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Square-wave voltammetric (SWV) determination of Captopril in reconstituted serum and pharmaceutical formulations

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Abstract

A simple, fast and sensitive square-wave voltammetric (SWV) method for the determination of trace amounts of Captopril in pharmaceutical formulation and reconstituted serum is reported. A three-electrode system containing the static mercury drop electrode (SMDE) working electrode, Pt auxiliary electrode and Ag/AgCl reference electrode was used throughout. Sodium sulfite was used as both supporting electrolyte and oxygen removing agent. No nitrogen purging is needed for oxygen removal from sample solution. Calibration graph showed good linearity in the concentration range of 0.5–50.0 $\mu g\,mL^{-1}$ of Captopril and regression coefficient of 0.9957 is obtained. R.S.D. for eight replicate measurements and LOD of the proposed method are 1.2% and 6.28×10^{-3} $\mu g\,mL^{-1}$, respectively. The effect of various parameters (equilibration time, scan increment, pulse height, drop size, frequency and sodium sulfite concentration) on the determination were investigated. The procedure was successfully applied to the determination of Captopril in pharmaceutical formulation and reconstituted serum.

Keywords: Captopril; SW voltammetry; SMD electrode; Determination

1. Introduction

Chemically, Captopril is 1-(3-mercapto-2-methyl-1-oxopropyl)-1-proline (Scheme 1). Captopril is used in the treatment of severe essential and renovascular, where other therapy has failed, and congestive heart failure. Its antihypertensive effect results from a decrease in peripheral vascular resistance. Captopril is initially administered in doses of 25 mg, two or three times each day.

Captopril is metabolized chiefly to disulphide conjugates with other sulfhydryl-containing molecules. The half-life of Captopril is less than 3 h. Blood levels correlate poorly with clinical response. Maximal blood pressure response is seen 2–4 h after the doses. At 1–2 weeks intervals, doses can be increased until blood pressure is controlled. Toxicity from Captopril is uncommon, but when it occurs, it may include bone marrow suppression and proteinuria. Neutropenia or pancytopenia usually occurs in the first month of therapy and resolves after the drug is discontinued, although fatal cases have been reported. Proteinuria is associated with minimal changes in kidney basal membranes and is reversible after stopping Captopril in most, but not all, cases.

Serious toxicity has occurred primarily when Captopril was given in high doses to patients with collagen vascular disease or renal insufficiency. Minor toxic effects which are seen include altered sense of taste, allergic skin rashes, and drug fever, which may occur in as many as 10% of patients [1,2].

As far as literature survey is concerned electrochemical behavior of Captopril is studied with some electrochemical methods [3–15]. Chromatographic, spectrophotometric, colorimetric, electrophoresis and atomic absorption spectrometric methods have been used for quantitative determination of Captopril [16–23].

Different electrochemical methods including selective membrane or graphite electrode analysis [11], square wave cathodic adsorptive stripping voltammetry [10], boron-doped

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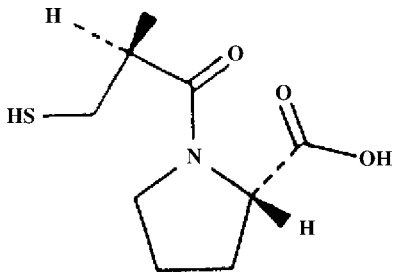

Scheme 1.

diamond thin film electrode voltammetry [12], amperometry and biamperometry [13–15] have been used for determination of Captopril.

Electrochemical methods, specially square wave voltammetry, make it possible to decrease the analysis time comparing to the time consuming spectrophotometric [16–20] and chromatographic methods [21–22].

Most of the mentioned methods are applied to the determination of Captopril in tablet. The present paper aims at establishing a square wave voltammetric method for determination of Captopril in tablet and reconstituted serum.

2. Experimental

2.1. Apparatus

The voltammetric measurements were carried out with a static mercury drop electrode (SMDE) working electrode in a three-electrode arrangement. A platinum wire was used as auxiliary electrode together with a silver–silver chloride reference electrode (Ag/AgCl), using 3 M KCl as electrolyte with a porous membrane. No nitrogen purge was needed since sodium sulfite was used as supporting electrolyte.

Measurements were carried out by a Princeton Applied Research (EG & G 273 A) electrochemical device. Electrodes and electrochemical vessels were parts of SMDE 303A EG & G PARC, which were controlled by the mentioned device. An IBM 325 T/S computer controlled all the settings and data processing of the system.

2.1.1. Reagents

All chemicals were of analytical grades and obtained from Merck. Pure Captopril was obtained from Sigma. Double distilled water was used throughout.

Sodium sulfite stock solution (2 M) was prepared by dissolving 25.2 g of sodium sulfite in water and diluting to 100 mL in a volumetric flask.

