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Original Paper

Preparation and characterization of a lamotrigine imprinted polymer and its application for drug assay in human serum

A molecularly imprinted polymer (MIP) against lamotrigine (LTG) was prepared, characterized, and its recognition properties were compared with a blank nonimprinted polymer (NIP). Two classes of binding sites were found in the MIP – high affinity (K_D = 16.2 μM) and low affinity (K_D = 161.3 μM). Selectivity of the synthesized MIP was examined using compounds with similar structures or therapeutic uses to LTG. In compounds which had structural similarity to LTG, the presence of amine groups appeared to affect binding to the MIP, however overall shape of the molecule was also important. Under the optimal conditions developed, other anticonvulsant drugs tested did not bind the MIP. A molecularly imprinted SPE (MISPE) procedure was developed which had a recovery of 84 – 89%, interday variation of less than 3.4% and intraday variation of less than 2.8%. The MISPE procedure was compared with a routine liquid – liquid extraction (LLE) procedure used for the HPLC determination of LTG in serum from patients. The data indicated that the MIP synthesized showed both good selectivity and high affinity for LTG and could be used for the extraction of the drug from serum samples or as the receptor layer for an LTG selective biosensor.

Keywords: Lamotrigine / Molecularly imprinted polymer / Serum samples / Solid-phase extraction

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1 Introduction

Molecular imprinting is a technique for preparing polymers with structure-selective adsorptive properties. This is achieved by arranging functional monomers around a template compound and then fixing the monomers in this spatial arrangement with a crosslinker [1]. An ideal molecularly imprinted polymer (MIP) has homogeneous binding sites which show no co-operative properties and no matrix effects [2]. Such an MIP could find uses as the sorbent in SPE [3–6], as the stationary phase in HPLC [7, 8], and as the receptor layer in biosensors [9–11]. It is probably the use of MIPs in biosensors which holds the greatest promise of enabling researchers to create analytical systems for highly selective, robust, and fast, one-step assays.

When an MIP is prepared, it is essential to characterize its template-binding properties. Two binding parameters have been recognized as important for this characterization [4, 7, 12]: binding selectivity and binding capacity. Equilibrium binding study followed by Scatchard analysis can help one to evaluate the selectivity and capacity of the polymer in binding its template [4, 13]. Dissociation constant (K_D) is a measure of the affinity of template for the MIP while maximum binding is related to the capacity of the polymer to adsorb the template. Scatchard analysis can also yield information on the heterogeneity of the binding sites [12, 14].

Therapeutic drug monitoring (TDM) has long been considered important for the appropriate use of many antiepileptic drugs. As such a number of chromatographic methods for the measurement of serum levels of phenytoin, carbamazepine, and phenobarbital have been developed [15–19], some of which rely on MIPs for the extraction and subsequent quantitation of the drugs [3, 4, 18, 20].

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Abbreviations: AIBN, 2,2′-azo-bis-iso-butyronitrile; **LLE,** liquid–liquid extraction; **LTG,** lamotrigine; **MAA,** methacrylic acid; **MIP,** molecularly imprinted polymer; **MISPE,** molecularly imprinted SPE; **NIP,** nonimprinted polymer

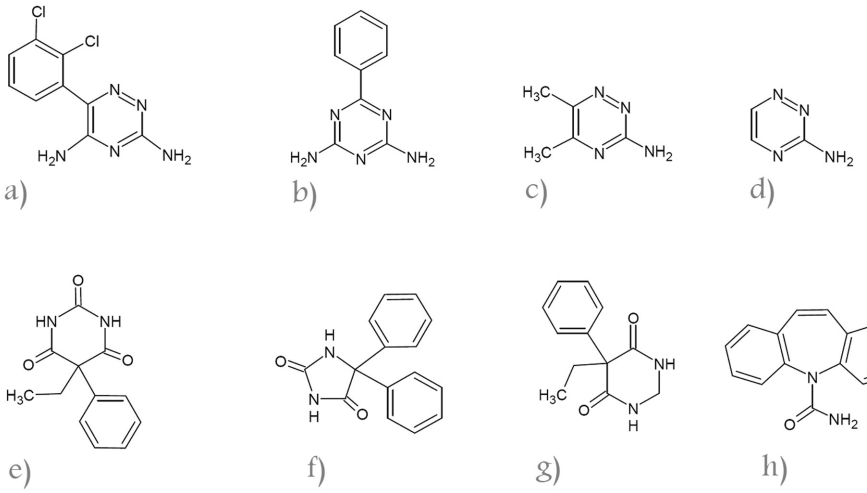

Figure 1. Structure of compounds which were examined for binding to MIP. (a) LTG; (b) 2,4-diamino-6-phenyl-1,3,5-triaizne; (c) 3-amino-5,6-dimethyl-1,2,4-triazine; (d) 3-amino-1,2,4-triazine; (e) phenobarbital; (f) phenytoin; (g) primidone; (h) carbamazepine.

