Potentiometric Sensing of Lamotrigine Based on Molecularly Imprinted Polymers

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Abstract
In the present work a novel potentiometric sensor, based on a noncovalent imprinted polymer, was developed for determination of Lamotrigine (LTG). At optimized conditions the electrode exhibited a Nernstian response (30.8 ± 1.0 mV decade^{-1}) in a concentration range of 1×10^{-6} to 1×10^{-3} M with a detection limit of 8×10^{-7} mol L^{-1}. The potential response of the electrode was constant in the pH range of 1.0–5.0. The electrode demonstrated a response time of ~30 s. The selectivity coefficient of the sensor toward a number of different drugs with molecular similarities and some metal ions was evaluated. The electrode was examined for determination of LTG in real samples.

Keywords: Molecularly imprinted polymers, Potentiometric sensors, Lamotrigine

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1 Introduction

Molecularly imprinted polymers (MIP's) are tailor-made materials with selective recognition properties toward a chosen guest molecule or related compounds similar to that displayed by antibodies but without their experimental restrictions such as obtainment and isolation, thermal and chemical stability [1–3]. Among the different methods available for the preparation of molecularly imprinted polymers (MIP's) the so-called noncovalent approach, which uses only non-covalent interactions between the template and the functional monomers, is probably the most flexible regarding the selection of the functional monomers and the possible template molecules. For this reasons, the non-covalent approach has been the most widely adopted [4]. The procedure for synthesizing an MIP is based on the chemical polymerization of a functional monomer and a cross-linking agent in the presence of a molecule used as a template. After the removal of the imprinted molecule, an imprinted polymer is obtained. This polymer contains sites with a high affinity for the template molecule, due to their shapes and the arrangement of the functional groups of the monomer units. The imprinted polymers are used as antibody-like materials for the selectivity and sensitivity, owing to their long-term stability, chemical inertness and insolubility in water and most organic solvents. These MIP's have shown to be useful in enantioseparations, catalysis, solid-phase extraction, drug delivery chromatography and in the preparation of sensors specific for the analytes of interest, using a variety of sensing methods. Over the past two decades, imprinted polymers have attracted a broad interest from the scientists engaged in sensor development. This attention can be attributed to the serious potential advantages of the MIP's usage in place of natural receptors and enzymes, such as their superior stability, low cost, high selectivity and easy preparation. A few examples of detection methods using imprinted polymers include: fluorescence [5], luminescence [6], surface Plasmon resonance [7], quartz crystal microbalance [8], impedance measurement [9], induced scintillation [10], field effect transistor [11], optical approaches [12,13] and acoustic wave sensors [14]. Currently, there is an increasing number of the MIP reports on electrochemical sensors with capacitive [15,16], conductometric [17], amperometric [18,19] and voltammetric [20–22] transduction. Despite the relatively simple transduction of the potentiometric signal, only limited reports on the design of the potentiometric sensors have been based on the molecular imprinted technology [23].

Lamotrigine (LTG), [6-(2, 3-dichlorophenyl)-1, 2, 4-triazine-3, 5-dimine], is an anticonvulsant drug (Figure 1). As an antiepileptic, it has been used successfully to treat epilepsy and bipolar disorder as monotherapy and as an adjunct with other antiepileptic for treatment of partial and generalized toxic-chronic seizures. It is also used to treat neurological lesions and as a tranquilizer [24,25].

The analysis of LTG in biological samples is abundantly described in the literature. Chromatographic techniques have been widely employed since they are power-

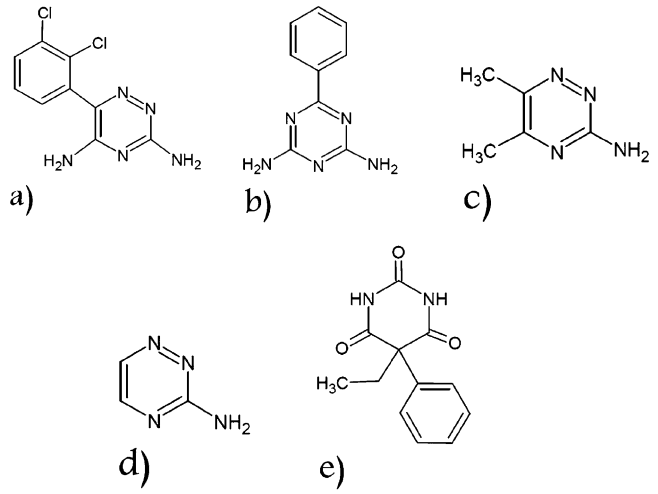

Fig. 1. Structure of compounds which were examined for binding to MIP. a) LTG; b) 2,4-diamino-6-phenyl-1,3,5-triazine; c) 3-amino-5,6-dimethyl 1,2,4-triazine; d) 3-amino-1,2,4-triazine; e) phenobarbital.

