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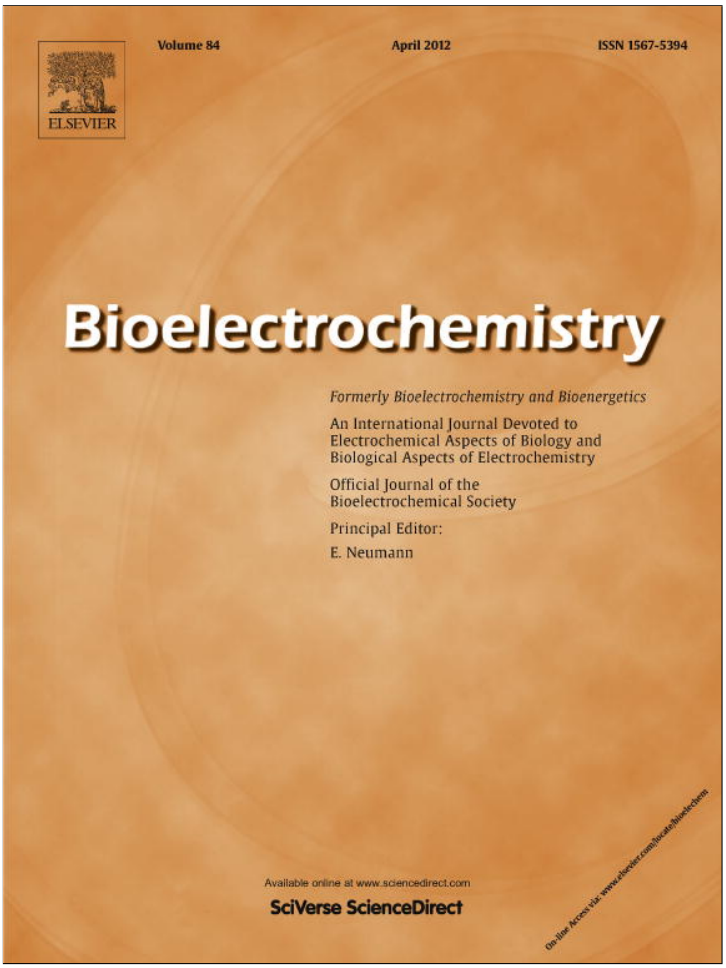

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Highly sensitive voltammetric determination of lamotrigine at highly oriented pyrolytic graphite electrode

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ABSTRACT

The electrochemical behavior of lamotrigine (LMT) at the pyrolytic graphite electrode (PGE) is investigated in detail by the means of cyclic voltammetry. During the electrochemical reduction of LMT, an irreversible cathodic peak appeared. Cyclic voltammetric studies indicated that the reduction process has an irreversible and adsorption-like behavior. The observed reduction peak is attributed to a two-electron process referring to the reduction of azo group. The electrode showed an excellent electrochemical activity toward the electro-reduction of LMT, leading to a significant improvement in sensitivity as compared to the glassy carbon electrode. The results of electrochemical impedance spectroscopy and cyclic voltammetry showed that edge-plane pyrolytic graphite electrode has excellent electrochemical response properties toward LMTs with respect to glassy carbon electrode modified with carbon nanotubes. High sensitivity, low detection limit and very good repeatability together with excellent recovery make the electrode as a powerful devise for accurate determination of LMT in pharmaceutical and biological samples.

1. Introduction

Lamotrigine (lamictal, LMT) is well known as an anticonvulsant, a medication for treating epilepsy. In addition, LMT has been approved by the U.S. food and drug administration (FDA) for the treatment of bipolar disorder. By decreasing electrical conduction or neurotransmitter activity in unstable brain cells, anticonvulsants play an effective role in controlling seizures and bipolar illness. In the case of overdoses, the most famous side effect of LMT is life-threatening skin rashes including a form called Stevens–Johnson syndrome, which is characterized by painful blistering of the skin and mucous membranes and is often fatal [1].

