Scientific paper

A New Voltammetric Method for the Determination of Lercanidipine in Biological Samples

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Abstract

Electrochemical behavior and adsorption-diffusion properties of lercanidipine (LCN) on a glassy carbon electrode (GCE) were investigated in a mixture of ethanol-Britton Robinson buffer (BR) using voltammetric methods. From experimental results LCN was found to be reduced irreversibly via a single four-electron process controlled mainly by diffusion with some adsorption contribution at about –0.65 V (vs. Ag/AgCl reference electrode). Therefore, a new, accurate, rapid, selective and simple square-wave cathodic adsorptive stripping voltammetric (SWCAdSV) method could be developed for direct determination of LCN in pharmaceutical preparations, spiked human urine and spiked human serum samples without time-consuming steps prior to drug assay. The peak current of the reduction wave linearly changed with the concentration of LCN in the concentration range between 4.0×10^{-8} molL^{-1} and 7.6×10^{-6} molL^{-1} in two different regions where optimum preconcentration potential and optimum preconcentration time were applied as –0.20 V and 90 s, respectively. The limit of detection (LOD) and the limit of quantitation (LOQ) values were found to be 2×10^{-8} molL^{-1} (0.01 mgL^{-1}) and 6×10^{-8} molL^{-1} (0.04 mgL^{-1}), respectively. The method was applied to determine the content of LCN in commercial pharmaceutical preparation, spiked human serum and spiked human urine. The method was found to be highly accurate and precise, having a relative standard deviation of less than 10% in all applications.

Keywords: Electrochemistry, Cyclic voltammetry, Square-wave adsorptive stripping voltammetry, Lercanidipine, Antihypertensive drug, Biological samples (spiked).

1.Introduction

Dihydropyridine derivatives are used as calcium channel blockers to control the blood pressure and chronic stable angina pectoris. Lercanidipine, (LCN) 2-[(3,3-diphenylpropyl)methylamine]-1,1-dimethylethylmethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5 pyridine dicarboxylic ester (Fig. 1), is a third generation dihydropyridine calcium channel antagonist that causes systemic vasodilatation by blocking the influx of calcium ions through L-type calcium channels in cell membranes. LCN is a highly lipophilic antihypertensive drug that is effective and well tolerated in patients with mild to moderate hypertension. It maintains adequate blood pressure control for more than a day with once-daily administration,

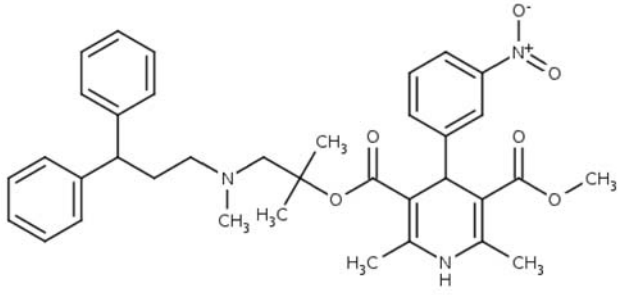


Figure 1. Chemical structure of LCN

without invoking unfavorable hemodynamic or sympathetic activity. Early clinical studies suggest that LCN has antiatherogenic potential and may also protect against end-organ damage.[1, 2]

Several analytical techniques such as high-performance liquid chromatography,[4–9] liquid chromatography,[10–16] thin layer chromatography and densitometry,[17] liquid-liquid extraction methods,[18] and spectrophotometric methods[19–24] have been devised for the determination of LCN in pharmaceutical samples or biological fluids. Some of these reported methods are either not sensitive or tedious and require highly sophisticated instrumentation even though they are sensitive. Additionally, there is a polarographic method[3] capable of determining LCN in commercial tablets based on its electroreduction. Reviewing the literature revealed that up to now there is no adsorptive stripping voltammetric method for the assay of LCN in pharmaceutical formulations and biological samples.

Voltammetric techniques such as cyclic voltammetry, differential pulse voltammetry and square-wave voltammetry have been proved to be very sensitive for the determination of electroactive molecules including organic and inorganic species, drugs and related molecules in pharmaceutical dosage forms and in biological fluids. These methods are faster, easier to operate and cheaper than most of the other analytical methods; the sensitivity increases upon applying stripping voltammetry. Adsorptive stripping voltammetry has been shown to be an efficient electroanalytical technique for determination of a wide range of electroactive species at nano molar level. Its remarkable sensitivity is attributed to the combination of an effective deposition step with an advanced measurement procedure that generates an extremely favorable signal to noise ratio. It usually involves a simple deposition step and most of the excipients used in pharmaceutical preparations do not interfere in the subsequent determination of the drugs.

There are many applications of stripping voltammetric methods.[25–29] In the present study the investigation of electrochemical reduction behavior of LCN using voltammetric methods was studied. The current study was also carried out to propose a tentative reaction mechanism. Development of a new validated square-wave cathodic adsorptive stripping voltammetric (SWCAdSV) assay method for direct determination of LCN in different samples including pharmaceutical preparations, human serum and human urine was one of the other goals of the present study.

2. Experimental

2. 1. Apparatus

All voltammetric measurements such as cyclic voltammetry (CV), controlled potential coulometry (CPC), square-wave voltammetry with and without cathodic adsorptive stripping mode were carried out using a BAS 100B-instrument electrochemical analyzer. A three-electrode cell system (C3 stand) comprising a glassy carbon electrode with a diameter of 3.0 mm as a working electrode (BAS MF-2012), a platinum wire as an auxiliary electrode (BAS MW-1034) and an Ag/AgCl (in 3.0 $molL^{-1}$ KCl) reference electrode (BAS MF-2052 RE-5B) was used in all experiments.

