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A Terbium Metal–Organic Framework Platform for Determination of Lamotrigine in Exhaled Breath Condensate

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Abstract

Background: Lamotrigine is widely used in the management of partial epilepsy, generalized tonic-clonic epilepsy and Lenox-Gastut syndrome and an add-on therapy in the treatment of complex and simple partial seizures and secondarily generalized tonic-clonic seizures resistant to multiple drug therapy. **Methods:** In the current study, a fluorometric nanoprobe based on metal–organic frameworks (MOF) was designed for the determination of lamotrigine in exhaled breath condensate (EBC). The MOF nanoprobe consisted of Tb^{3+} ions as metal part and dimethylformamide (DMF) and 1,10-phenanthroline (Phen) as organic parts of nanoprobe. **Results:** The used probe shows a week fluorescence in alkaline media owing to an energy transfer from nitrogen groups of DMF and Phen on carbonyl group of DMF as an antenna for Tb^{3+} luminescence. However, its fluorescence is enhanced in acidic conditions by protonation of DMF nitrogen atoms and Phen and deactivation of energy transfer pathways of nitrogen groups to carbonyl group. Lamotrigine addition to this fluorescent system leads to quenching in the fluorescence intensity due to reactivation of the above mentioned energy transfer pathways resulting in competitive interaction with H^+ ions. Moreover, the inner filter effect (IFE) of lamotrigine on DMF–Tb–Phen MOF NPs is considered as another reason for the observed quenching in the fluorescence of DMF–Tb–Phen MOF NPs. The intensity of the fluorescence was recorded at λ_{em} = 545 nm and the difference between fluorescence signal in the absence and presence of lamotrigine was the analytical response. The factors affected on experimental conditions were optimized utilizing a multivariate optimization technique. The validation of nanoprobe response to lamotrigine gives a linear relationship in the range of 0.05 to 2.0 $\mu g \cdot mL^{-1}$ with a detection limit of 11.0 $ng \cdot mL^{-1}$ for lamotrigine. **Conclusion:** The developed method reveals good repeatability and selectivity for lamotrigine in real samples.

Keywords: Lamotrigine; Metal–organic frameworks; Terbium; Response surface method; Central composite design.

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Introduction

Lamotrigine as an approved pharmaceutical is widely employed in the management of partial epilepsy, generalized tonic-clonic epilepsy and Lenox-Gastut syndrome. Lamotrigine is also suitaable as add-on therapy in the treatment of complex and simple partial seizures and secondarily generalized tonic-clonic seizures resistant to multiple drug therapy.[1] Thus, its therapeutic drug monitoring in the biological samples of patients is of an essential issue in the clinical application especially in co-therapy with other antiepileptic pharmaceuticals. The development of a fast, reliable and cost effective method for quantification of drug concentration is always a challenge for clinical circumstances. So far several analytical methods for the quantification of lamotrigine are published in the literature. Some of reports including immunoassay,[2] HPLC-UV,[3] HPLC-MS/MS,[4] GC-FID,[5] capillary zone electrophoresis,[6] spectrophotometry[7] and electrochemistry.[8] Chromatographic methods have excellent precision and accuracy for drug monitoring but these methods are sophisticated and time-consuming. Although the customary used optical methods are simple and quick, their main disadvantages are the high detection limit and low selectivity in comparison with the chromatographic methods. Nanomaterial-based optical methods could compensate for these limitations and present good performance in terms of stability, selectivity, sensitivity, linearity, reproducibility and response time.[9, 10] Nanomaterials based on the metal organic frameworks (MOFs) nanostructures with hybrid materials with inorganic and organic segements.[11] MOFs have a porous structure and are composed of an orderly array of metal ions with positive charge surrounded by organic molecules as a linker. The metal ions produce nodes that connect the arms of the linkers to produce a structure with cage-like, and repeating patterns.[12] MOFs with unique structural diversity, adjustable porosity, and flexibility in network geometry, topology, size, and modification can be applied in many fields such as liquid separation and extraction agent, gas storage and separation, catalysis, electrochemical energy storage and sensor.[13] Luminescent MOFs are composed of active elements such as light-emitting rare earths europium and terbium (Tb^{3+}) or luminophore ligands.[14-17] Luminescent MOFs have great potential to design different sensors based on their tunable luminescent properties. Most of the developed luminescence MOF systems mainly used for detection of volatile organic compounds such as determination of NH_3, triethylamine, N_2, ethylenediamine, H_2O by Zn_2(tetraphenylethylene) as ligand,[18] and DMF by (Eu_2(2,5-bis (methoxymethyl)-[1,1':4',1"-terphenyl]-4,4"-dicarboxylate) as ligand.[19]