ful separation techniques. The methods based on high-performance liquid chromatography (HPLC) [26–32], high-performance thin layer chromatography (HPTLC) [33] and gas-chromatography (GC) [34] have been described. There is an extensive literature on the determination of Lamotrigine in pharmaceuticals including planar chromatography [35], TLC and HPLC [36], HPLC and GC capillary electrophoresis [36–38]. The immunoassay techniques [39,40] have been developed for the determination of the drug in biological samples. UV-spectrophotometric method [41] was used to determination of LTG in tablets. Though the method is claimed to be selective, any N-containing basic moiety would definitely interfere with the assay.

Although many of the reported methods are sensitive and selective but they are time consuming, require expensive instrumental setup, and some require preliminary sample treatment. Considering these drawbacks, the construction and utilization of a potentiometric sensor as an alternative tool for determination of LTG in tablets and biological fluids can be of great interest. Two types of LTG-ion -selective electrode is reported by Gupta et al. [42] these electrodes are based on PVC membranes doped with LTG-tetraphenyl borate (TPB) or LTG-phosphotungstic acid (PT) ion-pair complexes as molecular recognition materials. The electrodes are used for determination of LTG in urine and plasma.

In this work we have developed a new ion selective electrode based on molecularly imprinted polymers, which to our knowledge is the first reported electrode of this kind.

2 Experimental

2.1 Materials and Instruments

LTG [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] was purchased from Hetro Drug (India), phenobarbital 2, 4-diamino-6-phenyl-1,3,5-triazine, 3-amino-5-6-dimethyl-1,2,4-triazine, 3-amino-1,2,4-triazine, methacrylic acid (MAA), and ethylene glycol dimethacrylate (EDMA) were obtained from Sigma-Aldrich (Milwaukee, USA). 2,2′-Azo-bis-iso-butyronitrile (AIBN) was obtained from Acros (Geel, Belgium). All solvents used, tetrahydrofuran (THF), Acetonitril (ACN), methanol, acetic acid were of HPLC grade. Poly(vinyl chloride) (PVC), dibutyl phthalate (DBP), dioctyl phthalate (DOP), sodium tetraphenyl borate (NaTPB), oleic acid (OA), and nitrobenzene (NB) were all purchased from Aldrich (USA).

The potential measurements were performed by means of Metrohm-692pH/mv meter. FTIR spectra were obtained by JASCO-410 FTIR instrument.

2.2 Preparation of LTG Imprinted Polymer

To prepare the MIP, a noncovalent molecular imprinting approach was used. LTG (0.4 mmol) as the template, MAA (2 mmol) as the functional monomer, EDMA (8 mmol) as the crosslinker, and AIBN (0, 06 mmol) as the initiator were dissolved in 7 mL THF/ACN (4 : 3 v/v) in a thick-walled glass tube. This solution was sparged with oxygen free nitrogen for 5 min. The tube was sealed and heated at 60 °C for 17 h. The polymer obtained was ground using a mortar and pestle. The ground polymer was passed through a 200 mesh sieve (particle size less than 75 μm). An NIP was synthesized, in the absence of LTG; following the same procedure described above. The best conditions for extraction of LTG from the MIP was found to be washing for 14 hours with THF, using a soxhlet extractor. The MIP was finally washed with THF/TFA 90 % (50–50 v/v) and then conditioned with water [43].

2.3 Electrode Preparation

The sensor was prepared by mixing thoroughly 6 mg of MIP or NIP, 1 mg of NaTPB, as additive, 61 mg of DOP as plasticizer and 32 mg of PVC in 2 mL of THF in a 2 cm diameter glass dish The resulting mixture was completely dissolved and it was left to evaporate slowly until a concentrated mixture was obtained. A Pyrex tube with about 5 mm (o.d.) was dipped into the mixture so that a membrane of about 0.3 mm thickness was formed. The tube was then pulled out from the mixture and kept at room temperature for about 12 hours. The tube was then filled with an internal filling solution and was conditioned for 24 hours.

