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ORIGINAL PAPER

Determination of tramadol in pharmaceutical products and biological samples using a new nanocomposite carbon paste sensor based on decorated nanographene/tramadol-imprinted polymer nanoparticles/ionic liquid

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Abstract In the present research, design and construction of a development potentiometric sensor based on a newly nanosensing layer for the sensitive determination of tramadol in various real samples were suggested. The proposed nanosensing layer was fabricated with the incorporation of a synthesized tramadol-imprinted polymer nanoparticles "as an efficient sensing agent" into the carbon paste matrix composed of graphite powder, decorated graphene nanosheets with silver nanoparticles, and a typically ionic liquid as the conductive pasting binder. The detection limit and the linear range of this study were found to be 2.04×10^{-9} and 3.50×10^{-9} to 1.00×10^{-2} M with a Nernstian slope of 59.85 ± 0.13 mV decade^{-1}, respectively. The presented modified carbon paste sensor was successfully applied for the determination of tramadol in pharmaceutical and biological samples.

Keywords Tramadol-imprinted polymer · Potentiometric sensor · Decorated nanographene · Silver nanoparticles · Ionic liquid

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Introduction

Determination of drug species in a wide variety of real samples such as biological and pharmaceutical samples, pharmaceutical industry products, and hospital residual waste is a very important branch of analytical chemistry for many applications (e.g., clinical, forensic science, quality production control, and environmental protection) [1–7]. Therefore, the introduction of new strategies for the design and construction of analytical tools for the accurate determination of drugs in trace level in these samples is an urgent need [4–7]. As a classic opioid and an analogue of codeine, tramadol (TRA) is a centrally acting analgesic in the treatment of moderate to severe pains [8]. Also, it is used as an anti-addictive by addicts and a severe dependence to TRA in addicts has been reported because of its effect on opioid receptors in central nervous system. According to the many reports in recent years, the overdose and poisoning of TRA have been significantly increased especially in young adults with a history of drug abuse and mental disorders [9]. The most common symptoms of TRA poisoning are including depression, nausea and vomiting, tachycardia, seizures, and prolonged hypoglycemia [10–12]. These serious side effects may occur because TRA can be accumulated in the body achieving critical levels of toxicity. Also, this toxic substance is a potent water polluter chemical agent which must be determined in wastewater in a water treatment process and then removed from it [13]. Consequently, it is desired to develop the simple, efficient, eco-friendly, and accurate methods with high sensitivity and selectivity for the determination of trace levels of TRA in real samples. Various quantitative instrumental techniques including high-performance liquid chromatography (HPLC) [14–16], liquid chromatography–mass spectroscopy (LC-MS) [17], gas chromatography–mass spectroscopy (GC-MS) [18–20], capillary electrophoresis with

electrochemiluminescence detection [21], spectrophotometry [22, 23], and fluorescence [24, 25] have been reported for the determination of TRA. However, most of these common instrumental techniques are too expensive that they are not applicable for in situ analysis and also need time-consuming procedures. On the other hand, the ideal analytical tool for analysis applications should provide a fast, reliable response, preferably at the point of sample collection, and should be advisedly affordable. These problems could be solved by the application of the electrochemical methods, which are relatively simple, low cost, and readily available techniques [26–42]. Among the various electrochemical methods, potentiometric carbon paste electrodes (CPEs) can offer the straight advantage of being able to distinguish one specific species in complex mixtures. Also, potentiometric CPEs are considered to have technical simplicity, good sensitivity, and stability in response, chemical inertness, robustness, and easy adaptability for in situ analysis with relatively inexpensive instrumental setups [3, 4]. The key component of any potentiometric CPEs is the sensing layer. The composition of the sensing layer is of unconditional importance in order to obtain a highly sensitive and selective potentiometric sensor. Potentiometric CPEs have been widely considered as efficient tools for the trace-level determination of various target species. The operation mechanism of such potentiometric CPEs depends on the properties of the modifier(s) used to improve the selectivity and sensitivity toward the target species. Recently, research activities are related to the optimization and rational design of the paste components and matrices targeted to particular applications [28–42]. On the other hand, due to the promotion of the performance of potentiometric CPEs, many attempts have been made by several alterations of the kind of methods. For example, various materials such new synthesized Schiff bases, ion exchangers, nanomaterials, molecularly imprinted polymers (MIPs), and ion-imprinted polymers (IIPs) into the paste structure of the potentiometric CPEs have been used as efficient modifiers [3, 4, 28–42]. A considerable number of research reports were raised recently, underlining the importance of utilizing graphene for potentiometric CPEs and proving the suitability of the material for the detection of species [4, 39–42]. But, there is still an urgent demand and new strategies for highly selective and sensitive modifiers in this field. A high degree of selectivity is particularly desirable for extraction of a single compound from a complex matrix [43].

In the present research, we introduced a new strategy for construction of a developed tramadol-selective potentiometric CPE based on a novel nanosensing layer including graphite powder, 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide "[BMP]Tf_2N" as the conductive pasting binder, synthesized tramadol-imprinted polymer nanoparticles (TRA-MIP NPs), and decorated graphene nanosheets with silver nanoparticles (GNS@Ag NPs). It was expected that replacing paraffin oil with [BMP]Tf_2N into the paste structure would improve the potentiometric response of the sensor. Also, the application of GNS@Ag NPs and TRA-MIP NPs in the paste composition could amplify the signal significantly.

Experiment

Instrumentation

The glass cell in which the potentiometry was carried out into contained an Ag/AgCl electrode (Azar electrode, Iran) as a reference electrode and fabricated modified TRA-selective potentiometric CPE as the working electrode for the determination of TRA. Both of the electrodes were connected to a digital millivoltmeter (HIOKI 3256.50). Voltammetric measurement was performed using an Autolab PGSTAT 302N potentiostat/galvanostat (302N, Utrecht, Netherlands). A Metrohm pH meter (CRISON GLP 22, Swiss) with a combined glass electrode was used for pH controlling, and a Heidolph type of stirrer (MR 2000, Germany) was used for stirring the solutions. Transmission electron microscopy (TEM; Philips, CM10 and 100 kV) was performed to assay the morphology and structure of the prepared TRA-MIP NPs, GNSs, and GNS@Ag NPs. Energy dispersive X-ray spectroscopy (EDX) analysis was performed using a FEI Quanta 250 FEG. A DIONEX HPLC instrument was used for chromatographic analysis of TRA samples.