Lamotrigine (LTG) is a third generation anticonvulsant drug used in the treatment of epilepsy [21]. The use of LTG has been associated with some side effects [22]. At levels of 10–15 μg/mL (39–59 mmol/L) 24% and above 20 μg/mL (78 mmol/L) 59% of patients showed signs of toxicity while others tolerated levels above 20 μg/mL [23]. Therefore, in order to achieve therapeutic drug levels without signs of toxicity, individual therapeutic thresholds should be defined for LTG. TDM has a place in the management of epilepsy with this drug and appears useful both for the establishment of individual reference ranges and the identification of the individual level-to-dose ratio [24–28].

Here we report, for the first time, the synthesis of an MIP for LTG and the optimization of conditions for LTG binding to and elution from this MIP. We studied its binding affinity and capacity in comparison with an appropriate nonimprinted polymer (NIP). In addition, we used this MIP as the sorbent in SPE of LTG and developed a sample preparation method for determination of LTG in human serum samples. We validated this MIP-SPE method against a liquid–liquid extraction (LLE) method routinely used as the sample pretreatment procedure before HPLC for the determination of LTG levels in human serum.

2 Experimental

2.1 Chemicals and materials

LTG (3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine) was purchased from Hetro Drug (Hyderabad, India); phenytoin, phenobarbital, primidone, carbamazepine, 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 (ACN, THF, methanol, acetic acid, TFA) were of HPLC grade. All work with human serum samples was carried out after approval and according to the guidelines of Iran University of Medical Sciences Ethical Committee. Written consent of all serum donors was obtained. Structures of LTG and other compounds assayed in this work are presented in Fig. 1.

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.

2.3 Batch adsorption procedure

Dry MIP or NIP (10 mg) was incubated, in a glass tube in 3 mL ACN at fixed temperature, with LTG. Tubes were centrifuged at 4000 × *g* for 10 min. Two hundred microliter of the supernatant was dried under a stream of nitrogen, redissolved in 200 μL of mobile phase and injected into the chromatograph. From the known initial concentration of LTG, the number of moles of the solutes in the solution phase at the start of the reaction was computed [13, 27] and the number of moles remaining in the solu-

tion after equilibration were subtracted to give the number of moles adsorbed on the polymer.

2.4 Optimization of the molecularly imprinted SPE (MISPE) procedure

Forty-five milligram polymer (MIP or NIP), in 3 mL ACN, was slurry packed into an empty polypropylene SPE cartridge. The column was washed and conditioned as previously described. LTG (5 μg) in 500 μL water was loaded onto the column. Five 1 mL aliquots of washing solvent were successively loaded onto the SPE column. Then the sample was completely eluted with 3 mL THF–TFA 90% (50:50 v/v). The solvent was removed from each washing, the residue was redissolved in 500 μL mobile phase and concentration of LTG was determined chromatographically. The amount of LTG in each aliquot of washing sample and THF–TFA 90% (50:50 v/v) eluent was calculated. Experiments were carried out in triplicate. Methanol, THF, ACN–THF (90:10 v/v), and ACN were applied as washing solvents in order to find a solvent resulting in maximum selectivity and recovery of LTG. Controls, where no LTG was loaded, were prepared in order to check template bleeding.

2.5 MISPE extraction procedure of LTG from human serum samples

Forty-five milligram imprinted polymer, in 3 mL ACN, was slurry packed into an empty polypropylene SPE cartridge, washed, and conditioned as before. ACN (800 μL) was added to a 200 μL serum sample in order to precipitate the serum proteins. After centrifugation at 14 000 × *g* for 10 min, 1.5 mL water was added to 500 μL of supernatant and the mixture was loaded onto the column. ACN (2 mL) was used for selective washing. LTG was eluted with 3 mL THF–TFA 90% (50:50 v/v). The solvent was dried under a stream of nitrogen. The residue was redissolved in 100 μL mobile phase and the concentration of LTG was determined chromatographically.

