ADSORPTIVE CATHODIC STRIPPING VOLTAMMETRIC DETERMINATION OF CURCUMIN IN TURMERIC AND HUMAN SERUM

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A simple, rapid, reliable and fully validated differential pulse adsorptive cathodic stripping voltammetric procedure has been developed for determination of the curcumin in human serum and turmeric, based on its electrochemical reduction at a hanging mercury drop electrode. The Britton–Robinson (BR) buffer of pH 9.5 was found to be reasonable as a supporting electrolyte for the assay of the compound. The effect of different parameters, such as pH, accumulation potential and accumulation time, on the sensitivity of method was evaluated. Under the optimized conditions (accumulation potential –0.3 V, accumulation time 50 s, BR buffer pH 9.5), curcumin was generated one irreversible cathodic peak. This cathodic peak showed a linear dependence on the concentration of curcumin over the range of 5.0×10^{-9}–2.8×10^{-7} mol l^{-1}. The obtained detection limit under the optimal experimental conditions is 1.5×10^{-9} mol l^{-1} after 50 s of the accumulation time. The relative standard deviation of 1.12% for concentration of 5.0×10^{-8} mol l^{-1} with 50 s accumulation time was obtained. The procedure was used successfully to the assay of the curcumin in turmeric and spiked human serum, and a good agreement was obtained between the results of the proposed method and high performance liquid chromatography (HPLC) analysis.
Keywords: Adsorptive cathodic stripping voltammetry; Curcumin; Human serum; Turmeric; HPLC.

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, the main constituent of the rhizomes of the plant curcumin longa, is a common ingredient used in spices, cosmetics and traditional Chinese medicine (Fig. 1). The studies revealed that a numerous biological effects have been associated with curcumin. Its capability to induce apoptosis, anti-inflammatory[1,2] and antioxidative[3] make it a promising compound in the prevention and treatment of cancer, cardiovascular diseases and inflamma-

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tory processes. It is currently used in clinical trials as a treatment for numerous cancers including multiple myeloma, pancreatic cancer and colon cancer[4]. It was also shown that curcumin acts as a powerful radical scavenger[5] for hydroxyl radicals[6] and superoxide anions[7]. The property of binding of curcumin to metals like iron and copper is considered as one of the useful requirements for the treatment of Alzheimer's disease[8,9]. Also, Barik and et al.[10] show a correlation between superoxide dismutase mimics and free radical scavengers properties with copper(II)–curcumin complexes. However, because a variety of spices, foods and traditional Chinese medicine are currently being consumed, the question arises as to how much curcumin is present in these compounds? Thus, although some of foods may contain enough curcumin to impart characteristic flavor, is the amount present sufficient to exert a beneficial pharmacological effect? Several techniques have been developed for determination of curcumin, such as thin-layer chromatography[11–13], high performance liquid chromatography[14–16], capillary electrophoresis[17], radio labeling and mass spectrophotometry[18], gas chromatography with supercritical fluid extraction[19], spectrofluorimetry[20] and UV Vis spectrophotometry[21]. However, these techniques have some disadvantages, such as complicated operation, high cost of maintenance, expensive apparatus and requiring well-controlled experimental conditions. For these reasons, the rapid, simple and accurate method with high sensitivity is expected to be established. An alternative method for determination of curcumin is voltammetry. The electroanalytical study of curcumin in acidic and basic media using carbon paste (CPE) and hanging mercury drop electrodes (HMDE) have been reported by Stanic and coworkers[22]. According to this report, the electrooxidation of curcumin at the surface of HMDE in negative potential region is not clear, but at the surface of CPE it yields a well-defined redox peaks in the potential range of 0.3–0.6 V, in which both anodic and cathodic peaks can be used for determination of curcumin. The short dynamic range and low limit of detection are drawbacks of this report. Recently curcumin has been determind by modified electrodes[23] which were coupled with flow injection analyzer. In spite of its

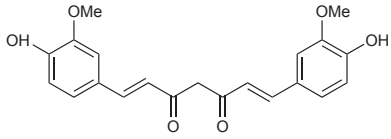


FIG. 1
Chemical structure of curcumin

sensitivity, the analysis time of the method is lengthy (from construction of modified electrode till measurement of target). In this paper, a simple, rapid and reliable differential pulse adsorptive cathodic stripping voltammetric (AdCSV) procedure was introduced for the determination of curcumin. This technique is based upon adsorptive accumulation of the target at the electrode and then scanning the potential of the electrode in the negative direction. It is a very sensitive method which can be used for determination of curcumin in real samples such as turmeric and human serum.