Reagents and materials

Natural graphite powder (98%, 50 mesh, and 2–5 mm in lateral size) was obtained from Hyundai Coma Ind. Co., Korea, high-purity paraffin oil (Aldrich, USA), and the room temperature ionic liquids)RTILs(of [BMP]Tf_2N (Merck Company) were used for the preparation of carbon pastes. Nanographites (NGPs) were supplied by Angstron Materials LLC, OH, USA. The thickness of the NGPs was smaller than 100 nm. Potassium permanganate ($KMnO_4$), sodium nitrate ($NaNO_3$), $NaBH_4$, ethylene glycol (EG), and silver nitrate ($AgNO_3$) were received from the Showa Chemical Co., Tokyo, Japan. Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 2-2′-azoisobutyronitrile (AIBN), chloroform, toluene, acetonitrile (ACN), methanol, and acetic acid from Merck (Darmstadt, Germany) were used. TRA tablets (Tehran Daru, Iran) were purchased from the local drugstore. Double-distilled water (DDW) was used throughout all experiments. For preparing of tramadol base (as the template for imprinting process), tramadol hydrochloride solution (100 mg mL^{-1}) was alkalinized with NaOH (1 M). In following, the resulted precipitate was washed several times with

DDW to remove the water-soluble impurities and finally dried at room temperature in darkness.

Preparation of graphene oxide from NGPs

Graphene oxide (GO) was prepared by a modified Hummers method [44, 45] as follows: 4 g of NGPs and 3 g of $NaNO_3$ (35 mmol) were combined in a four-necked 1-L flask with 600 mL of H_2SO_4 (98%). The mixture was magnetically stirred at 0 °C in an ice water bath. $KMnO_4$ (18 g, 113 mmol; purity 99%) was added to mixture slowly over 1 h, and then cooled for 2 h. The mixture was stirred for 2 days at room temperature to obtain a highly viscous fluid. The flask was placed in an oil bath at 60 °C to reduce viscosity, and 240 mL of DDW was added slowly. The resulting solution was added to 3 L of H_2O_2 (5% *w*/*v*) while stirred in an ice bath, and the resultant mixture was stirred for another 2 h. In order to remove oxidant ions, particularly manganese ions, the resultant liquid was purified by repeating the following procedure cycle 10 times: removal of the supernatant liquid, addition of a mixed aqueous solution of H_2O_2 (500 mL, 5% *w*), and centrifugation at 10,000 rpm for 30 min. In final, after completing 10 cycles, re-dispersed GO in DDW and a dispersive solution was obtained.

Preparation of GNSs and GNS@Ag NPs

According to the literature [46], 50 mg of GO, 0.9 mM of $AgNO_3$, and 200 mL of DDW were mixed in a 500-mL four-necked flask under a nitrogen atmosphere; the mixture was sonicated for 1 h. Then, 50 mL of $NaBH_4$ (13 mmol) was added slowly, and the reaction mixture was stirred at 100 °C for 24 h. The solid GNS@Ag product was isolated by 10 times repeated centrifugation (10,000 rpm). The GNSs were prepared using the same manner in absence of $AgNO_3$.

Synthesis of TRA-MIP NPs and non-imprinted polymer

TRA-MIP NPs were synthesized according to the literature [47] as follows: 0.36 mmol of TRA (as the template molecule) and MAA as the functional monomer (1.44 mmol) were dissolved in a solvent and stored at room temperature in the darkness for 12 h to form effective template–monomer complexes before polymerization. Then, 3.56 mmol of EGDMA (as the cross-linker) and 40 mmol of AIBN (as the initiator) were added to the solution. The mixture was sonicated in a bath sonicator at 60 °C for 15 min, purged with nitrogen gas for 5 min, and sealed while nitrogen gas was blown. After polymerization, the obtained particles were washed with 2 × 10 mL of acetone and then were washed with a methanol-acetic acid mixture (90–10, *v*/*v*). In order to remove the supernatant, the suspension was centrifuged at 15,000 rpm for 25 min. Washing step was continued to the extraction of the TRA from the polymer network. The template extraction of the polymer created the cavities, leading to the specific sorption of the template. Also, the removal of the residual of other compounds from the polymer took place. Finally, the particles were washed with methanol to remove residual acetic acid and dried at room temperature for 24 h. A control non-imprinted polymer (NIP) was prepared using the same manner without the presence of the template (TRA).

Fabrication of the TRA-MIP CPE

The proposed TRA-MIP CPE was prepared by thorough hand mixing different amounts of TRA-MIP NPs along with appropriate amounts of graphite powder (GP), paraffin oil (PO) or [BMP]Tf_2N RTIL, GNSs, and GNS@Ag NPs in a mortar using a pestle. A portion of the prepared nanocomposite mixture was packed firmly into an insulin syringe with an internal diameter of 2.5 mm and a height of 3 cm as a piston-driven CPE holder. The proposed homogenized paste was carefully packed into the syringe tip to avoid possible air gaps, which often enhance resistance of the electrode. A copper wire was inserted into the opposite end of the CPEs to establish electrical contact. The external surface of the carbon paste was smoothed with soft paper. A new surface was produced by scraping out the old surface and replacing the new carbon paste. The surface of fresh CPEs was preconditioned by exposure to a 1.00×10^{-3} M TRA solution for 1 h, and then, the electrode was rinsed with DDW. A fresh electrode surface was obtained by squeezing. The surplus of paste was cut out and the exposed end was polished on a paper until the surface showed shiny appearance.

Electrode system and EMF measurements

The performance of the presented potentiometric TRA-MIP CPE was assayed by measuring the EMFs of TRA solution in range of 1.00×10^{-10}–1.00×10^{-1} M. All EMF measurements with the proposed TRA-MIP CPE were carried out at 25 °C with the following potentiometric cell assembly:

Ag, AgCl, KCl (satd.) | sample solution | (GNS@Ag NPs / TRA-MIP NPs / [BMP]Tf_2N RTIL)-modified CPE

Results and discussion

The presented TRA-MIP CPE was used to the determination of TRA as a classic opioid drug. The species levels of TRA were sensed by a suggested potentiometric TRA-selective sensor. However, when MIP materials are used as a sensing agent, their strength and kind of binding to the target analyte species should be associated with the general behavior of the corresponding CPEs. In the present study, we synthesized TRA-MIP NPs by precipitation polymerization method and

were characterized by TEM technique (Fig. 1a) and finally used as a high selective sensing agent toward TRA into the newly TRA potentiometric CPE composed of a suggested novel nanocomposite. Also, for the fabrication of a new nanographene potentiometric CPE, the decorated GNSs with silver NPs (GNS@Ag NPs) were prepared as carbon base of CPE and characterized by TEM technique. The TEM images in Fig. 1b, c exhibit the stacking of GNS and GNSs coated with silver NPs, respectively. The TEM image of Fig. 1c shows that silver nanoparticles are well distributed at the GNS surface. Also, to investigate the Ag content in the prepared GNS@Ag NPs, we have used EDX. The elemental plot of Fig. 2 clearly indicates the presence of Ag NPs on graphene nanosheets, confirming preparation of coated GNSs with Ag NPs.