2.6 Preparation of standards and calibration standard samples

To prepare an LTG stock solution, 10 mg of the drug was dissolved in 10 mL methanol. The standard solutions (7.8, 19.5, 39, 78, 156, and 312 μM) used for batch adsorption test were prepared from this stock solution by dilution with ACN. The standard solutions (5, 10, 20, 50, and 100 μg/mL) used for spiking calibration samples were prepared from the stock solution by dilution with water. For calibration, 200 μL of the standard solutions was added to 1.8 mL of serum to obtain 0.5, 1, 2, 5, and 10 μg/mL calibration standards. The serum samples were frozen after spiking and stored at −20°C.

2.7 Chromatographic determination of LTG and other components

Chromatographic determination of components was carried out on a Micro-Tech Scientific microLC system (Vista, CA, USA). The column (25 cm × 1.00 mm, MM-25-C18W-1000) elute was monitored using a UV–VIS detector set to 220 nm. Data acquisition device was Autochro Data Module (Younglin, Korea) with Autochro-2000-0.1 software, for data collection and analysis. The injection volume was 5 μL and mobile phase flow rate was 70 μL/min. The column was thermostated at 45°C. A gradient method was used for chromatographic determination of LTG, phenytoin, phenobarbital, primidone, and carbamazepine in a mixed solution or extracted samples of serum. The mobile phase compositions were: A, H_2O 88%, ACN 11.3%, THF 0.6%, TFA 0.1% and B, H_2O 76.2%, ACN 17.7%, THF 6%, TFA 0.1%. The initial mobile phase composition was 100% A and 0% B which changed, linearly, to 0% A and 100% B at 5 min. An isocratic method was used for the determination of LTG, 2,4-diamino-6-phenyl-1,3,5-triazine, 3-amino-5,6-dimethyl-1,2,4-triazine, and 3-amino-1,2,4-triazine in water solution. The composition of mobile phase was: H_2O 89.3%, ACN 10%, THF 0.6%, and TFA 0.088%.

The MISPE method was compared with an LLE method, routinely used for the determination of LTG in human serum samples in a medical diagnostic laboratory. Briefly, the LLE method involved mixing of 200 μL of serum sample with 800 μL dichloromethane/isopropanol (19:1) and 50 μL of internal standard solution. After mixing for 1 min, 500 μL of the organic layer was evaporated to dryness, reconstituted in 100 μL mobile phase, and analyzed chromatographically as described above.

3 Results and discussion

3.1 Choice of functional monomer

High selectivity and strong interaction with template are two important characteristics of a good SPE medium. The monomer used for imprinting LTG was MAA. Due to the presence of two amine groups, LTG can easily bind this acidic functional monomer. The strong ionic bond between LTG and MAA at binding sites should make the MIP suitable for SPE procedure under aqueous conditions. The initiation of free radical polymerization reaction was effected by the use of AIBN as the catalyst. Many other workers have used AIBN, as it is soluble in most organic solvents used for the preparation of MIPs and stable enough to allow the reaction to proceed for a few hours at elevated temperatures [1–4]. It was found that at 60°C, the reaction produced visible polymer particles after about 45–60 min, which gradually became bigger and coalesced to form a monolithic polymer block. As

most other workers have done, for the reaction to reach completion, it was allowed to proceed for about 17 h at elevated temperature [1, 2].

3.2 Best remover solvent

Bleeding (*i.e.*, release of residual template from the polymer matrix) is a problem during batch adsorption tests and SPE. Therefore, a remover solvent is used to wash the MIP thoroughly and extract the template completely from the polymer.

In order to determine the best solvent system for removal of the template after polymerization and also separation of bound LTG during adsorption experiments, 20 mg of the MIP (LTG not extracted) was incubated at room temperature (25 ± 1°C) with 2 mL solvent for 20 h. After centrifugation at 4000 × *g*, 200 μL of the supernatant was dried under a stream of nitrogen, re-dissolved in 200 μL mobile phase and the amount of LTG was quantified, chromatographically, as described below. A number of solvents or mixtures were used to extract LTG from the MIP. The rank order of LTG extraction ability of the solvents tested was as follows: THF–TFA 90% (50:50 v/v) > THF > methanol containing 0.1% acetic acid > methanol/ACN (90:10 v/v) > methanol containing 0.1% HCl > ACN/methanol (90:10 v/v) > methanol/acetic acid (90:10 v/v) > ACN/acetic acid (90:10 v/v) > THF/ACN (57:43 v/v) > methanol > ACN containing 0.1% acetic acid > ACN > H_2O. In general, acidifying a solvent appeared to increase its ability to extract LTG from the MIP. This is not unexpected as the functional monomer is an acid which tends to lose its charge at low pH and make dissociation of LTG more likely. Some other workers who have synthesized MIP, also found that the addition of an acid to the remover solvent increased its extraction efficiency [29, 30]. Using this data, the best conditions for extraction of LTG from the MIP was found to be washing for 14 h with THF, using a Soxhlet extractor apparatus. Also, before each experiment, MIP was washed with 2 mL THF/TFA 90% (50:50 v/v) and conditioned with 4 mL water. When used for MISPE and after this template extraction and washing procedure, drug-free samples did not show a detectable LTG peak. This suggested that template bleeding was below the LOQ of the chromatograph.