2.4 Emf Measurements

The performance of the sensor was investigated by measuring the emf values of various LTG solutions. Potentiometric evaluation of the electrodes was carried out using the following cell:

Ref. electrode | | test solution | MIP membrane | internal solution | internal Ref. electrode.

Activity coefficients of ions in aqueous solutions were calculated according to the Debye–Hückel equation.

3 Results and Discussion

3.1 Optimization of the Membrane

The influence of membrane composition on the potential response was tested and the results are shown in Table 1. Since the plasticizer/PVC ratio of about two results in suitable performance characteristics [44], this ratio was kept constant in the optimization of membrane composition. Since lipophilic salts or ionic additives can decrease the membrane resistance, reduce anion interference and improve selectivity of the electrode, NaTPB was incorporated in the membrane. Experiments show that the response slope of the MIP based membrane can be improved to achieve theoretical value with the lipophilic borate. The selectivity and linearity of the membrane electrode also depends on the amount of the MIP which determines the number of the binding sites. Table 1 indicates that the membrane with 32 wt% of PVC, 60 wt% of DOP, 1.4 wt% of NaTPB and 6.6 wt% of MIP shows the best performance.

3.2 Characterization of Imprinted Polymer

After template extraction and washing procedure, the FT-IR spectra of control non-imprinted (NIP) and molecularly imprinted polymer (MIP), unwashed MIP and also the pure LTG were obtained. The spectra are shown in Figure 2. In the IR spectra the absorptions due to –OH stretch (~3500–3250 cm^{-1}), carbonyl group stretch (~1730 cm^{-1}), C–O stretch (~1260 cm^{-1}), C–H vibrations (~765, 1390, 1460, and ~2956 cm^{-1}), and NH_2 (2 bands), NH (1 band) at about 3530–3400 cm^{-1}) are observed. The most characteristic part of the spectra which can lead us to draw a correct conclusion is between 3000–4000 cm^{-1}. As can be seen the LTG has 3 pronounced bands which are due to NH and NH_2 group. Comparison of NIP spectrum with unwashed MIP shows that the band at about 3500 cm^{-1} is more intense than NIP, which indicates the presence of LTG in polymer. Whereas when the NIP and washed MIP spectra are compared the intensity of the bands in the same region are more or less the same, showing that the polymer (MIP) is sufficiently leached.

3.3 Effect of Internal Solution

The effect of the internal solution concentration on the potential response of the sensor was investigated. The concentration of LTG was changed in the range of 1×10^{-4} to 1×10^{-3} mol L^{-1} and the potential responses of the sensor were measured. It was found that the concentration variation of the internal solution showed a significant difference in the corresponding potential response. Therefore, the 1×10^{-3} mol L^{-1} internal solution which showed the best response to the target molecule was used for smooth functioning of the sensor Figure 3.

3.4 Effect of pH

The influence of sample pH on the potential response of MIP based Lamotrigine selective electrode was tested with LTG concentration of 10^{-4} M in the pH range of 1–12. The results are shown in Figure 4. It can be seen that the potential response is nearly constant in the pH range of 1–5, but potential decreases beyond this range. The higher and constant potentials observed in acidic range may be attributed to the formation of protonated form of LTG ($pK_a=5.5$) which is apparently better fitted into imprinted polymer. The potential drift in alkaline solution may be due to deprotonation of LTG. Therefore all the experiments were carried out at pH 4.0.

Table 1. Optimization of membrane ingredients.

Membrane No.	Polymer (mg)	PVC (mg)	AP (mg)	NB (mg)	DBP (mg)	DOP (mg)	OA (mg)	NaTPB (mg)	KT_pClPB (mg)	Slope (mV/decade)
1	6	30	60	–	–	–	4	–	–	–
2	6	30	60	–	–	–	–	4	–	3
3	6	30	60	–	–	–	–	–	4	2.85
4	6	30	–	60	–	–	4	–	–	4.45
5	6	30	–	60	–	–	–	4	–	12
6	6	30	–	–	60	–	–	–	4	–
7	6	30	–	–	–	60	4	–	–	10
8	6	30	–	–	–	60	–	4	–	15
9	6	30	–	–	–	60	–	–	4	–
10 [a]	6.6	32	–	–	–	60	–	1.4	–	29.5
11 [b]	6.6	32	–	–	–	60	–	1.4	–	5.5
12 [c]	–	32	–	–	–	60	–	1.4	–	–

[a] MIP; [b] NIP; [c] blank.