EXPERIMENTAL

Chemicals and Apparatus

All solvents and compounds obtained from Merck (Darmstadt, Germany) and Aldrich (Milwaukee, USA) were of the analytical grade and were used without further purification. Three distilled water was used throughout. Curcumin was supplied from Merck and was used without further purification. The 1.0×10^{-3} M stock solution of curcumin was prepared in ethanol and adequately protected from light. The BR buffer solutions in the pH range of 2–11 were prepared from 0.1 M (each) phosphoric, acetic and buric acids[24]. The polarographic investigations were carried out with a polarograph VA 797 Computrace (Metrohm). The polarograph consisted of a HMDE as a working electrode, an Ag|AgCl as a reference electrode (3.0 M KCl), and a platinum wire as an auxiliary electrode. A rotating Teflon rod stirred solution in the voltammetric cell. For preparation of the calibration curve and analysis of food samples, the analyzer was operated under optimized DPV parameters: initial potential –0.5 V, final potential –1.5, pulse amplitude 50 mV, pulse time 40 ms, voltage step 0.005 mV, voltage step time 0.125 s, resulting in a scan rate of 40 mV s^{-1}. Cyclic voltammogram was recorded when potential commenced from –0.7 V and its direction reversed at –1.5 V with different potential scan rates (20, 40, 100 and 200 mV s^{-1}). The pH values of the solutions were adjusted employing a Metrohm model 827 using a combined glass electrode. High performance liquid chromatography (HPLC) was performed on a KNAUER liquid chromatograph system employing EZ-Chrome Elite software. The variable wavelength UV-Vis detector was operated at 230 nm for curcumin determinations. A 20 µl injection loop was used. Separations were performed on a Eurospher 100-5C18 column (250 × 4.0 mm i.d.). The mobile phase was MeOH–0.02 M acetate buffer (80:20 v/v, pH 4.8) and the flow rate was maintained at 1.0 ml min^{-1}.

Serum and Turmeric Samples Preparation

Serum sample collected and stored frozen until assay. One ml of serum treated with a 0.5 ml methanol as a protein precipitating agent. After vortexing for 30 s the precipitated proteins were separated by centrifugation at 15000 rpm for 3.0 min. The clear supernatant layer (about 1.2 ml) was filtered to produce protein-free plasma, 0.5 ml of this serum was spiked with standard curcumin solution. An amount of about 0.1 g of turmeric was introduced into a dark bottle and 30 ml ethanol were added, then the mixture was shaked continuously for 24 h. The collected extracts were filtered and diluted to 50 ml with ethanol and stored at 4 °C.

Analytical Procedure

An amount of 9.9 ml of 0.01 M BR supporting electrolyte solution pH 9.5 was introduced into a dark electrolysis cell and deaerated with pure nitrogen for 5 min. A –0.3 V accumulation potential was then applied to HMDE for 50 s, while the solution was stirred at 1000 rpm. At the end of the accumulation period, the stirring was stopped and a 10 s rest period was allowed for the solution to become quiescent. Then, the differential pulse voltammogram was recorded by scanning the potential toward the negative direction using the scan rate of 40 mV s^{-1}. After recording the background voltammogram, 0.1 ml of the ethanolic solution containing different curcumin concentrations was introduced into the electrolysis cell (the final volume was 10 ml and 1.0% v/v EtOH–BR buffer) and the voltammogram was then recorded at a new mercury drop. The background of electrolyte was subtracted from the curcumin voltammogram and the peak currents at –1.1 V were evaluated. All data were obtained at room temperature.

RESULTS AND DISCUSSION

Curcumin is a diferuloyl methane having two *o*-methoxy and phenolic OH groups attached to the α,β-unsaturated β-diketone (heptadiene-dione) moiety. In order to observe the electrochemical activity of curcumin, its cyclic voltammograms (CV) were recorded in both acidic and basic media at different scan rates. Figure 2 shows the CV of 5.0×10^{-8} M curcumin in BR buffer containing EtOH (1.0% v/v) as supporting electrolyte in pH 6 and 9.5, respectively. The forward potential scan commences at an initial potential of –0.7 V, and its direction was reversed at –1.5 V. As it can be seen, two irreversible cathodic peaks were observed in acidic solution that may be due to the reduction of β-diketone moiety. In contrast, in basic solution only one cathodic peak without any anodic peak in the reverse scan was observed in the tested potential window. The same behavior for compounds containing carbonyl group has been reported in previous work[24]. Also as it is observed by increasing of the scan rate, the cathodic peak current (i_{pc}) of curcumin considerably increased and its peak potential (E_{pc}) was shifted to negative potentials in both solutions. These electrochemical behaviors demonstrated that the electrochemical reduction of curcumin is an irreversible process[25].