3.3 Batch adsorption measurements

3.3.1 Effect of incubation time upon LTG binding to the MIP and the NIP

It is important to allow adequate contact time between the ligand and the sorbent to reach binding equilibrium, *i.e.*, when no more net ligand binding occurs. In order to find the equilibrium time for adsorption of LTG, the time course of adsorption of a 10 μg/mL LTG solution was studied, at room temperature. The amount of bound LTG was plotted against time (Fig. 2). The data showed that about 70% binding of LTG to MIP occurred within the first 5 min and after 40 min, maximum binding was reached. With NIP, a similar picture emerged. This meant an incubation time of 180 min, which was used in saturation binding studies, would be more than sufficient to allow attainment of equilibrium for binding of LTG to the MIP and NIP.

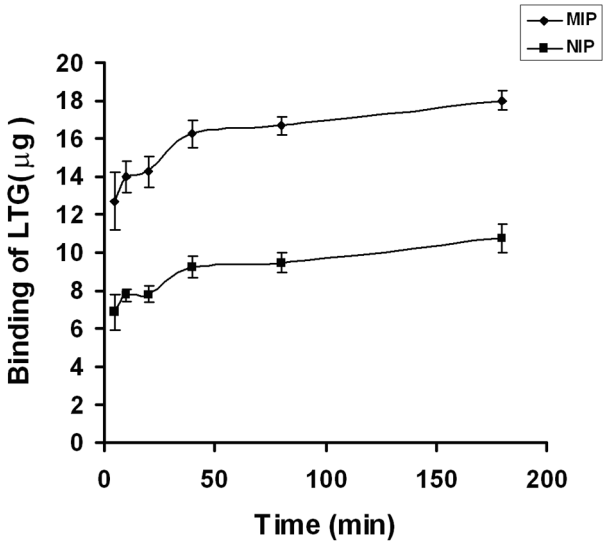

Figure 2. Binding of LTG to 10 mg of the MIP or the NIP at different times ($n = 4$). Each point represents mean ± SEM; LTG concentration in ACN was 10 μg/mL.

3.3.2 Effect of incubation temperature upon LTG binding to the MIP and the NIP

Any difference in template-binding behavior of the MIP and the NIP, at different temperatures, may yield information on the nature of binding of the template to the polymers. This, in turn, can give us further evidence that imprinting has indeed taken place during the polymerization. Effect of temperature on binding of 5 μg/mL LTG to the MIP and NIP was investigated at four different temperatures (Fig. 3). Binding of LTG to the NIP decreased when temperature was increased stepwise from 25 to 55°C. With the MIP, the picture was a little more complex. With increasing temperature, from 25 to 35°C, binding of LTG to the MIP increased. From 35 to 55°C, there was a progressive loss in binding by the MIP. Also, as can be seen in Fig. 3, at all temperatures tested, the amount of LTG bound to MIP was about 50% greater than that bound to NIP. These different behaviors of the MIP compared to the NIP support the idea that in the process of polymerization of the MIP, imprinting has occurred.

Increasing temperature leads to increased kinetic energy of molecules. In the NIP, this increased kinetic energy and thus motion of LTG molecules simply

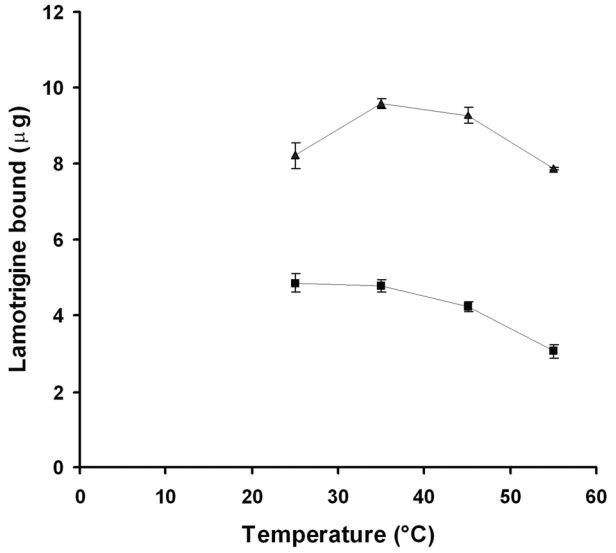

Figure 3. Effect of temperature on binding of LTG to 10 mg of the NIP (■) or the MIP (▲). Each point represents mean ± SEM ($n = 4$). LTG concentration in ACN was 5 μg/mL.

