CONTINUOUS WAVELET TRANSFORM FOR THE RESOLUTION OF THE OVERLAPPING VOLTAMMETRIC SIGNALS AND SIMULTANEOUS DETERMINATION OF LEVODOPA AND BENSERAZIDE

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ABSTRACT

The overlapping voltammetric signals of levodopa and benserazide in their binary mixture were processed by continuous wavelet transform and the transformed signals were used for the simultaneous determinations of these compounds in commercial pharmaceutical formulation. The Osteryoung Square Wave Voltammograms of levodopa and benserazide in Britton Robinson (BR, pH = 3) buffer were plotted in the potential range of -196 and 1200 mV using glassy carbon electrode versus Ag/AgCl reference electrode. To find an optimal signal processing method, various continuous wavelet family with different the scale parameter (a) was tested and Haar continuous wavelet transform method (a = 4) was found to be the optimal wavelet transform for the signal processing and determination. Calibration functions in the linear dynamic range of 4.0-18.0 μg mL^{-1} for levodopa and 2.0-10.0 μg mL^{-1} for benserazide were obtained by measuring the transformed voltammetric amplitude at 460 mV and 320 mV for levodopa

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and benserazide, respectively. The proposed analytical signal processing method was validated by analyzing the independent set of the synthetic binary mixtures containing levodopa and benserazide. The mean recoveries and relative standard deviations were found to be 98.9 % and 2.31 % for levodopa 101.6 % and 1.76 % for benserazide. This proposed method was applied to the real samples consisting of levodopa and benserazide in the commercial pharmaceutical as capsules. The experimental results obtained from the Haar continuous wavelet transform method were compared with those obtained by the literature method. A good agreement was observed for the obtained results.

Keywords: Levodopa, Benserazide, Simultaneous determination, Osteryoung square-wave voltammetry, Haar continuous wavelet transform method

INTRODUCTION

In quantitative analysis, one of the main problems of the analytical chemistry is the resolution of the overlapping signals such as spectral signals, voltammetric and chromatographic signals of mixtures containing two or more active compounds. The advancements on computer science, statistics and applied mathematics offer new possibilities for the mathematical resolution of the above analytical problems. Analytical chemists have categorized these signals processing techniques into chemometrics methods. In this context, wavelet transforms have been used for solving various problems in several areas of science and engineering. Wavelet transform is a powerful signal processing tool for data reduction, de-nosing, baseline correction and resolution of overlapping signals /1-5/. Wavelet transform methods are classified as discrete wavelet transform (DWT) and continuous wavelet transform (CWT).

Recently, CWT methods have been used for solving overlapped voltammetric peaks /6/ and overlapped absorption spectra /7-13/ to determine active compounds in complex mixtures.

Levodopa (LD) [3-(3,4-dihydroxyphenyl)-L-alanine] is a catecholamine, which easily enters the central nervous system. It is administered in an enantiometrically pure form as the racemic mixture has secondary effects

such as dyskinesia and granulocytopenia. It is used in the treatment of Parkinson's disease, usually associated with a peripheral aromatic-L-amino acid decarboxylase inhibitor, such as benserazide (BE) [N'-(2,3,4-trihydroxybenzyl)-D,L-serine], in order to increment the proportion that enters the brain and prevents its decarboxylation in extracerebral tissues, and prolong its antiparkinsonian effect. This LD and BE combination has good clinical efficacy in patients, in the treatment of night-time problems and early morning symptoms /14-18/.

Various analytical methods including spectrophotometry /19-25/, capillary electrophoresis /26-28/ have been reported for the determination of LD and BE in their mixtures in the literature.

In this study, quantitative analysis of mixtures of LD and BE was performed by the combined use of electrochemical and wavelet transform methods. The first step is to find the optimal experimental conditions for obtaining the Osteryoung Square Wave Voltammograms (OSWV) of LD, BE and their samples. In the second step various wavelets families were tested and Haar CWT method was found to be optimal wavelet transform for the signal processing. Calibration functions were obtained by measuring the transformed voltammetric amplitude at 460 mV and 320 mV for LD and BE, respectively. The Haar CWT method was tested by using the independent set of the synthetic binary mixtures containing LD and BE. The experimental results obtained from the proposed method were compared with derivative spectrophotometric method in the literature /24/.

