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## Original Article

# Determination of carnosic acid in *Rosmarinus officinalis* L. using square wave voltammetry and electrochemical behavior

Ümmihan Taşkoparan Yılmaz<sup>b,\*</sup>, Elif Calik<sup>a</sup>, Bayram Akdulum<sup>b</sup>,  
Hasim Yılmaz<sup>b</sup>

<sup>a</sup> Gazi University, Institute of Natural Sciences, Department of Chemistry, 06500, Ankara, Turkey

<sup>b</sup> Gazi University, Polatlı Science and Art Faculty, Department of Chemistry, 06900, Polatlı, Ankara, Turkey

## ARTICLE INFO

## Article history:

Received 13 February 2017

Received in revised form

10 April 2017

Accepted 11 April 2017

Available online 25 April 2017

## Keywords:

Carnosic acid

Hanging mercury dropping electrode

Voltammetric determination

Reduction

*Rosmarinus officinalis* L.

## ABSTRACT

A new, fast, sensitive and simple voltammetric method is established for the direct determination of carnosic acid (CA). And the electroreduction of carnosic acid (CA) was studied using electrochemical methods. The number of electrons transferred in electrode mechanisms were calculated for reversible and adsorption-controlled electrochemical reduction of CA at 17 mV versus Ag/AgCl at pH 7.0 in Britton–Robinson buffer (BR) on a hanging mercury drop electrode. Square-wave voltammetry was developed and validated for direct determination of CA. Square-wave parameters were optimized as accumulation potential = 0.0 mV, accumulation time = 5 s, frequency = 50 Hz, pulse amplitude = 50 mV, and staircase step potential = 5 mV. The developed method displays three linear responses from 2 to 9  $\mu\text{M}$ , 10 to 30 and 40 to 90  $\mu\text{M}$  for carnosic acid with a correlation coefficient of 0.996, 0.999 and 0.999. The detection limits were found to be 1.5  $\mu\text{M}$ , 4.0  $\mu\text{M}$  and 40.1  $\mu\text{M}$ , respectively. The interference effect of most common organic and inorganic species was investigated. Proposed method was successfully applied for determination of CA in natural extract of rosemary and the average content was determined as  $11.9 \pm 1.0$  ( $\mu\text{g CA}/1$  g rosemary). The results were in agreement with that obtained by HPLC-UV comparison method. The developed method can be widely used in routine quality control of herbal materials as well as other in foods, medicinal, pharmaceutical and environmental analysis.

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## 1. Introduction

Synthetic antioxidants are commonly used in the food industry to avoid or delay the oxidative deterioration, but in

more recent years, due to cause adverse health problems, there has been a growing interest in the use natural sources of antioxidants. The addition of antioxidants to foods in order to prevent rancidity has long been common practice to increase

\* Corresponding author.

E-mail address: [ummihan@hotmail.com](mailto:ummihan@hotmail.com) (Ü.T. Yılmaz).

<http://dx.doi.org/10.1016/j.jfda.2017.04.004>

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the shelf-life of good-quality food product [1]. Immensely plants, such as spices, fruits, especially vegetables, contain important protective agents for human health that have antioxidant properties [2,3].

Rosemary (*Rosmarinus officinalis*) is well-known for its antioxidant properties and in recent years their extracts have been used as antioxidants in food industry [4,5]. The most effective antioxidant in rosemary has been found to be a diterpene species, carnosic acid [6]. The determination of antioxidant compounds contained in the extract can be used to evaluate the quality of commercial extracts and could be important for industries. Therefore, the purpose of this study was to establish a new voltammetric method for the determination of low levels of carnosic acid (CA) in real systems, such as plant extracts, oils and fats, meat and meat products.

There are only a few analytical techniques for the quantitative determination of CA in different samples and most of them are based on chromatographic methods. The some of chromatographic detection methods, including high-performance liquid chromatography (HPLC) with electrochemical detection [7–9] or high-performance liquid chromatography (HPLC) with UV-diode array (DAD) [10–12] and ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS) [13] have been reported for determination of CA. These methods gave reproducible results but unfortunately are laborious, expensive, time-consuming and require sophisticated instrumentation for routine analysis. Therefore, a fast, simple, low cost, accurate, precise and sensitive method is very important especially for analysis of plant extracts and food additives which used as antioxidants in the food industry [14].

Electroanalytical techniques such as cyclic voltammetry (CV), square-wave voltammetry (SWV), have some advantages when compared to chromatography, e.g., their low cost, analysis without extraction or other pretreatments, and speed. These methods also make it possible to evaluate the redox characteristics, to propose the plausible mechanism pathways, to evaluate the adsorption–diffusion parameters of molecules and these parameters may have importance for their distribution, pharmacological [15], toxicological [16] and pharmacokinetic properties [17]. Voltammetric techniques are generally used for the quantitative determination of electroactive and electro inactive species.

Up to now, we found that only one paper is available for amperometric determination of antioxidant activity in herbs. Cosio et al. [18] described a amperometrically method for the determination of antioxidant activity in herb methanolic extracts using a flow injection (FI) system with an electrochemical detector. Herb methanolic extracts were analyzed with the proposed method and the results were expressed as mg of trolox equivalents/g of dried weight. However, in this study, carnosic acid have been reported as the compound having the highest antioxidant activity.

