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A novel electrochemical sensor based on a silver nanoparticle modified carbon ionic liquid electrode for selective and sensitive determination of levetiracetam in pharmaceutical tablets and blood plasma samples

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An electrochemical sensor based on a silver nanoparticle (AgNP) modified carbon ionic liquid electrode (CILE) was prepared for the determination of ultra trace levels of levetiracetam (LEV) in human plasma and pharmaceutical tablets. The AgNPs were electrodeposited on the CILE surface using a double pulse potentiostatic technique, which can suitably control the size and morphology of AgNPs electrodeposited on the electrode. The AgNPs deposited on the CILE surface revealed an excellent electrocatalytic activity towards the oxidation of LEV. The sensor exhibited a fast response towards LEV with a linear concentration range of 1–300 ng mL^{-1} and a limit of detection of 0.7 ng mL^{-1}. The possible interference from several common ions and drugs that usually accompany LEV was tested. The method was successfully applied to the determination of LEV content in real samples such as tablets and human plasma samples with good recovery, and the obtained results were checked by HPLC. The proposed sensor is suitable for routine analysis of LEV in plasma samples to monitor the therapeutic or toxic levels of LEV and for drug pharmacokinetic studies.

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1. Introduction

Levetiracetam, (*S*)-α-ethyl-2-oxo-1-pyrrolidine acetamide with a molecular formula of $C_8H_{14}N_2O_2$ and a molecular weight of 170.21, has been approved in the European Union as a drug used for monotherapy treatment for epilepsy in the case of partial seizures, or in adjunctive therapy for partial, myoclonic and tonic–clonic seizures.[1] Levetiracetam (LEV) has potential benefits for other psychiatric and neurologic conditions including autism, Tourette syndrome, bipolar disorder, anxiety disorder, and Alzheimer's disease.[2] LEV absorption is independent of food and dosage and its binding to a plasma protein is less than 10%. LEV's mechanism of action is yet to be fully elucidated, but it appears to interfere with a protein called synaptic vesicle protein 2A, which is found in the spaces between nerves and is involved in the release of chemical messengers from nerve cells. In fact, LEV could stabilize the electrical activity in the brain and prevent seizures. The plasma half-life of LEV is around 7 h depending on the dose, and 95% of the drug is eliminated *via* urine.[3] Since information about the LEV concentration in plasma is critical not only for adjusting its dose for patients to decrease adverse effects but also is useful in assessing compliance and managing patients in situations associated with pharmacokinetic alterations, there is still an urgent need for simple and rapid methods for therapeutic drug monitoring and drug analysis in pharmacokinetic studies after single oral administration.

Several analytical methods have been reported for the analysis of LEV in human serum, among which are GC (with nitrogen–phosphorus and mass detector),[4] LC-tandem mass spectrometry,[5] HPLC,[6] capillary electrophoresis[7] and electrochemical methods.[8] These methods usually possess low sensitivity, are time consuming, need a large sample volume and some of them require a pre-concentration step.[9] Recently, an ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method has been reported for the analysis of LEV in plasma of neonates.[10] The assay allowed quantification of LEV plasma concentrations in the range from 0.5 to 150 μg mL^{-1}. However,

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this method is cumbersome, expensive and possesses moderate sensitivity.

Based on the above background information, it seems that the search for a new electrochemical sensor possessing high sensitivity without the need for a treatment or pretreatment process to prevent electrode fouling is still of prime importance. It is worth mentioning that the electrochemical methods provide highly sensitive methods for the analysis of organic molecules including drugs in pharmaceutical formulations and human body fluids owing to their simplicity, low cost and relatively short analysis time, as compared to other routine analytical techniques such as chromatography. As LEV is an electroactive compound, it can be easily subject to oxidation on different working electrodes. Compared with other methods,[8,9] direct electrochemical methods have the advantages of being simple, sensitive, and selective even with small amounts of sample. Also electrochemical sensors can be fabricated with small dimensions suitable for placement directly into biological samples. Yet, it is still of prime importance to search for new electrodes that do not need any treatment or pretreatment for prevention of electrode fouling.

In recent years the carbon ionic liquid electrode has aroused great interest due to its advantages such as easy preparation, good reversibility, high sensitivity and the ability to lower the over potential of electroactive compounds.[11] In this paper, we describe a novel electrochemical procedure for the determination of low levels of LEV in pharmaceutical preparation and human plasma, without any sample pretreatment. The surface of a CILE based on the use of 1-ethyl-3-methylimidazolium hexafluorophosphate ($C_6H_{11}N_2PF_6$), as a suitable binder, was modified with electrodeposited silver nanoparticles and used for levetiracetam oxidation. It is interesting to note that $EMIMPF_6$, with a melting point of 58–62 °C, was found to be a very suitable IL in the preparation of carbon ionic liquid electrodes (CILEs) of high stability as high performance electrodes, by providing high rates of electron transfer processes.