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Furthermore, various luminescent MOFs have been reported for temperature sensing.[20] Qi and Chen developed a luminescent probe based on Tb^{3+} MOF for pH sensing.[21]

In the present study, a luminescence MOF based on light-emitting rare earths was synthesized and validated for the quantification of lamotrigine in exhaled breath condensate (EBC) samples. Here, the luminescence of Tb^{3+}-based MOF is quenched in the presence of lamotrigine owing to its interaction with the organic part of MOF which can act as an antenna for Tb^{3+} luminescence. Response surface method (RSM) was utilized for the optimum of the experimental conditions.

Experimental section

Reagents

Ultrapure deionized water (Ghazi Pharmaceutical Co. Iran), terbium (III) chloride hexahydrate ($TbCl_3.6H_2O$, Acros organics, USA), 1,10-phenanthroline (Phen, Janssen Chimica , Italy), dimethylformamide (DMF, Sigma-Aldrich, USA), and sodium dihydrogen phosphate (NaH_2PO_4, Scharlau, Spain) were used in this work. Lamotrigine (with a purity of 99.6%) was purchased from Arastoo Pharmaceutical Company (Tehran, Iran). A 1000 $mg{\cdot}L^{-1}$ stoke solution of lamotrigine was obtained using dissolving the proper value of it in methanol and a daily diluted working solution was used for experiments.

Apparatus and software

A JASCO FP-750 spectrofluorometer (Tokyo, Japan) for recording luminescence spectra, a pH meter (model 744, Metrohm Ltd., Switzerland) for pH adjustment, an ultrasonic bath (Alex Machine, Istanbul, Turkey) for reaction progressing and a CM30 transmission electron microscope (TEM) (Philips, The Netherlands) and MIRA 3 field emission scanning electron microscope (SEM) model (TESCAN, Czech Republic) for characterization of the shape and size of the prepared MOF were used in the current study. The chemical composition of the DMF–Tb–Phen MOF NPs was also acquired by energy dispersive X-ray (EDX) analyzer attached to the same SEM instrument. FT-IR spectra (4000 – 400 cm^{-1}) were recorded employing a Bruker-Tensor 270 spectrometer (Bruker, Germany, www.bruker.com) employing the KBr pellet method with a sample/KBr mass ratio of 1:100. The crystalline structure of the NPs was also characterized by a PHILIPS (PW1730, Holland) X-ray diffractometer. The size distribution of

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the NPs is observed using the Dynamic Light Scattering (DLS) measurements, Malvern particle size analyzer (Malvern, UK).

MINITAB (Minitab Inc. Release 17.0) statistical package was utilized for the experimental designing and analysis of obtained results.

Synthesis of DMF–Tb–Phen MOF nanoparticle

DMF–Tb–Phen MOF nanoparticles (NPs) were synthesized using a previously reported method.[21] Briefly, 0.4 mL of Phen in DMF solution (100 mmol.L^{-1}) was mixed to 8 mL of DMF and 0.8 mL of $TbCl_3$ aqueous solution (100 mmol.L^{-1}) was added to the above mixture. After 20 min stirring, the solution was placed in an autoclave and set at 160 °C for 2 h. After cooling to ambient conditions, the product was centrifuged at 6,000 rpm for 20 min. The obtained white precipitate was washed four times with methanol. Then, the precipitate was dispensed in 1 mL of deionized water to provide a suspension of DMF–Tb–Phen NPs with a concentration of 28.4 g.L^{-1}.