Useful information involving electrochemical mechanism can usually be acquired from the relationship between the peak current and scan rate. Therefore, the electrochemical behavior of curcumin at pH 9.5 at different scan rates was investigated. The obtained results showed a linear relationship ($I_p = 0.0454v - 0.222$ (I_p in nA, v in mV s^{-1})) with a correlation coefficient of $R^2 = 0.998$ obtained between the peak current and the square root of scan rate in the range of 25–200 mV s^{-1}, which revealed that the reduction of curcumin is a diffusion-controlled process. In the experiment, the

relationship between the reduction peak potentials and scan rates can be described as follows: E_{pc} = 0.065 log v – 0.981, R^2 = 0.995. According to Laviron's theory[26], the slope was equal to $2.303RT/\alpha n_\alpha F$. Then the value of αn_α was calculated as 0.91. As for a totally irreversible electrode reaction process, τ was assumed as 0.5. On the basis of the above discussion, the n_α

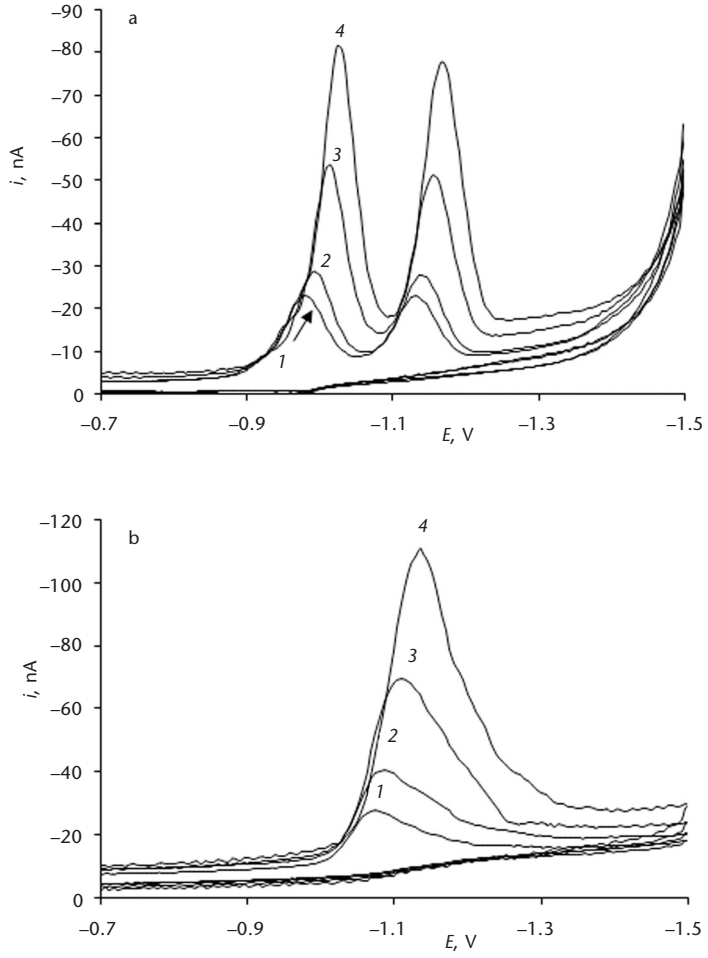


FIG. 2

Cyclic voltammograms of 5.0×10^{-8} M curcumin in 10 ml of electrolyte containing 1.0% v/v EtOH–BR buffer at pH 6.0 (a) and 9.5 (b) in different scan rates: 20 (*1*), 40 (*2*), 100 (*3*) and 200 mV s^{-1} (*4*). The conditions were as follows: drop size, medium; potential range –0.5 to –1.5 V; potential scan rates 20, 40 and 100 and 200 mV s^{-1}

was calculated to be 1.81 which indicated that two electrons were involved in the reduction process of curcumin at HMDE. Analysis of the currents (from Fig. 2b) using the Tomes' criteria $E_{3/4} - E_{1/4} = 1.857RT/\alpha n_\alpha F$ which is the difference between the three-quarter and quarter wave potentials, found it to be 56 mV suggesting an irreversible system with $n_\alpha = 2$ (using $\alpha = 0.5$) that confirmed the previous results.