Finally, the presented TRA-MIP CPE "(GNS@Ag NPs/TRA-MIP NPs/[BMP]Tf_2N RTIL)-modified CPE" was applied for the determination of TRA. For this purpose, the potentiometric responses of the conventional bare CPE and presented modified CPE toward TRA were studied in terms of linear range, detection limit, selectivity coefficients, response time, lifetime, and response stability which are important characterizations of every ion-selective CPE.

Surface area study

In order to obtain the electrochemical active surface areas of both the bare and modified CPEs, the cyclic voltammograms (CVs) of $K_4[Fe(CN)_6]$, as a redox probe, were performed using the Randles–Sevcik equation (Eq. 1):

$$\mathrm{Ipa} = \left(2.69 \times 10^5\right) n^{3/2} A C^* D^{1/2} v^{1/2} \quad (1)$$

The CVs for bare CPE and GNS@Ag NPs/TRA-MIP NPs/[BMP]Tf_2N RTIL)-modified CPE are shown in Fig. 3a, b, respectively. In the Randles–Sevcik equation, Ipa refers to the anodic peak current, n is the total number of electrons transferred (n = 1), A is the effective surface area of the electrode, D is the diffusion coefficient for $K_4[Fe(CN)_6]$ = 7.6 × 10^{-6} cm^2 S^{-1}, C^* is the concentration of $K_4[Fe(CN)_6]$, and v is the scan rate. The surface area of GNS@Ag NPs/TRA-MIP NPs/[BMP]Tf_2N RTIL)-modified CPE (0.19 cm^2) is higher than the CPE (0.062 cm^2), and the increase of the electroactive surface area of the modified electrode showed the influence of the modifiers that provides a large surface and facilitates the electron transfer between surface of the electrode and the solution. It was concluded that the functionalization of GNS with Ag NPs has made the realization of nanoscale composite electrodes possible.

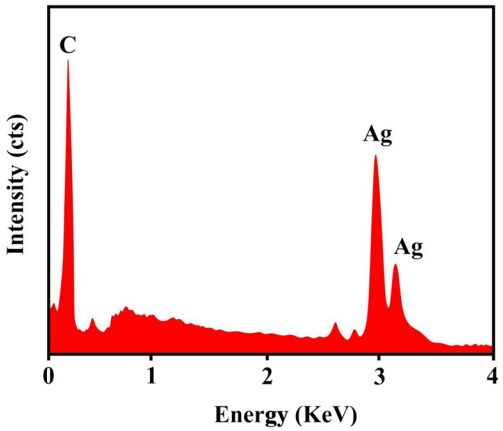

Fig. 2 EDX image of the graphene nanosheet coated with silver nanoparticles confirming the presence of Ag and C

Effect of the electrode composition

It has been well known that the potentiometric response of the CPEs depends significantly on nature and the amounts of the sensing agent or receptor in the paste mixture and also other components of the electrode composition [28–42]. Hence, in

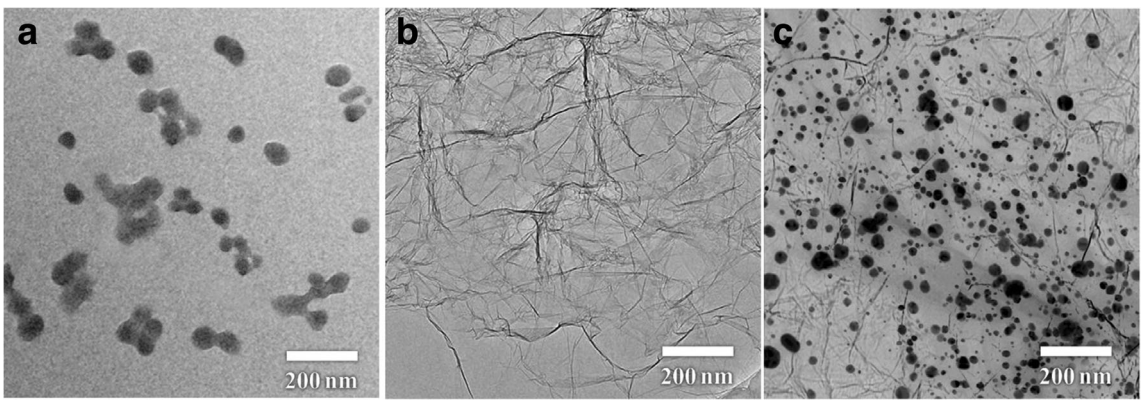

Fig. 1 TEM images of the tramadol-imprinted polymer nanoparticles (**a**), graphene nanosheet (**b**), and graphene nanosheet coated with silver nanoparticles (**c**)