Sample preparation

A lab-made collection device was used to collect EBC samples.[22] The collection instrument is composed of a cooling trap with tunable temperature (-25 - 0 °C). The instrument works by freezing exhaled air blown to trap, and finally sedimentation of exhaled aerosols onto a cold trap's surface. A photograph of the used devise is provided in the electronic supplementary material (ESM). Each volunteer was asked to breathe tidally into a mouthpiece of an instrument for 10 min. The exhaled breath into the cooling trap will be collected as a liquid phase. A pooled sample obtained from healthy individuals, directly and without any pretreatment was used for optimizing and validating the proposed technique. The EBC analyzed in the real sample section was obtained from 4 volunteers after the administration of lamotrigine. Sample donors signed a consent form confirmed by the Ethics Committee of the Tabriz University of Medical Sciences with the ethic code of IR.TBZMED.REC.1399.437.

General procedure

Detection was conducted in 2.0 mL microtubes; briefly, 50.0 µL of 0.1 mol·L^{-1} phosphate buffer (pH 5.0), and 22 µL MOF nanoparticles (1.136 g.L^{-1}) were added to 0.25 mL of real sample or sample spiked with a standard solution of lamotrigine at various concentrations of 0.05 to 2 µg·mL^{-1}. The volume was reached 0.5 mL with ultrapure water. After 15 min

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ultrasonication, the fluorescence response was written at λ_{em}= 545 nm with an excitation wavelength of 230 nm.

Results and discussion

Characterization of DMF–Tb–Phen MOF

SEM was employed to show the size and shape of the prepared MOF. As can be seen in Fig. 1A, the DMF–Tb–Phen MOF NPs have a polyhedral morphology with a mean size of <70 nm which is proved by DLS measurement. FT-IR was employed to characterize the surface chemistry of the DMF–Tb–Phen MOF NPs. FT-IR spectra are shown in Fig. 1B. Three characteristic peaks of 1587.08 (C–N stretching vibration peak related to DMF and C=C stretching vibration peak related to Phen), 1376.48 (bending vibration peak of NCH and CH_3 of DMF) and 757.22 cm^{-1} (out-plane bending vibration peak of C-H bond related to Phen) recorded for DMF–Tb–Phen MOF NPs are in accordance with FT-IR spectra reported in the literature[21] demonstrating the complete synthesis of nanoparticles. The successful synthesis of the DMF–Tb–Phen MOF NPs was further verified by EDX analysis. It can be seen in Fig. 1C, terbium, oxygen, nitrogen and carbon elements are distributed in the analyzed sample.

(Fig. 1 here)

A possible mechanism for employed nanoprobe

Qi and Chen[21] synthesized DMF–Tb–Phen MOF NPs for the first time and used them as fluorescent pH sensors. The sensor is composed of (i) DMF molecules with both an electron-acceptor segment and an electron-donor segment that can generate an electron exchange between the nitrogen and oxygen atoms *i.e.* an intramolecular charge transfer (ICT)[23] and weaken the antenna effect of DMF on Tb^{3+} luminescence and (ii) Phen molecules which combine with $–CH_3$ groups of DMF and induce a photoinduced electron transfer (PET) effect from nitrogen atom of pyridine to carbonyl by a transfer of hydrogen bonds and weaken the energy transfer to Tb^{3+}. So, ICT of DMF and PET of Phen leads to a week fluorescence for DMF–Tb–Phen MOF NPs in alkaline media. Whilst by protonation of DMF nitrogen atoms and Phen in acidic condition, PET effect from Phen toward DMF and the ICT effect within DMF molecule are deactivated and provide a stable fluorescent system (Figure 2S). In the current study, we used this fluorescent system as a new reported fluorescent probe for the determination of lamotrigine in EBC samples.