It is worth mentioning that the first reports on the preparation of carbon composite electrodes based on an ionic liquid as a binder and their application to the determination of some biologically important molecules were published in 2006 by Safavi and coworkers.[12] Later on, the extensive use of CILEs as high performance electrodes with improved resistance toward fouling and provision of high rates of electron transfer for low level detection of different biomolecules such as glucose,[13] NADH,[14] tryptophan,[15] dopamine[16] and hemoglobin[17] has been reported in the literature. In this work, the prepared silver nanoparticle modified carbon ionic liquid electrode (AgNP/CILE) was successfully applied to the determination of low levels of LEV. The Ag nanoparticles deposited on the CILE surface showed electrocatalytic activity towards the oxidation of LEV. Cyclic voltammetry and differential pulse voltammetry were used to explain the levetiracetam oxidation process at the modified CILE. For each investigated levetiracetam concentration in complex matrices, such as pharmaceutical drugs and human plasma samples, simultaneous HPLC determination was also performed.[18]

2. Experimental section

2.1. Reagents and chemicals

All reagents were of the highest purity available from Merck and Fluka chemical companies and used without further purification. LEV was obtained from Bakhtar Bioshimi (Kermanshah, Iran). Stock standard solution of LEV (1000 ng mL^{-1}) was prepared in water before use. Paraffin and graphite powder (mesh size < 50 μm) were supplied by Fluka. All the electrochemical studies were carried out with 0.1 M phosphate buffer saline (PBS) as a supporting electrolyte. The IL, 1-ethyl-3-methylimidazolium hexafluorophosphate ($EMIMPF_6$), was supplied by Merck. Distilled deionized water was taken from a Millipore water system and was used throughout. All solutions were stored at 4 °C where they were found to remain stable for at least 4 weeks. Nitrogen (99.99%) was used to remove dissolved oxygen.

2.2. Apparatus

Electrochemical experiments were performed on a computer controlled μ-Autolab electrochemical system (Eco-Chemie Utrecht, Netherlands) equipped with GPES software. Cyclic voltammograms of LEV in PBS were recorded at a scan rate of 30 mV s^{-1} from 0.0 to 0.5 V. All solutions were de-aerated by bubbling nitrogen prior to the experiments and the electrochemical cell was kept under a nitrogen atmosphere throughout the experiments. The electrochemical cell was assembled with a conventional three-electrode system consisting of a saturated calomel electrode (SCE) as the reference electrode, a platinum disk as the counter electrode and an AgNP/CILE as the working electrode. The surface morphology of the modified electrodes was characterized with a scanning electron microscope (SEM, Philips XL 30).

The UHPLC system used consisted of two pumps of a platin blue P-1 solvent delivery system, a diode array detector (PDA-1) operated at 205 nm, a column temperature manager (T-1) and an analytical column (Blue Orchid C_{18}, 1.8 μm, 50 × 2 mm), all from Knauer, Berlin, Germany. A mixture of acetonitrile and distilled water (20 : 80) was used as the mobile phase. The column oven temperature was set at 40 °C and the mobile phase was filtered and pumped at a flow rate of 0.2 mL min^{-1}.[18]

2.3. Fabrication of carbon paste electrodes

The traditional CPE was prepared by hand-mixing of graphite powder with paraffin at a ratio 70 : 30 (w/w) in an agate mortar. The homogeneous carbon paste was packed into a cavity of a PVC tube with a diameter of 2 mm. The electrical contact was provided by a copper wire connected to the paste in the end of the tube.

The CILE was prepared according to the following procedure: 1 g of graphite powder, 0.3 g of IL ($EMIMPF_6$, purity > 95%) and 0.15 g of paraffin, as a suitable co-binder, were mixed thoroughly in a mortar and heated to a temperature above the melting point of the IL (58–62 °C), in order to have better homogeneity in the composite and lower background current. A portion of the carbon paste was filled into one end of a PVC tube and a copper wire was inserted through the opposite end to

establish an electrical contact. The CILE surface was smoothed on a piece of weighing paper. Prior to electrochemical experiments, the electrode was rinsed thoroughly with water.