Captopril stock solution (1000 $\mu g\,mL^{-1}$) was prepared by dissolving 0.1000 g of Captopril (Sigma) in water and diluting to 100 mL in a volumetric flask.

2.1.2. Procedure

Into a 10-mL volumetric flask were added 5 mL of sodium sulfite (2 M) and appropriate amount of Captopril

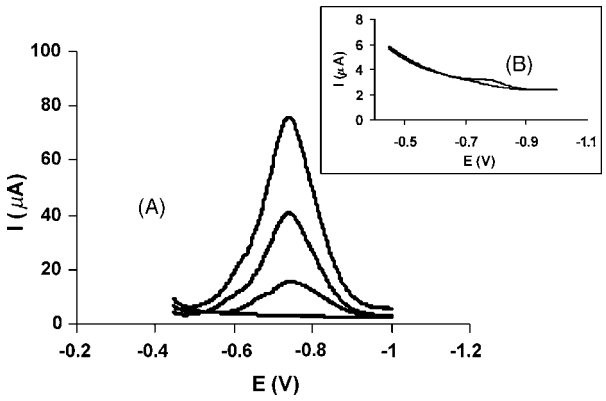

Fig. 1. (A) Square wave voltammograms of sample solutions containing 0, 0.5, 5, 12.5, and 25 $\mu g\,mL^{-1}$ of Captopril under optimum conditions and (B) voltammogram of 0.5 $\mu g\,mL^{-1}$ of Captopril under optimum conditions (which was masked when high concentration voltammograms are shown on A).

(1000 $\mu g\,mL^{-1}$) and the solution was diluted to the mark with distilled water. The SW voltammogram of the solution was recorded and the peak current of the obtained voltammogram was used for further calculations.

In order to determine Captopril in tablet, a tablet sample was introduced into a 1000-mL volumetric flask and 500 mL of distilled water is added. After shaking, the solution was diluted to the mark with distilled water. After filtration with an ordinary filtration paper, 1 mL of solution was transferred into a 10-mL volumetric flask. 5 mL of sodium sulfite solution (2 M) was added to the flask and then the solution was diluted to the mark with distilled water. The solution was transferred to the electrochemical vessel and the voltammogram of Captopril was obtained.

The analysis of reconstituted serum was done by adding 5 mL of sodium sulfite (2 M) to 5 mL of serum containing a fixed amount of Captopril and then the corresponding voltammograms were obtained.

3. Results and discussion

A typical SW voltammogram of Captopril is shown in Fig. 1. In order to obtain a wide calibration curve range with maximum slope (higher sensitivity) for the determination of Captopril in pharmaceutical formulations and also in reconstituted serum, different optimization procedures were used. Both instrumental (equilibration time (ET), pulse height, frequency, scan increment and mercury drop size) and chemical parameters were optimized.

In the first step, one parameter variable optimization method was used to observe the effect of each parameter on Captopril voltammogram peak. Variation of some parameters (equilibration time, scan increment, and mercury drop size) had low influence on voltammogram peak current and shape, also their effect on calibration graph was negligible. So the optimum value for these parameters was chosen as the value, which gave higher voltammogram peak current.

Table 1
Effect of frequency and pulse height variation on the voltammogram peak currents, linearity and slope of calibration graph for Captopril

Frequency (Hz)	Pulse height (mV)	Peak currents (μA)				Regression coefficient	Slope
		0[a]	5[a]	12.5[a]	25[a]		
150	25	0.1548	3.892	9.854	12.45	0.9517	0.4932
150	45	0.2588	5.648	14.34	18.78	0.9601	0.7440
150	**65**	**0.3510**	**7.021**	**16.54**	**22.42**	**0.9670**	**0.8801**
250	25	1.639	10.93	24.98	30.12	0.9417	1.137
250	45	2.997	16.60	37.01	46.02	0.9508	1.717
250	**65**	**3.862**	**24.99**	**43.02**	**56.98**	**0.9584**	**2.044**
350	25	6.502	31.01	54.69	64.81	0.9312	2.247
350	45	7.201	35.99	61.72	75.01	0.9372	2.602
350	**65**	**8.001**	**43.98**	**72.69**	**91.40**	**0.9436**	**3.181**
450	25	16.57	52.04	84.97	96.50	0.9187	3.064
450	45	29.30	74.51	119.3	136.4	0.9268	4.132
450	**65**	**37.30**	**92.20**	**150.4**	**172.4**	**0.9298**	**5.242**

[a] Captopril concentration (μg mL^{-1}).

The effect of equilibration time that controls a variable delay during which the cell is on and the initial potential is applied to the electrodes was studied. Equilibration times of 0, 5, 10, 20, 30 and 45 s were applied to the electrodes and the corresponding voltammograms were recorded. The results showed that ET values greater than 5 s have no considerable effect on the peak current and 5 s was chosen as optimum ET value.