A three-electrode combination system, consisting of a glassy carbon sieve (approximately 65 cm^2 area) as a working electrode, coiled platinum wire as an auxiliary electrode (23 cm) (BAS MW-1033) and Ag/AgCl (in 3.0 $molL^{-1}$ KCl) reference electrode (BAS MF-2052 RE-5B), was used for bulk analysis.

All pH measurements were made with a Thermo Orion Model 720A pH ion meter having an Orion combined glass pH electrode (912600) which had been calibrated with pH 4.13 and pH 8.20 stock buffer solutions before measurement.

Double-distilled deionized water was supplied from Human Power I^+, Ultra Pure Water System (Produced by ELGA as PURELAB Option-S). All the data were obtained at room temperature (21 ± 2 °C).

2. 2. Reagents and Solutions

A standard sample of LCN (99.0%, from Fako) was used to prepare the stock solution of LCN. This solution was prepared by dissolution of a precisely weighed amount of LCN in ethanol yielding a LCN concentration of 2.2×10^{-3} $molL^{-1}$ ($1.35gL^{-1}$). Calibration solutions were prepared by diluting the stock solution with the mixture of ethanol and Britton-Robinson buffer (BR) in the volume ratio of 20:80 as solvent-supporting electrolyte mixture, and adjusting the pH value of these solutions (using 0.2 $molL^{-1}$ NaOH) to desired values between 2.5–5.5 for pH.

Ortho-phosphoric acid (Riedel), boric acid (Riedel) and acetic acid (Merck) were used to prepare the BR solution; sodium hydroxide (Merck) for adjustment of the pH of the supporting electrolyte was of analytical reagent grade. Double-distilled deionized water was used in preparations of all the solutions.

All LCN solutions were protected from light and used within a day to avoid photochemical decomposition.

2. 3. Preparation and Analysis of Samples

Lercadip® (produced by Fako in Turkey) tablets were used in pharmaceutical dosage form containing 10 mg of LCN and some amount of excipients per tablet. Before preparation of tablet solutions, the average mass of a tablet was determined by weighing ten finely powdered tablets. A homogeneous sample, equivalent to one tablet, was weighed and transferred to a 100-ml calibrated flask to which approximately 50 mL of ethanol was added. The contents of the flask were sonicated for 30 min to achieve

complete dissolution of LCN. After dissolution the flask was filled to the mark with ethanol and the contents of the flask were centrifuged for 30 min at 1500 rpm. A 10-mL aliquot of the clear supernatant was withdrawn and quantitatively diluted to 100 mL with solvent-supporting electrolyte mixture; this LCN stock solution was kept refrigerated. Calibration solutions were prepared from this stock solution by pipetting volumes from 0.025 to 0.75 ml into the electrochemical cell containing 10.0 mL solvent-supporting electrolyte mixture; after pH adjustment to the desired value LCN was determined. For testing spiked serum and urine samples were used.

Actual serum and urine samples obtained from healthy individuals were stored frozen until assay. After gentle thawing, a 1-mL aliquot of serum (or urine) was added to the electrochemical cell containing 9 mL of solvent-supporting electrolyte mixture, followed by spiking with LCN stock solution (0.05–3 ml). After deareation, LCN in the cell was quantified using the least squares linear regression

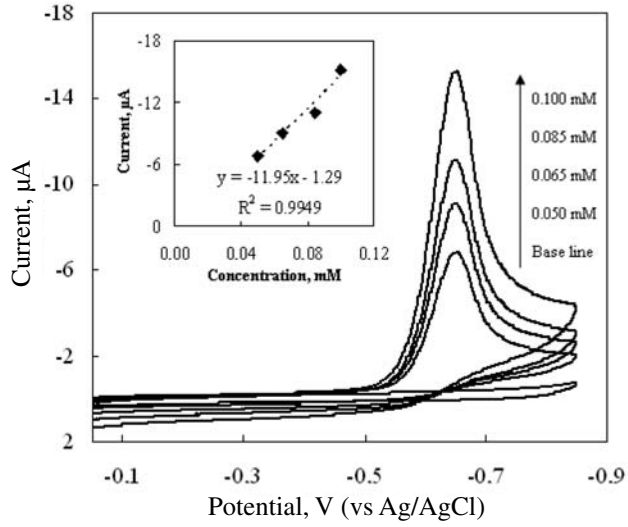


Figure 2. Cyclic voltammograms of different LCN solutions in given solvent-supporting electrolyte mixture at pH 4.0 at scan rate of 0.10 Vs^{-1} (inset: plot of current versus concentration)

2. 4. Voltammetric Procedure

In all voltammetric studies, 10 mL of LCN solution in solvent-supporting electrolyte mixture was placed into the electrochemical cell. A newly polished GC electrode was inserted into the system and then cell was deoxygenated with purified argon (99.99% purity) for 10 min before the first run and 30s between runs. After deareation and equilibration for 5s, voltammograms were recorded by applying a negative-going scan from 0.00 V to –0.90 V. Before each measurement the working electrode (GC) was polished with alumina and rinsed with ethanol for 30 s in an ultrasonic bath.