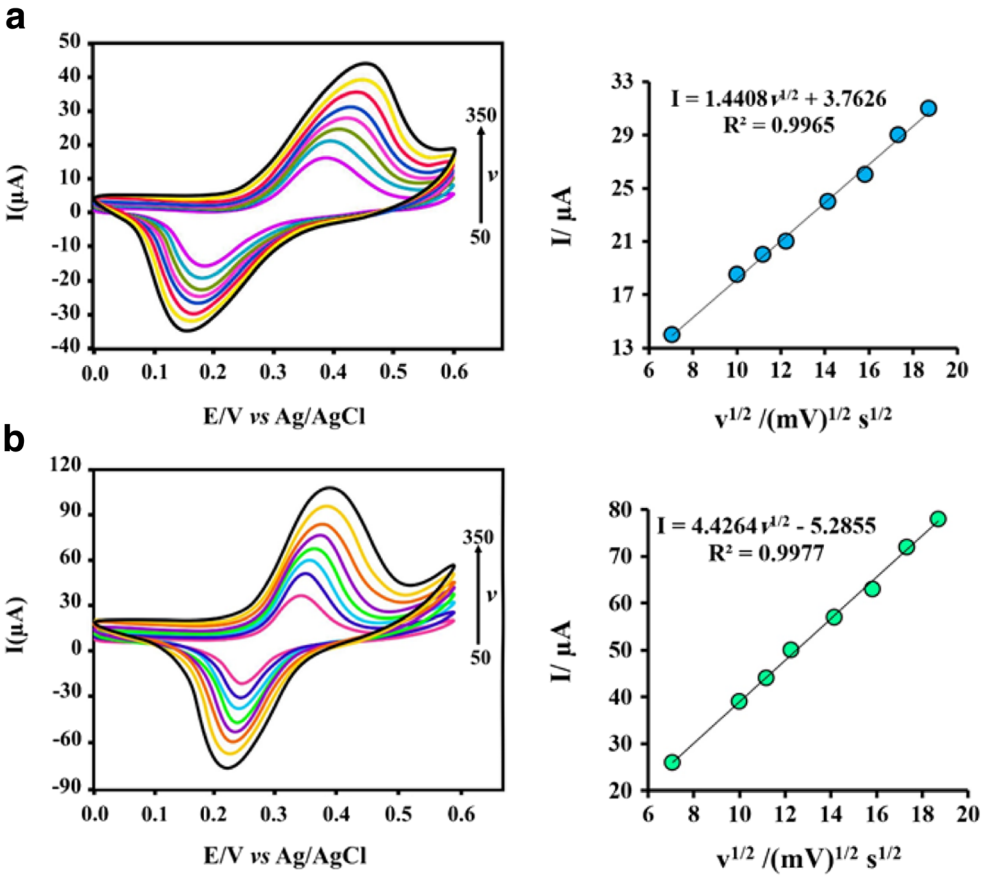

Fig. 3 Cyclic voltammograms and curves of I vs. $v^{1/2}$ of **a** bare CPE and **b** GNS@Ag NPs/TRA-MIP NPs/[BMP]Tf_2N RTIL)-modified CPE in 0.001 M $K_4[Fe(CN)_6]$ and 0.1 M KCl at different scan rates from 50 to 350 mV/s

order to obtain the best performance of the proposed electrode, several electrodes with different carbon paste compositions were prepared and investigated. The results are summarized in Table 1.

As can be seen from Table 1, the electrode without TRA-MIP modifier (no. 1) exhibited a low potentiometric response toward TRA. Clearly, an increase at the TRA-MIP level in the paste composition (nos. 1–3) caused a slope increase of the calibration curve. On the other hand, the potentiometric sensitivity of the proposed TRA-selective sensor was improved. This indicated that the TRA-MIP is the most important component in the paste composition for sensing of TRA. Further addition of the TRA-MIP, however, resulted in a little decrease in the response of the electrode. This phenomenon can arise

Table 1 Compositions of the paste used in the fabrication of the presented TRA potentiometric CPE

Electrode no.	Binder (%)	MIP (%)	GP (%)	GNSs (%)	GNS@Ag NPs (%)	Slope (mV/decade)
1	25- PO	0.0	75	0.0	0.0	6.91 ± 0.27
2	25- PO	10	65	0.0	0.0	24.88 ± 0.30
3	25- PO	12	63	0.0	0.0	33.52 ± 0.19
4	25- PO	13	62	0.0	0.0	32.79 ± 0.26
5	25- [BMP]Tf_2N	12	63	0.0	0.0	38.61 ± 0.31
6	30- [BMP]Tf_2N	12	58	0.0	0.0	37.29 ± 0.24
7	25- [BMP]Tf_2N	12	58	5.0	0.0	45.58 ± 0.17
8	25- [BMP]Tf_2N	12	53	10.0	0.0	52.84 ± 0.20
9	25- [BMP]Tf_2N	12	53	0.0	10.0	57.72 ± 0.18
10	25- [BMP]Tf_2N	12	52	0.0	11.0	58.65 ± 0.21
11	*25- [BMP]Tf_2N*	*12*	*51*	*0.0*	*12.0*	*59.85 ± 0.13*
12	25- [BMP]Tf_2N	12	50	0.0	13.0	59.47 ± 0.16

Optimized values are italicized

from the decrease of graphite powder/TRA-MIP ratio, thereupon decreases of paste homogeneities and conductivity. The same experiment was carried out in the case of the sensor fabricated with NIP. The results showed that the potentiometric response obtained for the MIP-CPE is noticeably higher than that of the NIP-CPE, indicating the existence and proper functioning of the selective cavities in the MIP, created in the polymerization step.

In recent studies, for improvement or the potentiometric performance of CPEs, the graphene compounds such as GNSs can be used as excellent candidates of modifier for the modification of CPEs owing to their superior electrical conducting ability and high specific surface area [4, 39–42]. On the other hand, GNSs increased the available surface area and also improved the conductivity properties of the electrode surface and, therefore, conversion of the chemical signal to an electrical one. The electrical conductivity of GNSs is due to the delocalized π-electrons throughout the entire structure [46]. The conductivity behavior of GNSs relates to the degree of graphene structure and to the heterocarbon component. In this study, a strategy was designed to improve the performances of GNSs though decorating silver NPs on GNSs, and then, it was incorporated into the paste composition. Silver NPs act as a useful nanospacer and conductor, which not only increase the interlayer distance but also improve the electrical conductivity between layers [42]. The results obtained in this study showed that the best potentiometric response of the proposed TRA-MIP potentiometric CPE was obtained for a 12.0 wt.% of the GNS@Ag NPs.

In the past years, RTILs have been proposed to be very interesting and efficient pasting binders in place of non-conductive organic binders such as PO for the fabrication of potentiometric CPEs [42, 32–38]. As can be seen from Table 1, using RTILs instead of PO in the paste composition indicates the better potentiometric response of the proposed TRA-MIP CPE. This is probably due to the much higher dielectric constant, high ionic conductivity, and good electrochemical and thermal stabilities of RTIL, and they may be a better binder compared to non-conductive organic binders. Also, when RTIL was mixed with GP and other components of the paste structure, it not only acted as a binder to bind the components together but also filled the void spaces between the components to form an excellent charge-transfer bridge in the bulk of the CPE [48]. A layer of RTIL was also formed on the surface of CPE. So, the significant improvement of the reversibility and sensitivity was achieved on the decorated nanographene/TRA-MIP NPs.

Consequently, the (GNS@Ag NPs/TRA-MIP NPs/[BMP]Tf_2N RTIL)-modified CPE percentage ratio of 25% [BMP]Tf_2N, 12% TRA-MIP NPs, 51% GP, and 12% GNS@Ag NPs (no. 11) indicated best performance with a Nernstian slope of 59.85 ± 0.13 mV/decade.

Calibration graph and detection limit of the presented TR-MIP potentiometric CPE

Measuring range and detection limit (DL) of the presented modified CPE for TRA (Table 1, no. 11) was evaluated in the concentration range of 1.00×10^{-10}–1.00×10^{-1} M. The applicable linear dynamic range of the proposed TRA-MIP potentiometric CPE extends from 3.50×10^{-9} to 1.00×10^{-2} M of TRA with the calibration equation of $y = 59.85x + 802.56$ and $R^2 = 0.997$, within the Nernstian slope of 59.85 ± 0.13 mV decade^{-1} at 25 ± 1 °C as seen in Fig. 4. The standard deviation for six replicates was 0.13 mV. The DL was 2.04×10^{-9} M, which was calculated by the extrapolating of the two segments of the calibration curve in Fig. 4.