To our present knowledge, no information about the voltammetric or electroreduction behavior of CA has so far been reported in the literature. Therefore, for the first time, this paper describes an electrochemical approach to CA. The aim of this work was to develop a new validated square-wave voltammetric assay method for the direct determination of CA on HMDE (hanging mercury dropping electrode). The developed method

was applied for the determination of CA in natural extract of rosemary and the obtained results were compared using HPLC–UV as a second method. In addition, to obtain valuable information about the electroreduction mechanism of CA at HMDE, the cyclic voltammetry (CV) technique was applied.

## 2. Materials and methods

### 2.1. Apparatus

A IviumStat model electrochemical analyzer (Potentiostat/Galvanostat, Netherlands) was used for square wave voltammetry (SWV) and cyclic voltammetry (CV) measurements. A three electrode system was used, consisting of a platinum counter electrode, an Ag/AgCl (3 M NaCl) reference electrode and a working hanging mercury drop electrode (HMDE) as a working electrode. pH values were measured using a Mettler Toledo pH/Conductivity Meter pH meter with combined glass electrode was used to measure pH of all the solutions.

### 2.2. Reagents

Carnosic acid (CA) was purchased from Aldrich (Germany) and a primary solution of  $1 \times 10^{-3}$  M CA was prepared daily in ethyl alcohol–water (1:1, v/v) solvent before every use in order to avoid aging of the solution. Working solutions were prepared by diluting the stock solution with distilled water and storing in the dark at 4 °C. All chemicals used for the supporting electrolyte, solvents and other reagents were of analytical reagent grade (Merck, Darmstadt, Germany). Britton–Robinson buffer (BR buffer) solutions were prepared from a stock solution containing 0.04 M phosphoric, boric and acetic acids (Sigma–Aldrich, Germany) by adding 2 M NaOH to obtain pH values ranging from 2 to 12.

### 2.3. Sample collection and preparation

According to the scientific literature [6,19], 99.8% ethanol (v/v) was selected as a solvent for the production of rosemary extracts. Rosemary extracts were obtained in September from fresh leaves of a wild rosemary plant by the following extraction protocol: After 20 g of air-dried leaves was placed in a glass long-necked flask, 250 mL of ethanol was added to macerate. And then the sample was boiled at 80 °C for 40 min. The solvent was removed with a rotary evaporator and sample was treated with hexane in the extraction flask. Hexane removes the insoluble residue of rosemary more effectively. And then, the extract was boiled at 80 °C for 20 min in 150 mL of water. The extract was analyzed for quantification of CA by using electrochemical method.

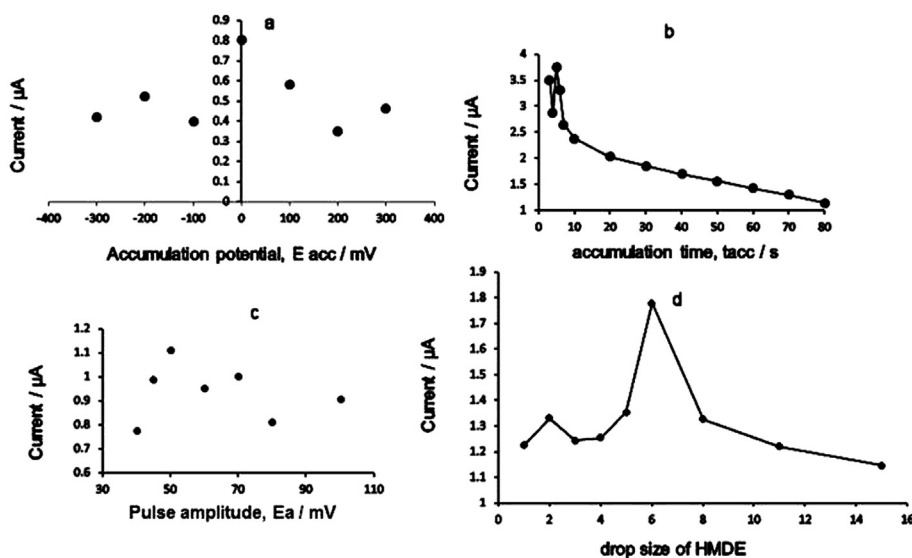
### 2.4. Voltammetric procedure

The voltammograms of CA were recorded in phosphate and B–R buffers within the pH range of 2.0–12.0. A certain volume of 10.0 mL of one of the buffers used was transferred to the voltammetric cell. After, the electrodes were put in the solutions through which pure nitrogen gas was passed for 15 min before obtaining the voltammograms. The square-wave

voltammograms of CA were received under the optimized conditions following the addition of the analyte into the voltammetric cell. An accumulation potential ( $E_{acc}$ ) of 0.0 mV was applied throughout the accumulation period accumulation time ( $t_{acc}$ ) = 5 s with stirring under a nitrogen atmosphere. Depending on the amount of CA, two peaks belong to reduction of CA were obtained. Determination of CA was carried out using these peaks at about 52 and  $-17$  mV (versus an Ag/AgCl reference electrode, pH 7.0,  $25 \pm 2$  °C). Quantifications were realized using the standard addition method. The voltammetric parameters were frequency ( $f$ ) 50 Hz, pulse amplitude ( $\Delta E$ ) 50 mV, and staircase step ( $\Delta E_s$ ) 5 mV.