EXPERIMENTAL

Apparatus

A BAS 100 B/W (Bioanalytical System, USA) electrochemical analyzer was used to plot voltammograms. The working electrode is a glassy carbon electrode. The platinum wire serves as an auxiliary electrode, while the reference electrode is Ag/AgCl with saturated 3 M KCI. Before each experiment, the glassy carbon electrode was polished manually with alumina on a smooth polishing cloth. Residual polishing material was removed from the surface and then the electrode was rinsed with water before starting voltammetric scan. The peak heights were automatically or manually

measured using the "tangent fit" capability of the instrument. All measurements were performed at room temperature.

All the calculations and data processing were carried out by using Microsoft Excel and wavelet toolbox in Matlab 7.0.

Chemicals and Solutions

LD and BE drugs were kindly supplied by Roche Industry. A commercial pharmaceutical formulation (Madopar® Capsule containing 100 mg LD and 25 mg BE/capsule, produced by Roche Pharm. Ind., İstanbul, Turkey) was studied. All solvents and chemicals used were analytical reagent grade.

The stock solutions of LD and BE (250 μg mL^{-1}) were prepared in 0.1 M HCl. Standard solutions of LD and BE were prepared in the working range of 4.0-18.0 μg mL^{-1} and 2.0-10.0 μg mL^{-1} using Britton Robinson (BR) buffer (pH = 3.0) (supporting electrolyte), respectively. A validation sets of 8 mixtures containing LD and BE in different concentration composition was obtained from above stock solutions.

RESULTS AND DISCUSSION

The main aim of this study is to improve a new application of the CWT method to the overlapping voltammetric peaks for the simultaneous quantitative determination of LD and BE in samples. As can be seen from Figure 1, the simultaneous determination of LD and BE in their binary mixture is not possible by classical voltammetric methods. To solve this problem, the simultaneous use of OSWV and CWT methods was proposed. Electrochemical and signal processing procedures are explained below.

By using glassy carbon electrode versus Ag/AgCl, BR buffers at different pH were tested for the optimization of experimental conditions to provide successfully electrochemical oxidation of our drugs. In our case, BR buffer (pH = 3.0) was found to be optimal for determination of LD and BE in samples.

Other instrumental conditions were selected as frequency (f) = 15 Hz, scanning increment (ΔE) = 4 mV and pulse amplitude (E_{SW}) = 25 mV.

In practice, voltammograms of LD and BE in the linear concentration range of 4.0-18.0 μg/mL and 2.0-10.0 μg/mL within BR buffer (pH = 3.0),

respectively, were plotted in the voltammetric range from -196 mV to +1200 mV as shown in Figure 1. The voltammograms obtained in the above step were transformed by using the CWT approach.