Effect of the pH solution on the electrode response

An important factor in the performance of potentiometric sensors is the pH value of the test solution [26–40]. To investigate the pH effect on the response of the presented TRA-MIP potentiometric CPE (Table 1, no. 11), the potentials were measured for fixed concentration of TRA solution (1.00×10^{-5} M) at different pH values (Fig. 5). The pH was varied from 2.00 to 10.00 by the addition of diluted solutions of nitric acid and/or sodium hydroxide. The electrode composition was kept constant during all experiments. It is clear from Fig. 5 that the potential remains constant in the range in the range of pH = 3.50–6.00, which may be taken as the working pH range of the electrode assembly, as no interference from H^+ or OH^- was observed in this range. The fluctuations at pH values lower than 3.50 can be attributed to the protonation of MIP active sites in the carbon paste.

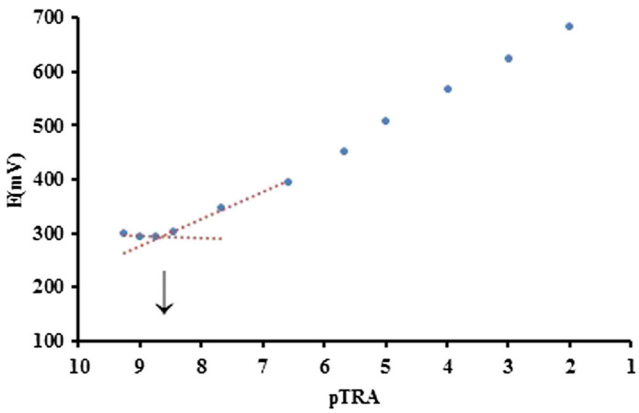

Fig. 4 Calibration curve of the presented TRA potentiometric CPE (no. 11)

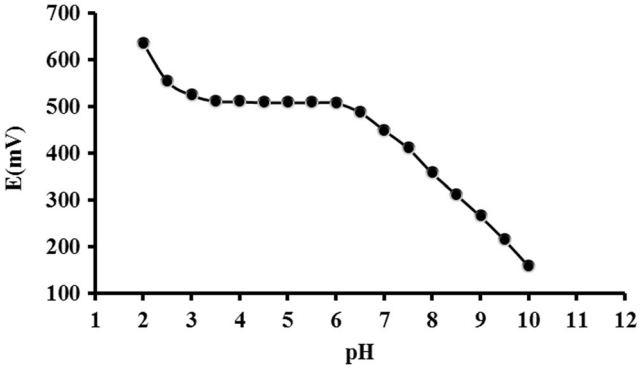

Fig. 5 Effect of pH on cell potential of the presented TRA potentiometric CPE (no. 11)

Response time and reversibility of the presented TR-MIP potentiometric CPE

The response time is a significant factor for any potentiometric sensor in analytical application [37–40]. The practical response time (the time required to achieve a 90% of the final steady potential value of the electrodes after successive immersion in TRA solutions, each having a 10-fold difference in concentration), has been recorded at different concentrations of TRA. In this study, the practical response time (for sensor no. 11) was recorded by changing the TRA concentration in solution, over a concentration range of 1.00×10^{-5} to 1.00×10^{-3} M (Fig. 6). As it is seen, the electrode reached the equilibrium response in a very short time of about 5 s. To evaluate the reversibility of the electrode, a similar procedure in the opposite direction was adopted. According to our previous papers [37–40], the measurements were performed in the sequence of high-to-low (1.00×10^{-3} to 1.00×10^{-5} M) concentrations. The results exhibited that the response of the electrode was reversible; although the time needed to reach equilibrium values (about 16 s) was longer than that for low-to-high sample concentrations (about 5 s), because residual analyte target will still be adsorbed on the surface of the

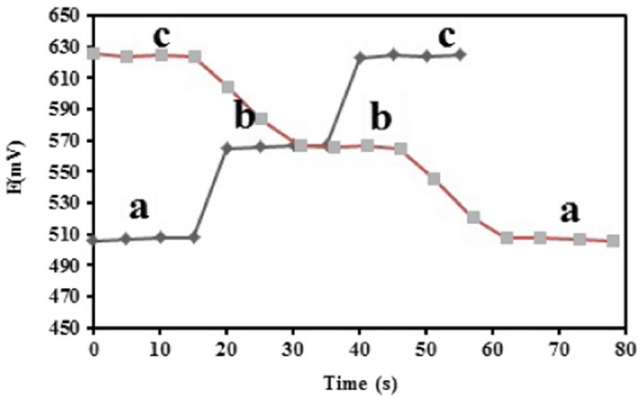

Fig. 6 Response time of the presented TRA potentiometric CPE (no. 11) for step changes in TRA concentration (from low to high and vice versa). **a** 1.00×10^{-5} M. **b** 1.00×10^{-4} M. **c** 1.00×10^{-3} M

presented modified potentiometric CPEs, and cause to occupy the site of surface of the indicator electrode significantly, the proposed electrodes cannot sense the analyte target that exists in the bulk of the solution promptly, which will lead to less response time.

Selectivity behavior of the (GNS@Ag NPs/TRA-MIP NPs/[BMP]Tf_2N RTIL)-modified CPE

As we know, an important characteristic of any potentiometric sensor is its response to the primary ion in the presence of other species in the test solution, which is expressed in terms of the potentiometric selectivity coefficient [28–42]. In this study, the potentiometric selectivity coefficients of the presented TRA-MIP potentiometric CPE toward different species were evaluated by the matched potential method (MPM) [42]. As shown in Table 2, all interfering species applied in this study would not affect the selectivity of the presented electrode and have a small value of selectivity coefficient in the most cases. On the other hand, the values in Table 2 reflect a very high selectivity of this electrode for reliable quantification of TRA over a wide variety of other species.

Lifetime and long-term stability of the (GNS@Ag NPs/TRA-MIP NPs/[BMP]Tf_2N RTIL)-modified CPE

Another important factor of the potentiometric sensor is the lifetime and long-term stability of the electrode that is generally evaluated in these methods [28–42]. The lifetime and long-term stability of the presented TRA-MIP potentiometric CPE were assayed by periodically recalibrating in series standard solutions of TRA and calculating the slope of the optimized CPE over a period of 24 weeks in a linear dynamic range of 3.50×10^{-9} to 1.00×10^{-2} M TRA solutions. During this period of time, the electrode was used weekly. The electrode was gently washed with distilled water, dried, and stored at room temperature when not in use. The results exhibited that the slope of the electrode responses before 21 weeks was reproducible, and no significant drift and also

Table 2 Selectivity coefficients ($K_{TRA,I}$) of various interfering species for the presented TRA potentiometric CPE

Interference cation (I)	$K_{TRA,I}$ by MPM
Diphenhydramine	1.63×10^{-6}
Dextromethorphane	3.29×10^{-6}
Bromhexine	1.37×10^{-6}
Gabapentin	4.51×10^{-6}
Imipramine	4.18×10^{-6}
Glucose	5.32×10^{-7}
Lactose	8.71×10^{-7}

no significant change in the performance of the electrode were observed and the electrode potential was stable for this period of time (Fig. 7). After this time, there was a slight gradual decrease in the slopes from 59.85 to 58.61 mV decade^{-1}. This shows that the lifetime of the presented TRA-MIP CPE was more than 5 months.