3. Results and Discussion

3. 1. Electrochemical Behavior of LCN

Electrochemical behavior, diffusion and adsorption properties of LCN were studied using the results of cyclic voltammetry, square-wave voltammetry, and controlled-potential electrolysis. In cyclic voltammetric studies a single reduction peak was observed at a potential of about 0.65 V at pH 4.5 (Fig. 2). There is no peak when a blank BR-ethanol mixture was scanned at the same conditions, and the peak current increases linearly with increasing concentration of LCN (Fig. 2; inset). Therefore, it can be concluded that this cathodic reduction peak is due to a reduction of LCN molecules on the GC electrode. As can be seen from Figure (2), there is no anodic peak at reverse scan, indicating that the electrode reaction is totally irreversible.

The influence of the potential scan rate on the cathodic peak current ($i_{p,c}$) was investigated for 5.0×10^{-5} mol L^{-1} LCN in the 0.005–1.000 Vs^{-1} range. In this range a linear dependency between cathodic peak current, $i_{p,c}$, and scan rate, υ, was found ($i_{p,c}$ (µA) = –30.03 υ(Vs^{-1}) –3.94; R^2 = 0.9937; Fig. 3, inset A). The linear relationship between peak current and scan rate confirms an adsorption-controlled mechanism. The plot of peak current versus square root of scan rate was also constructed (Fig. 3; inset B) and here too a linear relationship was found (i_p(µA) = $-32.47\sqrt{\upsilon} + 3.82$; R^2 = 0.9967). This relationship indicates that diffusion mechanisms are involved in the electrochemical reaction. Also a plot of the logarithm of the peak current (A) versus the logarithm of the scan rate (Vs^{-1}) was studied. This relationship was found to be linear with a slope of 0.621 (Fig. 3; inset C). The value of the slope is between the theoretical value of 1.0 for adsorbed species and 0.50 for a diffusion-controlled mechanism.[25] Some extra studies were carried out to control the adsorption phenomena with regard to the literature[30, 31] and as a result we found that i) the ratio of cathodic peak current-to-concentration ($i_{p,c}/C$) decreases with increasing concentration, ii) the ratio of the cathodic peak current-to-concentration times scan rate ($i_{p,c}/C\upsilon$) is nearly constant with increasing scan rate and iii) the ratio of cathodic peak current-to- concentration timesquare root of scan rate ($i_{p,c}/C\upsilon^{1/2}$) increases with increasing scan rate. Results of all these experimental investigations suggests that electroreduction of LCN molecules on the GC electrode is mainly controlled by diffusion with some adsorption contribution.

In the present study the effect of potential scan rate on the cathodic peak potential ($E_{p,c}$) was also investigated. The peak potential shifts to more cathodic values with increasing scan rate (Fig. 3). The relationship between the peak potential and the logarithm of the scan rate was found to be expressed by the following equation: E_p (V) =

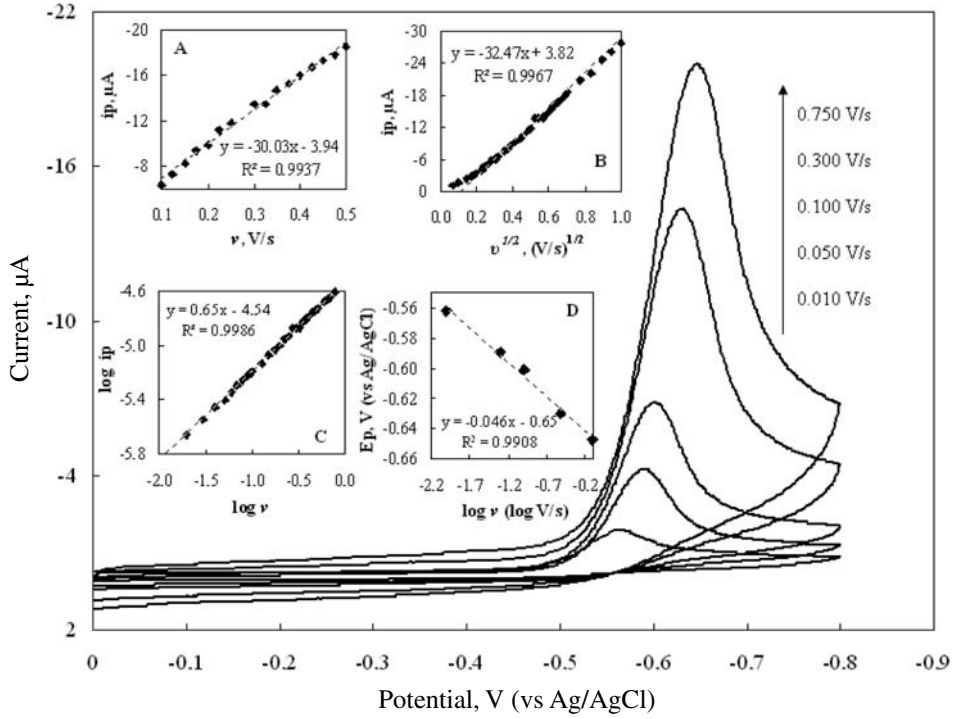


Figure 3. Influence of potential scan rate on both cathodic peak current and cathodic peak potential of 5.0×10^{-5} molL^{-5} LCN (inset: (A) curve of peak current versus scan rate, (B) curve of peak current versus square root of scan rate, (C) curve of logarithm of peak current versus logarithm of scan rate, (D) curve of peak potential versus logarithm of scan rate

