic buffer solutions. These peaks disappeared when the pH of the supporting electrolyte was increased. This showed that the reduction of VAL depended on hydronium ions. Various buffer systems were tried to investigate the electrochemical behavior of VAL at different pH values and different concentrations, such as acetate, phosphate, and Britton–Robinson (BR). When the obtained voltammograms were investigated, it was seen that the reduction in VAL showed similar trends with different acidic buffers, i.e. VAL gave two peaks. Symmetrical and sharp peaks were obtained in 0.05 M BR using square wave voltammetry. VAL gave two cathodic peaks at about –0.2 V and –0.8 V versus the Ag/AgCl reference electrode in this acidic medium. These peaks will be named peak I and peak II, respectively.

2.1.2. Effect of pH

The influence of pH on the peak current of VAL was investigated by using square wave voltammetry. For this purpose, the concentration of VAL was kept constant at 1.96 $\times$ 10^{-4} M and the voltammograms obtained were investigated at different pH values for BR buffer. Current values due to reduction of VAL versus pH values of BR solution are presented in Figure 2 for both peaks. It was seen that pH had an important effect on the reduction of VAL and reduction peaks of VAL were diminished totally in medium at pH higher than 6.0.

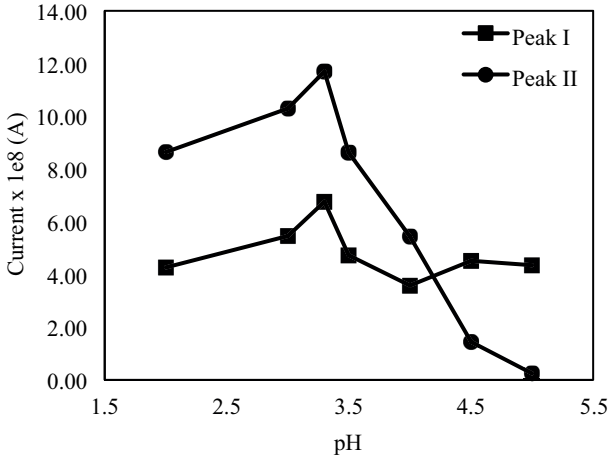

Figure 2. Effect of increasing pH on peak currents of 1.96 $\times$ 10^{-4} M VAL in 0.05 M Britton–Robinson buffer.

2.1.3. Cyclic voltammetry (CV) studies

Reduction of VAL was investigated by using CV to understand the type of current and reduction mechanism at different scan rates (V) between 10 and 1000 mV/s. A cyclic voltammogram is shown in Figure 3 for 3.33 $\times$ 10^{-4} M sodium valproate solution in 0.05 M BR buffer at pH 3.3 at a scan rate 100 mV/s.