It is noteworthy to mention that the current associated to the cathodic peak in basic solution is nearly equal to the current of each cathodic peak in acidic solution. This observation shows that the electrochemical reduc-

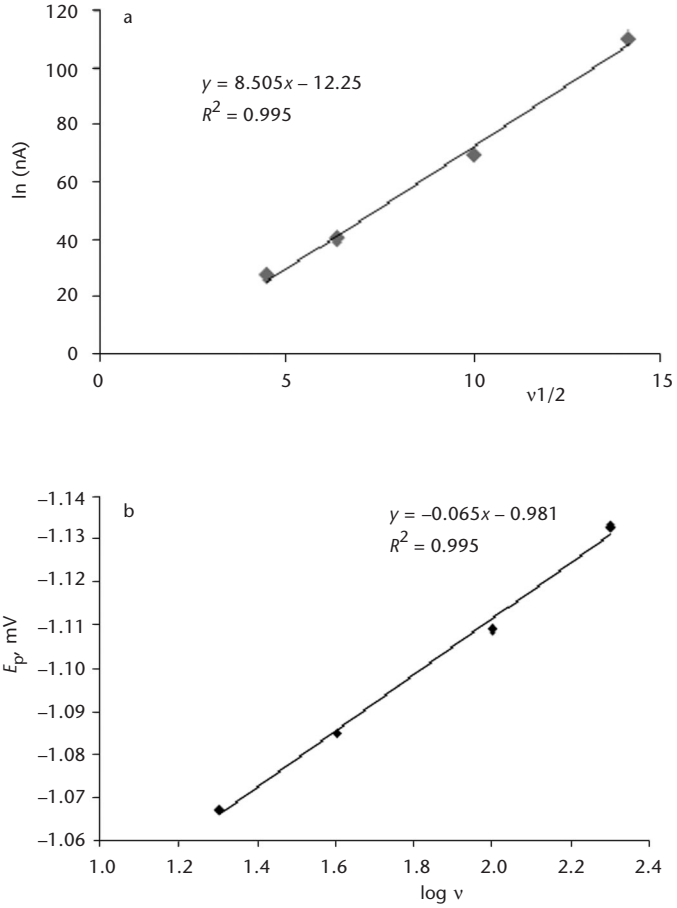


FIG. 3
The dependences of cathodic peak current I_p vs v (a) and potential E_p vs log v (b) derived from the voltammograms shown in Fig. 2