–0.046logυ – 0.65 with $R^2 = 0.9908$ (Fig. 3; inset D). Potential shifting with scan rate supports the irreversibility of the electrochemical reaction under investigation. According to the literature[32] the slope of the curve of the peak potential versus the logaritm of the scan rate has a value of 0.0296 V per unit ($n\alpha_c$), with α_c the cathodic charge transfer coefficient and n the number of electons, and the difference between the peak potential and the half peak potential is 0.0477 V per unit ($n\alpha_c$). As can be seen from Figure (3), inset D, the curve of the peak potential versus the logaritm of the scan rate has a slope value of –0.046. By using these experimental results the value of $n\alpha$ was calculated to be 0.64. This value was calculated to be 0.67 from the difference of the peak potential and the half peak potential. The value of $n\alpha$ was also calculated by using another relation given in the literature for CV studies and given below:[26]

$$E_p = k + \frac{RT}{(n\alpha)F}\log\upsilon \qquad (1)$$

This equation shows that the slope of the curve of the peak potential versus the logarithm of the scan rate has a value of $RT/(n\alpha_c)F$. The value of $n\alpha_c$ was calculated to be 0.61 from CV studies. In electrochemical studies, pH is one of the variables that commonly and strongly influences the electrochemical behavior of molecules. Therefore, the electrochemical behavior of LCN was studied as a function of pH in the pH range 2.15–5.0. At pH values higher than 4.55 the solubility of LCN decreases dramatically and formation of precipitation begins; more and more ethanol is needed to overcome the solubility problem and therefore effect of higher pH values could not be studied. As can be seen from the square-wave voltammograms (SWV) in Fig. 4, the potential of the cathodic peak shifts linearly to more negative values with increasing pH as can be expressed by the following equation: $E_{p,c}$(V) = –0.051 pH – 0.38 with $R^2 = 0.9690$ (Fig. 4; in inset). The experimental value of the slope of this curve was found to be 51 mV per unit pH in the studied pH range. The slope is very close to the theoretical value of 59 mV per unit pH required under assumption of the $2e^-/2H^+$ or $4e^-/4H^+$ process[25, 26] of the electro reduction of LCN. Based on the literature, the following equation was used to find the ratio of the number of protons to the number of electrons (∂/n) in the electrode mechanism:[29]

$$E_p = E^0 + \frac{RT}{nF}\ln\frac{[O]}{[R]} - \frac{\partial RT}{nF}\ln[H^+] \qquad (2)$$

In this equation ∂ and n are the number of protons participating in the reaction and the number of transferred electrons in the electrochemical step, respectively. The ratio of the number of protons to the number of electrons was found to be 0.95 from the slope of the plot of E_p versus pH value. As a result, the same number of electrons

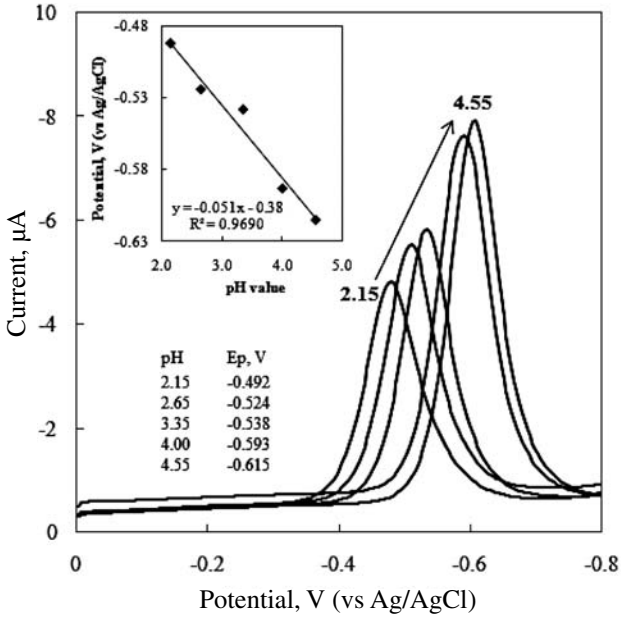


Figure 4. Influence of pH on square wave voltammograms of 1.2 × 10–6 molL^{-1} LCN (inset: Plot of peak potential versus pH value)

and protons are partipicating in the electroreduction of LCN molecules.

To find out the surface coverage of the adsorbed molecule, the following relationship proposed for adsorption in cyclic voltammetry was used[26]:

$$i_p = \frac{n^2 F^2 \Gamma A \upsilon}{4RT} \quad (3)$$

where Γ is the surface coverage of the adsorbed molecules (in mol cm^{-2}), and others are commonly known parameters with their values.[26, 29]

The surface coverage of the adsorbed molecule (Γ) was calculated from the slope of the curve of peak current (A) versus scan rate (Vs^{-1}) according to Eq. 3 and was found to be 2.88×10^{-11} mol cm^{-2} when 0.005 Vs^{-1} ≤ υ ≤ 0.050 Vs^{-1}, 1.43×10^{-11} mol cm^{-2} when 0.065 Vs^{-1} ≤ υ ≤ 0.275 Vs^{-1} and 6.19×10^{-11} mol cm^{-2} when 0.300 Vs^{-1} ≤ υ ≤ 1.00 Vs^{-1}.