Reproducibility of the (GNS@Ag NPs/TRA-MIP NPs/[BMP]Tf_2N RTIL)-modified CPE

In order to evaluate the reproducibility of presented TRA-MIP potentiometric CPE, a series of finally modified sensors (six) with an optimal composition of the paste (Table 1, no. 11) were fabricated and the responses of these sensors were assayed to TRA concentration. The results indicated the average of slopes, detection limits, and linear dynamic ranges were 59.85 ± 0.13 mV decade^{-1}, 2.04 (±0.17) × 10^{-9} M, and 3.50 (±0.19) × 10^{-9} to 1.00 (±0.22) × 10^{-2} M, respectively.

Precision and accuracy of the (GNS@Ag NPs/TRA-MIP NPs/[BMP]Tf_2N RTIL)-modified CPE

The precision of the proposed sensor was evaluated by caring out an analysis using standard working solutions, the same sensors, and the same conditions. The R.S.D. of <1% was observed for six measurements. The results indicate that the suggested method is capable of producing results with high precision. Also, the accuracy was expressed in terms of percentage of absolute errors obtained in the measurement of known concentrations. The obtained results are within the acceptable range of <1%.

Comparison studies with reported electrodes

A comparison between the analytical parameters of the presented TRA-MIP potentiometric CPE (Table 1, no. 11) and a few previous potentiometric [1, 49, 50] and voltammetric electrodes [26, 27] reported in the literature for the determinations of TRA are given in Table 3. It is clear that in all cases, the performances of the presented TRA-MIP potentiometric CPE exhibit superior behavior if compared with the some previously reported TRA electrodes.

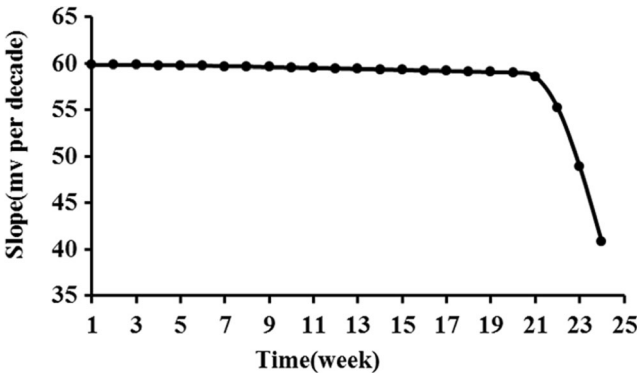

Fig. 7 The lifetime of the presented TRA potentiometric CPE (no. 11)

Evaluation of the analytical performance

In order to assay the analytical applicability of the presented TRA sensor, it has been applied for the direct analysis of TRA in different kinds of a pharmaceutical formulation (TRA tablet) and also biological samples (human urine).

To prepare the pharmaceutical samples of TRA, certain numbers of tablets were disintegrated to a homogeneous fine powder in a mortar. Then, a suitable amount of the tablet powder was accurately weighed and dissolved in 100 mL water by ultrasonication. After mixing completely, the mixture was filtered using filter paper, and 10 mL of each sample was subsequently transferred into a 100-mL volumetric flask and diluted to the mark with DDW. Then, an aliquot of the solution was transferred into the potentiometric cell for analysis.

Urine samples were stored in a refrigerator immediately after their collection. Of the sample, 10 mL was centrifuged for 20 min at 2000 rpm. The supernatant was filtered using a 0.45-μm filter, then diluted five times with DDW, and adjusted at optimal pH. Then, an aliquot of the solution was transferred into the potentiometric cell to be analyzed without any further pretreatment.

The potentiometric determination of TRA was carried out by the standard addition method. The obtained results are given in Table 4. According to this table, the satisfactory recoveries and the good agreements between the obtained value and the manufacturer's value (was given as 100 mg per tablet) for TRA tablet showed that the presented TRA sensor had great potential in the practical sample analysis.

The presented TRA sensor was also successfully applied to obtain recoveries of TRA in urine samples. According to results of Table 4, proposed sensor provides a good alternative for the detection of TRA in biological samples. In addition, HPLC method was used for the analysis of the samples to confirm the accuracy of the proposed method.

Conclusions

Herein, the excellent characteristics of nanographene-composite (decorated GNSs with silver nanoparticles) were used to construct a highly sensitive novel potentiometric TRA-MIP CPE for the determination of TRA. In summary,

Table 3 Comparison of some characteristics of the presented TRA potentiometric CPE with those for some previously reported TRA potentiometric electrode

DL (M)	Linear range (M)	Slope (mV/decade)	Lifetime (weeks)	Response time (s)	Reference
2.04×10^{-9}	3.50×10^{-9}–1.00×10^{-2}	59.85	21	About 5	Present work
6.00×10^{-8}	1.00×10^{-7}–1.00×10^{-3}	56.30	8	About 23	[1]
4.00×10^{-9}	1.00×10^{-8}–2.00×10^{-5}	–	Not reported	Not reported	[26]
3.60×10^{-9}	1.00×10^{-8}–9.00×10^{-6}	–	4	Not reported	[27]
1.80×10^{-6}	5.50×10^{-6}–1.00×10^{-1}	56.50	Not reported	About 8	[47]
2.10×10^{-6}	5.50×10^{-6}–1.00×10^{-1}	60.30	Not reported	About 8	[48]

Table 4 Results of determination of TRA in real samples by presented TRA potentiometric CPE

Samples	Added	Found[a]	Recovery (%)	HPLC method
Tablet (1)	–	98.76 ± 0.24 mg	–	98.79
	50 mg	147.87 ± 0.19 mg	98.2	147.96
	150 mg	250.92 ± 0.31 mg	101.4	251.11
Tablet (2)	–	98.21 ± 0.17 mg	–	98.25
	50 mg	147.53 ± 0.30 mg	98.6	147.58
	150 mg	251.35 ± 0.25 mg	102.1	251.41
Urine (1)	–	$4.52 (\pm 0.22) \times 10^{-5}$ M	–	4.55×10^{-5}
	2.50×10^{-5} M	$6.95 (\pm 0.26) \times 10^{-5}$ M	97.2	6.99×10^{-5}
	5.00×10^{-5} M	$9.58 (\pm 0.17) \times 10^{-5}$ M	101.2	9.60×10^{-5}
Urine (2)	–	$5.10 (\pm 0.20) \times 10^{-5}$ M	–	5.08×10^{-5}
	2.50×10^{-5} M	$7.56 (\pm 0.16) \times 10^{-5}$ M	98.4	7.53×10^{-5}
	5.00×10^{-5} M	$10.19 (\pm 0.29) \times 10^{-5}$ M	101.8	10.16×10^{-5}