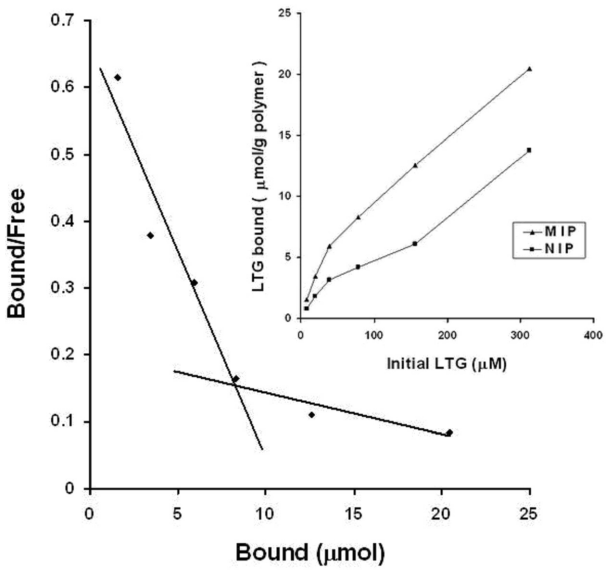

Figure 4. Scatchard plot of binding of LTG to the MIP. Inset: The adsorption isotherm obtained for the NIP and the MIP using batch adsorption test ($n = 4$). Each point represents mean ± SEM.

increased the chances of breaking of the LTG–NIP bonds. In the MIP, binding occurred at sites with a complementary spatial structure to LTG. This meant that LTG molecules would have to hit the MIP binding cavity at the right position, with the right orientation for any binding to happen. Therefore, increase in the temperature and giving LTG molecules more kinetic energy increased their chances of hitting the MIP at the right position and with a suitable orientation. This may explain why increasing the temperature from 25 to 35°C increased the binding of LTG to MIP. But as the system was given more kinetic energy, *i.e.*, increasing temperature beyond 35°C, drug molecules moved so much more as to escape the binding cavity of MIP and thus binding decreased.

3.3.3 Scatchard analysis of the MIP–LTG binding

Some workers have used the conventional batch adsorption method for the characterization of ligand–polymer interaction. This involves the incubation of solutions of the ligand at different concentrations with fixed amount of the sorbent. After an equilibration time period, the amount of the ligand bound is measured [2, 31, 32].

In the current work, binding of the LTG or phenobarbital to MIP and NIP was studied (Fig. 4). Phenobarbital was chosen as the second compound because it was structurally different from LTG and did not contain NH_2 functional groups. Therefore, it would not be capable of interacting efficiently with the active groups in the MIP binding sites (–COOH) and also, it was not expected to be able to bind differently to the MIP and the NIP.

When used at 86 μM, 2.76 ± 1.36 μmol phenobarbital was bound to each gram of the MIP. The figure for the NIP was 2.76 ± 1.41 μmol/g. There was no difference between the MIP and the NIP in adsorption of phenobarbital. This suggested that binding of phenobarbital probably occurred on sites other than LTG recognizing cavities and that these phenobarbital binding sites were present to similar extents on both the MIP and the NIP.

At 78 μM, LTG bound to NIP was 4.18 ± 0.77 μmol/g. This was greater than phenobarbital binding to NIP (2.76 ± 1.41 μmol/g NIP) at a similar concentration (86 μM). This could suggest that in the binding of LTG to NIP, and by implication to MIP, the presence of NH_2 groups might have played an important role.

From Scatchard plot (Fig. 4) two dissociation constants could be discerned, one representing high affinity binding sites with a K_D of 16.2 μM, and one representing low affinity binding sites with a K_D of 161.3 μM. Other researchers too have found that more than one class of binding sites were formed during imprinting [4, 7, 12] and suggested that the high affinity sites represent the template selective cavities formed during polymerization. The K_D values obtained by these workers ranged from low μM to M (sulfamethoxazole K_D = 18.8 μM [7], calculated K_D for phenytoin is 176 μM [4], theophylline K_D = 1.5 M [12]). The K_D value obtained in this study (16.6 μM) was in the lower end of this range, suggesting a relatively strong binding of LTG to MIP.