The effect of scan increment that determines the amount of potential changes between two data points in the experiment was investigated. Scan increments of 1, 2, 3, 4, and 5 mV were applied to the electrodes and corresponding voltammograms were recorded. The resulted peak currents showed that scan increments above 2 mV have no significant effect on the voltammograms peak current and so 2 mV was chosen as the optimum scan increment.

In order to determine the effect of mercury drop size on voltammogram peak current, three different drop sizes: small, medium, and large sizes were used. The volume ratio of mercury drops was 1:2:4 and their area ratio was 1:1.6:2.5 [24]. The results showed that by increasing the drop size, the peak current increases and therefore optimum drop size was chosen to be large drop size.

At the first optimization step it was found that variation of three other parameters (pulse height, frequency, and sodium sulfite concentration) have influenced the Captopril voltammogram peak to a much greater extent. Further investigations showed that higher value for these parameters although resulted higher voltammogram peak current, led to different effects on linearity of calibration graph and its slope. So multivariation experiments were planned to reach the optimum value for these parameter.

In the first experiment the effect of simultaneous variation of pulse height (25–65 mV) and frequency (150–450 Hz) in a range of Captopril concentration (0–25 μg mL^{-1}) was investigated.

The results (Table 1) showed that increasing of pulse height increases the linearity and slope of calibration graph, therefore 65 mV was chosen as optimum value for pulse height. Pulse heights over 65 mV were not used in this experiment because of their broadening effect on voltammogram peak.

Table 2
Effect of frequency and sodium sulfite concentration variation on the voltammogram peak currents, linearity and slope of calibration graph

Sodium sulfite concentration (M)	Frequency (Hz)	Peak currents (μA)			Regression coefficient	Slope
		5[a]	12.5[a]	25[a]		
0.6	150	10.0	21.85	20.92	0.7400	0.4818
0.6	250	30.21	59.05	69.47	0.9180	1.848
0.6	350	60.23	100.4	115.4	0.9207	2.600
0.6	450	93.87	152.7	177.0	0.9293	3.932
0.8	150	7.035	18.23	26.03	0.9698	0.9165
0.8	250	20.33	49.08	64.88	0.9524	2.129
0.8	350	53.50	102.2	127.2	0.9470	3.513
0.8	450	94.50	157.2	185.6	0.9368	4.322
1.0	150	4.321	14.57	35.06	0.9989	1.547
1.0	**250**	**16.02**	**33.80**	**77.01**	**0.9957**	**3.091**
1.0	350	47.20	113.8	165.4	0.9766	5.728
1.0	450	90.40	188.4	255.5	0.9687	7.960

[a] Captopril concentration (μg mL^{-1}).

Table 3
Composition of reconstituted serum[a]

Compound	Concentration (M)
Alanine	4.1×10^{-4}
Arginine	2.1×10^{-4}
Aspartic acid	8.8×10^{-4}
Cysteine	5.1×10^{-5}
Glycine	1.4×10^{-4}
Histidine	1.2×10^{-4}
NaCl	8.7×10^{-2}
Lysine	2.0×10^{-4}
Methionine	3.4×10^{-5}
Phenylalanine	1.6×10^{-4}
Serine	1.2×10^{-4}
Tyrosine	8.1×10^{-5}
Tryptophan	6.9×10^{-5}
$NaHCO_3$	7.9×10^{-3}
Albumin ($g\,dL^{-1}$)	3.2

[a] All amino acids are L or DL isomers.

In the other hand, increasing of frequency, decreased the linearity and increased the slope of calibration graph. These two different effects of frequency make it difficult to find the optimum value of frequency.

In the second experiment, frequency (150–450 Hz) and sodium sulfite concentration (0.6–1 M) were varied for different concentrations of Captopril (5–25 $\mu g\,mL^{-1}$).

As can be seen in Table 2, the best regression coefficient for calibration graph is achieved at higher sodium sulfite concentrations and lower frequencies. Increasing either of parameters led to increase the slope of calibration graph. The optimum values were chosen as 1 M for sodium sulfite concentration and 250 Hz for frequency. Above that the linearity and below that the slope of calibration graph decreased significantly.