[a] Average of four replicable measurements

we prepared a nanocomposite of graphene via three sequential steps. First, the GO was synthesized. In next step, the GNSs were prepared from GO, and finally, in the last step, the prepared GNSs were coated with silver NPs (GNS@Ag NPs). And also, TRA-MIP NPs were prepared as an efficient sensing element toward tramadol. In following, the prepared nanographene composite was incorporated into the proposed TRA potentiometric CPE consisting of graphite powder, [BMP]Tf_2N, and prepared TRA-MIP NPs. Finally, the high degree of TRA selectivity by the (GNS@Ag NPs/TRA-MIP NPs/[BMP]Tf_2N RTIL)-modified CPE makes it potentially useful for monitoring concentration levels of TRA in pharmaceutical and biological real samples without significant interactions from other interference species present in the samples. However, the presented new modified CPE has been shown to have good operating characteristics in terms of sensitivity, lifetime, response time, detection limit, and linear concentration range.

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References

1. Ganjali MR, Larijani B, Pourbasheer E (2016) Fabrication of an all solid state (ASS) polymeric membrane sensor (PME) for tramadol and its application. Int J Electrochem Sci 11:2119–2129
2. Bagheri H, Afkhami A, Panahi Y, Khoshsafar H, Shirzadmehr A (2014) Facile stripping voltammetric determination of haloperidol using a high performance magnetite/carbon nanotube paste electrode in pharmaceutical and biological samples. Mater Sci Eng C 37:264–270
3. Afkhami A, Shirzadmehr A, Madrakian T (2014) Improvement in performance of a hyoscine butylbromide potentiometric sensor using a new nanocomposite carbon paste: a comparison study with polymeric membrane sensor. Ionics 20:1145–1154
4. Bagheri H, Shirzadmehr A, Rezaei M (2015) Designing and fabrication of new molecularly imprinted polymer-based potentiometric nano-graphene/ionic liquid/carbon paste electrode for the determination of losartan. J Mol Liq 212:96–102
5. Khoshsafar H, Bagheri H, Rezaei M, Shirzadmehr A, Hajian A, Sepehri Z (2016) Magnetic carbon paste electrode modified with a high performance composite based on molecularly imprinted carbon nanotubes for sensitive determination of levofloxacin. J Electrochem Soc 163:B422–B427
6. Fouladgar M, Karimi-Maleh H (2013) Ionic liquid/multiwall carbon nanotubes paste electrode for square wave voltammetric determination of methyldopa. Ionics 19:1163–1170
7. Beitollahi H, Mohadesi A, Mostafavi M, Karimi-Maleh H, Baghayeri M, Akbari A (2014) Voltammetric sensor for