The number of high affinity and low affinity binding sites obtained from this analysis were found to be 10.7 and 33.1 μmol/g MIP, respectively.

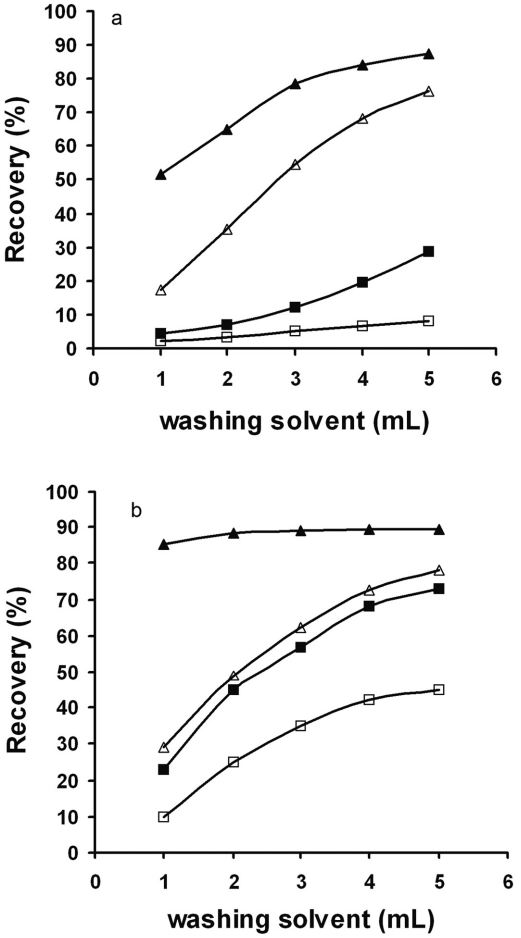

Figure 5. Recovery of LTG after percolation of increasing volumes of different washing solvents through an (a) MIP and (b) NIP column. ACN (□), ACN–THF (90/10) (■), methanol (△), THF (▲).

3.4 Optimization of the MISPE procedure

The application intended for this MISPE was the determination of LTG in human serum which is an aqueous medium. For this reason, water was selected as the loading medium. Several washing solvents were applied in order to find the one which yielded the maximum selectivity and recovery of LTG. Washing with different solvents was carried out in several steps. The percentage of washed LTG relative to the total loaded amount was calculated in each fraction and the cumulative recovery was plotted against the volume of washing solvent (Fig. 5).

Although THF, methanol, and ACN/THF (90:10 v/v) could disrupt nonspecific binding to the NIP, they could also effectively disrupt specific binding to the MIP. However, after washing the cartridges with 5 mL ACN only 8.2% of LTG was removed from the MIP, whereas 45% of LTG was removed from the NIP column. Therefore, ACN was selected as the washing solvent. ACN (2 mL) could be used to wash the polymer after sample loading without removing appreciable amount of LTG (<5%) from the MIP cartridge.

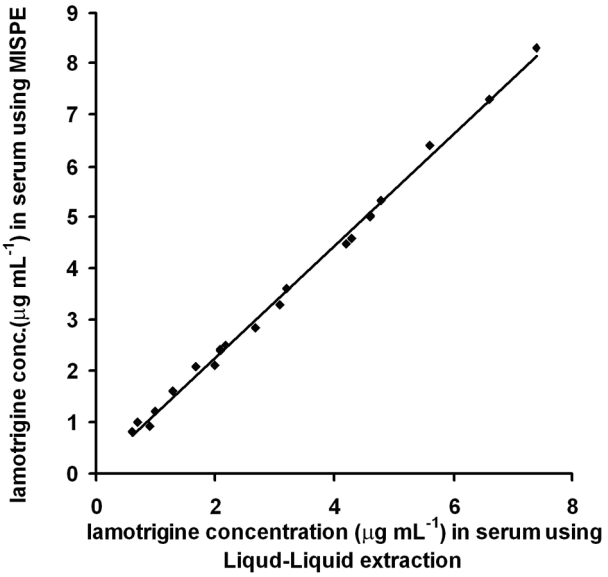

Figure 6. Correlation between LTG measured using MISPE and LTG measured using LLE for serum samples from 21 patients.