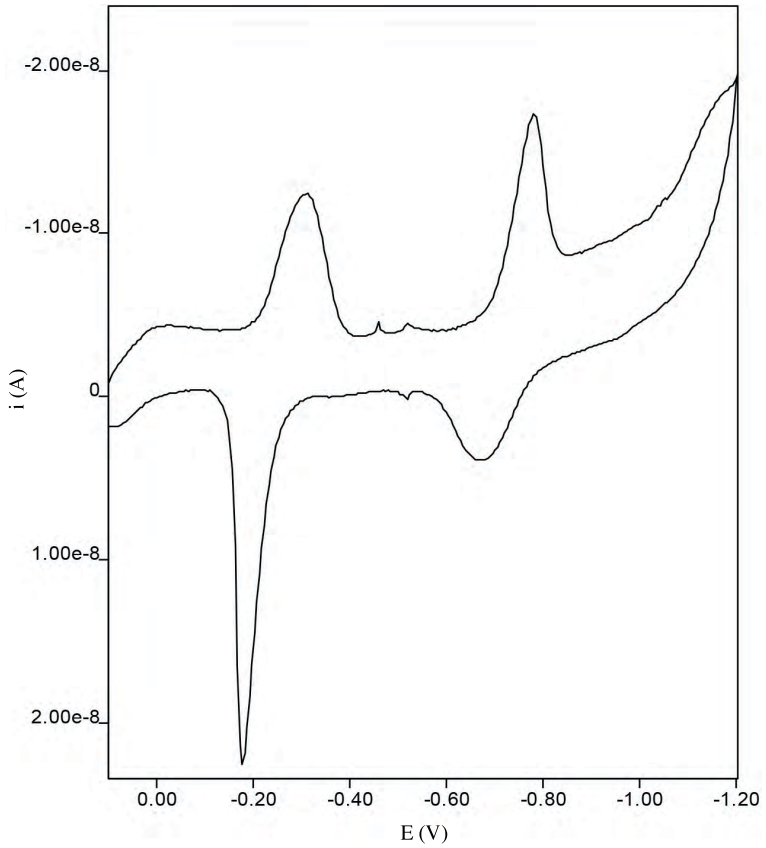

Figure 3. Cyclic voltammogram of 3.33×10^{-4} M sodium valproate solution in 0.05 M Britton–Robinson buffer at pH 3.3. Scan rate was 100 mV/s for this measurement.

Uslu et al. stated that the linear increase in peak current with the square root of the scan rate indicates a diffusion controlled process by working CV.[27] The plots of peak currents versus square root of scan rates gave correlation coefficients of 0.995 and 0.996 for peak I and peak II, respectively. These values showed that both electrochemical reactions giving peak I and peak II were diffusion controlled.

According to Bond,[28] when a graph is constructed as a logarithm of current versus logarithm of scan rate, if a slope value higher than 0.5 is obtained, it shows that the compound investigated is adsorbed on to the electrode surface. This study was carried out and related graphs were plotted as in the literature and slope values obtained were 0.54 and 0.71 for peak I and peak II, respectively; these values were higher than 0.5. As a result it can be said that VAL is reduced at –0.2 V vs. Ag/AgCl (3 M KCl) and then the produced form of VAL is both reduced at –0.8 V vs. Ag/AgCl (3 M KCl) and adsorbed on to the electrode surface.

Wang et al. describes another procedure for adsorption. If a plot of peak potentials versus logarithm of scan rate is linear, this situation indicates an adsorption controlled process.[29] In light of this information, related plots were constructed for both peaks and they gave correlation coefficients of 0.096 and 0.964 for peak I and peak II, respectively. The change was not linear for the first reduction peak but was linear for the second. It can be concluded that the reduction of VAL was diffusion controlled for the first reduction reaction, but the second reaction was affected by the adsorption process.

According to obtained data it can be understood that currents of both peaks were diffusion controlled but the effect of adsorption was more pronounced on peak II.

2.1.4. Reversibility

Reversibility tests were performed by using CV and direct current (DC) polarography. Cyclic voltammetric experiments were used to evaluate $\Delta E_p = E_p^c - E_p^a$ and i_p^a/i_p^c values at different scan rates. For a reversible process theoretically the $\Delta E_p = E_p^c - E_p^a$ value should be 58 mV at 298 K. These values were close to the values theoretically expected for a reversible reaction only at low scan rates. When scan rate increased, the reversibility rate for reactions decreased.[30]

Using the DC technique, a reversibility test was performed by plotting $\log i/(i_d - i)$ versus potential. The DC reversibility test was only applied to peak II, because peak I appeared under supporting electrolyte signals in the DC technique. For a reversible system the plot should be linear with a slope of n/0.059 at 25 °C.[31] The equation of this function for peak II was y = 17.113 x + 13.851 with a correlation coefficient of 0.93. The slope revealed that the electrochemical reaction of peak II undergoes one electron transfer.

From these results, it was concluded that the electrochemical reaction was reversible at low scan rates and the reaction was carried out by one electron transfer for peak II.