Owing to the dangerous side effect of LMT, the pharmaceutical quality control of LMT is vital. So, development of a sensitive and versatile analytical method is needed for its determination. Conventional methods reported for the analysis of LMT are HPLC [2,14–22], spectrophotometry [3,4], solid phase or liquid phase extraction/liquid chromatography [5,23,24], thin layer chromatography [25] and capillary zone electrophoresis/electrospray ionization mass spectrometry [6]. However, these methods suffer from some disadvantages such as requirement of special sample preparations, high cost, low sensitivity, insufficient selectivity and time-consuming. Therefore, the development of an alternative sensitive and simple analytical methodology for the determination of LMT is necessary.

Due to high efficiency, accuracy, sensitivity, simplicity and low cost, use of electrochemical techniques in pharmaceutical analysis attracted more attention. To our knowledge, electrochemical investigation of LMT is not completely done yet. This is limited to some articles from a Spanish group [7–9].

The technique which is used in this work is somehow a kind of *adsorptive striping voltammetry*. In adsorptive stripping analysis (AdSV), the adsorption process is purposely used as a preconcentration step. The principle of the method can be compared to that of anodic or cathodic stripping, except that no charge is transferred during the preconcentration step. Accumulation of the compound at the electrode surface is performed at open circuit or by applying a suitable potential at which no electrochemical reaction occurs. After an equilibration time, the potential is scanned anodically or cathodically, depending on the redox properties of the compound and on whether it contains a reducible or oxidizable group. Since a great number of organic compounds including pharmaceutical and biological substances exhibit surface-active properties, they can be determined at very low levels, generally ranging from 10^{-6} to 10^{-10} M [13].

Pyrolytic graphite is a polycrystalline form of carbon that has a high degree of orientation. The material is produced by vapor phase deposition [10]. Recently Pyrolytic graphite, in its edge-plane form, has attracted Compton group attention to discover the secret of the high sensitivity of this material toward electron transfer in comparison to the basal-plane form [12].

Taking into account the good performances of PGE in electroanalysis [11], in this work we attempt to investigate on electrochemical behavior of LMT, which based on our knowledge has been missed till now. In

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addition, due to the excellent performance of this electrode for LMT determination, compared to the glassy carbon electrode modified with carbon nanotube, there is no any modification needed. Simplicity of electrode preparation, wider linear range, low detection limit, high selectivity and very good stability of the response at the surface of PGE make it an effective sensor for LMT determination in pharmaceutical quality control or clinical applications.

2. Experimental

2.1. Chemicals and reagents

LMT was taken kindly from Sobhan Daru pharmaceutical company (Tehran–Iran). Solutions of LMT were prepared by dissolving appropriate amounts of it in water. Multi-walled carbon nanotubes (MWCNT, purity > 95%) with outer diameter less than 10 nm and tube length of 5–15 μm was prepared from Nanostructured & Amorphous Materials (USA). The appropriate amount of pure MWCNT was functionalized in 1:3 concentrated nitric–sulfuric acids at ca. 100 °C for 45 min in order to obtain more edge sites and better dispersion of nanotubes by creation of carboxylate groups. All other chemicals were of analytical reagent grade from Merck. Voltammetric experiments were carried out in the buffered solutions of LMT, deoxygenated by purging with pure nitrogen gas (99.999% from Roham Gas Company).

Tablets of LMT (50 mg per tablet) were purchased from Sobhan Daru pharmaceutical company (Tehran, Iran). Fresh human blood serum samples were obtained from Razi institute of Vaccine and Serum (Tehran, Iran). 3% methanol was added to serum sample, then serum samples were centrifuged, filtered and diluted 10 times with 0.1 M phosphate buffer solution of pH 7.0 and applied for the recovery tests in the spiked samples.

2.2. Apparatus

Voltammetric experiments were performed using a Metrohm potentiostat/galvanostat model 797VA. A conventional three-electrode system was used with a saturated Ag/AgCl reference electrode and a Pt wire counter electrode, a pyrolytic graphite electrode and GC as working electrodes (unmodified or modified). A digital pH/mV/Ion meter (Metrohm, 827 pH lab) was used for preparation of the buffer solutions.