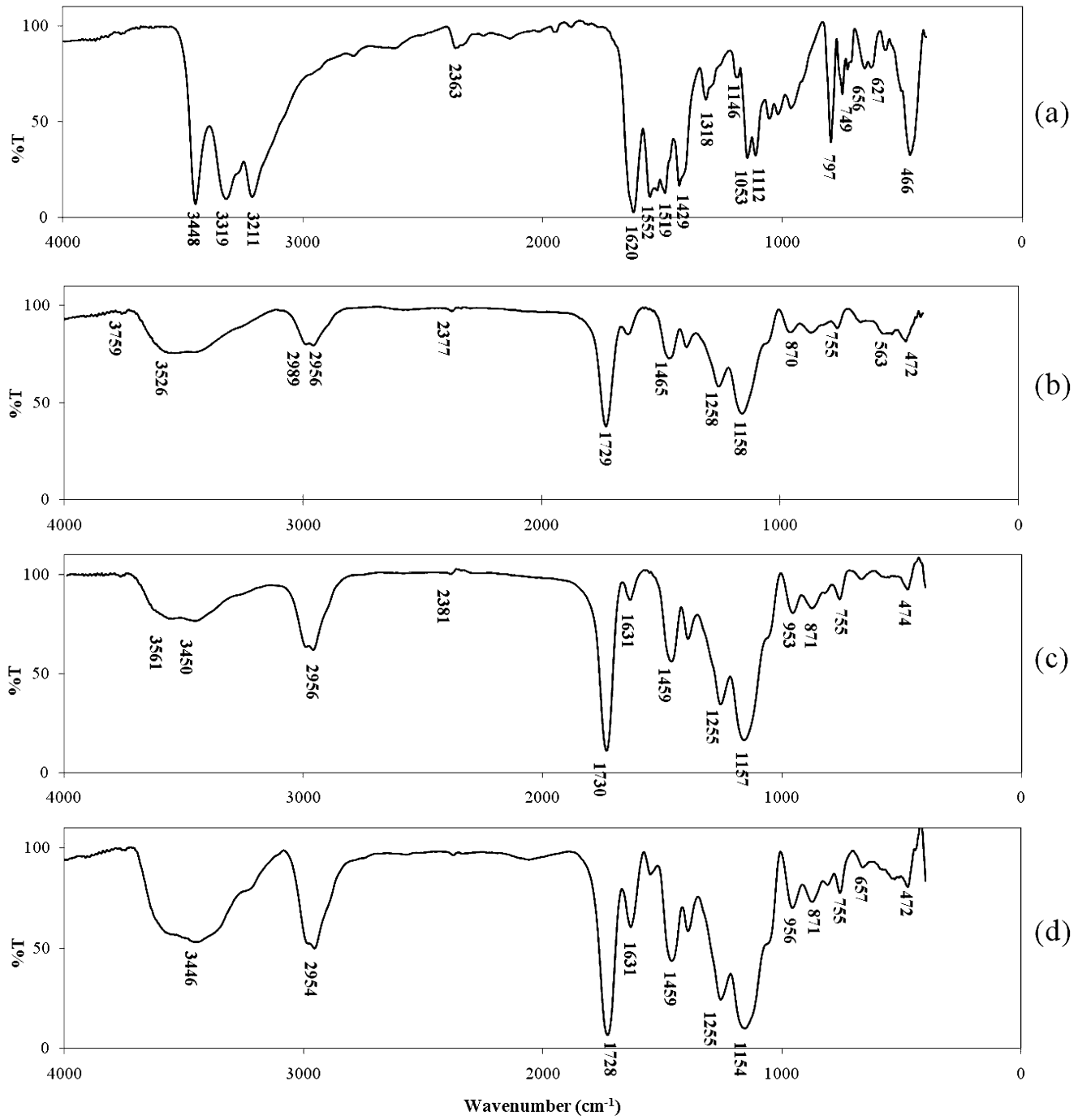

Fig. 2. FT-IR spectra of LTG (a), MIP (b), NIP (c) and MIP unwashed (d) in 400–4000 cm^{-1} range by KBr pellet method.