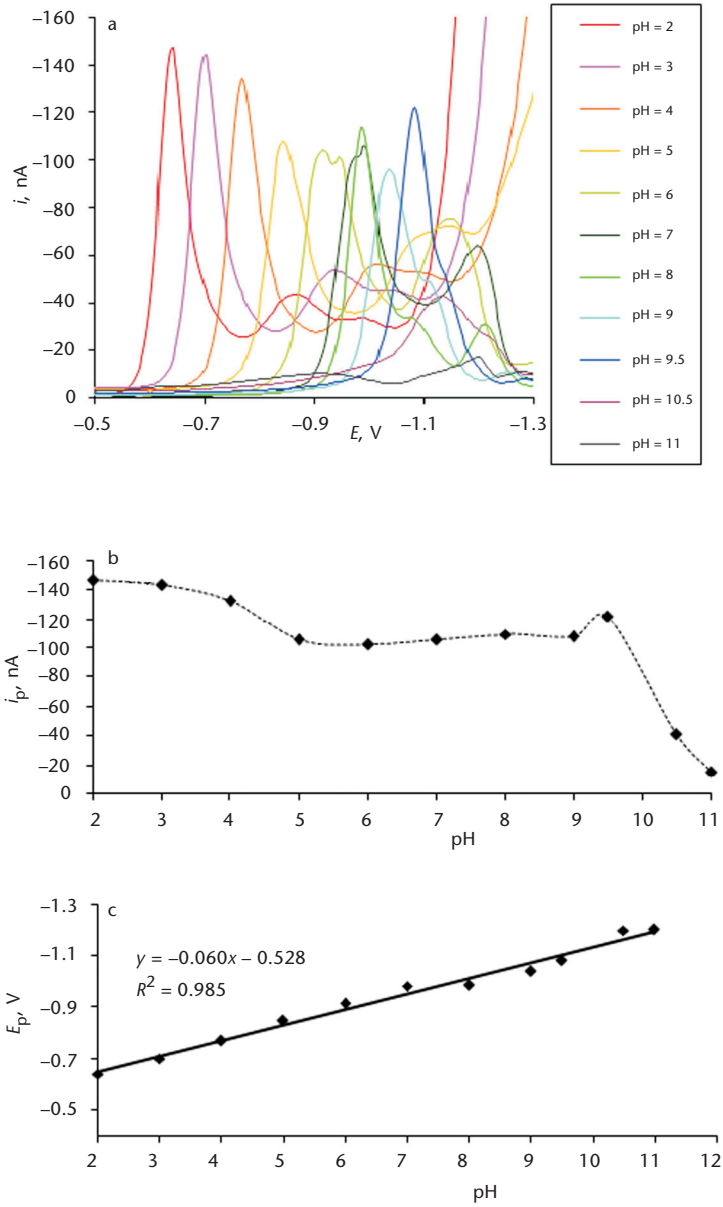
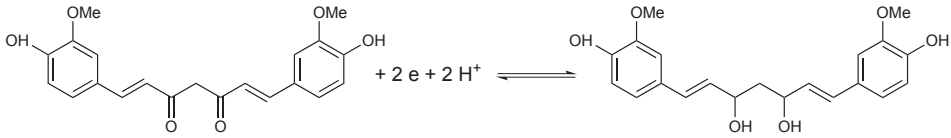


FIG. 4

Effect of pH on the reduction of 1.5×10^{-7} M curcumin: voltammograms (a), i_p vs pH (b) and E_p vs pH (c). The conditions were as follows: 10 ml of electrolyte containing 1.0% v/v EtOH–BR buffer, accumulation potential 0.0 V, accumulation time 20 s

tion of curcumin is irreversible and pH dependent. Therefore, the influence of pH on the peak current of curcumin was studied in the pH range of 2.0 to 11.0 for solution containing 1.5×10^{-7} M curcumin using differential pulse voltammetric (DPV) method. The voltammograms, peak current (I_p) and peak potential (E_p) of the first cathodic peak of curcumin as a function of pH were shown in Fig. 4. As it can be seen, not only both cathodic peaks potential shift toward negative values but the current of the first anodic peak decreases by increasing the pH of the test solution up to pH 9. In the alkaline solution, the second reduction peak was not observed in the potential window that was used for recording the DP voltammograms. In basic medium, the maximum peak current was obtained at pH 9.5. The voltammetric first peak potential versus pH plot was a straight line with a slope of 60 mV, which agrees well with two electron and two proton reduction process with transfer coefficient $\alpha = 0.5$. Therefore, the reduction mechanism of curcumin is proposed as follows.



In order to achieve a high sensitive method, the selection of a proper electrochemical technique is of great importance. So, differential pulse cathodic stripping voltammetry (DPCSV) was used for further investigation.

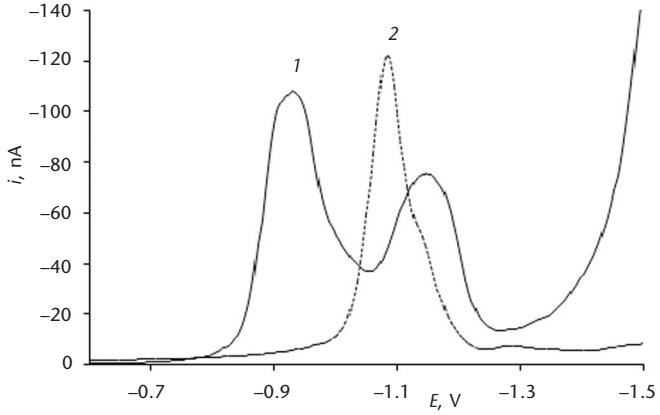


FIG. 5

Voltammograms of 1.5×10^{-7} M curcumin at pH 6.0 (*1*) and 9.5 (*2*). The conditions were as follows: 10 ml of electrolyte containing 1.0% v/v EtOH–BR buffer, accumulation potential 0.0 V, accumulation time 20 s