2.1.5. Proposed mechanism

According to the literature, the pH dependence of the potential is caused not only by the antecedent chemical acid-base reaction but also by the consumption of protons in the reduction process itself.[32] The pH dependence of the peak potential of reduction peaks of VAL were investigated to reveal the reduction mechanism and dE/dpH values were –48.3 mV and 27.0 mV. As seen from dE/dpH values, the reduction mechanism for both peaks involves consumption of protons.

Bhatti and Brown[33] suggested a theoretical mechanism as shown below for carboxylic acid. Wang and Hu[34] used this theoretical mechanism for explaining the electrochemical behavior of isonicotinic acid.

$$RCOOH + H^+ + e^- \rightleftarrows RC(OH)_{2(ads)} \tag{1}$$

$$RCOOH \rightleftarrows RCOO^- + H^+ \tag{2}$$

$$RCOO^- + H^+ + e^- \overset{slow}{\rightleftarrows} RC(OH)O^- \xrightarrow[fast]{H^+} RC(OH)_{2ads} \tag{3}$$

VAL has an acid–base equilibrium due to its weak fatty acid structure. Its pKa value was reported as 4.8 in the literature.[35] The concentration ratio of VAL to valproate can be calculated as 31.62 in the buffer solution of this work by using acid dissociation equilibrium (pH of BR buffer was 3.3). This result shows that the concentration of VAL is higher than the valproate concentration in the working medium.

Our suggestion for the electrochemical reduction mechanism of VAL is as follows:

$$C_7H_{15}COOH + H^+ + e^- \rightleftarrows C_7H_{15}C(OH)_{2(ads)} \tag{4}$$

$$C_7H_{15}COO^- + H^+ + e^- \rightleftarrows C_7H_{15}C(OH)O \xrightarrow{H^+} C_7H_{15}C(OH)_{2ads} \tag{5}$$

The conjugate acids were known to reduce at more positive potentials than conjugate bases. Therefore, it can be said that VAL is reduced at about –0.2 V following reaction (4) giving peak I, and its conjugate base valproate is reduced at about –0.8 V with reaction (5) forming peak II.

After revealing the mechanism some experiments were performed to determine whether adsorptive stripping voltammetry can be applied or not. However, relevant answers cannot be obtained using this type of voltammetry. The reason for this might be that the adsorbed species were electrochemical reduction products instead of valproate and VAL.

At the end of the investigations, peak II was selected for analytical purposes due to its wider linear concentration range.

2.2. Method validation

Validation of the developed was carried out according to the ICH regulation after optimization. For this purpose, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, dynamic range, and linearity parameters were investigated.

2.2.1. Selection of square wave parameters

2.2.1.1. Selection of potential step

A series of experiments was performed in order to optimize SqW parameters. For this purpose, an important parameter of SqW, potential step was changed while the others were kept constant and current values for both peaks were investigated. Concentration of VAL was 3.85×10^{-4} M during these investigations.

The variation in current for both peaks with potential step is shown in Figure 4. It was seen that as potential step increased, the current of peak I decreased, despite the current of peak II increasing. In order to optimize and obtain two well-defined peaks the potential step value was selected as 0.005 V.

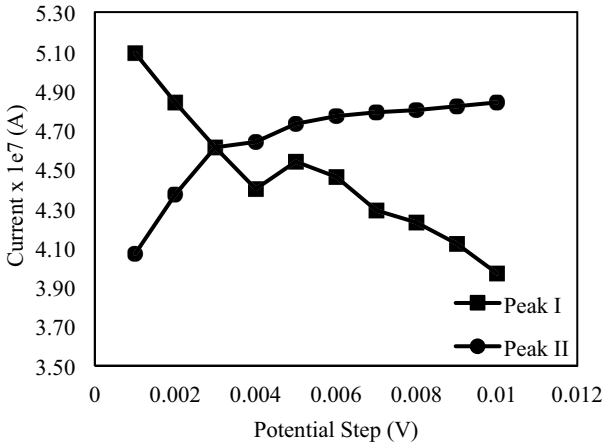

Figure 4. Variation in peak current values versus potential step for 3.85×10^{-4} M VAL in 0.05 M Britton–Robinson buffer pH 3.3.