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Lamotrigine is a drug that belongs to the class of 1,2,4-triazines with a triazene skeleton substituted by amino groups at positions 3 and 5, and by a 2,3-dichlorophenyl group at position 6. The addition of lamotrigine to a solution of DMF–Tb–Phen MOF NPs in acidic media (pH=5.0) leads to a decrease in the fluorescence of the probe proportionally with the lamotrigine concentration (Figure 2). The possible mechanism for this quenching can be related to competitive reaction (protonation) between nitrogen atoms on DMF–Tb–Phen MOF probe and those of lamotrigine molecule (*i.e.* two amine groups and triazens) with pK_a =5.7 in the presence of H^+ ions. This process activates ICT of DMF and PET of Phen and leads to a decline in the fluorescence intensity of DMF–Tb–Phen MOF probe (Fig. 2). Moreover, inner filter effect (IFE) is considered as another reason for the observed quenching in the optical response of DMF–Tb–Phen MOF NPs. Lamotrigine presents an absorption spectrum with maxima at 200 nm and 308 nm, and with very high absorption at 230 nm which is the excitation wavelength for investigated nanoparticles. As the absorption spectrum of the absorber (lamotrigine in this work) have enough overlap region with the emission and/or excitation band of the emitter (DMF–Tb–Phen MOF NPs in this work), the fluorescence intensity of the emitter affects by the absorber (Figure 3S). This means that lamotrigine will produce an IFE on DMF–Tb–Phen MOF NPs and decrease its fluorescence intensity. As a decrease in fluorescence of nanoprobe is proportional to lamotrigine concentration; a new MOF based nanoprobe is designed and validated for lamotrigine determination in EBC samples.

(Fig. 2 here)

Central composite design (CCD) employed for optimizing the reaction conditions

The independent variables involved in the experimental design are pH, reagent concentrations, and reaction time. CCD was used for designing the experimental based on four factors that affected the analytical response. RSM plots were utilized to study the effect of each parameter on the analytical response in the presence of the interactive effects between parameters. The parameters were assessed at five levels. Details of investigated parameters and constructed plots are given in figures and tables in the ESM. The optimum values for studied parameters to obtain the maximum response with lamotrigine concentration of 0.5 $mg.L^{-1}$ are: pH 5.0, [MOF NPs]= 0.05 g L^{-1}, [buffer]= 0.01 $mol.L^{-1}$, and time = 15 min. The correlated model in uncoded units is as follows:

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$$\boldsymbol{Y} = 29.6 + 9.98\,\boldsymbol{X_1} + 567.0\,\boldsymbol{X_2} - 465\,\boldsymbol{X_3} - 5.923\,\boldsymbol{X_4} - 43.83\,\boldsymbol{X_1X_2} + 0.7257\,\boldsymbol{X_1X_4} - 1262\,\boldsymbol{X_2X_3} - 13.34\,\boldsymbol{X_2X_4} + 151.9\,\boldsymbol{X_3X_4} - 0.454\,\boldsymbol{X_1^2} - 12420\,\boldsymbol{X_3^2} - 0.0694\,\boldsymbol{X_4^2} \quad (1)$$

Where X_1, X_2, X_3 and X_4 represent pH, [MOF NPs], [buffer] and reaction time, respectively. The obtained model demonstrates that the concentration of MOF NPs is an important factor with the maximum positive effect whilst buffer concentration shows the highest negative impact factor on the analytical response.

Interference investigation

To study the method selectivity, the impact of some co-administered and commonly used drugs such as over-the-counter drugs on the system response toward lamotrigine was investigated. In the optimum conditions, sample mixtures with a known concentration of lamotrigine (0.5 $\mu g \cdot mL^{-1}$) and different values of the interferents were determined and the results as a tolerance limit are reported in Table 1. The findings show that the validated MOF based nanoprobe has acceptable selectivity for the quantification of lamotrigine in EBC samples.

Moreover, the impact of different interfering inorganic ions and organic species concurrently existed in EBC samples on the quantification of lamotrigine by the validated technique under the optimized conditions is also studied and the results are given in Table 2. It can be seen that the amounts of most given coexisting compounds in EBC of patients and healthy subjects are lower than tolerable concentration. Thus, it can be said that the validated sensor has acceptable selectivity for lamotrigine quantification.