simultaneous determination of ascorbic acid, acetaminophen, and tryptophan in pharmaceutical products. Ionics 20:729–737
8. Leppert W (2009) Tramadol as an analgesic for mild to moderate cancer pain. Pharmacol Rep 61:978–992
9. Shadnia S, Soltaninejad K, Heydari K, Sasanian G, Abdollahi M (2008) Tramadol intoxication: a review of 114 cases. Hum Exp Toxicol 27:201–205
10. Marquardt KA, Alsop JA, Albertson TE (2005) Tramadol exposures reported to statewide poison control system. Ann Pharmacother 39:1039–1044
11. Mugunthan N, Davoren P (2012) Danger of hypoglycemia due to acute tramadol poisoning. Endocr Pract 18:e151–e152
12. Taghaddosinejad F, Mehrpour O, Afshari R, Seghatoleslami A, Abdollahi M, Dart RC (2011) Factors related to seizure in tramadol poisoning and its blood concentration. J Med Toxicol 7:183–188
13. Rúa-Gómez PC, Püttmann W (2012) Occurrence and removal of lidocaine, tramadol, venlafaxine, and their metabolites in German wastewater treatment plants. Environ Sci Pollut Res 19:689–699
14. Ardakani YH, Foroumadi RA, Rouini MR (2008) Enantioselective determination of tramadol and its main phase I metabolites in human plasma by high-performance liquid chromatography. J Chromatogr B 864:109–115
15. Campanero MA, Garcia-Quetglas E, Sadaba B, Azanza JR (2004) Simultaneous stereoselective analysis of tramadol and its primary phase I metabolites in plasma by liquid chromatography: application to a pharmacokinetic study in humans. J Chromatogr A 1031: 219–228
16. Gu Y, Fawcett JP (2005) Improved HPLC method for the simultaneous determination of tramadol and O-desmethyltramadol in human plasma. J Chromatogr B 821:240–243
17. Musshoff F, Madea B, Stuber F, Stamer UM (2006) Enantiomeric determination of tramadol and O-desmethyltramadol by liquid chromatography-mass spectrometry and application to postoperative patients receiving tramadol. J Anal Toxicol 30:463–467
18. Sha YF, Shen S, Duan GL (2005) Rapid determination of tramadol in human plasma by headspace solid-phase microextraction and capillary gas chromatography–mass spectrometry. J Pharmaceut Biomed Anal 37:143–147
19. Leis HJ, Fauler G, Windischhofer W (2004) Synthesis of d1-N-ethyltramadol as an internal standard for the quantitative determination of tramadol in human plasma by gas chromatography–mass spectrometry. J Chromatogr B 804:369–374
20. Gambaro V, Benvenuti C, De Ferrari L, Dell'Acqua L, Farè F (2003) Validation of a GC/MS method for the determination of tramadol in human plasma after intravenous bolus. Il Farmaco 58: 947–950
21. Li J, Ju H (2006) Simultaneous determination of ethamsylate, tramadol and lidocaine in human urine by capillary electrophoresis with electrochemiluminescence detection. Electrophoresis 27:3467–3474
22. Flores JR, Nevado JJB, Salcedo AMC, Diaz MPC (2004) Development of capillary zone electrophoretic method to determine six antidepressants in their pharmaceutical preparations experimental design for evaluating the ruggedness of method. J Sep Sci 27:33–40
23. Abdellatef HE (2002) Kinetic spectrophotometric determination of tramadol hydrochloride in pharmaceutical formulation. J Pharm Biomed Anal 29:835–842
24. Nobilis M, Pastera J, Anzenbacher P, Svoboda D, Kopecky J, Perlik F (1996) High-performance liquid chromatographic determination of tramadol in human plasma. J Chromatogr B 681:177–183
25. Ebrahimzadeh H, Tamini Y, Sedighi A, Rouini MR (2008) Determination of tramadol in human plasma and urine samples using liquid phase microextraction with back extraction combined with high performance liquid chromatography. J Chromatogr B 863:229–234
26. Afkhami A, Ghaedi H, Madrakian T, Ahmadi M, Mahmood-Kashani H (2013) Fabrication of a new electrochemical sensor based on a new nano-molecularly imprinted polymer for highly selective and sensitive determination of tramadol in human urine samples. Biosens Bioelectron 44:34–40
27. Afkhami A, Khoshsafar H, Bagheri H, Madrakian T (2014) Preparation of $NiFe_2O_4$/graphene nanocomposite and its application as a modifier for the fabrication of an electrochemical sensor for the simultaneous determination of tramadol and acetaminophen. Anal Chim Acta 831:50–59
28. Abbastabar-Ahangar H, Shirzadmehr A, Marjani K, Khoshsafar H, Chaloosi M, Mohammadi L (2009) Ion-selective carbon paste electrode based on new tripodal ligand for determination of cadmium (II). J Incl Phenom Macrocycl Chem 63:287–293
29. Ganjali MR, Khoshsafar H, Faridbod F, Shirzadmehr A, Javanbakht M, Norouzi P (2009) Room temperature ionic liquids (RTILs) and multiwalled carbon nanotubes (MWCNTs) as modifiers for improvement of carbon paste ion selective electrode response; a comparison study with PVC membrane. Electroanalysis 21:2175–2178
30. Ganjali MR, Khoshsafar H, Shirzadmehr A, Javanbakht M, Faridbod F (2009) Improvement of carbon paste ion selective electrode response by using room temperature ionic liquids (RTILs) and multi-walled carbon nanotubes (MWCNTs). Int J Electrochem Sci 4:435–443
31. Afkhami A, Madrakian T, Shirzadmehr A, Bagheri H, Tabatabaee M (2012) A selective sensor for nanolevel detection of lead (II) in hazardous wastes using ionic-liquid/Schiff base/MWCNTs/nanosilica as a highly sensitive composite. Ionics 18:881–889
32. Bagheri H, Afkhami A, Shirzadmehr A, Khoshsafar H (2014) A new nano-composite modified carbon paste electrode as a high performance potentiometric sensor for nanomolar Tl(I) determination. J Mol Liq 197:52–57
33. Afkhami A, Madrakian T, Shirzadmehr A, Tabatabaee M, Bagheri H (2012) New Schiff base-carbon nanotube–nanosilica–ionic liquid as a high performance sensing material of a potentiometric sensor for nanomolar determination of cerium(III) ions. Sens Actuators B Chem 174:237–244
34. Bagheri H, Afkhami A, Saber-Tehrani M, Shirzadmehr A, Husain SW, Khoshsafar H, Tabatabaee M (2012) Novel sensor fabrication for the determination of nanomolar concentrations of Ce^{3+} in aqueous solutions. Anal Methods 4:1753–1758
35. Afkhami A, Bagheri H, Shirzadmehr A, Khoshsafar H, Hashemi P (2012) A potentiometric sensor for Cd^{2+} based on carbon nanotube paste electrode constructed from room temperature ionic liquid, ionophore and silica nanoparticles. Electroanalysis 24:2176–2185
36. Bagheri H, Afkhami A, Shirzadmehr A, Khoshsafar H, Khoshsafar H, Ghaedi H (2013) Novel potentiometric sensor for the determination of Cd^{2+} based on a new nano-composite. Intern J Environ Anal Chem 93:578–591
37. Afkhami A, Shirzadmehr A, Madrakian T, Bagheri H (2014) Improvement in the performance of a Pb^{2+} selective potentiometric sensor using modified core/shell SiO_2/Fe_3O_4 nano-structure. J Mol Liq 199:108–114
38. Afkhami A, Khoshsafar H, Madrakian T, Shirzadmehr A (2014) A new nano-composite electrode as a copper (II) selective potentiometric sensor. J Iran Chem Soc 11:1373–1380
39. Afkhami A, Shirzadmehr A, Madrakian T, Bagheri H (2015) New nano-composite potentiometric sensor composed of graphene nanosheets/thionine/molecular wire for nanomolar detection of silver ion in various real samples. Talanta 131:548–555
40. Shirzadmehr A, Afkhami A, Madrakian T (2015) A new nanocomposite potentiometric sensor containing an Hg^{2+}-ion imprinted polymer for the trace determination of mercury ions in different matrices. J Mol Liq 204:227–235

41. Bagheri H, Shirzadmehr A, Rezaei M (2016) Determination of copper ions in foodstuff products with a newly modified potentiometric carbon paste electrode based on a novel nano-sensing layer. Ionics 22:1241–1252
42. Shirzadmehr A, Rezaei M, Bagheri H, Khoshsafar H (2016) Novel potentiometric sensor for the trace level determination of Zn^{2+} based on a new nanographene/ion imprinted polymer composite. Intern J Environ Anal Chem 96:929–944
43. Soleimani M, Ghaderi S, Ghahraman Afshar M, Soleimani S (2012) Synthesis of molecularly imprinted polymer as a sorbent for solid phase extraction of bovine albumin from whey, milk, urine and serum. Microchem J 100:1–7
44. Hirata M, Gotou T, Horiuchi S, Fujiwara M, Ohba M (2004) Thin-film particles of graphite oxide 1: High-yield synthesis and flexibility of the particles. Carbon 42:2929–2937
45. Hummers WS, Offeman RE (1958) Preparation of graphitic oxide. J Am Chem Soc 80:1339–1339
46. Tien HW, Huang YL, Yang SY, Wang JY, Ma CCM (2011) The production of graphene nanosheets decorated with silver nanoparticles for use in transparent, conductive films. Carbon 49: 1550–1560
47. Seifi M, Hassanpour Moghadam M, Hadizadeh F, Ali-Asgari S, Aboli J, Mohajeri SA (2014) Preparation and study of tramadol imprinted micro-and nanoparticles by precipitation polymerization: microwave irradiation and conventional heating method. Int J Pharm 471:37–44
48. Khani H, Rofouei MK, Arab P, Gupta VK, Vafaei Z (2010) Multi-walled carbon nanotubes-ionic liquid-carbon paste electrode as a super selectivity sensor: application to potentiometric monitoring of mercury ion(II). J Hazard Mater 183:402–409
49. Abu-Shawish HM, Abu Ghalwa N, Zaggout FR, Saadeh SM, Al-Dalou AR, Abou Assi AA (2010) Improved determination of tramadol hydrochloride in biological fluids and pharmaceutical preparations utilizing a modified carbon paste electrode. Biochem Eng J 48:237–245
50. Abu Shawish HM, Saadeh SM, Al-Dalou AR, Abu Ghalwa N, Abou Assi AA (2011) Optimization of tramadol–PVC membrane electrodes using miscellaneous plasticizers and ion-pair complexes. Mater Sci Eng C 31:300–306