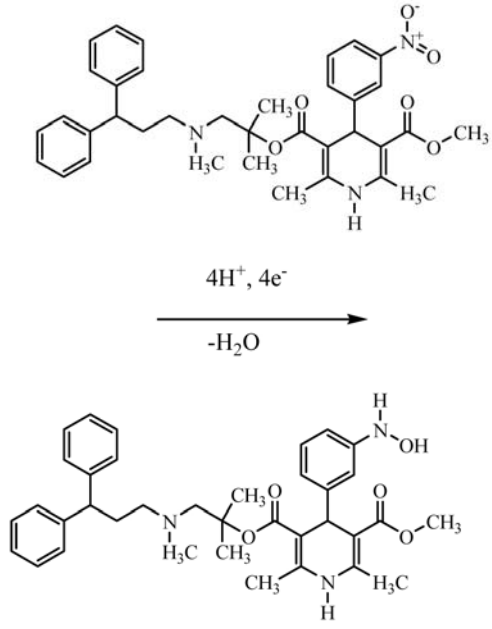
The following equation which expresses adsorption phenomena validated by Garrido[33] was used to calculate the diffusion coefficient of LCN:

$$i_p = 1.06 \times 10^6 n^2 A C \upsilon D^{1/2} t_p^{1/2} \quad (4)$$

The mean of the diffusion coefficient calculated from this equation was 2.19×10^{-6} cm^2 s^{-1}.

3. 2. Reduction Pathway

To propose a tentative electrode reaction mechanism for the reduction of LCN, the results of the voltammetric studies given above and also bulk electrolysis carried out at –1.15 V were evaluated. On the basis of all experimental results and according to literature dealing with the reduction of molecules contain nitro groups, it can be concluded that the NO_2 group in LCN is reduced to NHOH which can be expressed by the following reaction mechanism:



A similar type of mechanism was also given for the reduction of the CNO_2 group in the literature.[34–36]

3. 3. Electroanalytical Determination of LCN

In the present study, electrochemical assay of LCN was established with adsorptive techniques to achieve lower limits of detection then studies given in the literature. For this purpose, the instrumental parameters and experimental conditions such as pH, LCN concentration, deposition time, and deposition potential were optimized for the development of an assay method to determine LCN.

In order to obtain a well-defined square-wave voltammetric peak shape and high peak current, instrumental parameters such as frequency, f, scan increment, ΔE_i, and pulse-amplitude, ΔE_a, were optimized for 1.2×10^{-6} molL^{-1} LCN in a ethanol – BR mixture of pH 4.0. The optimum instrumental parameters were found to be f = 15 Hz, ΔE_i = 4 mV and ΔE_a = 25 mV.

The effect of pH on both peak current and peak potential was given in previous sections. In the optimization of the pH value, not only the peak current was chosen as an important parameter, but also peak shape, peak symmetry, linearity range and solubility of LCN were chosen as other important parameters. In order to get a useful peak shape and larger linearity range, a pH value of 4.0 was selected as optimum although peak current values are higher at higher pH values as can be seen from Fig. 4.

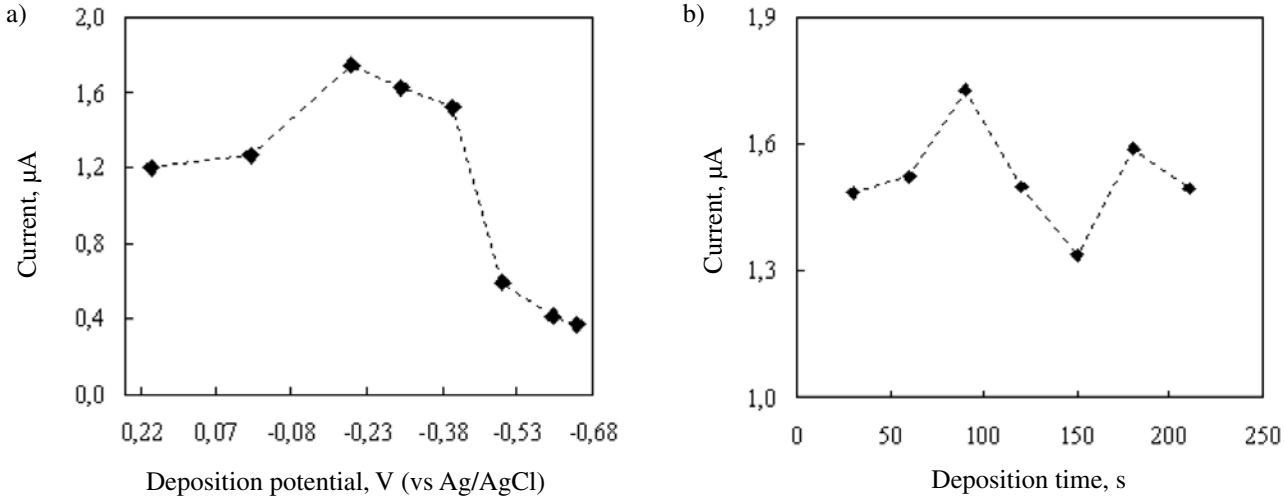


Figure 5. (A) Effect of deposition potential on peak current and (B) Effect of deposition time on peak current for the solution containing 5.0×10^{-7} molL^{-1} LCN in SWCAdSV

In the stripping method the influence of the deposition potential on the square-wave cathodic adsorptive stripping voltammetry (SWCAdSV) signal was studied for a 5.0×10^{-7} molL^{-1} LCN solution in the range from 0.0 V to 0.65 V. Variation of the peak current (i_p) versus deposition potential for 5.0×10^{-7} molL^{-1} LCN is given in Fig. 5A. As can be seen from this figure, the peak current decreases at more negative deposition potentials after –0.20 V. The maximum peak current in the deposition step was observed for the deposition potential of –0.20 V.