### 2.5. Optimization of voltammetric parameters

In the voltammetric method, the influence of the accumulation potential ( $E_{acc}$ ) on the CA peak ( $20 \mu\text{g mL}^{-1}$ ) signal was studied at pH 7.0 BR buffer in the range from +300 to  $-300$  mV. Variation of the peak current ( $i_p$ ) vs. the accumulation potential ( $E_{acc}$ ) is given in Fig. 1a. The maximum peak current in the accumulation step was observed for the  $E_{acc}$  of 0.0 mV. For optimization process of the accumulation time  $20 \mu\text{g mL}^{-1}$  CA was deposited on the HMDE by applying selected times 3–80 s at a fixed potential of 0.0 mV (Fig. 1b). Maximum peak current was observed at 5 s.



**Fig. 1** – The effect of experimental parameters on the SW voltammetric determination of  $20 \mu\text{M}$  CA in pH 7.0 B–R buffer solution (a) accumulation potential ( $E_{acc}$ ); (b) accumulation time ( $t_{acc}$ ); (c) pulse amplitude; (d) drop size of HMDE.

In order to obtain a well-defined voltammetric peak shape and high peak current, optimization process was proceeded with some square wave voltammetric instrumental parameters such as, namely, frequency ( $f$ ), pulse amplitude ( $\Delta E_a$ ) and step potential ( $\Delta E_s$ ). When  $E_a$  was varied in the range of 40–100 mV, the peak current changed with increasing  $E_a$  in Fig. 1c. Analysis of the data showed a linear increase in the peak current to 50 mV. When  $E_a$  was greater than 50 mV the peak width increased at the same time. The influence of step potential for  $20 \mu\text{g mL}^{-1}$  CA was evaluated by studying the step

height from 5.0 to 50.0 mV. The maximum peak current recorded at 5 mV step potential was applied for further experiments. The peak current was recorded at various frequencies ranging from 15 to  $100 \text{ s}^{-1}$ . Despite the increase in peak current up to  $50 \text{ s}^{-1}$ , the peaks became broader and combined with frequency higher than  $50 \text{ s}^{-1}$ . 50 Hz was used for the following analysis, because of the highest and the most proper responses there. As a result of these studies, the best peak definition was recorded when using 50 Hz square-wave frequency, 50 mV pulse amplitude 5 mV scan increment in SWV and without accumulation mode. The effect of the drop size of the hanging mercury drop electrode on the peak current of the carnosic acid was examined. The maximum peak current in the mercury drop size was observed for 6 as depicted in Fig. 1d.

## 3. Results and discussion

No former electrochemical work has been investigated concerning on hanging mercury drop electrode (HMDE) behavior and the sensitive voltammetric, cyclic voltammetric or square-wave voltammetric (SWV) properties of carnosic acid (CA).

### 3.1. Electrochemical behavior of CA on HMDE

Electrochemical behavior, diffusion and adsorption properties of CA were studied on HMDE using cyclic voltammetry (CV). Cyclic voltammograms of CA were recorded within the potential range from 150 mV to  $-100$  mV. As can be seen from Fig. 2a there were two peaks. The first of the peaks, reduction wave at about  $-21$  mV (vs. Ag/AgCl) in the initial cathodic scan and the second, upon scan reversal on the anodic branch, corresponding one oxidation peak at about 6 mV (vs. Ag/AgCl)

was observed (Fig. 2a). There is no peak when a blank BR solution was scanned at the same conditions. The effect of scan rate on peak potential was investigated while CA concentration was held constant as 100 μM. It is clear from Fig. 2a that potential of reversible reduction–oxidation couple is independent of scan rate and ratio of anodic peak current ( $I_p, a$ ) to cathodic peak current ( $I_p, c$ ) is unity as expected for reversible nature [20].