(Tables 1 and 2 here)

Analytical performance

In the given conditions by CCD, a linear relationship (R =0.999) is obtained between lamotrigine concentration and ΔF (subtraction of fluorescence intensity in the presence and absence of lamotrigine) in the concentrations of 0.05−2.0 $\mu g \cdot mL^{-1}$. The regression equation is I_{545} = 131.29 $C_{LTG.}$ + 63.143, in which ΔF is the response intensity in arbitrary units, and C_{LTG} is the lamotrigine concentration in $\mu g.mL^{-1}$. The limit of detection (LOD) and limit of quantification (LOQ) calculated by $3s_b/m$ and $10s_b/m$ (in which s_b is the standard deviation of the blank and m is the calibration curve' slope), for the proposed method are 11.0 and 38.0 $ng \cdot mL^{-1}$, respectively. For the investigation of the method's precision, a sample with a known concentration of lamotrigine (*i.e.* 1.0 $\mu g \cdot mL^{-1}$) was analyzed five times on the different days and

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the same day. The inter-day and intra-day relative standard deviations (%*RSD*) for five measurements are 4.5% and 3.9%, respectively. The analytical features of some previously developed techniques based on nanoparticles for the analysis of lamotrigine are listed in Table 3 and compared with the newly developed technique in the current study. As can be seen, the linear range and LOD of the present technique are compared with those of other published techniques.

(Table 3 here)

Analysis of EBC samples taken from patients

To investigate the applicability of the validated DMF–Tb–Phen MOF based method for lamotrigine in the real samples, the probe was utilized to quantify lamotrigine in EBC samples from four patients consuming lamotrigine. Table 4 shows the analysis results along with recovery experiments for the investigation of the method's accuracy. For this purpose, EBC samples obtained from patients are spiked with a known amount of lamotrigine. As shown, the relative recoveries are between 99.0% to 102.8%, which confirms the method's accuracy.

(Table 4 here)

Conclusions

A fluorescent nanoprobe based on DMF–Tb–Phen MOF NPs is validated for lamotrigine analysis in EBC samples. The novelty of this work falls under the utilization of the developed nanoparticles in lamotrigine sensing in EBC sample as a newly introduced biological sample after optimization reaction conditions with a chemometrics method. This nanoprobe has great potential for lamotrigine determination owing to many good properties including high reliability, high sensitivity, and short signal time. The MOF based method was used for the quantification of lamotrigine in EBC from epileptic patients treated with lamotrigine and the obtained concentration is found to be in the range of 0.55 – 0.99 µg·mL−1 for the investigated patients EBC samples.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Authors' contributions

Parisa Eydi: The acquisition, analysis, drafting the work**, Elaheh Rahimpour:** design of the work, interpretation of data for the work. **Maryam Khoubnasabjafari:** Sampling from patient individuals, preparation of collected biological samples for analysis. **Vahid Jouyban-Gharamaleki**: Management of mechanical part of study, **Abolghasem Jouyban:** Design of the work, interpretation of data for the work**,**

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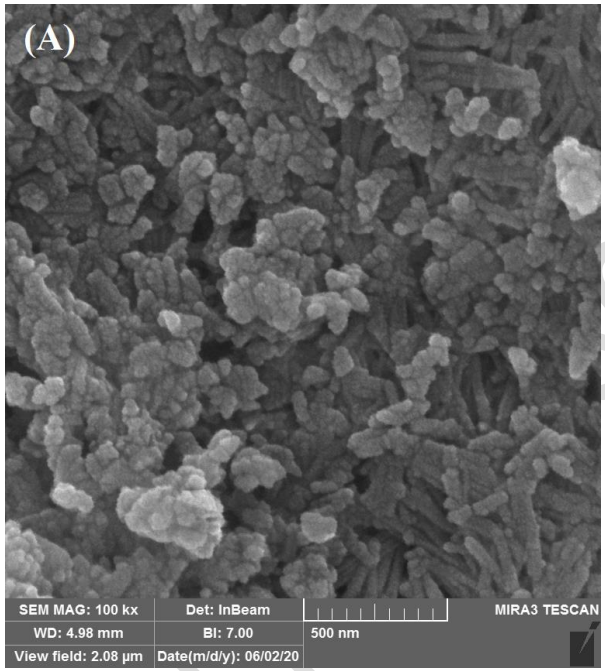