After washing with 2 mL ACN, 95–100% of phenobarbital, primidone, phenytoin, and carbamazepine was eluted from both the MIP and the NIP cartridges while LTG removed from the MIP column was less than 5%. Thus, optimized MISPE conditions were as follows: washing conditions, 2 mL ACN; elution conditions, 3 mL THF/TFA 90% (50:50 v/v).

3.5 Validation of the MISPE procedure

The analytical applicability of the MISPE procedure for the determination of LTG from human serum samples was validated using the method presented in Section 2.6. The linear calibration curve of the area under the UV absorption peak (220 nm) of serum spiked with known concentrations of LTG was plotted over a range of 0.5–10 μg/mL (slope = 381.0 ± 19.2, intercept = −22 ± 12, n = 5 for each data point, R^2 = 0.999) and compared with the linear standard curve of LTG in mobile phase (slope = 426.7 ± 26, intercept = −36 ± 15, n = 5 for each data point, R^2 = 0.999). The recovery of LTG calculated in this manner was found to be between 84 and 89%. At an S/N ratio of 3, the assay had an LOD of 0.03 μg/mL. At an S/N ratio of 20 – which represents a 5% error in the signal – the assay had an LOQ of 0.2 μg/mL which was much lower than the minimum therapeutic level in patient serum. Intraday variation was determined by measuring spiked serum samples at two concentrations. The intraday variation values, for LTG concentrations of 2 and 5 μg/mL, were 1.7 and 2.8%. The interday variation was

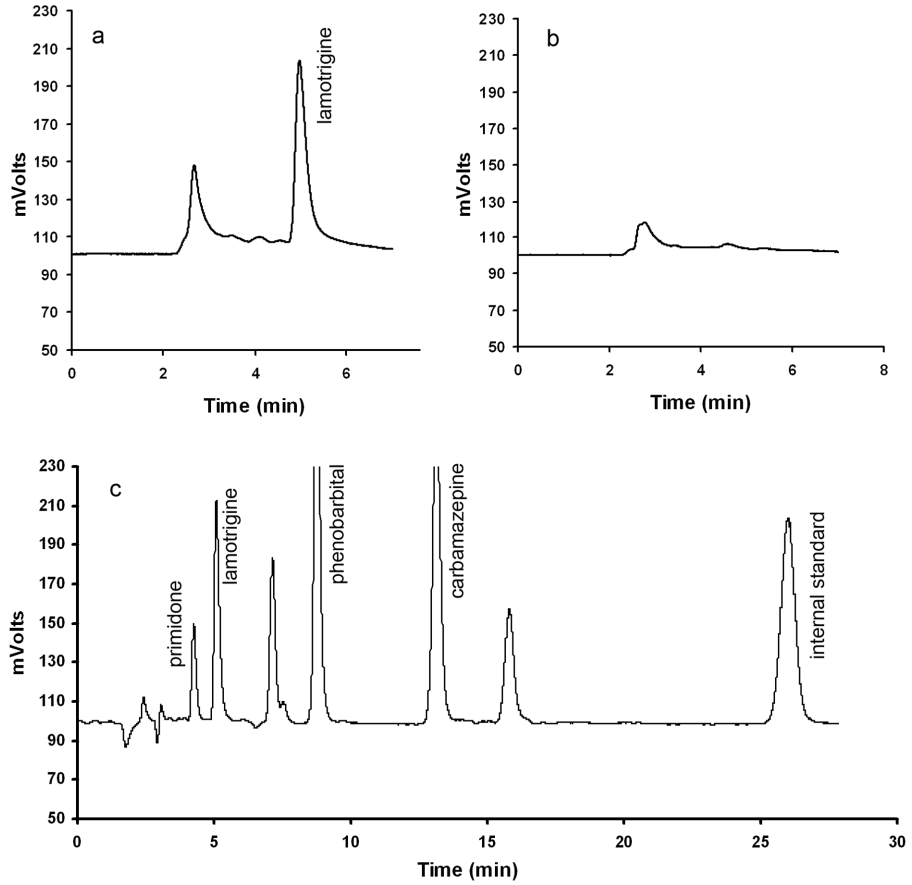

Figure 7. Typical chromatogram of a serum sample spiked with (a) 5 μg/mL LTG, (b) a blank serum sample, and (c) serum sample assayed using the LLE method. Washing condition for the MISPE method: 2 mL ACN, elution condition: 3 mL THF–TFA 90% (50:50 v/v).

determined on four different days using spiked serum samples at 2 and 5 μg/mL. In this case, variation values were 1.4 and 3.4%.