2.3. Preparation of the PGE

The PGE was polished on abrasive paper with different meshes. Then, it was put in buffer solution of pH 7.0 and potential cycles applied between −0.4 V to −1 V for 5 cycles at scan rate 100 mV/s.

3. Results and discussion

3.1. Electrochemical behaviors of LMT at PGE

The electrochemical behavior of 100 μM LMT was investigated at the PGE using cyclic voltammetry technique, recorded in the 0.1 M phosphate buffer solution (pH 7.0) at the scan rate of 100 mV s^{-1} (Fig. 1A). In the forward sweep a well-defined cathodic peak (I_c) was obtained at −952 mV, and in the reverse sweep an anodic peak (I_a) was appeared at the potential of 0.024 mV. More investigation on electrochemical behavior was done by potential sweep between −0.25 V to 0.5 V but no anodic peak was appeared which approved that the anodic peak (I_a) is due to oxidation of the product of

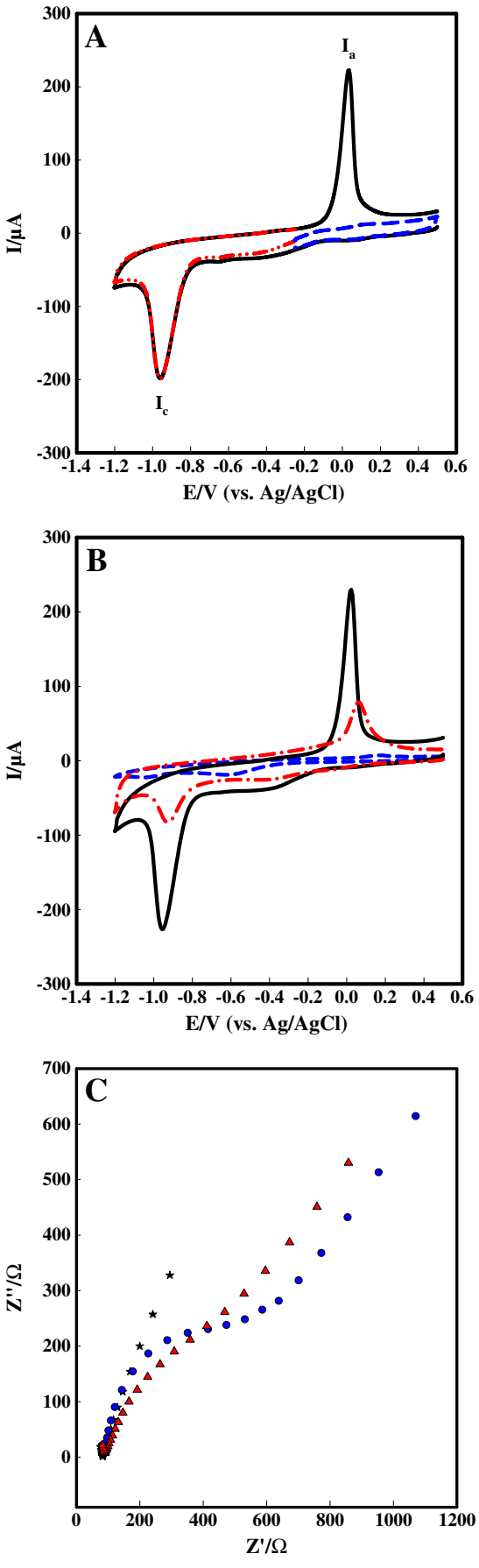


Fig. 1. Cyclic voltammetry of C(LMT) = 100 μM at the surface of (A) PGE at different sweep potential ranges and (B) PGE (——), bare GCE (- - - -) and CNT/GCE (-.-.-.). (C) Nyquist diagram (Z″/(Ω) vs. Z′/(Ω)) for the EIS measurements in 1 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ in 0.1 M KCl at the $E_{1/2}$ = 0.13 V for (●) bare GCE, (▲) CNT/GCE and (★) PGE.

electrochemical reaction in cathodic peak (I_c) which still appears in potential sweep between −0.25 V to −1.2 V. Based on above results, electrochemical reduction of LMT at PGE is an irreversible reaction.