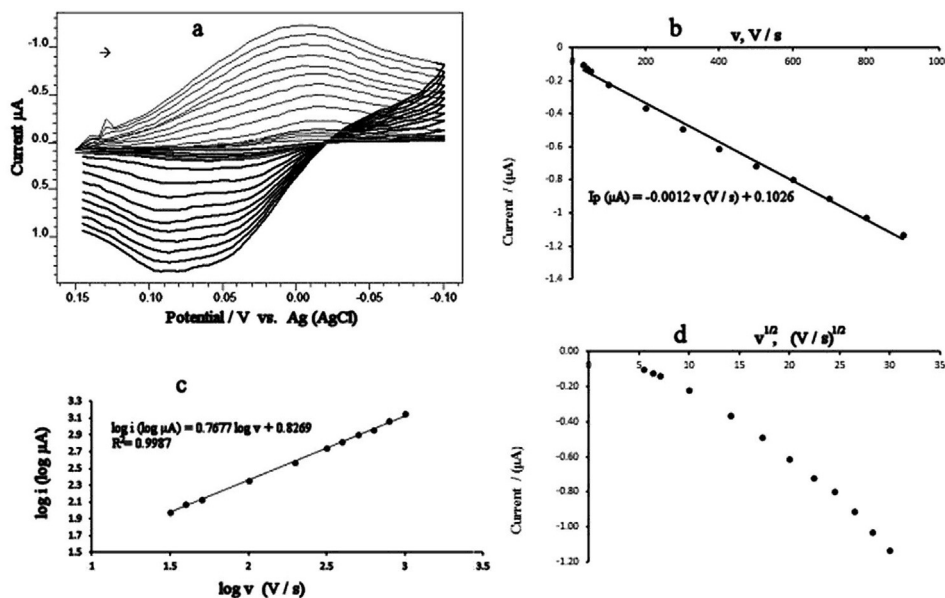


Fig. 2 – a. Cyclic voltammograms of 100 μM CA at various scan rates in B–R buffer of pH 7.0 (a) 30 mV s<sup>-1</sup>, (b) 40 mV s<sup>-1</sup>, (c) 50 mV s<sup>-1</sup>, (d) 100 mV s<sup>-1</sup>, (e) 200 mV s<sup>-1</sup>, (f) 300 mV s<sup>-1</sup>, (g) 400 mV s<sup>-1</sup>, (h) 500 mV s<sup>-1</sup>, (i) 600 mV s<sup>-1</sup>, (j) 700 mV s<sup>-1</sup>, (k) 800 mV s<sup>-1</sup>, (l) 900 mV s<sup>-1</sup>. b. The effect of peak current vs. scan rate. c. The graph for logarithm of peak current vs. logarithm of scan rate. d. Peak current vs. square-root of scan rate.

Effects of scan rate on peak current were also investigated. As scan rates ( $\nu$ ) increased from 30 mV s<sup>-1</sup> to 900 mV s<sup>-1</sup> at fixed concentration of CA, peak current was found to be changed linearly with scan rate (Fig. 2b), was found as given equation  $I_p, c (\mu A) = -0.0012 \nu (Vs^{-1}) - 0.1026$  with ( $R^2 = 0.995$ ), confirmed an adsorption behavior [21]. Linear plots of peak current versus square-root of scanning rate ( $I_p, c$  vs.  $\nu^{1/2}$ ) should be obtained for diffusing electroactive species, whereas species adsorbed on the electrode surface should result in linear plots of  $I_p, c$  versus  $\nu$  [20].

Also a plot of logarithm of peak current (Fig. 2c) versus logarithm of scan rate gave a straight line with a slope of 0.77 for CA, very close to the theoretical value of 1.0 (adsorption controlled) rather than 0.5. Relation between peak current and square root of scan rate is not linear at whole range but linear at relatively higher scan rates as depicted in Fig. 2d. This behavior may show the inefficient effect of diffusion to mechanism. Accordingly, the surface reaction could be mainly adsorption controlled electrode process.

### 3.2. Selection of a supporting electrolyte and validation of method

The selected electrolyte and its pH effects the sensitivity of the SW voltammetric measurements. The B–R buffer

(0.04 mol L<sup>-1</sup>) was chosen as a supporting electrolyte because of its wide pH range applicability. A single or double reduction peaks were observed depending on the concentrations. As shown in Table 1, the pH dependence reduction of 20 μM CA at the HMDE was studied systematically in the pH range between 2.0 and 12.0 (Fig. 3). In the measurements obtained for pH values until 5.0, it was observed that the peak's shape was distorted and the current value of the peak was lower. The

only pH range that both peaks could be observable was limited within pH 6.0–9.0. The peak potentials of both peaks shifted to more negative values with increasing pH between 6 and 9 and peak current of the first peak increased markedly until pH 6.0

Table 1 – SW voltammetric behavior of 20 μM carnosic acid in Britton–Robinson buffer electrolytes.

Organic compound	Electrolyte BR pH	Epeak (mV)	I (nA)	Peak shape
Carnosic acid	2.0	4	466	Very Broad
	4.5	-60	889	Broad
	5.0	-87	1084	Broad
	6.0	79	8103	Sharp
		-13	571	Sharp
	6.5	63	6192	Sharp
		31	5335	Sharp
	7.0	52	1914	Sharp
		-17	1732	Sharp
	7.5	25	802	Sharp
		-44	772	Sharp
	8.5	-60	614	Broad
		-113	557	Broad
	9.0	-76	320	Broad
	-151	313	Broad	
10.0	No peak	No peak	No peak	

and 6.5. But these peaks did not show proportional increments to standard additions. They were decreased at  $\text{pH} \geq 6$  and almost disappeared at  $\text{pH} \geq 9$ . Since CA SW voltammograms were well shaped and quite sensitive at pH 7.0 B–R buffers it was used as supporting electrolyte in the rest of the study.