3.5 Calibration Curve

The potential response curves of MIP- and NIP-based membranes and the blank membrane are shown in Figure 5. The MIP membrane shows a Nernstian response of 30.8 ± 1 mV per decade over the concentration range of 1×10^{-6} to 1×10^{-3} mol L^{-1} with a detection limit of 8×10^{-7} M. At high concentrations of LTG the imprinted polymer is saturated by LTG (template) and the calibration curve levels off At low concentrations the imprinted membrane can not detect the LTG and potential changes are not considerable.

3.6 Response Time

The response time of the MIP based membrane ISE is defined as the average time required for the sensor to reach ± 1 mV of the magnitude of the equilibrated potential signal after successively immersing in a series of LTG

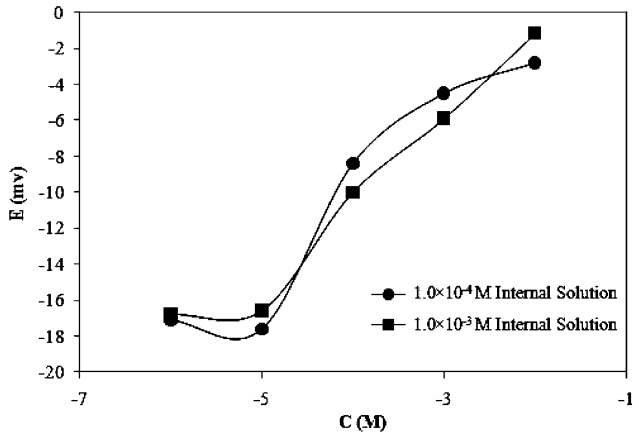

Fig. 3. Effect of internal solution concentration on electrode response.

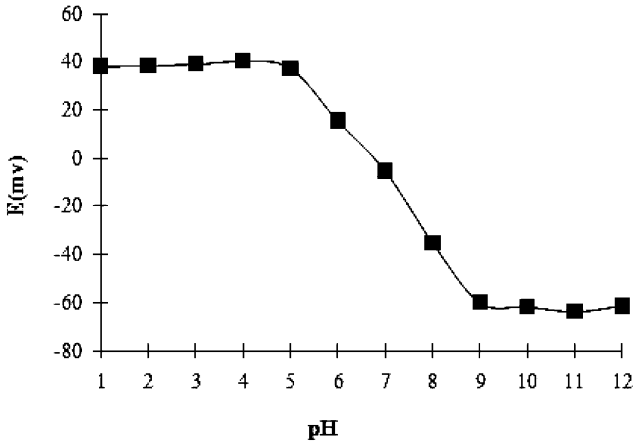

Fig. 4. Effect of pH on the response of optimized electrode.

ion solutions, each having a 10 fold concentration difference .The dynamic potential response with time is shown in Figure 6, where the LTG concentration is changed between 1×10^{-7} to 1×10^{-3} mol L^{-1}. It can be seen that the response of the MIP sensor is rapid (~30 s).

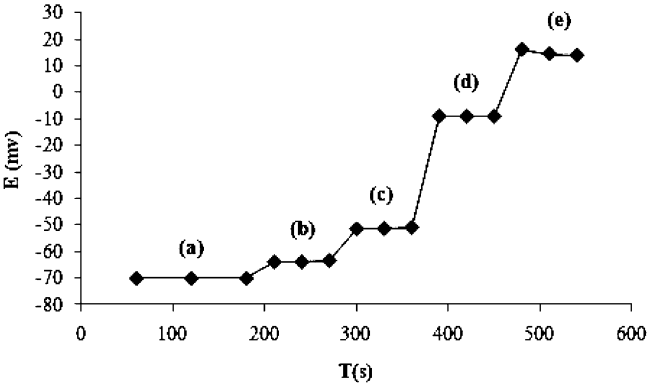

Fig. 6. Dynamic response of the electrode for step changes in LTG concentration, (a) 1.0×10^{-7} M, (b) 1.0×10^{-6} M, (c) 1.0×10^{-5} M, (d) 1.0×10^{-4} M, (e) 1.0×10^{-3} M.