There is more evidence on irreversibility of LMT reduction, since scan rate dependency experiments showed that the peak potential of I_c shifted negatively with increasing the scan rates (Fig. 2A). Also, the log of peak currents of I_c was linearly dependent on the log of scan rate with the slope of 0.8, suggesting an adsorption-diffusive controlled process.

The electrochemical behavior of LMT at PGE was compared with its behavior on bare and modified CNT/GCE (Fig. 1B). The results indicated in Table 1, show more effective application of bare PGE versus bare and modified GCE. Even though LMT reduces at lower potential at CNT/GCE surface, but the increased current on PGE is not comparable with the other electrodes which lead to better sensitivity toward LMT determination. Using the electrochemical impedance spectroscopy, the ability of LMT reduction with a faster kinetic at the surface of PGE was proved in comparison with bare GCE and CNT/GCE (Fig. 1C). There is a semi circle part in Nyquist diagram of the bare GCE, which reduces for CNT/GCE and disappears for PGE.

3.2. Optimization of experimental conditions

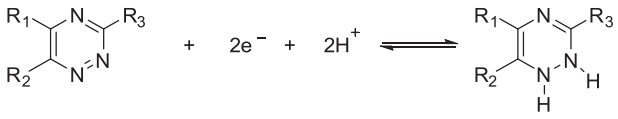
Since LMT reduction mechanism at PGE is adsorptive, the effect of accumulation time under open circuit was investigated in a buffered solution containing 100 μM of LMT using CV method (Fig. 2B and C). The peak current increased rapidly with increasing accumulation time in the first 50 s. The peak current reached the maximum after 100 s and then being nearly unchanged because of surface saturation (Fig. 2C). Taking account of sensitivity and also response repeatability, the accumulation time was set at 100 s in following experiments.

According to the reduction mechanism of LMT, the peak potential of LMT would be strongly pH dependent. The effect of pH on the electrochemical responses of LMT was investigated over the pH range 3.0–7.5. As shown in Fig. 3A, E_{pc} shifted negatively with pH rising based on Eq. (1):

$$E_{pc}/\mathrm{mV} = -523.2 - 59.00\ \mathrm{pH}\left(R^2 = 0.991\right). \quad (1)$$

The results indicated participation of equal numbers of electrons and protons in the electro-reduction of LMT. On the other hand, Fig. 3B shows the dependence of the peak current of the cathodic peak (I_c) on pH for cyclic voltammetry which introduce the maximum peak current at pH 7.0. Therefore, phosphate buffer with pH 7.0 was used as the supporting electrolyte in all voltammetric determinations.

In the light of the above results and similar investigations on LMT in the literature [7], the following mechanism can be suggested for the electro-reduction of LMT:



3.3. Analytical applications of PGE for LMT determination

Under the optimized experimental conditions, the cathodic peak currents in CVs were proportional to LMT concentration in two ranges of 0.1–10 μM and 10 to 100 μM. The break in the calibration curve probably reflects the formation of a sub-monolayer of LMT in the first range of calibration and the formation of a monolayer in the second range [27]. The detection limit based on the lower range is