The influence of deposition time on peak current was also optimized in the range from 15 to 210 s for 5.0×10^{-7} molL^{-1} LCN. The resulting peak current increased with increasing deposition time from 15 to 90 s, flowed by a decreasing signal (Fig. 5B). The optimum deposition time chosen was 90 *s*.

To establish the linearity range (working concentration range) of LCN in the proposed method, standard solutions having different LCN concentrations in the range from 1.0×10^{-8} molL^{-1} to 1.0×10^{-5} molL^{-1} were measured. Five replicate measurements were and the mean of these measurements was plotted against the corresponding concentration. The results these measurements showed the current of the reduction peak changes linearly with LCN concentration in two linear regions. In the first region (from 4.0×10^{-8} molL^{-1} (0.022 mgL^{-1}) to 3.0×10^{-7} molL^{-1} (0.183 mgL^{-1})) the following calibration equation is obeyed: $i_{p(\mu A)} = 1.62 \times C_{LCN\ (\mu M)} + 0.12$ with R^2

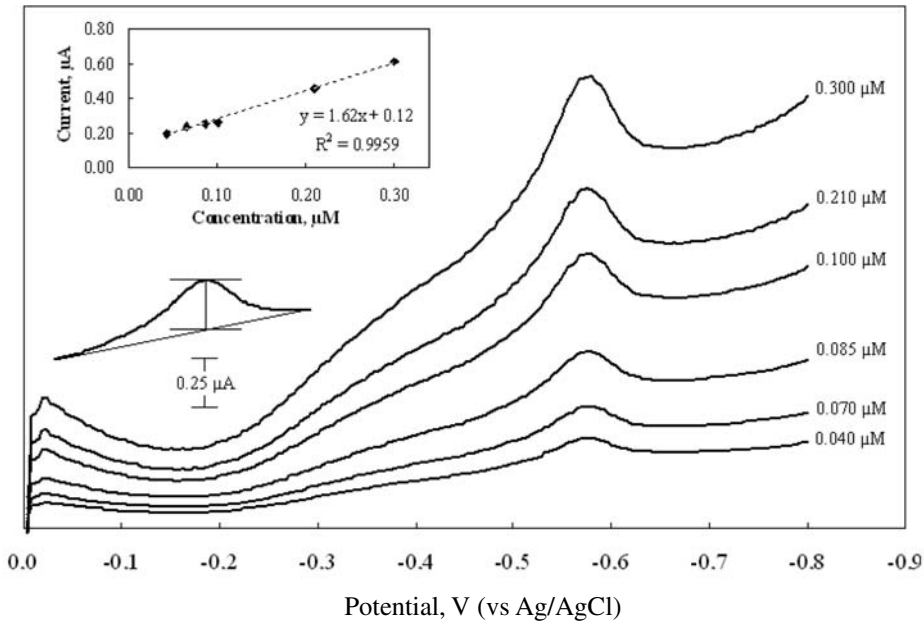


Figure 6. SWCAdSV of calibration solutions (inset: calibration curve for corresponding concentrations)

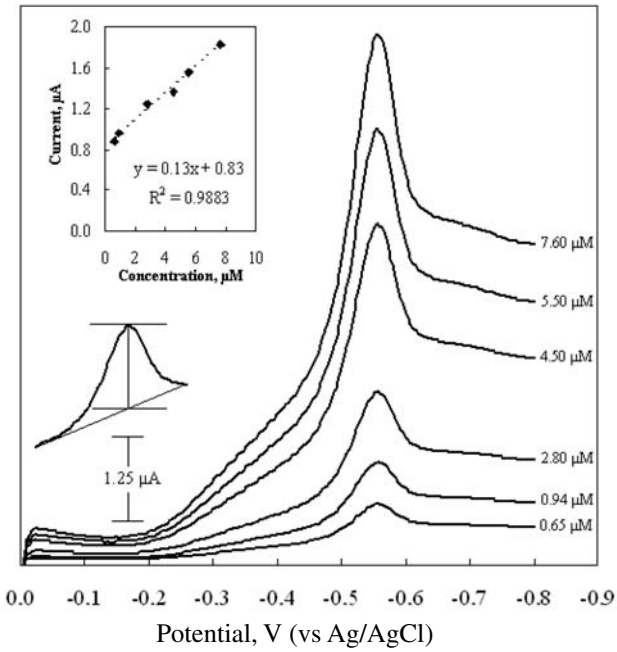


Figure 7. SWCAdSV of calibration solutions (inset: calibration curve for corresponding concentrations)

= 0.9959 (Fig. 6). In the second region (from 6.5×10^{-7} molL^{-1} (0.397 mgL^{-1}) to 7.6×10^{-6} molL^{-1} (4.646 mgL^{-1})) the following calibration is obeyed: $i_{p(\mu A)} = 0.13 \times C_{LCN\ (\mu M)} + 0.83$ with $R^2 = 0.9883$ (Fig. 7).

The characteristics of the calibration plots are summarized in Table 1.

3. 4. Application of Proposed Method to Dosage Form and Biological Samples

In order to evaluate the applicability of the proposed method, LCN was determined in pharmaceutical dosage forms. The results of the analysis of pharmaceutical preparations are given in Table 2 and of spiked human urine and spiked human serum in Table 3. The accuracy of the proposed method was determined by its recovery values.