2.4. Electrodeposition of silver nanoparticles on CILE

Among several different methods for preparation of silver nanoparticles such as chemical reduction of silver cation in the presence of stabilizers,[11] layer-by-layer adsorption,[12] template induction,[19] electrodeless preparation,[20] and electrochemical deposition,[21] electrodeposition is a simple, fast and inexpensive method for preparation of metal nanoparticles possessing unique properties such as high purity of the particles, higher control over the dimension and density of particles, lower particle size distribution and a very short time scale compared to other methods.[22] Thus, in this work, we used a double-pulse method for the electrodeposition of silver nanoparticles at the surface of a CILE based on IL EMIMPF$_6$ from a 0.1 M KNO_3 solution containing an optimized $AgNO_3$ concentration of 1.0 mM. The current–time behavior was registered using a computer controlled procedure. The optimized pulse parameters were as follows: nucleation pulse: $E_1 = 130$ mV *vs.* SCE and $t_1 = 5$ ms; growth pulse: $E_2 = 240$ mV *vs.* SCE and $t_2 = 50$ s. It should be noted that the above mentioned conditions were quite similar to those reported by Safavi *et al.*[23] Under these conditions minimum sizes of AgNPs were obtained. After its preparation, the modified AgNP/CILE was thoroughly washed with distilled water.

2.5. Preparation of pharmaceutical tablets and plasma samples

10 tablets were weighed and ground to a homogeneous powder in a mortar and the average mass per tablet was determined. A quantity of the powder equivalent to 1 mg of a tablet was dissolved in 200 mL of water, ultrasonicated and finally centrifuged. The working solutions were prepared by taking appropriate aliquots of the clear supernatant liquid and diluting with the selected supporting electrolyte in a calibrated flask. Each solution was transferred to a voltammetric cell and analyzed under the same experimental conditions that were used to obtain a calibration graph.

The present method was also applied to assay the LEV content of blood plasma samples. The study protocol was approved by the Medical Ethics Committee of Kermanshah University of Medical Sciences. Twenty-four male healthy volunteers aged 27.2 ± 3.1 years and weighing 67.7 ± 8.3 kg with normal biochemical parameters were enrolled in this study. All the subjects received a single oral dose of 500 mg LEV from Bakhtar Bioshimi (Kermanshah, Iran). All blood donating volunteers were asked to refrain from food or water consumption for 3 h after drug administration.

Blood sampling was carried out at suitable intervals up to 24 h. Blood samples were collected into four 10 mL K_2EDTA tubes, inverted 10 times, and stored at room temperature, until centrifugation. The blood was centrifuged at 1300 rpm for 10 min at 2–6 °C. The supernatant (plasma) is transferred to a new tube and centrifuged at 2400 rpm for 15 min at 2–6 °C. The samples were stored frozen until their assay. In order to remove its proteins more effectively, 1 mL of plasma sample containing LEV was dissolved in 2.5 mL acetonitrile. After mixing for 30 s, the mixture was centrifuged for 10 min at 5000 rpm to get rid of plasma protein residues, and the supernatant was taken carefully. Appropriate volumes of this supernatant were transferred into a 25.0 mL volumetric flask and diluted to the mark with 0.1 M PBS of pH 7.4.

All experiments on human subjects were performed in compliance with the relevant laws and institutional guidelines approved by the Medical Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran. The required consent was obtained for any experimentation with human subjects.

3. Results and discussion

3.1. Characteristics of the modified AgNP/CILE

The properties of prepared AgNP/CILE were then investigated by different methods such as scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV).

The response of a modified electrode is related to its physical morphology. The SEM images of the CPE, CILE and AgNP/CILE are shown in Fig. 1. Significant differences in the surface structure of the CPE and CILE are observed. The surface of the CPE was predominated by isolated and irregularly shaped graphite flakes and separated layers were seen (Fig. 1A). The SEM image of the CILE (Fig. 1B) showed a more uniform surface so that no separated carbon layers could be observed which was attributed to the viscosity and good adherence of IL EMIMPF$_6$ to the graphite powder in the carbon paste. After electrodeposition of AgNPs on the surface of the CILE, the SEM pattern revealed many spherical nanoparticles with an average diameter of 80 nm (Fig. 1C), indicating that nano-sized Ag particles are successfully deposited on the surface of the CILE with high density, homogeneous diameter and good-distribution.

EIS is well known to provide information on the interface of the electrode surface during the modification process.[24,25] Thus, the Nyquist plots for different modified electrodes were obtained by using a 5 mM $[Fe(CN)_6]^{3-/4-}$ redox as an electrochemical probe, over a frequency range of 10 kHz to 100 mHz at an amplitude of 5 mV and a working potential of 0.17 V, and the results are shown in Fig. 2. The diameter of the semicircle is usually equal to the electron transfer resistance (R_{et}). The values of charge transfer resistance (R_{ct}) can be estimated by fitting the impedance data to the Randles equivalent circuit.[24,25] It can be seen that the charge transfer resistance (R_{ct}) value for the bare CPE was 750 Ω (curve A). While, on the CILE, the R_{ct} value was decreased to 500 Ω (curve B), indicating that the presence of the conductive IL in the carbon paste could greatly enhance the conductivity of the electrode. Meanwhile, in the case of the CILE modified with electrodeposited AgNPs, the R_{ct} value further decreased to 300 Ω (curve C) emphasizing that the highly conductive AgNPs have been successfully immobilized on the CILE surface. The synergistic effect of nano-sized Ag particles with the ionic liquid on the modified electrode effectively