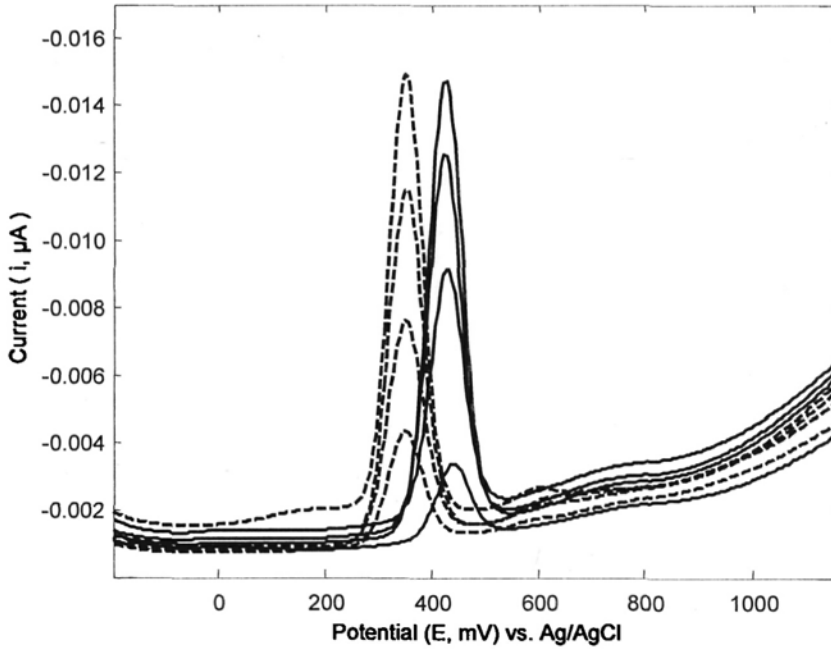

Fig. 1.: Voltammograms of 2, 4, 8 and 10 µg mL^{-1} BE (----) and 4, 10, 16 and 18 µg mL^{-1} LD (—) in BR buffer at pH 3.0

Optimization and data processing

Voltammograms of standard series of LD, BE and their samples were recorded in between -196 and 1200 mV under the above mentioned voltammetric conditions. Data vectors corresponding to the voltammograms were transferred via Microsoft Excell into wavelet domain. In order to find to an optimal signal processing technique, various wavelet families at different scale parameters (a) were applied to the voltammetric data vectors. In our case Haar CWT method with scale parameter, $a = 4$ was found as an optimal signal processing approach. This optimization was carried out by the experimental observation according to the method recovery results. For example, when the original voltammograms of pure 16 µg mL^{-1} LD, pure 4

μg mL^{-1} BE and their mixture were processed by Haar CWT method, the CWT voltammograms were obtained as indicated in Figure 2. The examination of this Figure shows that the CWT voltammograms of pure drugs and their mixtures were coincided at 460 mV and 320 mV. These points were selected as working potential points, 460 mV for LD and 320 mV for BE to obtain calibration graphs.

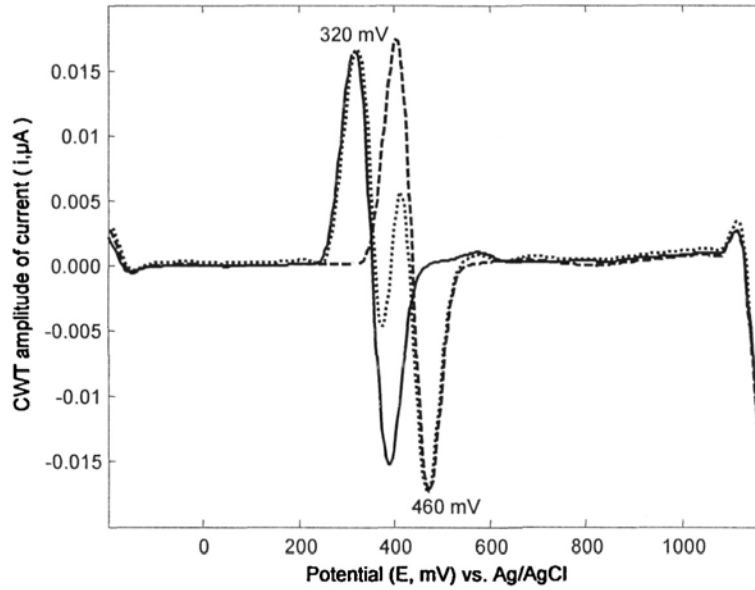

Fig. 2.: Transformed voltammograms of pure 4 μg mL^{-1} BE (—), pure 16 μg mL^{-1} LD (----) and their mixture (·····)

Method Validation

Haar CWT method was applied to the original voltammograms as shown in Figure 1 and the CWT voltammograms were obtained as given in Figure 2. Calibration functions in the linear dynamic range of 4.0-18.0 μg mL^{-1} for LD and 2.0-10.0 μg mL^{-1} for BE were obtained by measuring the transformed voltammetric amplitude at 460 mV and 320 mV for LD and BE, respectively (Figure 2 and Figure 3). These calibration graphs are based on the use of the relationship between concentration and electrochemical CWT amplitude. Linear regression functions and their statistical results are presented in Table 1.