3.7 Interference Studies

The potentiometric selectivity coefficients were determined by matched potential method (MPM). The coefficients describe the preference of the suggested electrode for interfering ions X, with reference to primary ion. This method is recommended by IUPAC to overcome the difficulties associated with the method based on the Nicolsky–Eisenman equation [45,46].

According to the MPM, the specified activity (concentration) of the primary ions is added to a reference solution and the potential is measured. In another experiment, the interfering ions (X) are successively added to an identical reference solution, until the measured potential matches that obtained before the addition of the primary ions .The $K^{MPM}_{LTG,X}$, is then given by the resulting primary ion activity (concentration) to the interfering ion activity ratio:

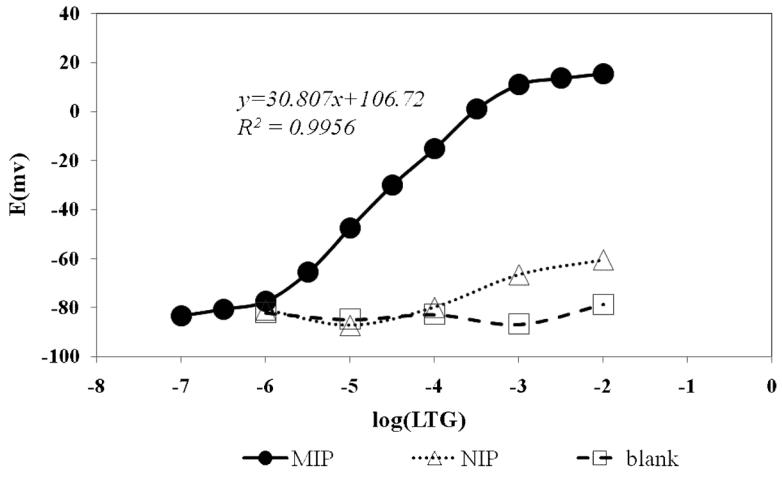

Fig. 5. Calibration curve for the proposed electrode with optimized composition based on MIP, NIP, and blank (with no polymer).

Table 2. Selectivity coefficient values using matched potential method.

Interference	log $K^{MPM}_{LTG,X}$,	Interference	log $K^{MPM}_{LTG,X}$,
Cu^{+2}	−2.4	Pheno barbital	−3.074
Na^{+2}	−2.75	3-Amino-1,2,4 triazin	−3.074
Mg^{+2}	−3	2,4-Diamino-6 phenyl-1,2,4 triazin	−2.666
Ca^{+2}	−2.9	3-Amino-5,6 dimethyl-1,2,4 triazin	−2.666

$$K^{MPM}_{LTG,X} = a_{LTG}/a_x \quad (1)$$

The MPM selectivity coefficient for the LTG ion-selective electrode at the constant pH value of 5.0 is listed in Table 2. In this study, the rebinding was achieved by non-covalent interactions, such as hydrogen bonding and the hydrophobic effect. As it is obvious from Table 2, when the MIP sensor is applied to determine LTG, all the other substances, even drugs with molecular similarity (Figure 1) hardly interfere with the determination. As a result, the MIP molecular recognition is based both on the template molecular structure (shape) and on the interaction between the print molecule and the imprinted polymer.

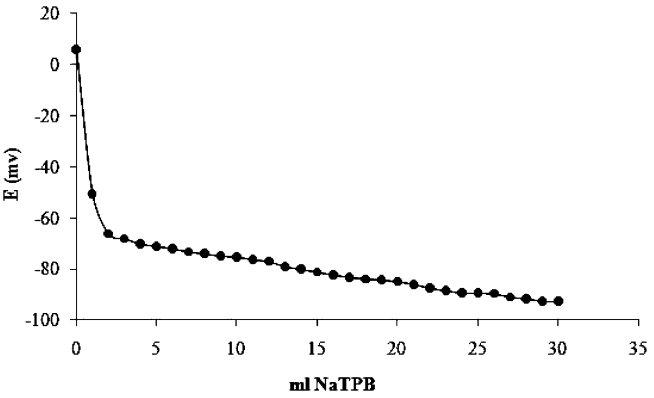

Fig. 7. Potentiometric titration curves for 25 mL of solution of LTG with 0.1 M NaTPB.

3.8 Accuracy

The accuracy of the measurement by means of the described potentiometric sensor was checked by calculating the recovery of a known LTG concentration (1×10^{-4} mol L^{-1}). The mean percentage recovery, obtained by applying the calibration curve method, was 94.59 % ($n=6$).