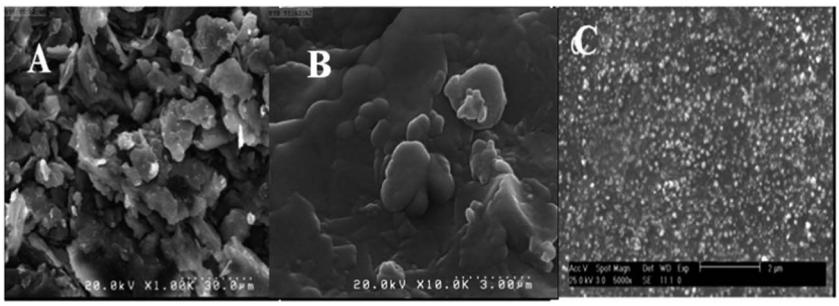

Fig. 1 SEM images of the (A) CPE, (B) CILE and (C) AgNP/CILE.

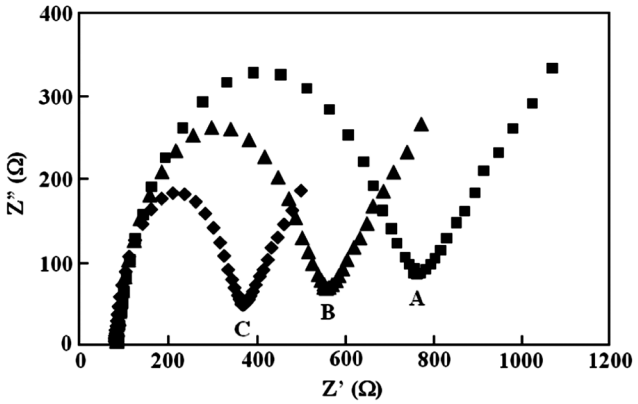

Fig. 2 Electrochemical impedance spectroscopy image for the (A) CPE, (B) CILE and (C) AgNP/CILE in 5 mmol L^{-1} solution of $[Fe(CN)_6]^{3-/4-}$ in the presence of 0.1 M KCl. Experimental conditions: frequency range, 10 kHz to 100 mHz; amplitude, 5 mV; working potential, 0.17 V.

increased the conductivity of the electrode and promoted the oxidation of LEV.

The cyclic voltammograms of different electrodes, including the CPE, CILE and AgNP/CILE, in PBS buffer solutions of pH 6.5 in the absence and presence of 30 μM LEV are shown in Fig. 3. As clearly seen from Fig. 3, while no redox signal is observed on the bare GCE (Fig. 3Aa) and the CILE (Fig. 3Ba) in the absence of LEV, the GCE shows a weak oxidation signal at about 2 V in the presence of LEV most possibly due to the oxidation of the drug. Meanwhile, as clearly seen from Fig. 3Ca, in the absence of LEV, the modified AgNP/CILE revealed a couple of quasi-reversible signals, located at about 0.18 V (oxidation) and 0.05 V (reduction) relative to the SCE electrode due to the presence of AgNPs at the surface of the AgNP/CILE. However, in the presence of 30 μM of LEV (Fig. 3Cb), the oxidation peak current increased significantly compared to that of the AgNP/CILE while the reduction one corresponding to AgNPs remained more or less the same as that in the absence of the drug. This behavior is most possibly due to the presence of Ag nanoparticles at the electrode surface, which enhances the conductivity and catalytic activity of the modified electrode towards the oxidation of LEV. It is well known that, in the case of metal nanoparticle modified electrodes (especially for AgNP/CILE[23]), the electroactive area can be increased significantly so that the electrocatalytic redox reactions at the modified surfaces will be facilitated.