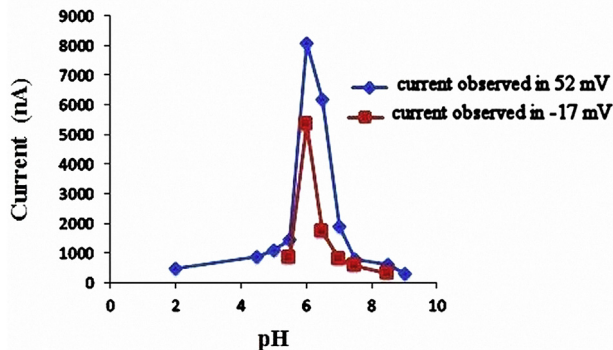


Fig. 3 – Variation of peak current depending on pH for 20  $\mu\text{M}$  carnosic acid.

The square wave voltammetric measurements of CA showed that the peak current increased linearly upon increasing the analyte concentration under the optimal conditions and method parameters. As shown in Fig. 4, CA exhibited two reduction peaks at about 52 and  $-17$  mV in pH 7.0 BR buffer solution. While the first of these peaks (52 mV) showed proportional increments for the first three standard additions (Fig. 4), the second peak at about  $-17$  mV performed using fourth or more standard additions.

While the corresponding first peak at 52 mV peak current was linearly proportional to CA concentration in the range of

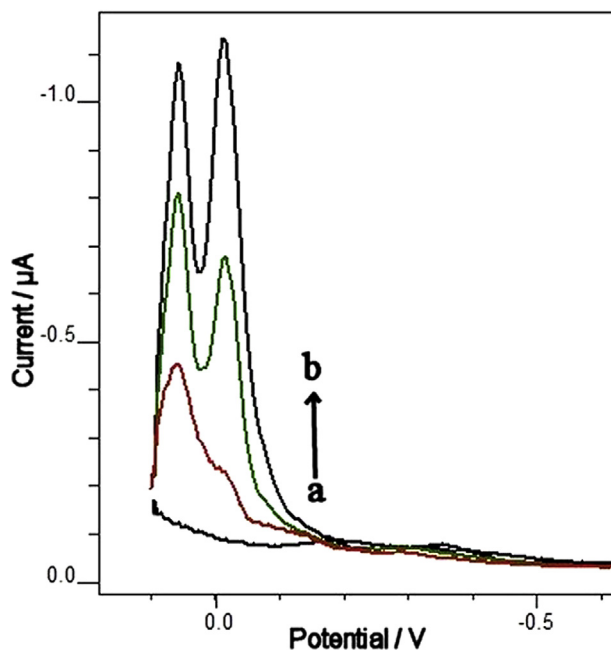


Fig. 4 – Determination of CA by standard additions using SW voltammetric method. (a) 10 mL pH 7 B–R buffer, (b) a + 0.1 mL  $1 \times 10^{-3}$  M CA, (c) b + 0.1 mL  $1 \times 10^{-3}$  M CA, (d) c + 0.1 mL  $1 \times 10^{-3}$  M CA.

10–30  $\mu\text{M}$  with the analytical equation given by:  $I_p$  ( $\mu\text{A}$ ) =  $0.0214 C$  ( $\mu\text{M}$ ) +  $0.0807$  ( $R^2 = 0.999$ ) ( $n = 6$ ) (Fig. 5a–I), the belong to second peak at  $-17$  mV peak current versus concentrations plot were rectilinear over the range from 40 to 90  $\mu\text{M}$  with the analytical equation given by:  $I_p$  ( $\mu\text{A}$ ) =  $0.0113 C$  ( $\mu\text{M}$ ) +  $0.4711$  ( $R^2 = 0.9997$ ) ( $n = 6$ ) (Fig. 5a–II). As can be seen in