It can be seen from these tables that average recovery values are in good agreement with RSD values less than 10 %, which is good evidence for the validity of the method. Thus, the precision is very satisfactory for the analysis of biological samples as well as bulk formulations. These results indicate that the content of LCN in the pharmaceuticals and biological fluids can be safely determined by using the proposed voltammetric method without interference from other substances in the samples after a simple dilution step.

3. 5. Validation of Method

Validation of an analytical method is the process by which it is established that the performance characteristics of the method meet the requirements for the intended analytical applications. The elements required for method validation are: linearity range, limits of detection and quantification, precision, accuracy, reproducibility, stability, selectivity and robustness.[37]

Results of concentration studies were given in early stages of the manuscript. Limit of detection (LOD) and limit of quantification (LOQ) values were calculated using the following relationships: LOD = 3 *s*/*m* and LOQ = 10 *s*/*m*,[38] with *s* is the standard deviation of the intercept of the calibration curve and *m* the slope of the related calibration curve; the LOD and LOQ values found were 2×10^{-8} molL^{-1} (0.01 mgL^{-1}) and 6×10^{-8} molL^{-1} (0.04 mgL^{-1}), respectively. Both LOD and LOQ values confirmed the sensitivity of the proposed methods.

The accuracy of the measurement by means of the described procedure was checked by calculating the recovery of a known concentration of LCN following the proposed method at optimum instrumental and experimental conditions. Recovery values range from 97.2% to 101.4% for tablet analysis, from 98.4%

Table 1: Regression data of the calibration curve for assay of LCN by SWCAdSV

Calibration Parameter	First Linear Region	Second Linear Region
Linearity Range, mol L^{-1}	$4.0 \times 10^{-8} - 3.0 \times 10^{-7}$	$6.5 \times 10^{-7} - 7.6 \times 10^{-6}$
Calibration Equation	$i_p(\mu A) = 1.624 \times C_{LCN\ (\mu M)} + 0.119$	$i_p(\mu A) = 0.131 \times C_{LCN\ (\mu M)} + 0.825$
Slope of Calibration Curve, AL mol^{-1}, (m)	1.624	0.131
Intercept, A	1.19×10^{-7}	8.25×10^{-7}
SD (Standard Deviation) of Calibration, A	1.34×10^{-8}	4.72×10^{-8}
SD of Slope, AL mol^{-1}	0.065	0.008
SD of Intercept, (s), A	9.79×10^{-9}	3.44×10^{-8}
Limit of Detection (LOD), mol L^{-1}	1.81×10^{-8}	7.88×10^{-7}
Limit of Quantification (LOQ) mol L^{-1}	6.03×10^{-8}	2.63×10^{-6}
Regression Coefficient, R^2	0.9952	0.9883
Repeatability of peak current[a], (RSD, %)	6.59	4.28
Repeatability of peak potential[a], (RSD, %)	2.87	1.97

[a] Calculated for 5 serial measurements

Table 2: Results of proposed method for determination of LCN from the solution of lerkcadip® tablets

Sample[a]	Nominal value per tablet, mg	Found values per tablet, mg	Recovery value[b], %	RSD[c], %
I	10	9.62, 9.67, 9.70, 9.75, 9.86	97.23 ± 1.14	0.94
II	10	9.87, 9.96, 10.16, 10.34, 10.38	101.42 ± 2.81	2.22

[a] Samples given the name I is in the first linear region and II is in the second linear region [b] Results of recovery values are given as mean ± ts/√N (at 95 % confidence level) [c] RSD is relative standard deviation

Table 3: Results of proposed method for determination of spiked standard LCN and spiked tablet samples into various biological media

Sample[a]	Spiked amount, µg	Found values, µg	Recovery value[b],	RSD[c], %
Standard in Urine I	0.50	0.45, 0.48, 0.49, 0.51, 0.53	98.40 ± 7.54	6.16
Standard in Urine II	15.00	13.41, 14.35, 14.67, 15.19, 16.00	98.16 ± 7.98	6.55
Tablet in Urine I	1.00	0.92, 0.93, 0.94, 1.05, 1.11	99.04 ± 10.62	8.60
Tablet in Urine II	20.00	17.95, 18.82, 19.52, 21.18, 22.00	99.47 ± 10.38	8.35
Standard in Serum I	0.50	0.46, 0.47, 0.50, 0.54, 0.56	101.20 ± 10.78	8.57
Standard in Serum II	10.00	9.25, 9.36, 9.43, 10.52, 11.10	99.32 ± 10.32	8.35
Tablet in Serum I	1.00	0.89, 0.91, 0.98, 1.02, 1.12	98.40 ± 11.48	9.39
Tablet in Serum II	30.00	26.75, 26.86, 28.32, 30.50, 35.25	94.45 ± 11.78	9.97

[a] Samples given in I is in the first linear region and II is in the second linear region b Results of recovery values are given as mean ± ts/√N (at 95 % confidence level) c RSD is relative standard deviation

to 99.5% for urine analysis and from 94.4% to 101.2% for serum analysis (Table 2, Table 3). From these recovery values it is concluded that proposed method is highly accurate. From the results of t- and F-tests the variance between two methods were found to be insignificant at the 95% confidence level indicating that no significant differences exist between the performances of the two methods regarding their accuracy, precision and recoveries (Table 4). As a result the proposed method might be an alternative for the methods given in the literature.