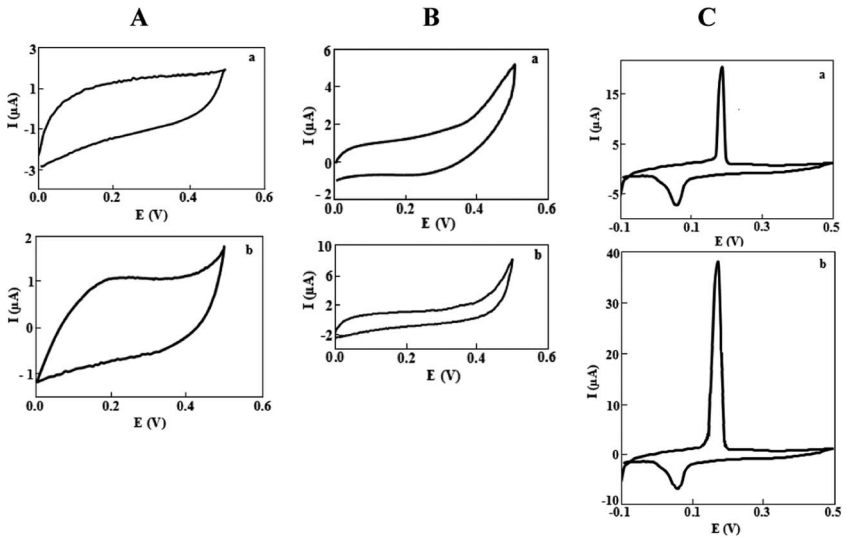

Fig. 3 Cyclic voltammograms of different electrodes at a scan rate of 30 mV s^{-1} in buffer solution in the absence (curves a) and presence of 30 μM of LEV (curves b) at the CPE (A), CILE (B) and AgNP/CILE (C).

3.2. Cyclic voltammetric studies of LEV at AgNP/CILE

The cyclic voltammograms of the AgNP/CILE in PBS solutions of pH 6 containing varying concentrations of LEV at a scan rate of 30 mV s^{-1} are shown in Fig. 4. As clearly seen, the electrochemical oxidation of LEV is represented by an anodic peak between 0.0 and 0.5 V, so that the corresponding oxidative reaction is in direct correlation with the increased drug concentration in solution. As it is quite obvious from the inset of Fig. 4, it was found that the intensity of the oxidative peak current of LEV (located at 0.18 V, under a scan rate 30 mV s^{-1}) has a linear function of the drug concentration in the range 0–130 μg mL^{-1}. The strong electrocatalytic activity of the AgNPs deposited on the CILE resulted in a greater oxidation peak current of LEV and higher sensitivity for its detection. However, the bare CILE did not show any significant response in the studied potential range.

3.3. Effects of scan rate and solution pH

Fig. 5 shows the cyclic voltammograms of a 100 μM solution of LEV at the modified AgNP/CILE at different scan rates, in the range of 10–100 mV s^{-1}. As seen, with the increase of the scan rate the redox peak currents increased gradually. The peak currents of levetiracetam oxidation were found to be linearly proportional to the square root of the scan rate ($\nu^{1/2}$) with a correlation coefficient of 0.9869 (Fig. 5A). Such linear behavior indicates that the electrochemical reaction rate is fast and the oxidation of LEV at AgNP/CILE is a typical diffusion-controlled process.[26] Meanwhile, a plot of $I_p/\nu^{-1/2}$ *versus* ν needs to have characteristic behavior if the process is catalytic.[27,28] This profile (Fig. 5B) possesses the characteristic shape of a typical electrocatalytic oxidation process for LEV at the surface of the modified electrode used, as reported in the literature.[28,29]

The effect of pH of the buffer solution on the response of the AgNP/CILE to LEV was investigated. The voltammetric responses of the sensor were studied in 0.1 M PBS of different pHs from 3.0 to 10.0 in the presence of 100 μM LEV. The resulting change in peak potential with solution pH is shown in Fig. 6. As seen, the anodic peak current increased with increasing pH values in the range of 3.0–5.0, then remained almost constant from pH 5.5 to about 7.4 and, finally, continued to decrease with increasing pH up to 10. Therefore, a biological pH of 7.4 was selected as the best value to get the most sensitive results in further studies.

Meanwhile, the peak potential showed a continuous linear decrease with increasing pH of solution, with a linear regression equation of $E_p = -0.051\text{pH} + 0.49$ ($n = 5$, $r = 0.994$).

As is obvious, the slope was −0.051, which is close to the theoretical value of −0.0576. According to the equation $-0.0576x/n = 0.051$, where n is the number of electrons transferred and x is the number of hydrogen ions participating in the reaction, the uptake of electrons was found to be accompanied by an equal number of hydrogen ions.

Based on the above mentioned information obtained from the scan rate and solution pH studies of the proposed modified

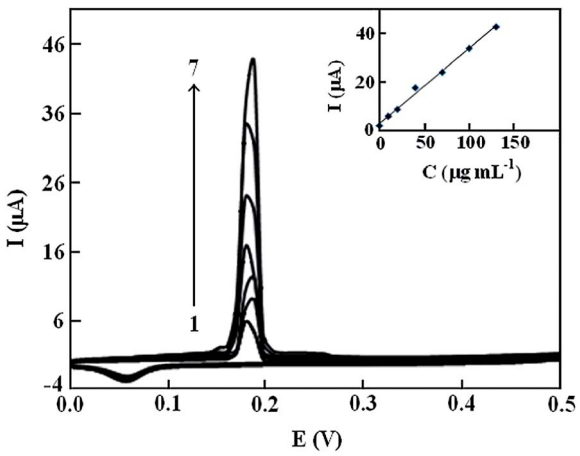

Fig. 4 Cyclic voltammograms of the AgNP/CILE in a PBS of pH 6.5 containing (1) 0, (2) 10, (3) 20, (4) 40, (5) 70, (6) 100 and (7) 130 μg mL^{-1} of LEV at a scan rate of 30 mV s^{-1}. The inset shows the corresponding linear plot of I_p (μA) *vs.* [LEV] (μg mL^{-1}).