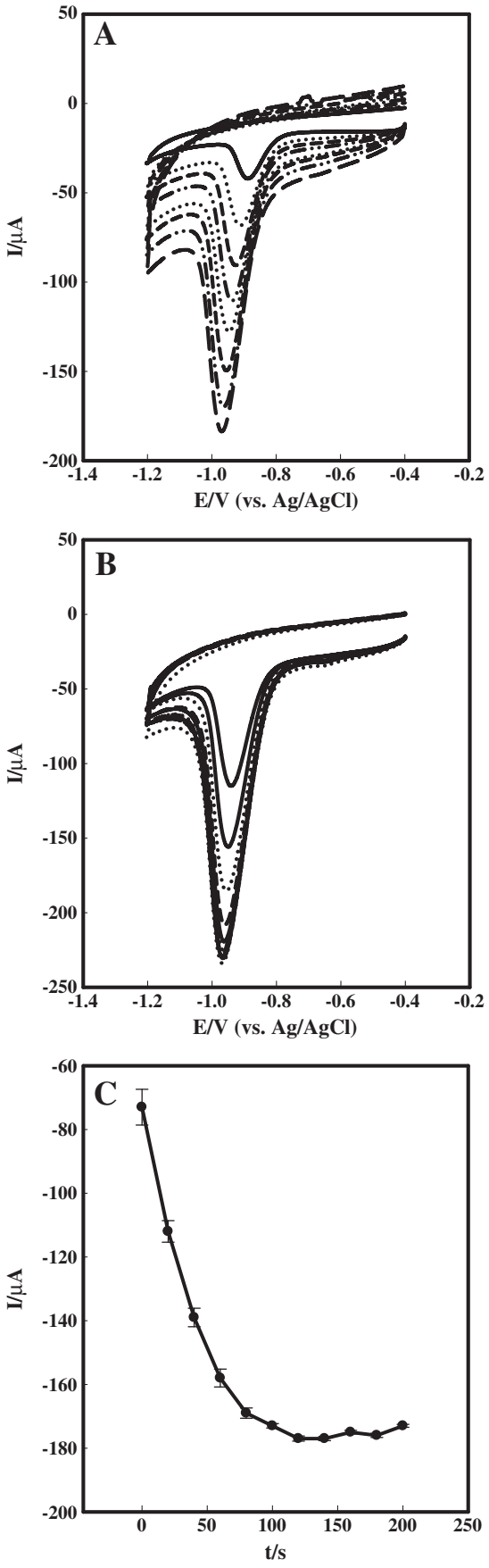


Fig. 2. Cyclic voltammograms of C(LMT) = 100 μM at PGE (A) in different scan rates from 25 to 200 mV s^{-1} and (B) different accumulation time from 0 to 200 s. (C) Dependence of the reduction peak current (I_{pc})/μA with accumulation time (s); scan rate 100 mV s^{-1}.

Table 1
Peak potentials and peak currents obtained for LMT on the surface of various electrodes.

Type of electrode	E_{pc} (mV)	I_{pc} (μA)
PGE	−952	−161
GCE	−1040	−3.37
CNT/GCE	−928	−43.5

estimated to be 80 nM (based on S/N = 3) (Fig. 4). The linear regression equation for the first range was:

$$I_{pc}/\mu A = -3.35(\pm 0.12)C(LMT)/\mu M - 1.05(\pm 0.11)(R2 = 0.990 \pm 0.004). \quad (2)$$