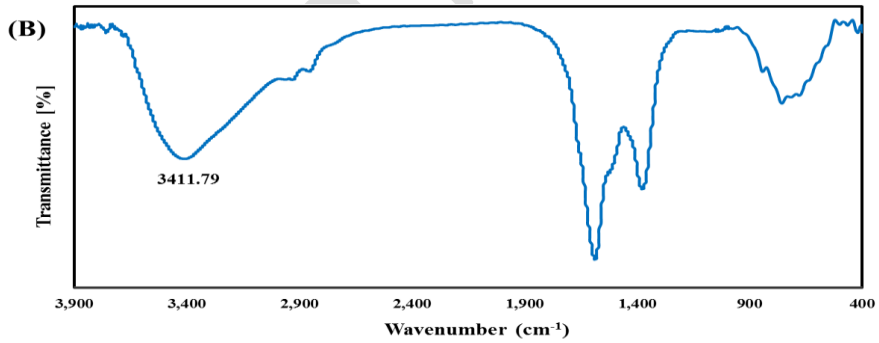

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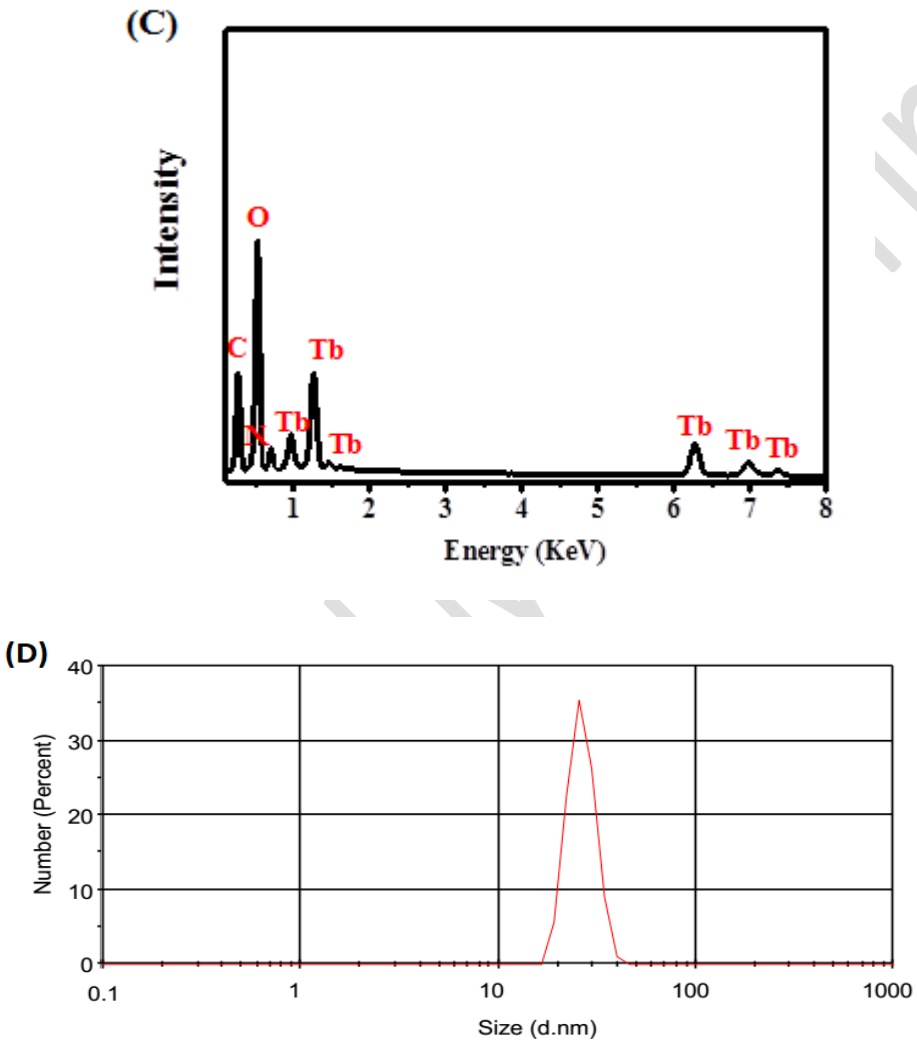

Figure 1 (A) SEM image; (B) FT-IR spectrum; (C) EDX (D) DLS of the DMF–Tb–Phen MOF NPs.