Table 4: Statistical analysis of the results obtained by different methods for LCN.

Method	Mean Recoveries, %	RSD, %	*N*
SWCAdSV	98.7	6.9	10
LC-MS[15]	94.9	6.8	16
F-Test significance	1.03	F (tabulated) = 2.03	
t-Test significance	0.41	t (tabulated) = 1.71	

The high sensitivity of an analytical method is usually accompanied by very good reproducibility. This analytical performance was evaluated from five replicate measurements of different LCN solutions following the proposed method. The precision of the proposed procedure is excellent with a relative standard deviation (RSD) of the recovery values ranging between 0.94% and 9.97% for all measurements, including tablets, urine and serum samples (Table 2, Table 3).

The stability of LCN in an ethanol-BR mixture at pH 4.0 was evaluated under the optimal procedural conditions by monitoring the changes in both the cathodic peak potential and the cathodic peak current of a standard LCN solution. Maximum values for the RSD of peak current and peak potential for five series of measurements were found to be 5.59% and 2.87%, respectively (Table 1). As a result, there is no significant change in peak potential and peak current, confirming the stability of LCN over the time period of the measurement.

Upon applying the proposed method to biological samples and tablets, before adding a standard solution of LCN, the voltammetric base line of the biological medium was measured with the same procedure as applied to calibration studies with standard samples. In such applications no additional voltammetric signals were found in the studied potential window (Fig. 8), indicating that there are no significant interferences from inorganic cations, anions and some organic substances found in pharmaceutical preparations (tablets) and biological media (human urine and human serum). These results show that the reduction peak is specific to LCN and that this peak can be used selectively to determine LCN in biological fluids.

The robustness[39] of the measurements by means of the described SWCAdSV procedure to assay LCN was examined by studying the effect of small variations in some important procedural conditions such as pH value, accumulation potential, accumulation time and room temperatures of different days. Small changes (±1%) in such conditions do not affect the performance of the proposed method significantly.

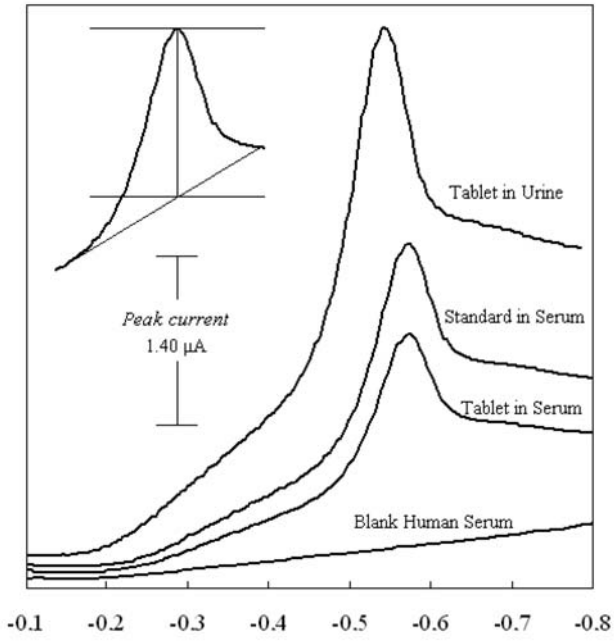


Figure 8. Voltammetric responses of blank human serum, spiked human serum and spiked human urine

4. Conclusion

In this study the electrochemical reduction behavior of LCN was studied on a GC electrode for the first time. The electrochemical behavior of pharmaceutical compounds is valuable to either get insight into understanding the mechanism of their action or determining their concentration in living organisms at various intervals after intake. The developed method provides a sensitive, fast, cost-effective, high-throughput and simple approach to the determination of LCN in tablet dosage forms, spiked human serum and spiked human urine samples. When applied to actual serum and urine samples, the proposed method offers the advantage that no prior extraction procedure is required. Furthermore, the proposed method has distinct advantages over other existing methods, epecially with regards to a higher sensitivity, shorter analysis time, lower limit of detection and interference-free analysis avoiding a separation step.

5. References

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Povzetek

Z voltametričnimi tehnikami. so bile določene nekatere elektrokemijske in adsorpcijsko-difuzijske lastnosti lerkanidipina (LCN) na elektrodi iz steklastega grafita v mešanici etanola in Britton. Robinsonovega pufra. Ugotovljeno je bilo, da se LCN na elektrodi reducira ireverzibilno. Redukcija, pri kateri sodelujejo 4 elektroni, poteka v eni stopnji pri –0,65 V proti Ag/AgCl elektrodi in je difuzijsko kontrolirana, deloma pa poteka tudi adsorpcija. Na osnovi teh ugotovitev je bila izdelana preprosta, hitra, selektivna in zanesljiva metoda na osnovi katodne adsorpcijske stripping voltametrije (SWCAAdSV) za neposredno določanje LCN v farmacevtskih preparatih, humanemu urinu in humanemu serumu. Maksimalni tok je linearno odvisen od koncentracije LCN v koncentracijskem območju med 4.0×10^{-8} molL^{-1} in 7.6×10^{-6} molL^{-1} pri potencialu predkoncentriranja –0,20 V in času akumulacije 90 s. Meja zaznave (LOD) je bila 2×10^{-8} molL^{-1} (0.01 mgL^{-1}) in meja kvantifikacije 6×10^{-8} molL^{-1} (0.04 mgL^{-1}). Metoda daje pravilne rezultate in je natančna z relativnim standardnim odmikom manj kot 10 %.