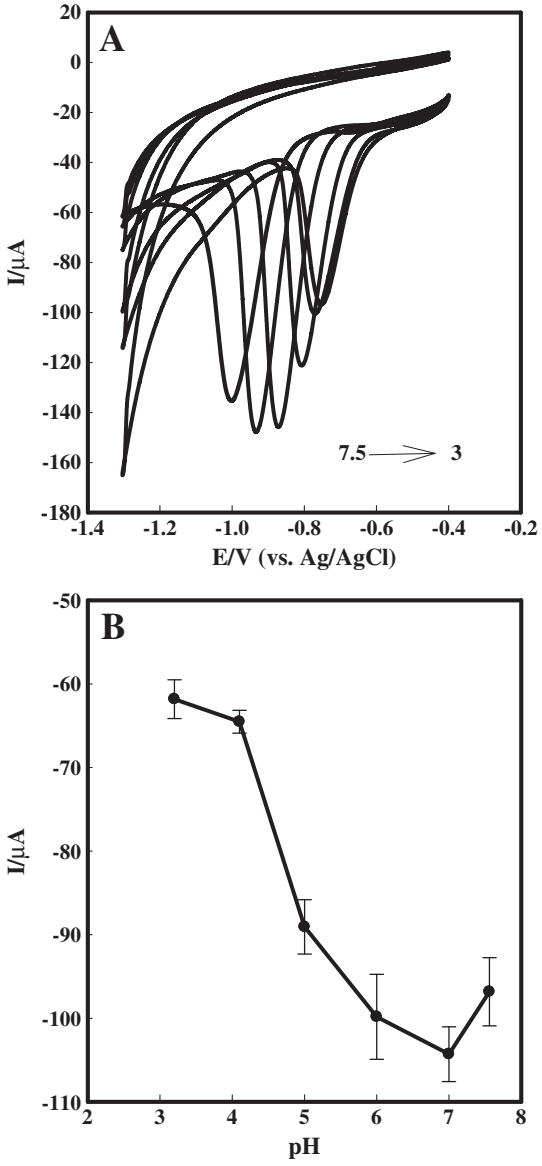


Fig. 3. (A) Cyclic voltammograms of C(LMT) = 100 μM at PGE in various pHs (from 3 to 8: 3, 4, 5, 6. 7, 8), (B) Dependence of the reduction peak current (I_{pc})/μA with pH solution; scan rate 100 mV s^{-1}. 40 s accumulation time.

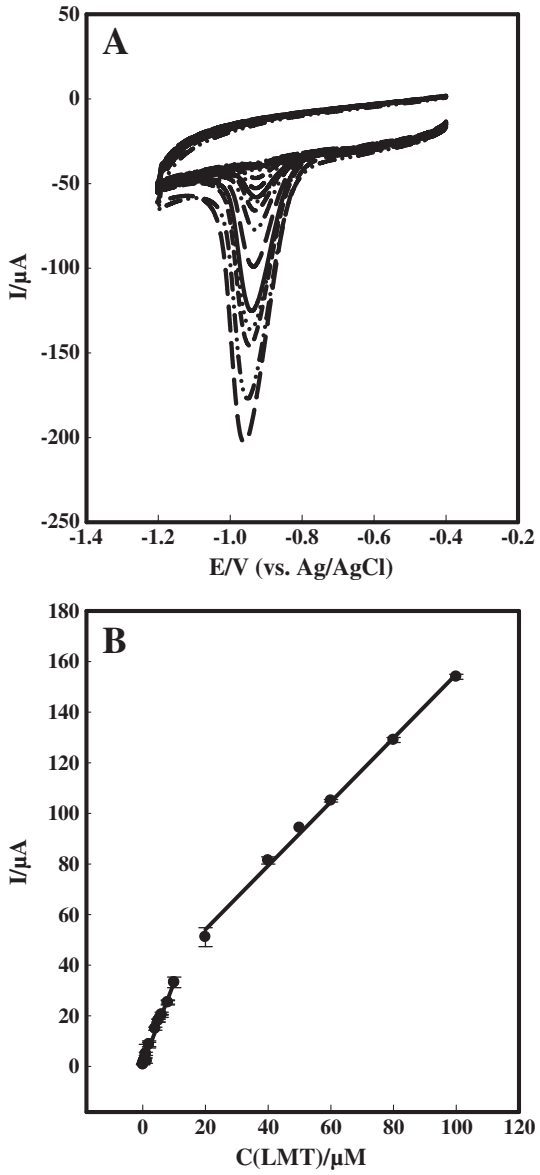


Fig. 4. (A) CVs for various concentrations of LMT in the range of (down to up), C(LMT) = 0.1 to 100 μM in 0.1 M PBS (pH 7); (B) Corresponding linear calibration curve of peak current I_p/μA versus C(LMT)/μM;100 s accumulation time.

The amount of Lamotrigine for epilepcy therapy could be between 1 and 4 mg/L but it could reach to 10 mg/L too. The detection limit in this work is 80 nM which is equal to 0.02 mg/L [26].