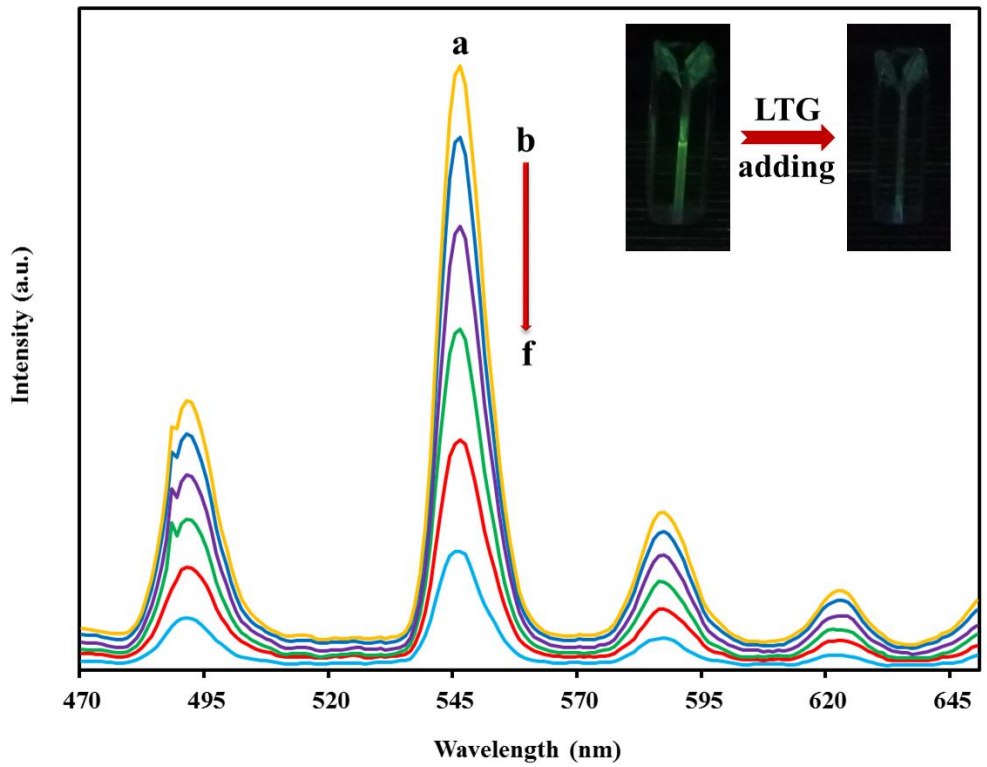

Figure 2. Fluorescence spectrum of the DMF–Tb–Phen MOF probe in the absence (a) and presence of lamotrigine in the concentration range of 0.05–2.0 µg mL^{-1} (b-f). Conditions: pH 5, [phosphate buffer]=0.01 mol L^{-1}, [MOF NPs]= 0.05 g L^{-1}, and time = 15 min, $\lambda_{ex}/\lambda_{em}$: 230/ 545 nm. Inset is the selected photograph of DMF–Tb–Phen MOF NPs in the absence and presence of lamotrigine.