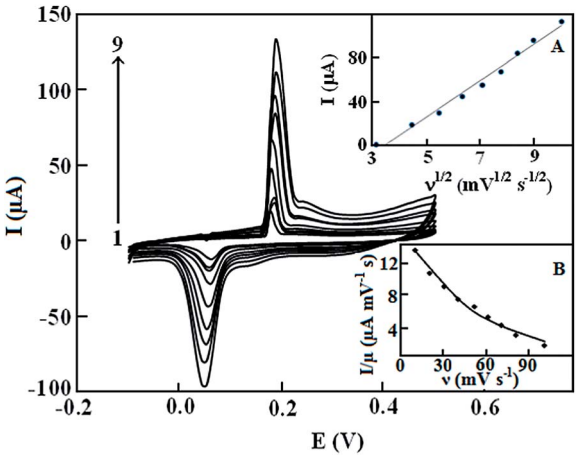

Fig. 5 Effect of scan rate from 10 to 100 mV s^{-1} on electrooxidation of 100 μM LEV in a 0.1 M PBS at the AgNP/CILE. Scan rates are: (1) 10, (2) 20, (3) 30, (4) 40, (5) 50, (6) 60, (7) 70, (8) 80 and (9) 100 mV s^{-1}. Inset A: plot of peak current I *versus* scan rate ν. Inset B: plot of $I_p/\nu^{-1/2}$ *versus* ν.

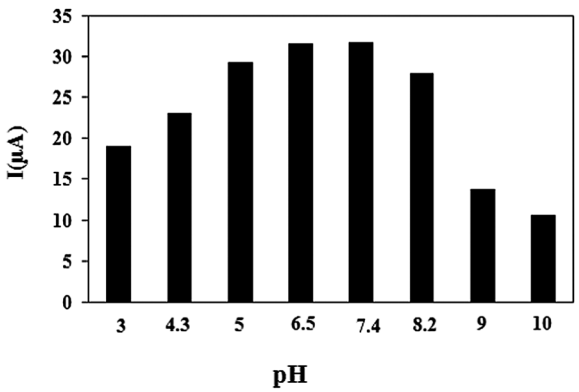

Fig. 6 pH dependence of the peak current for oxidation of 100 μM LEV at a scan rate of 30 mV s^{-1} at the surface of the AgNP/CILE in 0.1 M PBS of varying pH from 3.0 to 10.0.

electrode, a plausible mechanism for the oxidation of LEV is proposed in Fig. 7.

3.4. Figures of merit of the proposed electrochemical sensor for determination of LEV

The analytical features of linearity, stability and repeatability of the AgNP/CILE for the determination of traces of LEV were investigated. Differential pulse voltammograms were recorded at the electrode in 0.1 M PBS of pH 6.5 containing different concentrations of LEV. Potential was scanned from 0.1 to 0.3 V at a scan rate of 20 mV s^{-1} and a pulse amplitude of 50 mV. As it is seen from Fig. 8, all voltammograms possess a sharp anodic peak positioned at approximately 0.18 V, which significantly increased with increasing drug concentration in solution. In fact, a calibration graph of the oxidation peak current *vs.* LEV concentration resulted in linear behavior with LEV concentrations in the range of 1–300 ng mL^{-1} (see the inset of Fig. 8), according to the following regression equation: $I\,(\mu A) = 2.3651 + 0.0793\ [LEV]\ (ng\ mL^{-1})$, $R = 0.9948$.

The detection limit was calculated by using the relation $3S_b/m$, where S_b is the standard deviation of the blank and m is the slope of the calibration curve, which was found to be 0.7 ng mL^{-1}. The limit of quantification (LOQ), which is defined as the minimum level at which the analyte can be readily quantified with accuracy (signal to noise ratio of 10 : 1), was then established with a value of 2.33 ng mL^{-1}.

Precision has to be determined at different concentration levels. Thus, the electrochemical experiments were repeatedly performed 10 times at LEV concentrations of 10, 50, 150 and 200 ng mL^{-1} using the AgNP/CILE as a drug sensor. The relative standard deviation was found to be 2.1, 2.65, 3.6 and 4.3%, which revealed the excellent repeatability of the electrochemical system designed. The stability of the modified electrode was also investigated. After using the same AgNP/CILE 20 times over 30 days for determination of 200 ng mL^{-1} LEV, only a small decrease of current sensitivity of about 8% was observed, which can be attributed to the high stability of the AgNP/CILE.