Therefore, the DPCS voltammograms of 1.5×10^{-7} M curcumin at pH 6.0 (line 1) and pH 9.5 (line 2) after 20 s accumulation at 0.0 V were recorded. The results are presented in Fig. 5. As it can be seen, in basic solution (pH 9.5) one cathodic peak was observed which its current is greater than those obtained in acidic solution. The same experiment was carried out under the identical conditions but at pH 2 and different concentrations of curcumin. The obtained results showed that decreasing the pH of the solution and increasing the concentration causes to reduce the cathodic peaks separation and enhances the amount of overlapping of both cathodic peaks presented in acidic medium. This resulted a non linear dependency between peak currents and curcumin concentrations. Therefore, in order to overcome the above problem, the reduction peak current of curcumin at basic pH 9.5 was used for its monitoring.

Effect of Accumulation Parameters on the Peak Current

To determine the optimal accumulation potential, the potential range of 0.0 to –0.4 V was examined for 1.5×10^{-7} M curcumin in BR buffer at pH 9.5, followed preconcentration of 20 s (Fig. 6a). As it is obvious, the peak current of curcumin is independent on accumulation potential at potentials more negative than –0.3 V. Therefore, –0.3 V (vs Ag|AgCl) was chosen for all subsequent measurements. The dependence of the peak current intensity of the curcumin on accumulation time was also investigated. Figure 6b exhibits the effect of the deposition time upon AdCSV signals at 1.5×10^{-7} M curcumin. We found an optimal deposition time of 50 s. At longer deposition times, the signal began to level off. In our previous works, the same behavior was observed and we believed it may be attributed to the equilibrium attainment at the electrode surface–solution interface[25,27–33].

Validation of the Analytical Procedure

Under the selected conditions, the reduction peak current of the curcumin yields well defined concentration dependence. The voltammograms for solutions of increasing curcumin concentration after 50 s accumulation at –0.3 V are presented in Fig. 7a. The peak current of DPASVs was linearly related to the curcumin concentration according to the regression equation of the calibration curve: $i_p = -0.758C$ (C in nmol l^{-1}) – 16.98m ($R^2 = 0.995$. The reduction peak current of curcumin at –1.1 V was found to be directly proportional to the curcumin concentration in the range of 5.0×10^{-9}–

2.8×10^{-7} mol l^{-1} (Fig. 7b). As the curcumin concentration was extended above 2.8×10^{-7} mol l^{-1}, a more or less pronounced deviation from the linearity appeared, which may be attributed to reach the maximum equilibrium at the mercury electrode surface. Validation of the optimized procedure for the quantitative assay of the curcumin was examined via evaluation of the limit of detection (LOD), limit of quantitation (LOQ), selectivity and repeatability. The LOD and LOQ were calculated from the calibration graphs (after preconcentration of the curcumin onto the MHDE for 50 s), using the below equations[34]