Table 1
Linear regression function and its statistics

E (mV)	Regression function	r	SE (m)	SE (n)	SE (r)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
460	$S=1.24x10^{-3}\ C_{LD} - 1.99x10^{-3}$	0.9989	$7.87x10^{-05}$	$1.04x10^{-03}$	$8.62x10^{-04}$	0.54	1.79
320	$S=1.24x10^{-3}\ C_{BE} - 1.99x10^{-3}$	0.9960	$1.87x10^{-03}$	$1.75x10^{-03}$	$6.51x10^{-04}$	0.85	2.84

r: Correlation coefficient of the regression function,
SE (r): Standard error of the correlation coefficient,
SE (m): Standard error of the slope,
SE (n): Standard error of the intercept,
LOD: Limit of detection,
LOQ: Limit of quantitation.

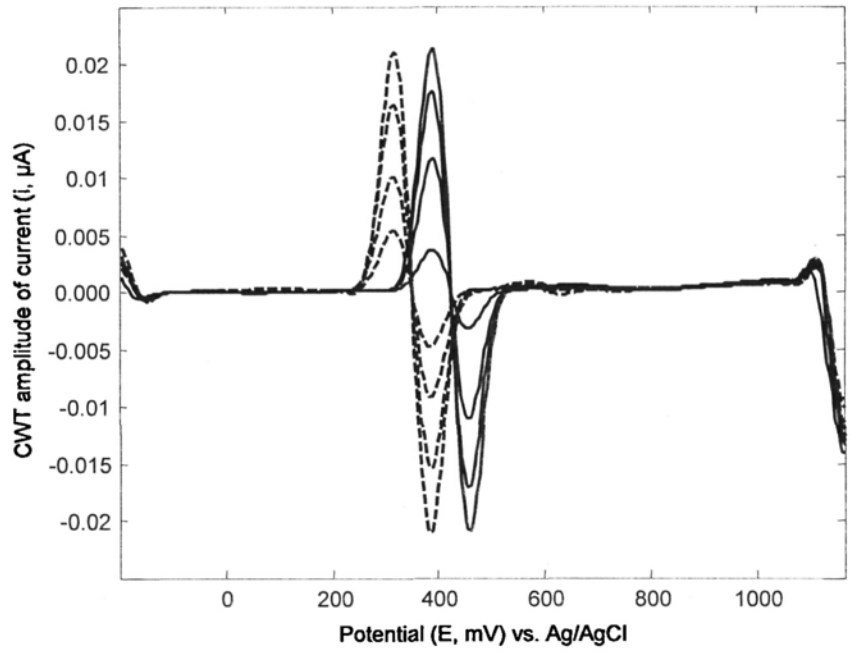

Fig. 3: Transformed voltammograms of 2, 4, 8 and 10 µg mL^{-1} BE (----) and 4, 10, 16 and 18 µg mL^{-1} LD (—) in BR buffer at pH 3.0

It was observed that high correlation coefficients (r) and satisfactory slope, intercept, the limit of detection and the limit of quantitation provided by application of the Haar CWT with the selected optimal parameters. This hybrid approach was applied to the samples in the above mentioned optimized experimental conditions.

According to ICH /29/, the calculations of the limit of detection (LOD) and quantification (LOQ) were achieved by using the standard deviation of the response and the slope of the calibration equation (Table 1).

The proposed approaches in this study were validated by using synthetic samples and standard addition technique. In the recovery study, mean recoveries and their relative standard deviations were calculated and presented in Table 2. The mean percentage recoveries in these synthetic mixture of LD and BE were found as 98.88 ± 0.81 and 101.63 ± 0.68 with RSD of 2.31 and 1.88 % for LD and BE, respectively. The experimental

results indicate that the proposed approaches are suitable for the determinations of LD and BE in samples.