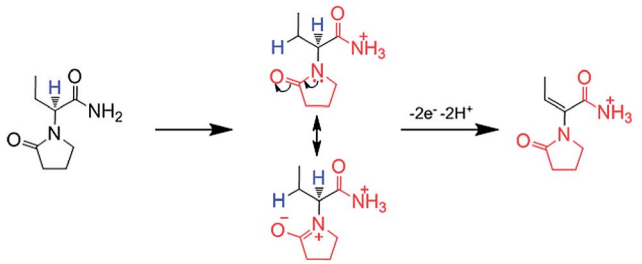

Fig. 7 Proposed mechanism for oxidation of levetiracetam at the AgNP/CILE.

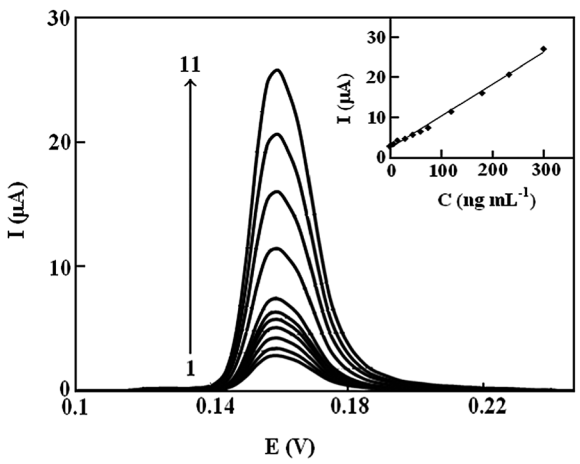

Fig. 8 Differential pulse voltammograms for oxidation of varying concentrations of LEV in 0.1 M PBS of pH 6.5 at a scan rate of 20 mV s^{-1} and a pulse amplitude of 50 mV on the AgNP/CILE. LEV concentrations, from bottom to top, are: (1) 1.0, (2) 7.5, (3) 15, (4) 30, (5) 45, (6) 60, (7) 75, (8) 120, (9) 180, (10) 230 and (11) 300 ng mL^{-1}. The inset shows the calibration graph of I_p (μA) against [LEV] (ng mL^{-1}).

3.5. Interference study

Possible interference for the detection of LEV at AgNP/CILE was investigated by addition of various drugs and some other compounds to a 0.1 M PBS of pH 7.4 in the presence of 30 ng mL^{-1} of LEV. A 100-fold excess concentration of the common drugs such as gabapentin, acetaminophen and valproic acid, which are usually consumed in combination with LEV by patients, did not show any measurable interference in LEV detection. As for the common interferences in biological samples for the determination of LEV, the influence of 100-fold excess concentration of ascorbic acid, glucose, tyrosine and cysteine were examined. In all cases, the change in LEV signal was found to be below 5%, suggesting the high selectivity of the proposed sensor towards the determination of LEV in the presence of the above mentioned potential interferences.

3.6. Comparison of the sensitivity of the AgNP/CILE with that of other methods

In Table 1 the linear calibration range and limit of detection of several previously published methods are compared with those of the proposed AgNP/CILE electrochemical sensor for the

Table 1 Comparison of the linear range and limit of detection of the proposed electrochemical sensor with those of other reported methods for LEV determination

Detection method	Linear range (μg mL^{-1})	Limit of detection (μg mL^{-1})	References
LC-MS/MS[a]	1–40	1.0	5
LC-MS/MS[a]	0–50	0.05	4
HPLC-UV[b]	4–80	2	6
CE[c]	10–100	3	7
HRP-SPCE[d]	0.17–1.41	0.03	8
HRP-PP-SPCE[e]	0.85–8.5	0.02	9
UPLC-MS/MS[f]	0.5–150	0.06	10
AgNP/CILE	0.001–0.3	0.0007	This work

[a] Liquid chromatography-tandem mass spectrometry. [b] High performance liquid chromatography-ultraviolet detection. [c] Capillary electrophoresis. [d] Horseradish peroxidase covalent grafting onto screen-printed carbon electrode. [e] Peroxidase–polypyrrole immobilized onto the screen-printed carbon electrode. [f] Ultra performance liquid chromatography-tandem mass spectrometry.

determination of LEV. As is obvious from the summarized data, the proposed differential pulse voltammetric AgNP/CILE sensor possess the highest sensitivity and lowest limit of detection among previous methods. Moreover, the proposed electrochemical sensor possesses a short response time, low cost and could be used for months without any significant divergence in its response. This sensor is suitable for routine analysis of LEV in plasma samples of patients to monitor their therapeutic or toxic levels as well as for pharmacokinetic studies and measurement of LEV in plasma samples of epileptic patients taking the drug.