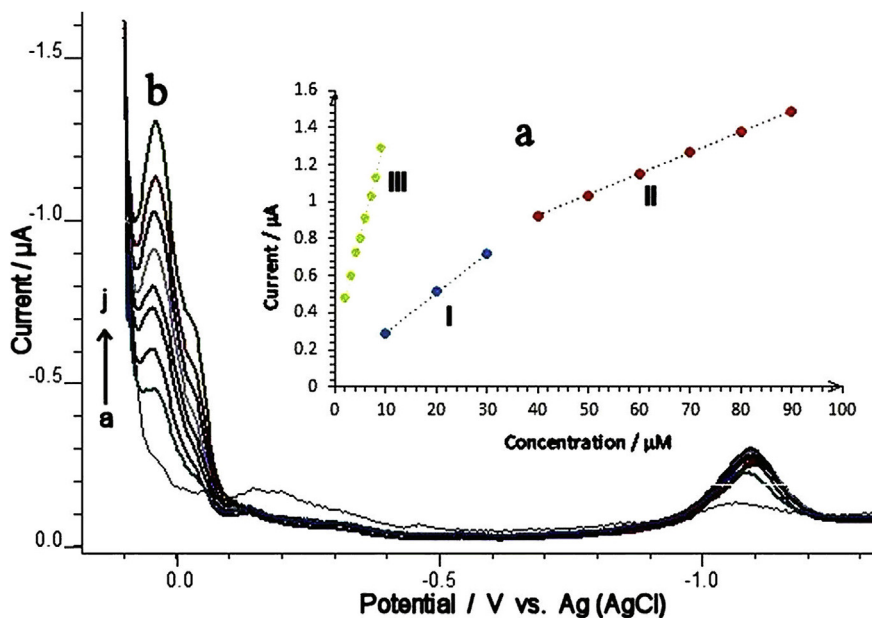


Fig. 5 – a. The calibration curves of CA in B–R buffer solution (pH = 7) obtained at different concentration values; b. SW voltammograms for the calibration curve III of CA (a) 10 mL pH 7 B–R buffer, (b) a + 1  $\mu\text{M}$  CA, (c) b + 2  $\mu\text{M}$ , (d) c + 3  $\mu\text{M}$ , (e) d + 4  $\mu\text{M}$ , (f) e + 5  $\mu\text{M}$ , (g) f + 6  $\mu\text{M}$ , (h) g + 7  $\mu\text{M}$ , (i) h + 8  $\mu\text{M}$ , (j) i + 9  $\mu\text{M}$ .



Fig. 5b, when working with more dilute solution of CA concentration in the range of 2–9  $\mu\text{M}$  with the calibration graph was obtained as  $I_p (\mu\text{A}) = 0.1109 C (\mu\text{M}) + 0.2629$  ( $R^2 = 0.996$ ) ( $n = 10$ ) (Fig. 5a–III). Three linear calibration graphs were obtained with slopes of 0.0214, 0.0113 and 0.1109 ( $\mu\text{M}/\mu\text{A}$ ), respectively (Fig. 5a). The three good linear calibration curves were constructed and the detection limits were found to be 4.0  $\mu\text{M}$ , 40.1  $\mu\text{M}$  and 1.5  $\mu\text{M}$  for CA, respectively.

**Table 2 – Recovery of CA determination in various synthetic samples using SW voltammetry.**

	Peak potential (mV)	[CA] ( $\mu\text{M}$ ) added	[CA] found ( $\mu\text{M}$ )	N	RSD%
1	52	1	$0.95 \pm 0.03$	4	2
2	52	2	$2.01 \pm 0.21$	4	9
3	52	10	$10 \pm 1.0$	4	8
4	-17	20	$19 \pm 1.5$	4	6

Confidence interval 90%.

Limit of detections were calculated using the following equations [22], where S is the standard deviation and m is the slope of the calibration plot:  $\text{LOD} = 3S/m$ . LOD indicates the high sensitivity of the proposed voltammetric method. The square wave voltammograms were recorded at various concentrations of CA under the optimum conditions. The quantity of CA could be determined by additions of standard CA solution. The obtained results are summarized in Table 2. The evaluation of the results obtained from these experiments show quite good precision, accuracy and repeatability.

### 3.3. Proposed mechanism

In SWV studies between the pH values of 6.5 and 9.0, first and second peak potentials change linearly with the pH value and the regression equations were  $E_p = -0.0613 \text{ pH} + 0.4732$  with  $R^2 = 0.971$ ,  $E_p = -0.0705 \text{ pH} + 0.4841$  with  $R^2 = 0.996$ , respectively. The experimental values of the peak potential slope against pH curves in SWV studies were found to be 0.061 and 0.070 V per unit pH value in the given pH range. The value of