Table 2
Recovery data obtained in the synthetic mixtures by Haar CWT approach

Added ($\mu g\ mL^{-1}$)		Found ($\mu g\ mL^{-1}$)		Recovery (%)	
LD	**BE**	**LD**	**BE**	**LD**	**BE**
4.00	4.00	3.88	4.17	96.89	104.25
10.00	4.00	9.56	4.03	95.56	100.63
16.00	4.00	15.70	4.11	98.15	102.78
18.00	4.00	18.51	4.11	102.82	102.78
16.00	2.00	15.70	1.98	98.15	98.83
16.00	4.00	16.03	4.11	100.17	102.78
16.00	8.00	16.11	7.94	100.67	99.20
16.00	10.00	15.79	10.18	98.66	101.76
			Mean ± SE	98.88 ± 0.81	101.63 ± 0.68
			SD	2.28	1.91
			RSD %	2.31	1.88

SE: Standard error
SD: Standard deviation,
RSD: Relative standard deviation.

In addition, in order to evaluate the effect of the excipients for the LD and BE analysis the standard addition technique was applied and its results were given in Table 3. The mean percentage recoveries of LD and BE were found as 98.61 ± 0.89 and 101.11 ± 1.01 with RSD of 2.55 and 2.82 % for LD and BE, respectively. No interferences and systematical errors were observed during quantitative analysis procedure.

Table 3
Experimental results of standard addition technique

	Recovery (%)	
No	**LD**	**BE**
1	99.2	98.6
2	99.7	97.6
3	102.7	105.5
4	98.7	100.6
5	99.2	98.1
6	94.6	102.1
7	95.6	103.5
8	99.2	102.9
Mean ± SE	98.61 ± 0.89	101.11 ± 1.01
SD	2.51	2.86
RSD %	2.55	2.82

SE: Standard error
SD: Standard deviation,
RSD: Relative standard deviation.

Sample analysis

The proposed Haar CWT approach was applied to the determination of LD and BE in Madopar® Capsule. The experimental results of commercial preparation are shown in Table 4. The experimental results obtained from the developed method were compared with those obtained by a derivative spectrophotometric method in the literature /24/. For comparisons of the results obtained by OSWV-CWT method and the literature method, t- and F-tests were applied. The t_c and F_c values at 95 % level found as 1.06 and 1.39 for LD and 0.52 and 1.11 for BE, respectively ($t_t = 2.18$, $F_t = 5.82$, $p > 0.05$). No significant difference between the two methods for LD and BE. A good agreement was observed for the obtained results.

Table 4
Experimental results of commercial pharmaceutical formulation by Haar CWT approach

	Proposed Method mg/capsule		Compared Method /24/ mg/capsule	
No	**LD**	**BE**	**LD**	**BE**
1	101.89	25.94	98.88	25.54
2	99.16	24.39	101.70	24.48
3	99.67	25.74	100.10	25.14
4	98.66	25.16	101.81	25.12
5	99.16	24.53	98.30	25.73
6	96.50	25.53	99.38	24.13
7	99.16	25.12	99.74	25.11
Mean ± SE	99.17 ± 0.60	25.20 ± 0.22	99.99 ± 0.51	25.04 ± 0.21
SD	1.58	0.59	1.34	0.56
RSD %	1.60	2.33	1.34	2.24
CL (α : 0.05)	99.70 - 100.64	24.66 - 25.74	98.74 - 101.24	24.53 - 25.55
	$t_c = 1.06$, $F_c = 1.39$ for LD ($p > 0.05$)			
	$t_c = 0.52$, $F_c = 1.11$ for BE ($p > 0.05$)			

Label claim: 100 mg LD and 25 mg BE per capsule
CL: Confidence limit
t_c: calculated t value, t_t: tabulated t value, $t_t = 2.18$
F_c: calculated F value, F_t: tabulated F value, $t_t = 5.82$.

CONCLUSION

Although the individual voltammogram of BE and LD overlap in the potential range of -196 and 1200 mV, the Haar CWT approach gave successful results for the quantitative resolution of the binary mixtures and a commercial sample consisting of two components.

Finally, this CWT method can be strongly applied to a routine analysis, quality control of binary mixtures, and commercial products containing these two drugs.

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