3.7. Applications to determination of LEV in pharmaceutical tablets and plasma samples

The working solutions, prepared in accordance with the Experimental section, were transferred to a voltammetric cell and analyzed under the same experimental conditions as those used to obtain a calibration graph. The mean amount of LEV in the tablet obtained from triplicate measurements by the proposed sensor was 497.0 ± 3.2 mg, which is in satisfactory agreement with that obtained from the HPLC analysis, 506.0 ± 2.9, within the experimental errors present, and compared with the claimed content of 500 mg by the manufacturer.

The clinical studies have revealed that the consumption of LEV can be associated with some adverse effects on the central nervous system, which are classified as somnolence and fatigue, coordination difficulties and behavioral abnormalities.[7,30] In addition, when LEV is given with other antiepileptic drugs, some other adverse events such as asthenia, infection and dizziness can also happen.[31] The drug may also reduce hyperactivity, impulsivity, mood instability, and aggression in patients. Thus, the information about the steady state pharmacokinetics is of critical importance. In this work, the analyses were carried out using the standard addition method, and the resulting differential pulse voltammetric responses for plasma samples, taken at different times after LEV consumption, are shown in Fig. 9.

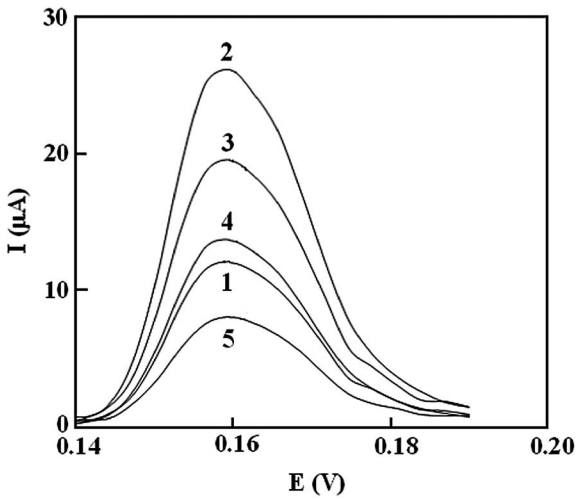

Fig. 9 Differential pulse voltammograms of LEV in human serum samples of a patient taken at different times after the drug consumption (*i.e.*, (1) 2 h, (2) 5 h, (3) 10 h, (4) 12 h and (5) 24 h) in 0.1 M PBS at a scan rate of 20 mV s^{-1} and a pulse amplitude of 50 mV on the AgNP/CILE.

Table 2 Determination of LEV in human plasma samples at different times after consumption of drug by HPLC and the proposed electrochemical sensor

Time after consumption of LEV (h)	LEV concentration by HPLC (ng mL^{-1})[a]	LEV concentration by proposed sensor (ng mL^{-1})[a]	Difference (%)
2	133 ± 4.2	130 ± 8.3	−2.25
5	280 ± 3.6	289 ± 12.2	+3.11
10	213 ± 2.7	211 ± 9.6	−0.99
12	155 ± 3.9	159 ± 8.9	+1.25
24	93 ± 5.2	90 ± 7.4	−3.33

[a] Mean ± SD ($n = 3$).

The results thus obtained together with those evaluated from HPLC determinations are summarized in Table 2. As is obvious from Table 2, the LEV contents of the plasma samples obtained by the use of the proposed AgNP/CILE electrochemical sensor are in satisfactory agreement with those from HPLC analyses, within the experimental errors. In addition, the data shown in Table 2 clearly indicated the dependence of the plasma LEV content on the time elapsed after the drug consumption. In fact, maximum LEV in the plasma was observed 5 h after the drug consumption and then diminished gradually with increasing time period.

4. Conclusions

In this study, an electrochemical sensor based on the AgNP/CILE was used to investigate the electrooxidation behavior of LEV in 0.1 M PBS of pH 7.4. Compared with the unmodified CILE, the peak current for electrooxidation of the drug was increased significantly at the modified AgNP/CILE. Under the optimized experimental conditions, the calibration range and limit of detection of the proposed sensor were 1–300 ng mL^{-1} and 0.7 ng mL^{-1}, respectively, which showed a significant improvement over those of the previously reported methods including LC-tandem mass spectrometry,[5] HPLC,[4] capillary electrophoresis,[7] and electrochemical[8] and ultra performance liquid chromatography-tandem mass spectrometric methods.[10] The sensor revealed considerable selectivity for LEV over several common drugs (*e.g.*, gabapentin, acetaminophen and valproic acid) that are usually consumed in combination with LEV, as well as the common interferences present in biological samples (*e.g.*, ascorbic acid, glucose, tyrosine and cysteine) for the determination of LEV in biological fluids. The proposed voltammetric sensor, possessing high sensitivity, short response time, low cost and high stability for months without any significant divergence, was successfully applied to the determination of tablets and, more importantly, to monitoring the therapeutic or toxic levels of the drug and to investigate its pharmacokinetics in plasma samples.

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