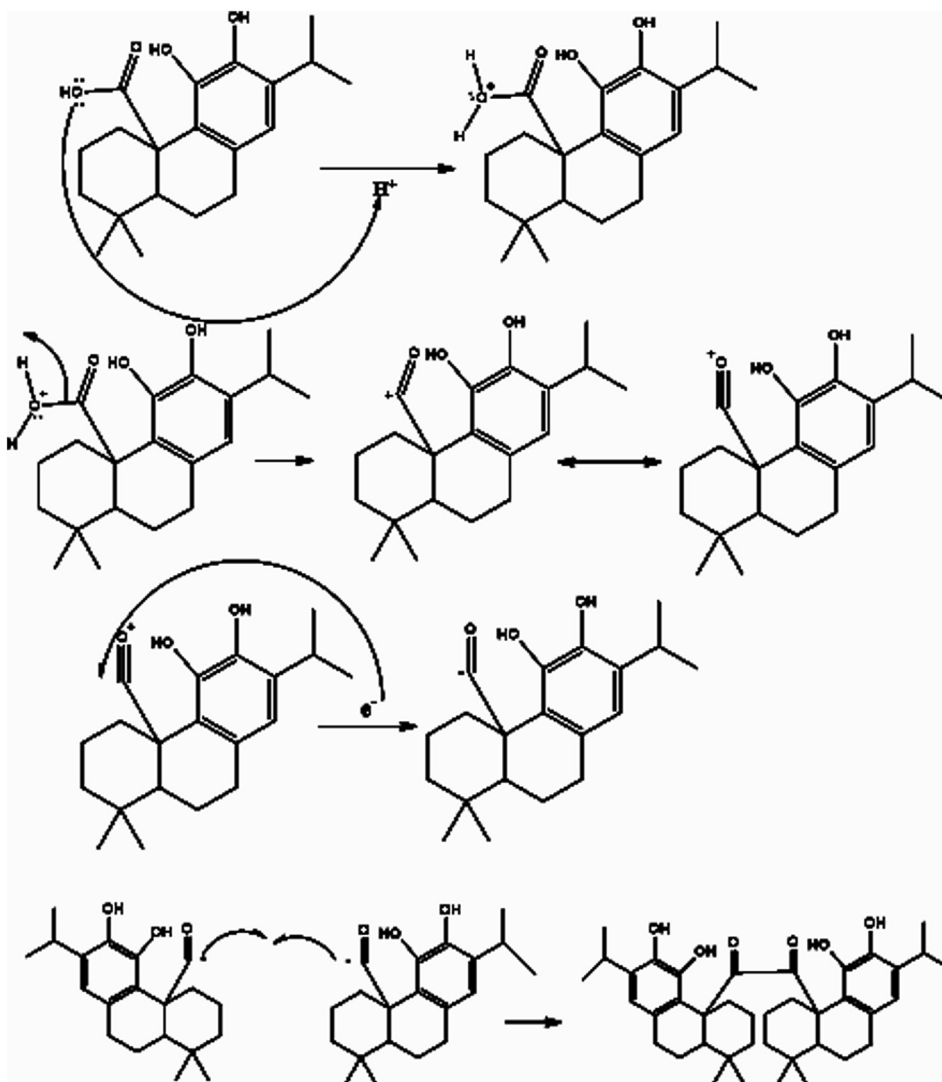


Fig. 6 – The proposed electrode reaction mechanism for the reduction of CA on the HMDE.

the slope is very close to the theoretical value of 0.0592 V per unit pH [23,24]. When the slopes of these relations were applied in Eq. (1) [25], the ratio of proton to electron participated in mechanism was calculated as 1.0:

$$E_p = E^0 - \frac{RT}{nf} \ln \frac{[Ox]}{[Red]} + \frac{2.303\theta RT}{nf} \text{pH} \quad (1)$$

Here,  $E^0$  is standard peak potential in V; [Ox] and [Red] are equilibrium concentrations of oxidized and reduced species, respectively, and  $\theta$  is number of proton participated in mechanism and others are common abbreviations. In alkaline media, the peaks totally disappeared, indicating that proton plays a role in the reduction process. This is due to the participation of protons in electrode reaction [26,27].

According to results obtained from the experiments, carnosic acid reduced on the electrode surface using one proton, one electron. In the proposed mechanism illustrated in Fig. 6, the reaction first stage involving an attack of oxygen's unpaired electrons in the hydroxyl group in carboxylic acid to proton accompanied by water elimination. Composed cation can be two resonance form. Then electron incorporated into molecule to generate radical. In turn radical species can give dimerization reaction immediately.

### 3.4. Interference effect

The selectivity and applicability of the proposed method for the determination of CA in the presence of some coexisting inorganic ions and organic compounds were investigated. The selectivity was established using recovery tests. The magnitude of interference effects was evaluated by comparing the assay results in the presence of the coexisting species to that in their absence. The amounts corresponding to the peak current responses for 20  $\mu\text{M}$  M CA in the presence of inorganic ions or organic compounds in a 1:1 mass ratio are presented in Table 3. They did not show serious interfering effects on the determination of CA.

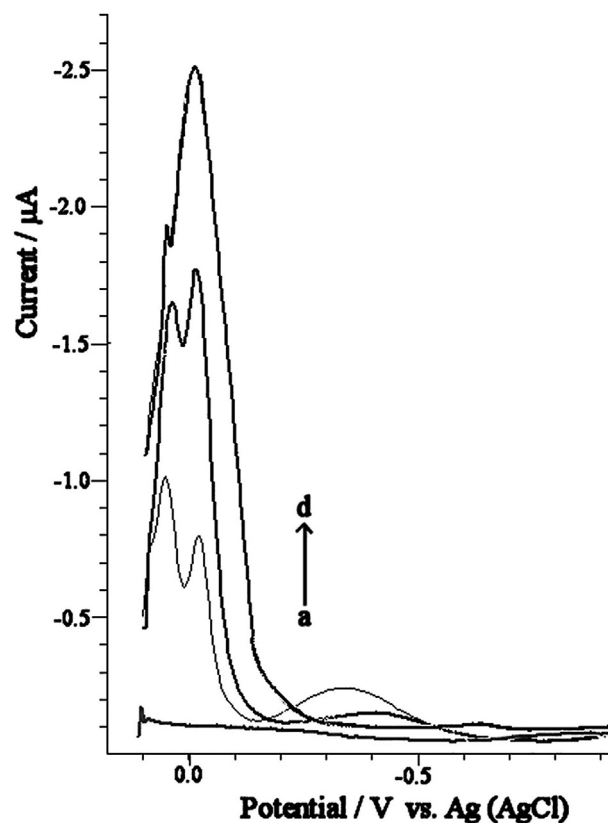
### 3.5. Direct determination of carnosic acid (CA) in natural extract of rosemary (*Rosmarinus officinalis*)