Under the optimum conditions, the calibration graph for the determination of Captopril is obtained in the concentration range of 0.50–50 $\mu g\,mL^{-1}$ of Captopril with a correlation coefficient of 0.9957. The regression equation $\Delta(i_d) = 2.995C - 1.7591$ was obtained (n = 8), where C is the concentration of Captopril in $\mu g\,mL^{-1}$ and $\Delta(i)$ is the difference between voltammogram peak currents of sample and that of blank solution in μA. The slope of the line $b = 2.995 \pm 8.28 \times 10^{-2}$ which shows a R.S.D. = 3.0% and the intercept of the line $a = -1.7591 \pm 0.05$ with a R.S.D. = 2.9% were also obtained. R.S.D. for 12 replicate measurements and LOD of the proposed method were 1.2% and 6.28×10^{-3} $\mu g\,mL^{-1}$, respectively. Limit of quantitation (LOQ) of the proposed method is 0.250 $\mu g\,mL^{-1}$ [25]. The repeatability and reproducibility evaluations for peak current and peak potential of

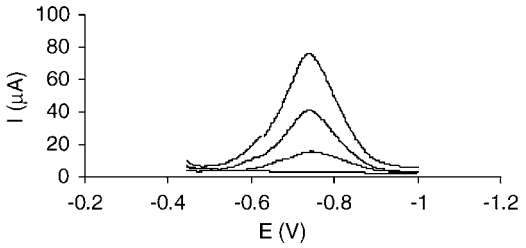

Fig. 2. Square wave voltammograms obtained from spiked serum samples for different concentrations (5, 12.5, and 25 $\mu g\,mL^{-1}$) of Captopril (standard addition) under optimum conditions. The baseline is also shown.

Captopril were carried out. For 16 measurements of peak current and peak potential of 25 $\mu g\,mL^{-1}$ of Captopril by recommended square-wave voltammetric (SWV) method on one day, the R.S.D. values of 3.13% and 1.07% are obtained, respectively. Sixteen measurements of peak current and peak potential were also carried out on different solutions containing the same amount of Captopril (25 $\mu g\,mL^{-1}$) on different days and R.S.D. values of 2.80% and 3.70% are obtained, respectively.

Sodium sulfite is used as supporting electrolyte and oxygen removing agent and therefore no nitrogen gas purging for elimination of soluble oxygen in the sample solution is required and so, decreasing the analysis time. The proposed method needs no sample pretreatment with respect to the previous reported methods.

4. Application

In order to investigate the matrix effect of a complex sample on the method of analysis, experiments were performed to determine the feasibility of using the proposed method to measure Captopril in reconstituted serum samples. Recovery studies were conducted with samples containing 5, 10, and 15 $\mu g\,mL^{-1}$ of Captopril. The composition of the reconstituted serum is listed in Table 3. The concentration of each component of reconstituted serum sample was chosen to match its normal level in human serum. The results of the recovery studies are summarized in Table 4. Excellent recovery was observed indicating that the constituents of the reconstituted serum samples do not interfere in any way with the detection of Captopril. Therefore, the proposed voltammetric method could be used for the determination of Captopril in serum samples. The SW voltammograms obtained from spiked serum samples for different concentrations of Captopril (standard addition) are shown in Fig. 2.

Table 4
Recovery test of Captopril added to reconstituted serum sample

Captopril added ($\mu g\,mL^{-1}$)	Captopril found ($\mu g\,mL^{-1}$)[a]	Percentage of recovery	R.S.D. (%)
5.0	4.9	98	2.0
10.0	9.7	97	1.2
15.0	15.3	102	2.1

[a] Average of three determinations.

Table 5
Comparison of the results for the analysis of 25-mg tablets of Captopril with proposed SWV method and standard spectrophotometric method

Sample	SWV method		Standard spectrophotometric method	
	mg (n = 3)	Percentage of recovery	mg (n = 3)	Percentage of recovery
A	25.11	100.4	25.42	101.7
B	24.65	98.5	24.52	98.1
C	25.30	101.2	25.65	102.6
D	24.85	99.4	24.98	99.9
E	25.50	102.4	25.30	101.2
Mean	25.08	100.3	25.17	100.7
%R.S.D.	1.4		1.8	

To evaluate the validity of the proposed method for the analysis of pharmaceutical preparations, Captopril tablets were assayed. The results were compared with those obtained from standard spectrophotometric method [16]. As it is seen from the results of Table 5, there is well agreement between standard spectrophotometric method [16] and proposed SWV method. Both methods were compared using paired *t*-test and *F*-test. Statistical evaluation of results showed that there is no significant difference between two methods for 95% confidence level.

5. Conclusion

The overall time of analysis in this method is quite short (less than 1 min), since there is no gas purging for dissolved oxygen removal as other electrochemical method, and/or preconcentration time as in stripping methods [10]. The method is also faster than chromatographic methods [21,22] due to use of SWV technique. The proposed method needs no careful adjustment of pH as in spectrophotometric methods. The analytical range of analysis (0.5–50 $\mu g\,mL^{-1}$) is wider than most of the previous works. The LOD = 6.3×10^{-3} $\mu g\,mL^{-1}$ and R.S.D. = 1.2 $\mu g\,mL^{-1}$ of the method is better than most other electrochemical methods.

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