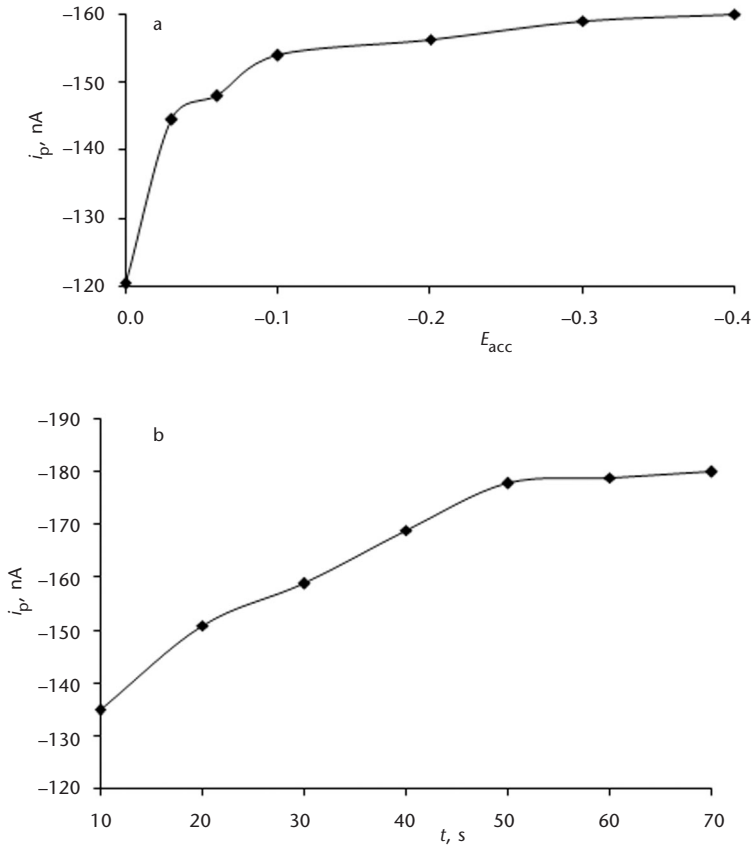


FIG. 6
Effect of accumulation parameters on the peak current at accumulation potential (a) and accumulation time (b). The conditions were as follows: 10 ml of electrolyte containing 1.0% v/v EtOH–BR buffer, pH 9.5

$$\text{LOD} = 3\,\frac{\text{SD}}{b} \quad \text{and} \quad \text{LOQ} = 10\,\frac{\text{SD}}{b}$$

where SD is the standard deviation of the blank (intercept) and b is the slope of the calibration graph. LOD) and LOQ were found to be 1.5×10^{-9} and 3.1×10^{-9} mol l^{-1}, respectively. Repeatability was examined by perform-

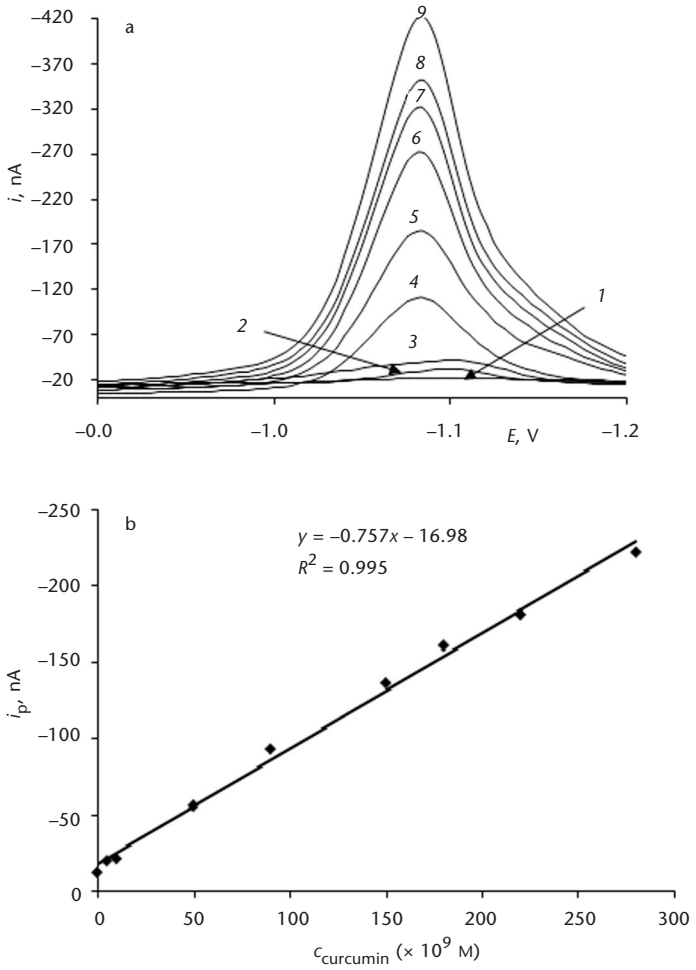


FIG. 7

Adsorptive stripping voltammograms of 0 (*1*), 5 (*2*), 10 (*3*), 50 (*4*), 90 (*5*), 150 (*6*), 180 (*7*), 220 (*8*) and 280 (*9*) × 10^{-9} M curcumin (a) and calibration graph (b). The conditions were as follows: accumulation time 50 s, pH 9.5, accumulation potential –0.3 V

ing five replicate measurements for 5.0×10^{-8} M curcumin followed preconcentration for 50 s and the relative standars deviation (RSD) of 1.12% was achieved. To evaluate the effect of foreign ionic and organic species commonly found in serum and foods on the determination of 1.0×10^{-7} mol l^{-1} (37 ng ml^{-1}) of curcumin, a systematic study was carried out. A 20 μg ml^{-1} level of potentially interfering species was tested first and if interference occurred, the interfering amount was reduced progressively until its effect was less than 5% of tolerance. The tolerance was defined as the amount of foreign species that produces an error not exceeding than 5% in the determination of the analyte (Table I). As it can be seen from the data given in Table I, the method offers a practical potential for trace determination of curcumin with high selectivity, sensitivity, simplicity and speed, except for curcuminoied[35] and Fe^{3+}, Cu^{2+} and Zn^{2+} cations. The effect of transition metal ions was significantly reduced by addition of EDTA.