LTG from serum of 21 patients who took LTG and other anticonvulsant drugs were used for comparison. The LTG concentration value obtained using the MISPE was plotted against the corresponding value obtained by LLE (Fig. 6). The line of best fit had a slope of 1.09 and a correlation coefficient of 0.996. Student's *t*-test carried out on the two sets of data showed that the difference between the two sets of data was statistically nonsignificant ($p = 0.58$).

Figure 7 shows typical chromatograms obtained for LTG extracted by the MISPE procedure from LTG spiked serum and that of a serum blank. As can be seen from serum blank chromatogram, there is no bleeding detectable from the MIP matrix. Also a chromatogram obtained after LLE of a spiked serum sample is presented in Fig. 7.

3.6 Selectivity of the synthesized MIP against some structurally related compounds

In order to study selectivity of LTG imprinted polymer, 1 mL water containing 5 μg of each structurally related compounds (2,4-diamino-6-phenyl-1,3,5-triazine, 3-amino-5,6-dimethyl-1,2,4-triazine, and 3-amino-1,2,4-triazine) was loaded onto the MIP column. Figure 8 shows the effect of ACN washing on the recovery of each compound. After 3 mL washing, 12% of 3-amino-1,2,4-triazin, 16% of 3-amino-5,6-dimethyl-1,2,4-triazine, and 27.4% of 2,4-diamino-6-phenyl-1,3,5-triazine remained on the MIP cartridge, whereas 91% of LTG was still bound to the MIP (Fig. 8). Examining the structures of these compounds suggested that the strength of binding to MIP was related to the number of amine groups in the structure. However, although LTG and 2,4-diamino-6-phenyl-1,3,5-triazine both have two amine groups, they showed very different affinities toward the MIP, as deduced from the ability of ACN to remove them from the matrix. These findings indicated that the MIP synthesized in this work did not act as a simple ion-exchange resin and that both amine groups and appropriate structure were important in the binding of LTG to the MIP.

4 Concluding remarks

LTG imprinted polymer was prepared using MAA as a functional monomer. The polymer was characterized and its recognition properties were compared with a blank polymer (NIP). Effects of time and temperature

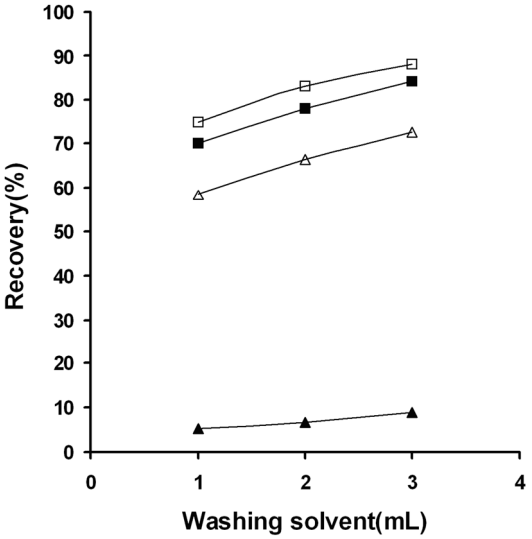

Figure 8. Recovery of LTG and similar compounds loaded onto the MIP cartridge after washing with increasing volumes of ACN as washing solvent. Legend: LTG (▲), 2,4-diamino-6-phenyl-1,3,5-triazine (△), 3-amino-5,6-dimethyl-1,2,4-triazine (■), 3-amino-1,2,4-triazin (□).

upon binding of the template to the polymer were studied. Affinity of the imprinted polymer for the template was investigated using Scatchard analysis and two classes of binding sites were found. The number of binding sites in each gram of polymer and the dissociation constants (K_D) of template to high affinity and low affinity binding sites were determined. The imprinted polymer was then used as the sorbent in an SPE procedure for the determination of serum LTG. The MISPE procedure was optimized with regards to washing condition. Selectivity of the MISPE procedure was investigated using LTG and some anticonvulsant drugs that could be present, simultaneously, in serum of patients. The MISPE procedure was compared with a routine LLE procedure. Also binding of some compounds that were structurally related to LTG and to the MIP was investigated. The results indicated that the MIP synthesized in this work was selective for LTG and had a high affinity for binding this drug. This MIP was found to be suitable for use in MISPE of LTG from human serum. Also, this MIP may be a good starting point for the preparation of a receptor layer for an LTG selective biosensor.

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