Carnosic acid (CA) in rosemary (*Rosmarinus officinalis*) has been used as natural antioxidants [28]. In addition to its antioxidant activity, CA also has antiinflammatory, antiplatelet, antibacterial, anticancer and photoprotective activities [29]. Therefore, determination of trace carnosic acid is very important. In order to evaluate the accuracy and applicability of the proposed method in determining CA in natural rosemary, the square wave (SW) voltammograms were recorded after a direct transfer of 500  $\mu\text{L}$  of extracted samples to the voltammetric cell containing 10 mL of BR buffer, pH 7.0. As shown in Fig. 7, rosemary extract sample gave a well-defined reduction peaks at around  $-17$  mV and CA in samples was determined by standard additions (Fig. 7). The results were summarized in Table 4.

The considerably low standard deviations are in good agreement with the RSD values less than 10% for rosemary

**Table 3 – Interference effect of some organic and inorganic species on the determination of 20  $\mu\text{M}$  CA.**

Interfering species 20 $\mu\text{M}$	Recovery of CA (II) (%)
Zn <sup>2+</sup>	90
Na <sup>+</sup>	97
NO <sub>3</sub> <sup>-</sup>	97
Mn <sup>2+</sup>	102
SO <sub>4</sub> <sup>2-</sup>	102
Cl <sup>-</sup>	102
Pb <sup>2+</sup>	92
Al <sup>3+</sup>	109
Se(IV)	100
Mg <sup>2+</sup>	108
Fe <sup>3+</sup>	110
Ca <sup>2+</sup>	102
As <sup>3+</sup>	97
Cr <sup>3+</sup>	97
Hg <sup>2+</sup>	103
Co <sup>2+</sup>	105
Ni <sup>2+</sup>	102
K <sup>+</sup>	103
Histamine	105
Dopamine	99
Cholic acid	98
Ascorbic acid	103
Melamine	86
Rosmarinic acid	91



**Fig. 7 – Determination of CA in rosemary extract. (a) 10 mL of B–R buffer (pH = 7), (b) a + 0.5 mL rosemary extract sample, (c) b + 0.1 mL 10<sup>-3</sup> M CA, (d) c + 0.1 mL 10<sup>-3</sup> M CA.**

**Table 4 – Comparison of the developed SW voltammetric with HPLC-UV method for the determination of carnosic acid (CA) extracted with ethanol in rosemary sample.**

Rosemary sample	$X_{\text{found}}$ ( $\mu\text{g CA}/1 \text{ g dry rosemary}$ )	${}^a\bar{X} \pm ts/\sqrt{N}$	
		The developed SWV method	HPLC-UV method
Ethanol extract	11.9 (SWV)	$11.9 \pm 1.0$	$11.0 \pm 2.3$
	11.0 (HPLC-UV)	N = 4 RSD% = 7 $F^b = 0.19$ $t^c = 1.79$	N = 4 RSD% = 12

<sup>a</sup> 90% confidence interval.

<sup>b</sup> Tabulated F-values for 95% confidence level and is 9.28.

<sup>c</sup> Tabulated t-values for 90% confidence level and six degrees of freedom are 1.94; N: number of analyses.

extract, which provided evidence for the high accuracy and precision of the recommended square wave voltammetric method. The performance of the developed new method was also evaluated by calculation of t- and F-values compared with the HPLC-UV method. These results displayed no considerable difference between the performance of the two methods. The content of CA in the rosemary can be safely determined using the proposed SW voltammetric method, without interference with other substances in the extract and extraction or filtration step. The developed method can be applied to rosemary extract added to foods, cosmetics and pharmaceutical products for the determination of CA.

#### 4. Conclusions

Electrochemical behavior of CA, antioxidant, was investigated and electrode-reduction mechanism on mercury electrode was presented in this study for the first time, to the best of our knowledge. According to these investigations, CA was reduced on HMDE by reversible mechanism with contribution of adsorption. From the CA peak produced on HMDE, highly reproducible and accurate results can be achieved. The applicability and selectivity of the developed method in the presence of some coexisting organic compounds and cations–anions were also investigated. Square-wave voltammetric methods for direct determination of CA in real systems, such as oils and fats, meat and meat products, natural extract of rosemary and biological samples were developed. The developed SW voltammetric method provides simple, less influence of the matrix effect, rapid, selective, and accurate analysis of carnosic acid in *R. officinalis* L. extracts. The analytical results obtained from in rosemary extract are compared with t-test and F-test against the HPLC-UV method and the results showed that the new method is reliable. These results indicate that the quantity of CA in the extracts of herbs used in food formulations can be confidently determined using the proposed voltammetric method without the need for any time-consuming and polluting pre-processing steps such as extraction, cleanup, derivatization, interferences or pre-concentration.

#### Conflicts of interest

All contributing authors declare no conflicts of interest.

#### Acknowledgements

We would like to thank “TUBITAK” for supporting this project (Grant No. TUBITAK-115Z711).

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