Assay of Curcumin in Spiked Human Serum and Turmeric Samples

The optimized procedure was successfully used for determination of curcumin in protein-free spiked human serum. The reliability of the proposed procedure for the determination of curcumin was checked using different spiked human serum samples. The free human serum was used for evaluation the signal of blank. The results revealed that no peak current was observed at potential of –1.1 V. The mean recovery of curcumin based of the average of five replicate measurements for 5.0×10^{-8}, 1.0×10^{-7} and 2.0×10^{-7} mol l^{-1} with 50 s accumulation time was found to equal 97.5, 102.5 and 97.8% with RSD of 3.1, 2.8 and 2.6%, respectively. The amount of cur-

TABLE I
Effect of foreign species on the determination of 37 ng ml^{-1} of curcumin

Foreign species	Tolerance level ng ml^{-1}	Effect on signal
NO_3^-,Cl^-, Na^+, K^+	20000	NE[a]
Ca^{2+}, Mg^{2+}, Al^{3+}	1000	negative
Fe^{3+}, Cu^{2+}, Zn^{2+}	200	negative
Benzoic acid	20000	NE[a]
Curcuminoied	50	positive

[a] NE, not effect.

cumin in human serum without separation of protein was also analyzed. The mean recovery of curcumin based on the average of three replicate measurements for foregoing concentrations under the same conditions was found to be between 46–50%, with RSD less than 8.7%. The proposed procedure was also successfully applied for the assay of curcumin in turmeric. The results are shown in Table II. The mean percentage recovery of curcumin obtained by AdCSV procedure is based on the average of five replicate measurements. The effect of curcuminoied in tumeric samples on the curcumin signals was less than 5% (see Table I). The same results were achieved by HPLC analyses of the turmeric samples[36].

TABLE II
Determination of curcumin in turmeric sample

Sample	Concentration of curcumin added, mol l^{-1}	Found by AdSV mol l^{-1}	Found by HPLC mol l^{-1}	Recovery, %	RSD, %
1	0	1.2×10^{-7}	1.21×10^{-7}	–	2.3
2	5.0×10^{-8}	1.6×10^{-7}	1.54×10^{-7}	94.0	2.5
3	1.0×10^{-7}	2.3×10^{-7}	2.2×10^{-7}	104.5	2.3

[a] The relative standard deviations were calculated for AdSV method.

TABLE III
The comparison of proposed procedure with other methods

Method	Linear range, mol l^{-1}	LOD, mol l^{-1}	Ref.
Quenching fluorescence of Eu^{3+}–tryptophan complex	1.0×10^{-8}–1.2×10^{-4}	9.0×10^{-10}	20
Capillary electrophoresis-SPE	3.0×10^{-6}–7.0×10^{-4}	3.0×10^{-8}	17
HPLC-SPE	5.3×10^{-6}–7.6×10^{-4}	3.8×10^{-6}	16
UV-Vis	3.8×10^{-8}–1.1×10^{-6}	3.0×10^{-8}	21
AdsASV	5.76×10^{-8}–483×10^{-6}	NR[a]	22
Fast Fourier transformation square wave voltammetry	2.0×10^{-9}–1.0×10^{-6}	5.0×10^{-10}	23
AdsCSV	5.0×10^{-9}–2.8×10^{-7}	1.5×10^{-9}	this work

[a] NR, not reported.

CONCLUSION

The present study demonstrates the adsorptive cathodic striping voltammetric determination of curcumin. The electrochemical reduction of curcumin under the conditions described in this work is an irreversible process. Therefore an adsorption process of curcumin occurs on the hanging mercury drop electrode surface that can be used as an effective preconcentration step prior the voltammetric measurement. The method offers a practical potential for trace determination of curcumin with high selectivity, sensitivity, simplicity and speed that have not been presented together in the previously reported systems (Table III). The obtained results show that the proposed method may be used to determine curcumin in human serum and turmeric samples.

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