The measurements were done for RSD determination intra-day and inter-days for 100 μM LMT. RSD for 3 measurements for one day is 1.35% and RSD for 3 different days is 5.53%.

The PGE was applied for the determinations of different amounts of tablet samples spiked in buffered solution of pH 7.0. The excellent recoveries and RSD, which are reported in Table 2, indicate adequate

Table 2
Recovery results of real samples spiked in buffered solution of pH 7.0.

Real Sample	LMT amount added (μM)	LMT amount found (μM)	Recovery (%)	RSD (%)	n
Tablet	3	2.94	98	0.41	3
Tablet	30	29.7	99	0.66	3
Plasma	4	3.79	95	5.57	3

precision and accuracy of the presented method for the determination of LMT in pharmaceutical preparations.

Besides, the recovery studies of the spiked LMT in a human blood serum sample showed average values 95% suggesting the successive applicability of the electrode for the determinations in clinical samples. Biological assay in plasma is always a challenge for AdSV techniques, since during accumulation blocking of the electrode surface could happen due to adsorption of plasma ingredient. This problem could be solved by plasma dilution or deactivation of plasma proteins. In this work plasma is diluted 10 times and plasma proteins are deactivated by adding 3% methanol to plasma.

The selectivity of this electrode toward carbamazepine as an antiepileptics medicine is investigated and there is no any electrochemical activity for carbamazepine in the range of 0.5 V to −1.2 V at the surface of PGE (data not shown).

Based on our knowledge, there are just a few works on LMT determination. Even though the limit of detection obtained by using HMDE is 4.68 nM [7] but our work using PGE which is safer and more convenient than HMDE while still there is a good detection limit around 80 nM at the surface of PGE. In the other work, the same group used a silver nanoparticle-modified carbon screen-printed electrode for LMT determination [8]. The detection limit for LMT obtained by this electrode is 0.372 μM which is much higher than the detection limit in our work. The other advantage of our work to this one is using a simple PGE without any modification which is not time consuming for electrode preparation. Even though there is no any modification the detection limit for this simple electrode for LMT determination is 80 nM which shows the high sensitivity of PGE. The last work from this group is done for LMT determination at the surface of mercury coated carbon screen-printed electrodes [9] and the limit of detection is 2 μM which shows that our work is much better than previous ones (Table 3).

4. Conclusions

For the first time, electrochemical behavior of lamotrigine (LMT) was investigated at the surface of pyrolytic graphite electrode (PGE). The high applicability of this electrode for lamotrigine determination was proved in comparison with bare glassy carbon electrode and CNT-modified glassy carbon electrode by cyclic voltammetry and electrochemical impedance spectroscopy. It was demonstrated that LMT can be accumulated into a PGE surface. The results showed that this simple electrode without any modification can dramatically affect the kinetics and sensitivity of the electrochemical responses toward LMT. In these investigations, the electrochemical response corresponding to the reduction of the azo functional group was used as a sensitive procedure for the determination of LMT in the concentration range of 0.1–100 μM with a detection limit of 80 nM.

Table 3
Comparison of reported sensors for LMT.

Electrode	Technique	Dynamic Range/μM	LOD/nM	RSD (%)	Accumulation time/s	Ref.
AgNP-CSPE[a]	DPAdSV[b]	0.33–1.5	0.000372	2.58	200	[8]
HMDE[c]	DPAdSV	0.004–0.12	4.68	5.21	N	[7]
	SWAdSV[d]	0.003–0.019	5.02			
Hg-CSPE	DPASV	2–18	0.002	9.83	300	[9]
PGE	CASV[e]	1–100	80	5.53	100	This work

[a] AgNP-SCPE: silver nanoparticles modified carbon screen printed electrode.
[b] DPADSV: Differential pulse adsorptive striping voltammetry.
[c] HMDE: Hanging mercury drop electrode.
[d] SWAdSV: Square wave adsorptive striping voltammetry.
[e] CASV: Cyclic adsorptive striping voltammetry.

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