3.9 Reproducibility

This analytical performance was evaluated with six repeated potentiometric measurements of the 1×10^{-4} mol L^{-1} LTG solution. The precision of the described procedure in terms of relative standard deviation was 0.4 %.

3.10 Analytical Application

3.10.1 Potentiometric Titration

It should be noted that the LTG-selective membrane electrode introduced not only can be used for the direct determination of the LTG ions, but also it may have potential applications in a variety of fields. We successfully applied the MIP-based electrode as an indicator electrode in potentiometric titration of 25 mL 1×10^{-4} mol L^{-1} LTG against 0.1 M sodium tetraphenyl borate solution and the resulting titration curve is shown in Figure 7. As seen, the amount of LTG in solution can be determined with this electrode.

3.10.2 Analysis of LTG Tablets

The proposed described potentiometric procedure was successfully applied for the LTG determination in tablets. Ten tablets, each containing 100 mg of Lamotrigine and some common excipients, bought from local pharmacist were weighed accurately and powdered using a mortar and pestle. A portion of the powder (0.321 g, average tablet weight) was dissolved in 500 mL distilled water and sonicated for 20 minutes to aid dissolution and then filtered. A 10 mL of the filtrate was diluted further to 50 mL with water so that the concentration of LTG in the final solution was within the working range of the electrode (about 1.56×10^{-4} mol L^{-1}) and then analyzed by the proposed MIP electrode . One of the ways to check the accuracy of the measurements is spiking the sample in which the background analyte is measured by the same procedure and subtracting from the total (sample + spike) value to obtain recovery. The spiked samples were prepared at three levels and each level was prepared in triplicate. The resulting data, using the calibration curve procedure, were statistically compared with the labeled amounts on the tablets Table 3. To demonstrate that method is comparable with other methods reported for determination of Lamotrigine, the performance characteristic of the proposed electrode in comparison with other methods is presented in Table 4. As seen the results are

Table 3. Determination of LTG in tablet.

Sample No.	Claimed value in each tablet (mg)	Amount added (mg)	Found (mg) [a]	*RSD* %
1	100	–	96.50	1.12
2	100	50	143.20	1.20
3	100	100	198.15	1.80
4	100	150	258.30	2.10

[a] Average of three measurements

Table 4. Comparison of different methods for determination of Lamotrigine.

Method	Recovery (%)	*LOD* (μg/mL)	Working concentration range (μg/mL)	Reference
HPLC-MIP	84	0.03	10–30	[43]
Sensor (LTG-TPB)	99.6	0.08	0.13–256	[42]
UV	98.33	0.43	40–208	[41]
TLC	96	0.5	0.5–10	[35]
SPME-LC	–	0.05	40–	[47]
LC-Tandom-MS	97.9	0.5	0.025–10	[48]
Present work	94.59	0.2	0.26–256	–

Table 5. Recoveries of spiked Lamotrigine in human urine and plasma samples.

Sample	Added LTG (mg)	Amount found (mg)	Recovery (%)	*RSD* (%)
Urine	2.56	2.47	96	2.18
Plasma	0.256	0.235	91	3.45

[a]Average of three measurements

quite satisfactory and it can be concluded that the method is sensitive and accurate.

3.10.3 Analysis of Spiked Plasma and Urine

Blood sample from a healthy volunteer was collected in a heparin-containing tube .Plasma was obtained after blood centrifugation at 1800 g for 5 min and 1 mL of plasma was diluted 10 times with water and brought to pH 4.0 before potentiometric study. Urine sample (10 mL), from healthy volunteer was deproteinated with TFA (100 μL), vortexed for 3 min, and centrifuged for 5 min at 1500 g, and was diluted 100 times with water and brought to pH 4.0 prior to potentiometric analysis with proposed LTG electrode results and recovery studies are summarized in Table 5. Studies were also carried out in very low and very high concentrations of LTG, but results were not very satisfactory.

4 Conclusions

The new polymeric membrane ion-selective electrode based on MIP for Lamotrigine showed good analytical performance, especially in relation to selectivity towards large number of drugs and metal ions. The analytical applicability of proposed sensor was checked by the determination and recovery of Lamotrigine in tablets, urine and plasma and also titration method.

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