2.2.1.2. Selection of frequency

Effect of frequency was investigated for both peaks. Peak I current increased with frequency proportionally. However, peak II showed a linear relationship with frequency until 60 Hz value, and after this value the upward tendency of current changed. Moreover, peak shapes were regular below 60 Hz frequency value. Due to these reasons a 60 Hz frequency value was selected for the SqWV wave parameter.

2.2.1.3. Selection of amplitude

When the effect of amplitude on current was investigated, both peak currents increased with amplitude until 0.08 V, but after this value the current values did not change prominently. For that reason, 0.08 V was selected as amplitude for the SqW parameter.

2.2.2. Dynamic range

Dynamic range of the method developed was evaluated by measuring standard solutions of VAL under optimized SqWV conditions. VAL gave two cathodic peaks when it was measured by using SqWV in pH 3.3 0.05 M BR buffer as shown in Figure 5. The peak currents increased linearly with increasing concentration. When the calibration curves were constructed, it was seen that the curve was linear according to the correlation coefficients in a large concentration range (0.98 and 0.99 for peak I and peak II, respectively). However, the linearity of the method developed should be checked and for this purpose Mandel's test was used. Mandel's test evaluates the change in data sets by comparing a constructed linear graph with a polynomial graph statistically and at the end of the calculations F values are compared with theoretical F values. According to this test the current values obtained did not change with concentration linearly determined by using calibration curve studies. For the studied data set the theoretical F value was 10.13 at 95% confidence interval and the F value for peak I was calculated as 11.84 by using Mandel's test. However, the current of peak II showed a linear change with increasing concentration and according to this test that F value for peak II was calculated as 1.40. Peak II was used for analytical purposes and further investigations were conducted based on peak II. Related parameters for these investigations are given in Table 1.

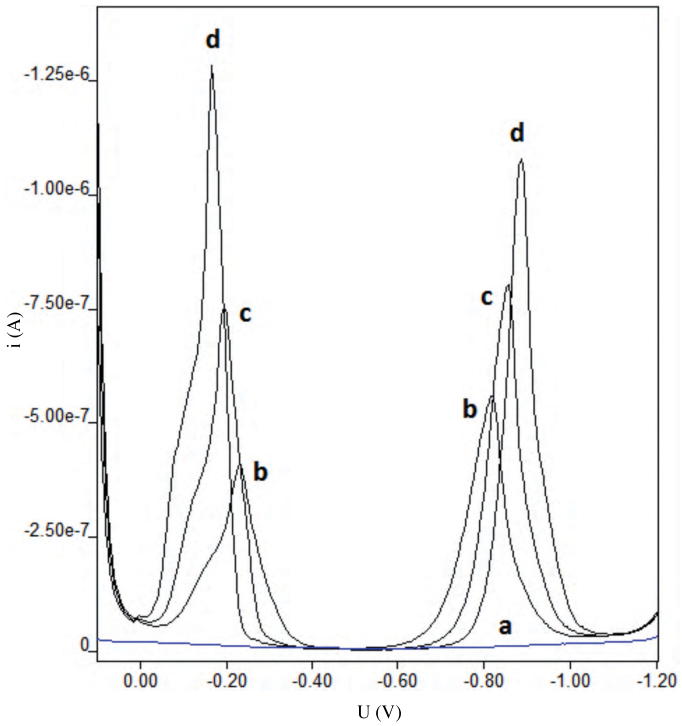

Figure 5. SqW voltammograms of standard solutions of VAL at different concentrations. a: 0.05 M pH 3.3 BR buffer, b: 4.95×10^{-4} M, c: 6.52×10^{-4} M, d: 8.33×10^{-4} M of VAL solution.

Table 1. Calibration curve results for VAL.