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Table 1. Tolerance limits of some prescribed and over-the - counter drugs in the determination of 0.5 µg.mL^{-1} of lamotrigine

Interfering substance	Tolerance limit (× 0.5 µg.mL^{-1})
Rivaroxaban, Topiramate	250
Valproate sodium	150
Cetirizine, Celecoxib	100
Asprin, Lovastatin	50
Phenobarbital	30
Sildenafil citrate	20
Phenytoin	15
Bosentan, Nicotinamide, Diltiazem, Acetaminophen, Oxazepam, Clonazepam, Chlordiazepoxide, Ibuprophen	10
Ascorbic acid, Hydrochlorothiazide, Alprazolam, Pantoprazole, Carbamazepine	5
Diazepam,	2

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Table 2. Effect of coexisting inorganic ions and organic compounds on the determination of 0.5 $\mu g.mL^{-1}$ of lamotrigine using the developed method.

Compounds	Concentration range		Examined concentration of coexisting substance (µg/mL)	RE[a]%
	Healthy subjects (µg/mL)	Patient subjects (µg/mL)		
Acetone	0.10-2.60	0.10-19.80	40.0	2.4
Phosphate	0.09-14.24	-	16.0	4.3
Ammonia	0.40-1.30	0.81-14.70	30.0	3.8
Urea	-	0.04-4.19	8.0	4.2
Malondialdehyde	0.0004-0.0006	0.0004-0.0006	0.3	4.9
Arginine	0.022	0.02	10.0	5.0
NH_4^+	3.74-9.21	2.30-11.93	15.0	3.9
K^+	0.78-3.43	0.04-0.60	38.0	4.8
Ca^{2+}	0.20-0.59	0.08-1.254	40.0	3.6
Mg^{2+}	0.02-0.04	0.01-0.049	38.0	4.5
NO_2^-	0.01-0.08	0.05-0.52	20.0	4.2
NO_3^-	0.02-0.24	0.07-1.10	50.0	4.8
SO_4^{2-}	0.05-0.21	0.02-0.25	15.0	3.9
Fe^{3+}	0.02-0.16	0.004-7.25	30.0	3.8

[a] Relative error

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Table 3. Comparison of the analytical characteristics of the presented method with other reported techniques in the literature for lamotrigine determination.

Method	Nanomaterial	Real sample	Linear range ($\mu g \cdot mL^{-1}$)	LOD ($\mu g \cdot mL^{-1}$)	Reference
DPAdSV[a]	Ag NPs-CSPE[b]	Capsules	0.084 – 0.384	0.0952	24
DPV[c]	MMIP[d] NPs	Urine and plasma	2.56 – 256.09 ($\times 10^{-6}$)	0.768×10^{-6}	25
SWV[e]	GR-CPE[f]	Urine, plasma and capsules	0.013 – 76.8	0.389×10^{-3}	26
Spectrophotometery	ASA[g]-Ag NPs	EBC	0.02 – 0.4	0.005	9
Spectrofluorometery	ASA-Ag NPs and Au QDs[h]	Plasma	0.5– 6.0	0.3	10
Spectrofluorometery	N-doped graphene quantum dots	Serum and urine	0.002 – 0.045	0.00039	27
Spectrofluorometery	DMF–Tb–Phen MOF NPs	EBC	0.05 – 2.0	0.011	This work

[a] Differential pulse adsorptive striping voltammetry; [b] Silver nanoparticle-modified carbon screen-printed electrode; [c] Differential pulse voltammetry; [d] Magnetic molecularly imprinted polymer; [e] Square wave voltammetry; [f] Graphene-modified carbon paste electrode; [g] Amidosulfonic acid; [h] Gold quantum dots.

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Table 4. Details of the real samples and found concentration of lamotrigine in the EBC samples of patients receiving lamotrigine.

No.	Gender	Age (year)	Receiving dosage (mg)	Added (µg.mL^{-1})	Found (µg.mL^{-1})	Recovery (%)[a]
1	Female	50	25	–	0.55	–
				0.2	0.74	99.0
2	Female	48	25	–	0.68	–
				0.2	0.89	102.8
3	Female	41	75	–	0.99	–
				0.2	1.19	100.9
4	Female	39	50	–	0.74	–
				0.2	0.94	98.2

[a] Recovery (%)=[(Found–Base)/Added] × 100. "Base" and "Found" refer to the amount of the analyte in samples before and after spiking, respectively.

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