Parameter	Value
Concentration range	3.27×10^{-4}–8.33×10^{-4} M
Slope	1.3473×10^{-3}
$F_{theoretical}$	10.13
$F_{calculated}$	1.40
LOD	1.09×10^{-4} M
LOQ	1.10×10^{-4} M
Correlation coefficient	0.996

2.2.3. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined using peak II by the following equations:

$$LOD = \frac{3s}{m} \quad LOQ = \frac{10s}{m},$$

where s, the noise estimate, is the standard deviation of the peak currents of the sample (five runs), and m is the slope of the calibration curve. For VAL LOD and LOQ were 1.09×10^{-4} M and 1.10×10^{-4} M, respectively.

2.2.4. Accuracy

Recovery studies were carried out to investigate the accuracy of the developed method. For this purpose standard triplicate experiments of three independent samples of VAL at 4.60×10^{-4} M concentration value were performed and the recovery value was calculated by calibration curve. The mean recovery was 109.6 ± 4.85%. This result showed that the accuracy of the developed method was appropriate for the analysis of VAL.

2.2.5. Precision

Precision of the method was evaluated by comparing peak potentials and peak current values for peak II at different concentrations. For this purpose VAL solutions at three different concentration levels were measured and the data were compared using standard deviation. The standard deviations of peak potentials and peak currents are presented in Table 2. The results obtained showed that the developed method produced very precise results.

Table 2. Results of precision study for VAL in 0.05 M pH 3.3 BR buffer.

Concentration (M)	RSD of E (V)	Average current (A)	RSD of i (A)
2.38×10^{-4}	0.0028	3.53×10^{-7}	3.15
4.95×10^{-4}	0.0034	7.30×10^{-7}	2.41
8.68×10^{-4}	0.0050	1.22×10^{-6}	4.05

2.2.6. Selectivity

Selectivity of the method developed was investigated in the presence of some excipients like talc, lactose, sodium saccharin, and starch. There was no interaction preventing the signal of VAL except for a small potential shift. Selectivity experiments showed us that VAL can be analyzed in the presence of these excipients. Furthermore, the standard addition method allowed the analysis of VAL in the presence of these excipients. Figure 6 shows the voltammograms obtained by using the standard addition method for analysis of VAL in tablets.

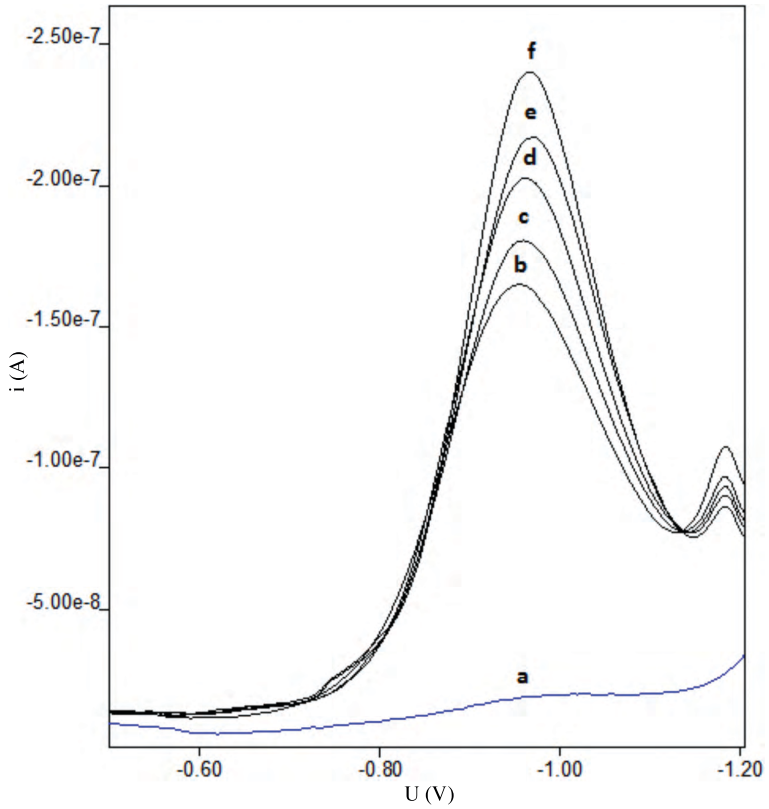

Figure 6. SqW voltammograms of a standard addition study. a: supporting electrolyte, b: tablet solution containing 3.70×10^{-4} M VAL, c: 100 μL, d: 200 μL, e: 300 μL, f: 400 μL of 5×10^{-3} M standard solution added.

2.2.7. Analysis of commercial preparations

The proposed method was performed for VAL determination in syrup and a tablet. For this purpose, three different bottles of syrup and tablets were obtained. Table 3 presents the results of analysis using the developed SqW method. Pharmaceuticals were examined for the amount claimed on the label and the results were 93.7 $\pm$ 8.22% for syrup and 92.8 $\pm$ 11.87% for the tablet.

Table 3. Analysis results of some commercial pharmaceutical preparations for VAL in 0.05 M pH 3.3 BR buffer.

Product	Claimed amount	Found amount
Syrup 1	200 mg/mL	187.16 $\pm$ 7.69
Syrup 2	200 mg/mL	170.08 $\pm$ 16.36
Syrup 3	200 mg/mL	202.99 $\pm$ 9.77
Tablet 1	500 mg per tablet	506.00 $\pm$ 63.00
Tablet 2	500 mg per tablet	422.00 $\pm$ 44.00

3. Experimental

3.1. Apparatus

Experiments were carried out using 757 VA Computrace (Metrohm, Switzerland), which is a PC controlled system for voltammetry and consists of a VA Computrace stand, add-on board for PC, connecting cable, and VA Computrace software 1.0. The stand includes a Ag/AgCl (3 M KCl) reference electrode and a platinum

wire auxiliary electrode. The working electrode was a multimode electrode combining the most important polarographic and voltammetric mercury electrodes in a single construction: hanging mercury drop electrode (HMDE), static mercury drop electrode (SMDE), and dropping mercury electrode (DME).

The optimum operation conditions for square-wave voltammetry were chosen as follows: pulse amplitude, 80 mV; frequency, 60 Hz; potential step, 5 mV.

3.2. Reagents and solutions

Sodium valproate (NaVAL) was kindly provided by Sanofi Doğu Pharmaceuticals Inc. (İstanbul, Turkey). Chemicals for buffer solutions, i.e. potassium chloride, potassium nitrate, sodium hydroxide, and orthophosphoric acid, were purchased from Merck (Darmstadt, Germany). Acetic acid was purchased from Sigma (Steinheim, Germany). Tablet and syrup samples for investigation were bought from local pharmacies.

A Jenway 3040 Ion Analyzer digital pH meter was used to measure the pH values of buffer solutions. For all procedures ultrapure water was used, obtained from the Elga water purification system.

A stock solution of VAL (1×10^{-2} M) was prepared by dissolving a calculated amount of sodium valproate in water and it was kept in the refrigerator. The working solutions for the voltammetric investigations were prepared by dilution of stock solution with water between 1×10^{-4} and 1×10^{-3} M VAL (14.42–144.20 μg/mL).

3.3. Procedure

In voltammetric cell a 10.0-mL volume of supporting electrolyte was deoxygenated with pure nitrogen for 5 min. After the voltammogram of this solution was recorded, VAL standard solution was added by micropipette. Then the solution was mixed and nitrogen was passed through the solution for 2 min. The voltammogram was recorded again.

Cyclic voltammetric experiments were performed in pH 3.3 BR buffer solution as supporting electrolyte at different scan rates.

3.4. Assay for pharmaceuticals

Tablet and syrup samples containing VAL were analyzed. Twenty tablets were weighed for tablet samples. A known amount of powdered solid corresponding to one tablet weight was taken and dissolved in water. After complete dissolution, the supernatant was filtered and diluted. The diluted solutions of tablets were used for analysis by using the standard addition method. The syrup samples were directly diluted with water and used for voltammetric analysis.

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